RECENT TRENDS IN DIAGNOSIS AND CONTROL OF IMPORTANT ZOONOSES AND FOODBORNE INFECTIONS

ICAR SUMMER SCHOOL
JUNE, 5-25, 2000

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Director
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Foreword

It is heartening to note that the Summer School on “Recent Trends in Diagnosis and Control of Important Zoonoses and Foodborne Infections” has got immense response for participation from Universities, State Departments, Public Health authorities, Research Institutes and Food quality control agencies. This, in itself, speaks the worth and utility of the course.

It is common knowledge that about 80% of our rural population is exposed to the risk of zoonotic diseases due to their intimate association with animal. Many zoonoses like Brucellosis, Tuberculosis, Rabies, Leptospirosis, Plague, Listeriosis and Colibacillosis etc. have caused great economic losses and heavy morbidity and mortality in human and animal population. Furthermore, some of the emerging viral zoonoses like Hanta virus and Yellow fever infections have great potential to cause epidemic in this region. Similarly, on food front, rapid industrialization, mass food processing, centralized production and lack of proper quality control has posed newer challenges to public health scientists and quality enforcement agencies. Environmental pollution due to non-biological contaminants is another area of immense significance to public health workers and the Faculty needs to be congratulated to have included this aspect also in their programme.

The major problems today, lies in confirmation of diagnosis and identification of weak areas of zoonoses control. There is utmost need of rapid and sensitive ‘on line’ testing procedure to meet growing challenges of foodborne infections. I am sure, the school will prove a milestone in providing latest trends in diagnosis and control of zoonoses and foodborne infections.

I am especially happy to note that Dr. V.N. Bachhili, Director, Summer School and the highly experienced faculty have brought out the compendium well in time. I am sure, the participants will be greatly benefitted by deliberations and practical demonstrations.

Izatnagar
June 5, 2000

(M.P. Yadav)
Director, IVRI
PREFACE

Between human and animal medicine, there is no dividing line—nor should there be. This age old observation by Rudolph Virchow has become more significant in recent times, when it has been realised that about 80% of all the described human infections are shared in nature between man and other vertebrate animals. Today more than 300 clinical entities are grouped as zoonoses. Emerging Zoonoses are further complicating and compounding animal and human health problems. Similarly foodborne infections and intoxications are other major problems of developing countries like India. Rapid industrialization of food processing has further added to the risks of foodborne hazards. Of all the foodborne diseases, bacteria alone account for 85% of the cases. Diarrhoeal diseases, which are mostly food and waterborne, take a toll of more than 7 lakh infants in India and the etiologies mostly remain obscure and not diagnosed. Thus we need to learn rapid diagnostic tests to exercise proper quality control to reduce foodborne hazards.

In recent times, another important group of non-biological contaminants has emerged as a major public health problem. Environmental pollution through pesticides, insecticides, other non-biological substances, poisonous metals and other toxic substances have serious public health implications. These have been given due emphasis in this programme.

Our competent faculty has taken lot of pains to formulate an appropriate and exhaustive syllabus covering about 30 lectures and 15 demonstrations. I am sure the participants will be greatly benefitted by this and it will also help in creating awareness about these problems.

I am extremely thankful to our Director, Dr. M.P. Yadav for his unabated encouragement for successful completion of the course. The contributions made by the guest speakers, scientists and other staff are gratefully acknowledged. I am extremely thankful to ICAR for financial grants without which it would not have been possible to conduct this Summer School.

Izatnagar
June 5, 2000

(V.N. Bachhil)
Director,
Summer School
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ZOONOSES: AN OVERVIEW

V.N. Bachhil, R. Banerjee and S. Ghatak

"Between animal and human medicine there is no dividing line—nor should there be. The object is different but the experience obtained constitutes the basis of all medicine" (Rudolf Virchow). The experience of zoonoses is one of the foundation of this comment because the agents of approximately 80% of all described human infection are shared in nature by other vertebrate animals. The origin of the word ‘Zoonoses’ could be traced back to Greek ‘zoon’ meaning animal, ‘nosos’ meaning disease and it was Rudolf Virchow who first used the term in 1855 in his famous “Handbook of Communicable Diseases” to describe the animal diseases secondarily transmissible to man. However, the definition of zoonoses, after a long journey through debates and discussions was finalised by WHO Expert Committee (1959) as “those disease and infections that are naturally transmitted between vertebrate animals and man”.

Impact of Zoonoses

At present more than 300 clinical entities are grouped as zoonoses. Although there is no systematic data on any zoonotic disease currently available to accurately evaluate its socio-economic impact in India zoonotic diseases are not new to this country. Plague killed 12 million people since 1898 (Shegal and Bhatia, 1981), rabies causes approximately 30000 deaths annually (WHO, 1999), brucellosis costs India at least Rs 350 million annually (Schwabe, 1984). Keeping in mind the vast array of zoonoses prevalent in India, these telling figures seems to be only the tip of the iceberg. It is not surprising because about 80% of our people lives in rural areas in close contact with animals. Furthermore, besides causing morbidity and mortality in humans, zoonoses cause considerable loss of livestock, dairy products and protein food by affecting animal population. Thus human health is inextricably linked to animal health and production.

Zoonoses Classification

A. Based on maintenance cycle in nature:

1. Direct zoonoses: require a single vertebrate species to be perpetuated, e.g. rabies, brucellosis.

2. Cyclozoonoses: Require more than one vertebrate species:
   i) Type I: obligatory cyclozoonoses, man must be one of the host e.g. taeniasis.
   ii) Type II: non-obligatory cyclozoonoses, man is sometimes involve e.g. hydatidosis.

3. Metazoonoses: Require both vertebrates and invertebrates and transmitted biologically by invertebrate vectors in which the agent multiplies (plague), develop (Malayan filaria) or both (Babesiosis).

Depending upon the hosts required, metazoonoses are type I requiring one vertebrate and one invertebrate (yellow fever); type II with one vertebrate and two...
invertebrates (Paragomimiasis); type III, two vertebrates and one invertebrate (chlonorchiasis); type IV representing transovarian transmission (tick borne encephalitis)

3. Saprozoanoses: Require an inanimate reservoir or development site as well as vertebrate Host.

Type I: The agent may propagate in non-animal site e.g. histoplasmosis.

Type II: The agent may undergo essential development only in non-animal site e.g. *Ancylostoma brasilense* infection.

Type III: The agent require an invertebrate host e.g. Fascioliasis.

B. Based on direction of transmission:

1. Anthropozaonoses: Animal to man transmission e.g. rabies

2. Zooanthroponoses: Man to animal transmission e.g. human tuberculosis.

3. Amphixenoses: Both way transmission e.g. streptococcus.

C. Based on infection sharing

1. Wild animal-man shared zoonoses e.g. brucellosis.

2. Domiciliated animal-man shared zoonoses e.g. plague.

3. Domestic animal-man shared zoonoses e.g. brucellosis.

Zoonotic diseases/ infections caused by various organisms are listed below (based on etiologic agents)

A. Zoonotic diseases/infections of bacterial origin:


B. Zoonotic diseases of viral origin

Herpes simplex infection, Herpes virus simiae Encephalomyelitis or B virus infection, Buffalo, camel, cow and monkey pox, Vaccinia virus infection, Bovine papular stomatitis, Orf, Pseudocow pox, Tonapox, Yaba pox, Venezuelan and haemorrhagic fever, Lassa fever, Lymphocytic choriomeningitis, Bolivian haemorrhagic fever, California encephalitis, Hanta virus haemorrhagic fever and Hanta virus pulmonary syndromes, Granjame disease, Crimean Congo haemorrhagic fever, Rift valley fever, Marburg virus disease, Ebola haemorrhagic fever, Japanese encephalitis, Kyasanoor Forest disease, Louping ill, Murray valley encephalitis. Omsk haemorrhagic fever, St. louis encephalitis, European tick borne and Russian spring summer encephalitis, Wessalbron disease, West Nile fever, yellow fever, Influenza, New caste disease, FMD,

C. Parasitic zoonoses

i) Protozoan: Amoebiasis, Babesiosis, Balantidiasis, Cryptosporidiosis, Giardiasis, Iodamoeba infection, Leishmaniasis (cutaneous, mucocutaneous and visceral), Pneumocystis pneumonia, Sarcosporidiosis, Simian malaria, Toxoplasmosis, Trypanosomiasis (African sleeping sickness and chagas disease).

ii) Trematodal zoonoses: Amphistomiasis, Clonorchiasis, Dicrocoeliasis, Echinostomiasis, Fascioliasis, Haplorchiasis, Heterophyiasis, Homasthla infection/Metagonimiasis, Metorchiasis, Paragonimiasis, Opisthorchiasis, Prohemistomiasis and Schistosomiasis.

iii) Cestodal zoonoses: Bertielliasis, Coenuriasis, Diphyllobothriasis, Dipyldiasis Hymenolepiasis, Hydatidosis, Hymenolepiasis, Hymenolepiasis, Inermicapsiferiasis, Mesocestoidiosis, Paragonimiasis, Onchocerciasis, Oesophagostomiasis, Oesophagostomiasis, Schistosomiasis.

iv) Nematodal zoonoses: Ancylostomiasis, Anisakiasis, Ascariasis, Capillariasis, (Dioctophymosis, Filariasis and Tropical eosinophilia), Dracunculiasis, Eosinoophilic meningocerebralitis, Gnathostomiasis, Gongylonemiasis, Lagochelias-cariasis, Cutaneous and visceral larva migrans, Loaasis, Mamomenoganiasis, oesophagostomiasis, Onchocerciasis, Physaloptera infection, Strongyloidiasis, Thelexiasis, Trichinosis, Hirudimiasis, Acanthocephalid infection.

v) Arthropodal zoonoses: Dust allergy (Dermatophagoides, Euroglyphus and Otosdesctus pp. etc.) Mange (Ascarasis), Myiasis, Pentastomidae, Scabies, Tick paralysis (Dermacentor, Ixodes etc.)

D. Mycotic Zoonoses: Actinomycosis, Adiaspiromycosis, Aspergillosis (Pneumomycosis), Blastomycosis, Candidiasis, Coccidioidomycosis, Cryptococcosis, Dermatophytosis, Histoplasmosis, Maduromycosis, Histoplasmosis-Nocardiosis, Phycomycosis (Mucor and Zygomycoses), Rhinosporidiosis, Ringworm (Dermatophytosis and Dermatomyositis) and Sporotrichosis.

E. Rickettsial Zoonoses: Ehrlichiosis, Q fever, Rickettsial pox, Rickettsial spotted Fevers (Indian, Siberian, Queensland and Rocky mountain spotted fevers), Rickettsial typhus (Murine, scrub and epidemic typhus caused by R. typhi cmooseri), R.tsutsumushami and R. prowazekii respectively

F. Chlamydial Zoonoses: Psittacosis (ornithosis in birds) caused by C. psittaci.

Emerging Zoonoses

Emerging Zoonoses are defined as those zoonotic diseases caused either by apparently new agents or by previously known microorganisms, appearing in places or species in which the disease was previously unknown (Neslin, 1992). Ecological changes, changes in human demographics and behavior, international travel and
trade, technology and industry microbial adaptation and change, breakdown of public health measures and deficiencies in public health infrastructures contribute in an interwoven ways to the emergence many new zoonoses. It has been estimated that out of 22 major etiologic agents causing infectious diseases identified between 1973 and 1994, 14 were zoonotic (Chomel, 1998). Furthermore, all the 4 newly discovered pathogenic viruses (Nipah virus, Australian bat lyssa virus, Menangla virus and Hendra virus) between mid-1994 and 1999 have been found to be zoonotic. Emerging zoonoses, thus, are continuously complicating and compounding the existing problems of zoonotic diseases.

Control strategies
1. Establishment of diagnostic services: - Clinical/pathological
   - Laboratory
   - Epidemiological
2. Control in animals
   - Control of animal populate
   - Reduction of susceptible
   - Maintenance of disease-free status
3. Control of vehicles
   - Establishment of food hygiene
   - Ensuring safety of other animal production
   - Safe disposal or use of animal carcasses and wastes
4. Prevention and treatment in man
   - Preventing infection
   - Diagnosing infections
   - Treating disease

AN ANNOTATED LIST OF ZOONOSES

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<td>Disease /Infection</td>
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<td>Anthrax</td>
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<tr>
<td>Arizona enterobacterial infections</td>
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<td>Bartonellosis</td>
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<td>Brucellosis</td>
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<tr>
<td>Campylobacter infection</td>
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<tr>
<td>Cat-scratch fever</td>
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<tr>
<td>Cholera</td>
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<tr>
<td>Clostridial diseases</td>
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## VIRAL ZOONOSES

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<th>Virus name</th>
<th>Disease/infection</th>
<th>Principal transmission mode(s)</th>
</tr>
</thead>
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<td><strong>DNA Viruses</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>F:Herpesviridae</td>
<td><em>Herpes simplex</em> virus type 1</td>
<td>Herpes Simplex infection</td>
<td>Contact (man-to-monkey)</td>
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<tr>
<td>Cercopithecid herpes</td>
<td><em>Herpesvirus simiae</em> virus 1(B)</td>
<td>Herpesvirus simiae encephalomyelitis B-virus infection</td>
<td>Contact-bite, scratch, droplet or (monkey-to-man)</td>
</tr>
<tr>
<td>F:Poxviridae</td>
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<tr>
<td>G:Orthopoxivirus</td>
<td><em>Buffalo pox virus</em></td>
<td>Buffalopox</td>
<td>Contact</td>
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<tr>
<td></td>
<td><em>Camelpox virus</em></td>
<td>Camelpox</td>
<td>Contact (milk vehicle ?)</td>
</tr>
<tr>
<td></td>
<td><em>Cowpox virus</em></td>
<td>Cowpox</td>
<td>Contact</td>
</tr>
<tr>
<td></td>
<td><em>Monkeypox virus</em></td>
<td>Monkeypox</td>
<td>Contact, inhalation (man-to-)</td>
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<td></td>
<td>Vaccinia virus (T)</td>
<td>Vaccinia virus infection</td>
<td>Contact</td>
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<td>G:Parapoxivirus</td>
<td><em>Bovine papular stomatitis virus</em></td>
<td>Bovine papular stomatitis</td>
<td>Contact</td>
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<tr>
<td></td>
<td><em>Orf virus (T)</em></td>
<td>Orf (contagious ecthyma; contagious pustular dermatitis; scabby mouth; sore mouth)</td>
<td>Contact</td>
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<td></td>
<td><em>Pseudocowpox virus</em></td>
<td>Pseudo cowpox (milker's module)</td>
<td>Contact</td>
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<td>G: Yatapoxivirus</td>
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<td>Tanapox</td>
<td>Contact</td>
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<td><em>Yaba monkey tumour virus (T)</em></td>
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<td>Unknown</td>
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<tr>
<td>F:Arenaviridae</td>
<td><em>Guanarito virus</em></td>
<td>Venezuelan haemorrhagic fever</td>
<td>Contact (saliva; faecal oral?)</td>
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<td>G:Arenavirus</td>
<td><em>Junin virus</em></td>
<td>Argentine haemorrhagic fever</td>
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<td><em>Lassa virus</em></td>
<td>Lassa fever</td>
<td>Contact (ingestion, inhalation) -do-</td>
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<td><em>Lymphocytic choriomeningitis virus</em></td>
<td>Lymphocytic choriomeningitis</td>
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<td></td>
<td><em>Machupu virus</em></td>
<td>Bolivian haemorrhagic fever</td>
<td>Contact</td>
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<tr>
<td>F: Bunyaviridae</td>
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<tr>
<td>(formerly Group C Arbovirus)</td>
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<tr>
<td>G:Bunavirus</td>
<td><em>California encephalitis virus</em></td>
<td>California encephalitis</td>
<td>Mosquito (Aedes spp.) (LaCrosse encephalitis)</td>
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<td>Virus name</td>
<td>Disease/infection</td>
<td>Principal transmission mode(s)</td>
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<tr>
<td><strong>G: Hantavirus</strong></td>
<td>Dobrava-Belgrade virus</td>
<td>Haemorrhagic fever with renal syndrome</td>
<td>Inhalation mainly; also through broken skin or conjuctiva, ingestion</td>
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<td></td>
<td>Hantaan virus (T)</td>
<td>(Korean haemorrhagic fever)</td>
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<td>Isla Vista virus</td>
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<td>Puumala virus</td>
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<td>Seoul virus</td>
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<td>Bayou virus</td>
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<td>Black Creek Canal virus</td>
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<td>New York Virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sin Nombre Virus (T)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>G: Nairovirus</strong></td>
<td>Nairobi virus</td>
<td>Nairobi sheep</td>
<td>Tick-bite (Haemaphysalis intermedia in India; Rhipicephalus appendiculatus in Africa)</td>
</tr>
<tr>
<td></td>
<td>(identical to Ganjan virus)</td>
<td>disease (Ganjam)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Crimean-Congo haemorrhagic fever virus</td>
<td>Crimean-Congo haemorrhagic fever</td>
<td>Tick-bite (Hyalomma spp.)</td>
</tr>
<tr>
<td><strong>G: Phlebovirus</strong></td>
<td>Rift valley fever virus (T)</td>
<td>Rift valley fever</td>
<td>Mosquitoes (Culex, Aedes, Mansonia, Anopheles)</td>
</tr>
</tbody>
</table>

- **F: Filoviridae**
  - **G: Marburg-like viruses**
    - Marburg virus (T)
    - Côte d'Ivoire Ebola virus
    - Reston Ebola virus
    - Sudan Ebola virus
    - Zaire Ebola virus (T)
  - **G: Ebola-like viruses**
    - Japanese encephalitis virus
    - Kyasanur forest disease virus
    - Louping ill virus
    - Murray Valley encephalitis virus
    - Omsk haemorrhagic fever virus
    - St. Louis encephalitis virus

- **F: Flaviviridae**
  - **G: Flavivirus**
    - formerly (Group B Arbovirus)
    - Kyasanur forest disease virus
    - Louping ill virus
    - Murray Valley encephalitis virus
    - Omsk haemorrhagic fever virus
    - St. Louis encephalitis virus
    - European (central) tickborne & Russian spring summer encephalitis

- **F: Bunyaviridae**
  - **G: Hantavirus**
    - Dobrava-Belgrade virus
    - Hantaan virus (T)
    - Isla Vista virus
    - Prospect Hill virus
    - Puumala virus
    - Seoul virus
    - Andes virus
    - Bayou virus
    - Black Creek Canal virus
    - New York Virus
    - Sin Nombre Virus (T)
  - **G: Nairovirus**
    - Nairobi virus
    - Crimean-Congo haemorrhagic fever virus
  - **G: Phlebovirus**
    - Rift valley fever virus (T)

- **F: Filoviridae**
  - **G: Marburg-like viruses**
    - Marburg virus (T)
    - Côte d'Ivoire Ebola virus
    - Reston Ebola virus
    - Sudan Ebola virus
    - Zaire Ebola virus (T)
  - **G: Ebola-like viruses**
    - Japanese encephalitis virus
    - Kyasanur forest disease virus
    - Louping ill virus
    - Murray Valley encephalitis virus
    - Omsk haemorrhagic fever virus
    - St. Louis encephalitis virus

- **F: Flaviviridae**
  - **G: Flavivirus**
    - formerly (Group B Arbovirus)
    - Kyasanur forest disease virus
    - Louping ill virus
    - Murray Valley encephalitis virus
    - Omsk haemorrhagic fever virus
    - St. Louis encephalitis virus
    - European (central) tickborne & Russian spring summer encephalitis
<table>
<thead>
<tr>
<th>Family</th>
<th>Genus</th>
<th>Virus Name</th>
<th>Disease</th>
<th>Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>F: Orthomyxoviridae</td>
<td>Orthomyxovirus</td>
<td>Influenza A virus (T)</td>
<td>Influenza</td>
<td>Airborne</td>
</tr>
<tr>
<td>G: Influenzavirus A</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>F: Paramyxoviridae</td>
<td>Paramyxovirus</td>
<td>Newcastle disease virus</td>
<td>Newcastle disease</td>
<td>Airborne</td>
</tr>
<tr>
<td>G: Rubulavirus</td>
<td></td>
<td></td>
<td></td>
<td>(or faecal contact)</td>
</tr>
<tr>
<td>F: Picornaviridae</td>
<td>Picornavirus</td>
<td>Foot-and-mouth disease virus (T)</td>
<td>Foot-and-mouth disease</td>
<td>Contact</td>
</tr>
<tr>
<td>G: Apthovirus</td>
<td></td>
<td></td>
<td></td>
<td>Food vehicle</td>
</tr>
<tr>
<td>G: Cardiovirus</td>
<td>Cardiovirus</td>
<td>Encephalomyocarditis virus (T)</td>
<td>Encephalomyocarditis</td>
<td>Food vehicle, faecal-oral</td>
</tr>
<tr>
<td>G: Hepatovirus</td>
<td>Hepatovirus</td>
<td>Hepatitis A virus</td>
<td>Hepatitis A</td>
<td></td>
</tr>
<tr>
<td>F: Reoviridae</td>
<td>Reovirus</td>
<td>Colorado tick fever virus</td>
<td>Colorado tick fever</td>
<td>Tick-bite</td>
</tr>
<tr>
<td>G: Collivirus</td>
<td></td>
<td></td>
<td></td>
<td>(Dermacentor)</td>
</tr>
<tr>
<td>G: Rotavirus</td>
<td>Rotavirus</td>
<td>Rotavirus A (T)</td>
<td>Rotavirus enteritis</td>
<td>Contact, vehicle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rotavirus B</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rotavirus C</td>
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<tr>
<td>F: Rhabdoviridae</td>
<td>Rhabdovirus</td>
<td>Australian bat lyssavirus</td>
<td>Rabies like disease</td>
<td>Bat-bites &amp; scratches</td>
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<tr>
<td>G: Lyssavirus</td>
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<tr>
<td></td>
<td>Duvenhage virus</td>
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<td>European bat lyssavirus</td>
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<td></td>
<td>European bat lyssavirus 1</td>
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<tr>
<td></td>
<td>European bat lyssivirus 2</td>
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<tr>
<td></td>
<td>Lagos bat virus</td>
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<tr>
<td></td>
<td>Mokola virus</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Rabies virus (T)</td>
<td></td>
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<tr>
<td></td>
<td>Chandipura virus</td>
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<tr>
<td>G: Vesiculovirus</td>
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<tr>
<td></td>
<td>Vesicular stomatitis</td>
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<tr>
<td></td>
<td>Indiana virus</td>
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<tr>
<td></td>
<td>Vesicular stomatitis</td>
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<tr>
<td></td>
<td>New Jersey virus</td>
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<tr>
<td>F: Togaviridae</td>
<td>Togavirus</td>
<td>Chikungunya virus</td>
<td>Chikungunya fever</td>
<td>Mosquito bite</td>
</tr>
<tr>
<td>G: Alphavirus</td>
<td></td>
<td></td>
<td></td>
<td>(Aedes spp.)</td>
</tr>
<tr>
<td>(formerly Group A Arbovirus)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Eastern equine encephalitis virus</td>
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<tr>
<td></td>
<td>Eastern equine encephalitis</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Mosquito-bite</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Culiseta spp., Aedes spp.)</td>
</tr>
<tr>
<td>Disease / Infection</td>
<td>Etiologic agent(s)</td>
<td>Principal transmission mode(s)</td>
<td></td>
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<tr>
<td>-------------------------------</td>
<td>------------------------------------------</td>
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<tr>
<td>Amoebiasis</td>
<td>Entamoeba histolytica</td>
<td>Vehicle (rarely animal to man)</td>
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<tr>
<td>Babesiosis</td>
<td>Babesia microti</td>
<td>Tick bite (Boophilus, Ixodes, Dermacentor)</td>
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</tr>
<tr>
<td></td>
<td>B. bosis</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>B. divergens</td>
<td></td>
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<tr>
<td>Balantidiasis</td>
<td>Balantidium coli</td>
<td>Ingestion</td>
<td></td>
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<tr>
<td>Cryptosporidiosis</td>
<td>Cryptosporidium spp.</td>
<td>Contaminated food and drink</td>
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<tr>
<td>Giardiasis</td>
<td>Giardia lamblia (intestinalis)</td>
<td>Ingestion, person-to-person, faecal-oral</td>
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<tr>
<td>Iodamoeba infection</td>
<td>Iodamoeba butchii</td>
<td>Vehicle</td>
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<tr>
<td>Leishmaniasis</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>* Cutaneous</td>
<td>Leishmania aethiopica</td>
<td>Sand fly (Lutzomyia) bite</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>L. major</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>L. mexicana</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>L. tropica</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Mucocutaneous</td>
<td>L. brasiiliensis</td>
<td>-do-</td>
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<tr>
<td>* Visceral</td>
<td>L. donovani</td>
<td>Sand fly (Phlebolomas spp. Lutzomyia spp.) -bite</td>
<td></td>
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</tr>
<tr>
<td>Pneumocystis pneumonia</td>
<td>Pneumocystis carinii</td>
<td>Aerosol ? (common in AIDS patients)</td>
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</tr>
<tr>
<td>Sarcosporidiosis (Sarcocystosis)</td>
<td>Sarcocystis hominis, Isospora hominis, S.suihominis</td>
<td>Ingestion of raw beef</td>
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<tr>
<td>Simian malaria</td>
<td>Plasmodium brasilianum</td>
<td>Mosquito bite (Anopheles)</td>
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<tr>
<td></td>
<td>P. simium</td>
<td></td>
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<tr>
<td></td>
<td>P. cynomaogi</td>
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<td></td>
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<tr>
<td></td>
<td>P. inui</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>P. knowlesi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P. schwetzi</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Newly recognised zoonotic agents Nipah virus, Menangle virus & Hendra virus proposed in Paramyxoviridae family.

**Parasitic Zoonoses**

**Protozoan Zoonoses**

<table>
<thead>
<tr>
<th>Disease / Infection</th>
<th>Etiologic agent(s)</th>
<th>Principal transmission mode(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayaro virus</td>
<td></td>
<td></td>
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<tr>
<td>Mayaro fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ross River virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidemic polyarthritis</td>
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<td></td>
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<tr>
<td>Sindbis virus (T)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sindbis fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venezuelan equine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>encephalitis virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venezuelan equine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>encephalitis virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western equine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Western equine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>encephalitis virus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Mansonla, Aedes, Culex)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Culex, Aedes spp.)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

T - Type species

* Newly recognised zoonotic agents Nipah virus, Menangle virus & Hendra virus proposed in Paramyxoviridae family.
<table>
<thead>
<tr>
<th>Disease / Infection</th>
<th>Etiologic agent(s)</th>
<th>Principal transmission mode(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphistomiasis</td>
<td>Gastrodiscoides</td>
<td>Snail (?)</td>
</tr>
<tr>
<td>Chlonorchiasis</td>
<td>Clonorchis sinensis</td>
<td>(raw or under cooked fish)</td>
</tr>
<tr>
<td></td>
<td>(Chinese liver fluke)</td>
<td></td>
</tr>
<tr>
<td>Dicrocoeliasis</td>
<td>Dicrocoelium dendriticum</td>
<td>Land snails, ants (ingestion of)</td>
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<tr>
<td>Echinostomiasia</td>
<td>Echinostoma spp.</td>
<td>Ingestion of uncooked/raw fresh water molluscs or fish</td>
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<tr>
<td>Fascioliasis</td>
<td>Fasciola hepatica</td>
<td>Ingestion of contaminated herbage</td>
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<tr>
<td></td>
<td>F. gigantica</td>
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<tr>
<td>Fasciolopsiasis</td>
<td>Fasciolopsis buski</td>
<td>- do -</td>
</tr>
<tr>
<td>Haplorchiasis</td>
<td>Haplorchis spp.</td>
<td>Snail vector</td>
</tr>
<tr>
<td>Heterophylasis</td>
<td>Heterophyes heterophyes</td>
<td>Ingestion of uncooked fish</td>
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<tr>
<td>Himasthla infection</td>
<td>Himasthla muchlensi</td>
<td>Snail</td>
</tr>
<tr>
<td>Metagonimiasis</td>
<td>Metagonimus yokogawai</td>
<td>Ingestion of undercooked fish</td>
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<td>Troglotrema salmincola</td>
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</tr>
<tr>
<td>Metorchiasis</td>
<td>Metorchis conjunctus</td>
<td>Snail</td>
</tr>
<tr>
<td>Paragonimiasis</td>
<td>Paragonimus westermanii</td>
<td>Ingestion of raw or improperly cooked fresh water crustaceans</td>
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<tr>
<td>(Lung fluke disease)</td>
<td>P. africanus</td>
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<td>P. mexicana</td>
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<tr>
<td>Nanophyetus infection</td>
<td>Nanophytus schikhalovii</td>
<td>Snail</td>
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<tr>
<td>Opisthorchiasis</td>
<td>Opisthorchis felineus</td>
<td>Ingestion of uncooked fish</td>
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<tr>
<td></td>
<td>(Cat liver flukes)</td>
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<td></td>
<td>O. viverrni</td>
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<tr>
<td></td>
<td>O. pseudofelineus</td>
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<tr>
<td>Prohemistomiasis</td>
<td>Prohemistomum vivax</td>
<td>Snail</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>Schistosoma haematobium</td>
<td>Waterborne</td>
</tr>
<tr>
<td>* Vesical or urinary schistosomiasis</td>
<td>S. mansoni</td>
<td>Waterborne</td>
</tr>
<tr>
<td></td>
<td>S. intercalatum</td>
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</tr>
<tr>
<td>* Intestinal schistomiasis</td>
<td>S. japonicum</td>
<td>Waterborne</td>
</tr>
<tr>
<td></td>
<td>S. mekongi</td>
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<tr>
<td>* Oriental or Asiatic schistosomiasis</td>
<td>Schistosome cercaria</td>
<td>Waterborne</td>
</tr>
<tr>
<td>* Schistome cercarial dermatitis (Swimmer’s itch)</td>
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</tr>
<tr>
<td>Disease / Infection</td>
<td>Etiologic Agent(s)</td>
<td>Principal transmission mode(s)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------------------------------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>Bertielliases</td>
<td>Bertiella studeri</td>
<td>Ingestion of infected mite</td>
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<td>B. mucronata</td>
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<tr>
<td>Coenurosis</td>
<td>Taenia multiceps</td>
<td>Ingestion</td>
</tr>
<tr>
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<td>T. serialis</td>
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<td>T. brazuni</td>
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<tr>
<td>Diphyllobothriasis</td>
<td>Diphyllobothrium latum</td>
<td>Ingestion of raw or undercooked fish / copepod crustacean</td>
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<tr>
<td></td>
<td>(Fish tape worm or broad tape worm)</td>
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<td>D. pacificum</td>
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<tr>
<td>Dipylliasis</td>
<td>D. caninum</td>
<td>Ingestion of dog or cat flea</td>
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<tr>
<td></td>
<td>(Dog tape worm)</td>
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</tr>
<tr>
<td>Hymenolepiasis</td>
<td>Hymenolepis nana</td>
<td>Ingestion of tape worm eggs</td>
</tr>
<tr>
<td></td>
<td>(Dwarf tape worm)</td>
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</tr>
<tr>
<td></td>
<td>H. diminuta</td>
<td>Ingestion of cysticercoids in flea, meal worms etc. in food</td>
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<tr>
<td></td>
<td>Mouse or rat tape worm</td>
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<tr>
<td>Hydatidosis</td>
<td>Echinococcus granulosus</td>
<td>Ingestion</td>
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<tr>
<td>(Echinococcosis)</td>
<td>E. multilocularis</td>
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<td>E. vogeli</td>
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<tr>
<td>Inermicapsiferiasis</td>
<td>Inermicapsifer madagaskariensis</td>
<td>Ingestion of infected arthropod</td>
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<tr>
<td>Mesocestoidiasis</td>
<td>Mesocestoides liniaitura</td>
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<tr>
<td></td>
<td>M. variabilis</td>
<td>Coprophagus arthropod vector (also carnivorous mammals as meat vehicle)</td>
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<tr>
<td>Raillietiniasis</td>
<td>Raillietina spp.</td>
<td>Ingestion of infected arthropod</td>
</tr>
<tr>
<td>Sparganosis</td>
<td>Diphyllobothrium spp.</td>
<td>Ingestion of infected cyclops</td>
</tr>
<tr>
<td></td>
<td>Spirometra spp.</td>
<td>on raw infected animal flesh</td>
</tr>
<tr>
<td>Taeniasis and cysticercosis</td>
<td>Taenia saginata</td>
<td>Ingestion of measly beef and measly park</td>
</tr>
<tr>
<td></td>
<td>(Beef tape worm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T. solium (Pork tape worm)</td>
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</tr>
</tbody>
</table>

### Helminthic (Nematodal) Zoonoses

<table>
<thead>
<tr>
<th>Disease / Infection</th>
<th>Etiologic Agent(s)</th>
<th>Transmission Mode</th>
</tr>
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<tbody>
<tr>
<td>Ancylostomiasis</td>
<td>Ancylostoma ceylanicum</td>
<td>Skin penetration</td>
</tr>
<tr>
<td>Anisakiasis</td>
<td>Anisakis marina</td>
<td>Marine fish vehicle</td>
</tr>
<tr>
<td></td>
<td>A. simplex</td>
<td></td>
</tr>
<tr>
<td>Ascariasis</td>
<td>Ascaris suum</td>
<td>Ingestion of soil, inhalation of dust</td>
</tr>
<tr>
<td>Capillariaasis</td>
<td>Capillaria philippinensis</td>
<td>Ingestion of infected fish (intestinal)</td>
</tr>
<tr>
<td></td>
<td>C. hepatica (hepatic)</td>
<td></td>
</tr>
<tr>
<td>Dioctophymosis</td>
<td>Dioctophyma renale</td>
<td>Ingestion of infected fish (Giant kidney worm)</td>
</tr>
<tr>
<td>Filariasis</td>
<td>Dirofilaria immitis</td>
<td>Mosquito bite</td>
</tr>
<tr>
<td></td>
<td>D. tenuis</td>
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<td></td>
<td>D. repens</td>
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<tr>
<td>Disease / Infection</td>
<td>Etiologic Agent(s)</td>
<td>Principal transmission mode(s)</td>
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<tr>
<td>-------------------------------------</td>
<td>----------------------------------------------------</td>
<td>----------------------------------------------------</td>
</tr>
<tr>
<td>* Malayan filariasis</td>
<td>Bridia malagi</td>
<td>-do-</td>
</tr>
<tr>
<td>* Tropical eosinophilia</td>
<td>Microfilaria of Bridia pahangi, Dirafilaria spp.</td>
<td>-do-</td>
</tr>
<tr>
<td>Dracunculiasis (Dracunciasis, Guinea worm disease)</td>
<td>Dracunculus medinensis (serpent worm)</td>
<td>Ingestion of infected cyclops in water</td>
</tr>
<tr>
<td>Eosinophilic meningocencephalitis</td>
<td>Angiostrongylus cantonensis</td>
<td>Ingestion of infected animal or secretion of crustacean and molluscs</td>
</tr>
<tr>
<td>Gnathostomiasis</td>
<td>Gnathostoma spinigerum</td>
<td>Ingestion of infected fish or amphibians</td>
</tr>
<tr>
<td>Gongylonemiasis</td>
<td>Gongylonema pulchrum</td>
<td>Ingestion of infected arthropods</td>
</tr>
<tr>
<td>Lagocheliasmariasia</td>
<td>Lagocheliascaris minor</td>
<td>Meat vehicle</td>
</tr>
<tr>
<td>Larva migrans</td>
<td>Larva of Ancylostoma brasiliense (mainly), A.caninum Uncinera stenocephala Bunostomum phlebotomum Strongyloides spp.</td>
<td>Contact with infected larvae (skin penetration)</td>
</tr>
<tr>
<td>* Cutaneous</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Visceral</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loaiasis</td>
<td>Loa loa</td>
<td>Deer flies</td>
</tr>
<tr>
<td>Mammonomonogamiasis (syngamosis)</td>
<td>Mammonomonogamus laryngeus</td>
<td>Ingestion of soil or paratenic hosts</td>
</tr>
<tr>
<td>Larva of Toxacara canis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. cati</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angiostrongylus cantonensis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loaiasis</td>
<td>Loa loa</td>
<td>Deer flies</td>
</tr>
<tr>
<td>Mammonomonogamus laryngeus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(syngamosis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Zoonoses Of Annelid Origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hirudiniasis</td>
<td>Limnatis allotica and other leaches</td>
<td>Direct contact</td>
</tr>
<tr>
<td>Acanthocephalid infection</td>
<td>Macrocanthorhynchus hirudinaceus</td>
<td>Coprophagus beetle vector</td>
</tr>
<tr>
<td>Oesophagostomiasis (Nodular worm disease)</td>
<td>Oesophagostomum spp. Terdens deminutes</td>
<td>Ingestion of soil or vegetations</td>
</tr>
<tr>
<td>Onchocerciasis</td>
<td>Onchocerca volvus</td>
<td>Bite of Simulium fly</td>
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<tr>
<td>Physaloptera infection</td>
<td>Physaloptera caucasica</td>
<td>Coprophagus insect vector</td>
</tr>
<tr>
<td>Strongyloidesis</td>
<td>Strongyloides stercoralis</td>
<td>Skin penetration</td>
</tr>
<tr>
<td>S. fuelleborni</td>
<td></td>
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</tr>
<tr>
<td>Thelaziasis</td>
<td>Thelazia callipeda</td>
<td>Fly vector</td>
</tr>
<tr>
<td>T. californiesis</td>
<td></td>
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<tr>
<td>T. rhodesi</td>
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</tr>
<tr>
<td>Trichinosis</td>
<td>Trichinella spiralis</td>
<td>Meat vehicle</td>
</tr>
<tr>
<td>Zoonoses Of Annelid Origin</td>
<td></td>
<td></td>
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<tr>
<td>Acanthocephalid infection</td>
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</table>
### Arthropod Zoonoses

<table>
<thead>
<tr>
<th>Disease</th>
<th>Etiologic Agent(s)</th>
<th>Principal transmission mode(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dust allergy</td>
<td><em>Dermatophagoides pteromissinu</em>&lt;sup&gt;*&lt;/sup&gt;, <em>D. farinae</em>&lt;sup&gt;**&lt;/sup&gt;, <em>Euroglyphus maynei</em>, <em>Oribatula sylvirum</em>, <em>Otodectes cynotis</em> and others</td>
<td>Aerosol</td>
</tr>
<tr>
<td>Mange (Acariasis)</td>
<td><em>Demodex</em> spp.</td>
<td>Contact</td>
</tr>
<tr>
<td>Myiasis</td>
<td><em>Sarcopotes</em> spp.</td>
<td>Contact</td>
</tr>
<tr>
<td></td>
<td><em>Cochliomyia hominivoran</em></td>
<td>Deposition on body of eggs by fly; ingestion</td>
</tr>
<tr>
<td></td>
<td>(screw worm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Calliphora</em> spp.</td>
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<tr>
<td></td>
<td><em>Callitrotis</em> spp.</td>
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<tr>
<td></td>
<td><em>Chrysomyia</em> spp.</td>
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</tr>
<tr>
<td></td>
<td><em>Codylora</em> spp.</td>
<td></td>
</tr>
<tr>
<td>Pentastomidiasis</td>
<td><em>Linguatula serrata</em></td>
<td>Meat or other vehicle</td>
</tr>
<tr>
<td></td>
<td><em>Armillifer</em> spp.</td>
<td></td>
</tr>
<tr>
<td>Scabies</td>
<td><em>Sarcoptes scabei</em></td>
<td>Contact</td>
</tr>
<tr>
<td>Tick paralysis</td>
<td><em>Envenomization of ticks like</em></td>
<td>Direct contact (attachment with ticks)</td>
</tr>
<tr>
<td></td>
<td><em>Dermacentor</em> spp.</td>
<td></td>
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<tr>
<td></td>
<td><em>Ixodes</em> spp.</td>
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<tr>
<td></td>
<td><em>Haemaphysalis</em> spp.</td>
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<tr>
<td></td>
<td><em>Rhinocephalus</em> spp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Argus</em> spp.</td>
<td></td>
</tr>
<tr>
<td>Tungiasis</td>
<td><em>Tunga penetrans</em> (Sand flea or zigger)</td>
<td>Skin penetration</td>
</tr>
<tr>
<td>Disease / Infection</td>
<td>Etiologic agent(s)</td>
<td>Principal transmission mode(s)</td>
</tr>
<tr>
<td>---------------------</td>
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</tr>
<tr>
<td>Ehrlichiosis</td>
<td>* Ehrlichia canis</td>
<td>Tick-bite</td>
</tr>
<tr>
<td></td>
<td>(Tropical canine</td>
<td></td>
</tr>
<tr>
<td>pancytopenia)</td>
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<td></td>
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<tr>
<td>Infectious nephrosis</td>
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<td></td>
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<tr>
<td>nephrosonephritis</td>
<td></td>
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<tr>
<td>Q fever</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rickettsial pox</td>
<td>* Rickettsia</td>
<td>Ticks, fleas</td>
</tr>
<tr>
<td>(Vesicular rickett-</td>
<td>pavlovskyi</td>
<td></td>
</tr>
<tr>
<td>siosis)</td>
<td></td>
<td>Contact; vehicle; vector</td>
</tr>
<tr>
<td>Rickettsial spotted</td>
<td>* Rickettsia</td>
<td>Mite (Allocreata sanguinei)</td>
</tr>
<tr>
<td>fever</td>
<td>akari</td>
<td>bite</td>
</tr>
<tr>
<td>* Boutonneuse fever</td>
<td>* R. conori</td>
<td>Ticks</td>
</tr>
<tr>
<td>(Indian tick typhus)</td>
<td></td>
<td>(Rhipicephalus spp.,</td>
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<tr>
<td></td>
<td></td>
<td>Boophilus spp., Ixodes spp.)</td>
</tr>
<tr>
<td>* North-Asian tick-</td>
<td>* R. siberica</td>
<td>-do-</td>
</tr>
<tr>
<td>borne rickettsiosis</td>
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<td></td>
</tr>
<tr>
<td>(Siberian tick typhus)</td>
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<td></td>
</tr>
<tr>
<td>* Queensland tick</td>
<td>* R. australis</td>
<td>Tick-bite (Dermacentor spp.,</td>
</tr>
<tr>
<td>typhus</td>
<td></td>
<td>Amblyomma spp., Rhipicephalus</td>
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<td></td>
<td></td>
<td>spp.)</td>
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<tr>
<td>Rickettsial typhus</td>
<td>* R. typhi (moose)</td>
<td>Flea-bite (Xenopsylla cheopes)</td>
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<td>* Murine typhus</td>
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<td></td>
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<tr>
<td>(Endemic typhus;</td>
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<td></td>
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<tr>
<td>rat typhus; urban</td>
<td></td>
<td></td>
</tr>
<tr>
<td>typhus)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Scrub typhus</td>
<td>* R. tsutsugamushi</td>
<td>Mite-bite</td>
</tr>
<tr>
<td>(Tropical typhus;</td>
<td></td>
<td>Leptotrombidium akamushi</td>
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<tr>
<td>flood fever; rural</td>
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<td>L. deliense</td>
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<tr>
<td>typhus)</td>
<td></td>
<td></td>
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<tr>
<td>* Epidemic typhus</td>
<td>* R. prowazekii</td>
<td>Squirrel fleas or ticks bite</td>
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<tr>
<td>(Classical typhus;</td>
<td></td>
<td></td>
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<tr>
<td>war fever; jail</td>
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<tr>
<td>fever)</td>
<td></td>
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<tr>
<td>Psittacosis</td>
<td>Chlamydia psittaci</td>
<td>Ingestion; inhalation</td>
</tr>
<tr>
<td>(ornithosis in birds)</td>
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**RICKETTSIAL AND CHLAMYDIAL ZOOLOSES**
References


EMERGING ZOONOTIC AND FOOD-BORNE DISEASES IN INDIA

R.K. Agarwal¹, V.N. Bachhila² and K.N. Bhilegaonkar³

The emerging disease may be defined as a disease whose occurrence has increased in past two decades or threats to increase in near future (Ledurbugal et al., 1992). The emergence of disease may be due to appearance of a completely new disease or entry of a known disease into a new geographic area. Emergence may also denote reappearance after a decline. There are over 3000 zoonotic and foodborne diseases of which some have emerged recently. Some of the emerging diseases important in Indian context are discussed below.

A. BACTERIAL

(1) Anthrax

Anthrax is an ancient, rapidly fatal infectious disease. In some tropical countries incidence is as high as 1,00,000 cases a year. Anthrax is enzootic among animals and endemic in certain areas of India. In recent years, the disease has shown increasing trends in human beings. Reports indicate that southern state especially the trijunctural zone of Andhra Pradesh, Karnataka and Tamil Nadu is the focus of emergence of human anthrax. An outbreak of anthrax affecting over 30 persons, in Chittoor, district of A.P., occurred in 1989 (Chandershakher et al., 1990). A recent hospital based study has indicated whereas only 12 cases were noticed between 1977 to 1993, 23 cases occurred in 1994-95 (John, 1996).

(2) Arcobacter

Arcobacter are Campylobacter like bacteria which are aerotolerant and grow at 150°C. There are three species linked to clinical illness. A. cryaerophilus, A. skirrowii and A. butzleri, of which the later may be primary pathogen. In human beings it causes enteritis whereas in animals it is associated with abortions and enteritis. Arcobacter spp. has been reported from wide variety of sources viz., faeces of calves with diarrhoea, cattle with mastitis, preputial swabs of boars, meat, chicken carcasses, turkey, tortoise and pork. Arcobacter may be transmitted by (i) water, (ii) food or (iii) person-to-person contact.

(3) Campylobacteriosis

Campylobacter in recent years has received worldwide recognition. The thermophilic campylobacters including C. jejuni and C. coli are now considered to be one of the most common cause of gastroenteritis. Other clinical manifestations associated with these organisms are Guillian Barre syndrome, meningitis and haemolytic ureamic syndrome. In India, the reports of C. jejuni and C. coli infections in man, animal reservoirs and foods have come from some places which provides fragmentary knowledge on the occurrence of this infection in this part of the world. In general isolation from poultry meat is higher than mutton (Table-1). Further, C. jejuni has been isolated in almost equal proportion from patients with diarrhoea as well as healthy persons.

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4. **Helicobacter pylori**

In 1983, Warren and Marshall reported an identified curved, spiral bacilli overlaying the gastric mucosa in stomach biopsy samples (Warren and Marshall, 1983), which was later identified as belonging to a new genus *Helicobacter*, with *H. pylori* as type species. Now *H. pylori* is well established human pathogen world over including India. It causes a spectrum of human gastrointestinal diseases viz. chronic intermittent vomiting, peptic ulcer, multifocal atropic gastritis-metaplasia-dysplasia gastric carcinoma complex and gastric lymphoma. Until 1994, the only recognized non-human reservoirs for *H. pylori* were macaque species, especially rhesus monkeys and possible baboons. But now it has been isolated from cats and also successfully transmitted to cats (Fuse et al., 1995). Natural transmission between man and animal is yet to be established.

5. **Klebsiella**

*Klebsiella*, an opportunistic pathogen has now emerged as leading cause of mortality in newborns, aged and immunosuppressed (Singh and Sharma, 1999). *Klebsiella* stands second to *E. coli* in causing diarrhoea in children and stands among top 5 killers of human race due to broncho pneumonia. The two important pathogenic species are *K. pneumoniae* and *K. oxytoca*. Contaminated foods, water, inanimate objects and hands of the carriers mainly transmit *Klebsiella*.

6. **Leptospirosis**

Leptospirosis is caused by *L. interrogans* whose principal reservoir is rodent. It is considered to be occupational disease in workers coming in contact with water contaminated with rodent urine. Tropical climate in India provides favorable conditions for the survival of the causative agent throughout the year. Its presence has been noted in India since the beginning of 20th century. However, in 1980’s it reemerged in many parts of South India (Madras and Kerala). A large outbreak of the disease occured in Andamans. Recently, the disease has been reported from Bulsar and Surat Districts of Gujarat.

7. **Listeriosis**

Listeriosis caused by *Listeria monocytogenes* is a serious invasive disease which may lead to septicaemia, abortion, still birth, meningitis and meningoencephalitis. The disease has now been recognized as an emerging foodborne zoonosis.

In India, the outbreak of listeriosis in animals have been reported from time to time by several workers. The reports on listeriosis cases in human are very scanty which seems to be due to lack of systemic work. However, in recent years, reports on isolation of *L. monocytogenes* from foods in India have started pouring in.

8. **Melioidosis**

Melioidosis is also recognized as occupational disease of those who work in moist soil. The disease is caused by *Burkholderia pseudomallei* which was earlier known as *Pseudomonas pseudomallei*. Reports of its occurrence in India have started trickling in from 1990s. As on today, the disease is considered to be endemic in Kerala, Tamilnadu, Maharasthra, Orissa, Assam, West Bengal and Tripura (John et al., 1996). Systemic studies are needed to unearth its real magnitude.
(9) Plague

Plague is a classical example of re-emergence of a disease due to ecological changes. The earthquake in Latur, Maharashtra in 1994 gave the opportunity for rodents to migrate from sylvatic area to human habitation, which resulted in outbreak of plague in certain parts of India. Thus outbreak occurred almost 25 years after the last report of human plague in 1967 (John, 1996) and caused an estimated loss of Rupees 900 crores. A continuos watch on the organism is required.

(10) Verotoxic Escherichia coli (VTEC)

VTEC has emerged as an important human pathogen producing conditions like haemorrhagic colitis (HC) or haemorrhagic enteritis and haemolytic uraemic syndrome (HUS). E. Coli 0157:H7 is considered to be prominent human EHEC serotype, though other serotypes may also play important role. EHEC has been reported from India from the cases of HUS (Kishore et al., 1992) as well as HC (Kulsherstha et al., 1994; Kapoor et al., 1995).

B. VIRAL

(1) Buffalo pox

Buffalo pox is an infectious emerging viral disease of persons coming in close contact with buffaloes. A single or multiple pocks on the hands and forearm manifest the disease. It was first reported in India in 1934 among buffaloes, since then it has been described from several states. Recent surveys by National Institute of Virology, Pune, indicate the presence of buffalo pox in certain parts of Maharashtra. A close watch is needed on the organism.

(2) Influenza

Influenza is considered to be most important emerging viral zoonoses. Among the three major varieties of influenza (A, B and C), A is most important. Genetic mutation is an important factor in perpetual emergence of the disease. Animals and birds act as host for influenza virus. An outbreak of influenza was recorded in Delhi in 1993 and Influenza A sub-type H3N2 virus was found to be responsible (Singh et al., 1994). Similarly, another outbreak occurred in Pune in 1996 (Rao et al., 1997).

(3) Japanese encephalitis

Japanese encephalitis (JE) has emerged as a major public health problem inflicting heavy losses of precious human lives in most countries of East and South Asia. Ever since the disease appeared in India, its epidemics have occurred in many parts of the country and the disease has become endemic in some of the states. The cases have been reported from Bihar, Uttar Pradesh, Madhya Pradesh, Karnataka, Tamil Nadu, Kerala, Goa and Pondicherry. Mammals and birds play the role in transmission but swine are the principal hosts. The principal vectors are Culex spp. including C. tritaeniorhyncus, C. vishnui and C. pseudovishnui in India. The epidemics of JE usually coincide with rainy season, the period of high mosquito prevalence. However, sporadic occurrence has also been observed throughout the year in endemic areas.
(4) **West Nile**

In India, serological evidence for the existence of West Nile virus came from the reports of Banker (1952) and Smithburn et al. (1954). Since then several strains of this virus have been isolated from mosquitoes and human (Banerjee, 1996). The infection is probably missed due to its immunological relationship with some of the other arboviruses prevalent in the country. The West Nile virus needs to be monitored effectively.

(5) **Dengue fever**

Two severe clinical manifestations of dengue fever are Dengue shock syndrome (DSS) and dengue haemorrhagic fever (DHF). Aedes aegypti is reported to be most important vector. Although the disease is known since the last century, recent outbreaks in different parts of India with haemorrhagic manifestations have raised new interest in the disease. All the four serotypes of dengue virus have been isolated in India.

(6) **Kyasanur forest disease (KFD)**

The KFD virus is ontogenetically and clinically related with Omsk haemorrhagic fever virus. The disease was first reported in the Kyasanur forest and Shimoga district of Karnataka state in 1983, presumably due to changes in the ecosystem of the forest which brought the infected ticks (H. spinigera) in contact with human population. The infection is maintained in monkeys.

(7) **Rift valley fever (RVF)**

RVF virus belongs to Phlebovirus group under Bunyaviridae family. The disease is wide spread in African continent. The presence of RVF was first time reported in India in 1990, from some parts of Rajasthan. Antibodies against RVF or a closely related virus was demonstrated by Joshi et al. (1995). An epizootic among sheep flock coinciding with febrile illness among shepherds in Tamilnadu was suspected to be RVF (Banerjee, 1996). A close watch is needed on the disease.

(8) **Hanta virus**

The Hanta virus was discovered in Korea (Lee et al. 1990). Since then viruses similar to Hanta virus have been isolated from different parts of the world. The haemorrhagic fever with renal syndrome caused by this group of viruses is fairly wide spread. The viruses are carried by different species of rodents and excreted through urine and faeces. Lee (1988) has described three epidemiological patterns, viz., rural, urban and animal house type. Thottapalyam virus belonging to Hanta-virus family has been reported from a shrew in South India (Banerjee, 1996).

(9) **Congo crimean haemorrhagic fever (CHF)**

The disease was first recorded in USSR. The virus isolation and serological surveys indicate wide distribution of the virus in parts of Africa, Europe and Asia. An outbreak has been reported in Pakistan. In India, antibodies against this virus have been demonstrated in man and domestic animals in Trivandrum, Pondicherry and Mysore. The vector ticks of genus *Hyalomma* plays an important role in transmission of CHF. These ticks are abundant in Indian subcontinent which indicate the need for caution.
(10) Ganjam virus

A virus identical to Nairobi sheep disease virus of Africa was isolated from adult *Haemaphsalis intermedia* ticks collected from sheep in Ganjam district of Orissa State (Dandawate and Shah, 1969) and laboratory staff working on the virus (Mohan Rao et al., 1981). The virus appears to be an important emerging zoonotic agent.

(11) Chandipura virus

Since the first isolation of Chandipura virus from a febrile patient at Nagpur (Bhat et al., 1967), the serological surveys conducted all over India indicated its wide spread presence in humans, horses, donkeys, cattle, sheep, goats and monkeys. The virus is transmitted by sandfly (Dhanda et al., 1970) and A. aegypti (Rao et al., 1967).

In addition to emerging diseases discussed above, certain other pathogens like multi-drug resistant tuberculosis, salmonellosis, methicillin resistant staphylococcosis etc. are also important. A close watch is also required for Lyme borreliosis and *Legionella* whose prevalence is not yet recorded in India.

There is a need to develop strategies to combat emerging diseases. Surveillance is of paramount importance to know the disease pattern. When a new disease is suspected, measure should be immediately instituted for its diagnosis and control. It requires research and development activities aiming at providing data base, reagents, specimen and serum banks. A close linkage between health and veterinary authorities, research institutions and field staff is essential.

REFERENCES


* * *


Effective communicable/zoonotic disease control relies on effective surveillance. A functional national communicable disease surveillance system is essential for action on important and priority zoonotic diseases. The present description on GIS and surveillance standards has been abstracted and reproduced from WHO Document No.CDS/CSR/ISR/99.2.

**Surveillance** is defined as “the continuous scrutiny of the factors that determine the occurrence and distribution of disease and other conditions of ill health. However WHO (1999) defined surveillance as the process of systematic collection, orderly consolidation and evaluation of pertinent data with prompt dissemination of results to those who need to know, particularly those who are in position to take action.

**Main objectives of surveillance are:**

a) To provide information about new and changing trends in the health status of population e.g. morbidity, mortality etc.

b) To provide feedback which may be expected to modify the policy and system.

c) Provide timely warning of public health disasters. So that interventions can be mobilized.

Thus surveillance requires professional analysis and sophisticated judgement of data leading to recommendations for control activity. Several countries have developed surveillance activities for communicable diseases to monitor diseases with high burden, to detect outbreaks of epidemic-prone diseases and to monitor progress towards national/international control and eradication targets. Thus surveillance of communicable diseases is a national function and all surveillance activities represent “National communicable disease surveillance system”.

**Multi-disease approach to surveillance:**

A multiple disease approach to communicable disease surveillance involves looking at all activities. These activities involve similar function and very often use the same structures, processes and personnel. In disease surveillance only that information is collected which is required to achieve the control objectives. The data required may vary from disease to disease.

Surveillance system can function as “Early warning system” if reporting confirmation, decision-making and response are rapid.

**The core functions** in surveillance are:

* Case detection
* Reporting
* Investigation and confirmation
* Analysis and interpretation

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The Support functions of in surveillance are:

* Setting of standards
* Training and supervision
* Setting up laboratory support
* Setting up communications
* Resource management
* Setting priorities

One of the most important components of national surveillance plans is making list of priority zoonotic diseases for surveillance. Close participation of national health/veterinary authorities is essential for making priority list. Various points to be considered while prioritizing list are:

* Does disease result in high impact eg. morbidity, mortality, disability
* Does it have epidemic potential
* Does this disease is a target of national, international control programme.

Once priority diseases have been selected, a plan of action for surveillance should be developed. An integrated approach to coordinate and streamline all surveillance activities is needed.

**Fig. 1: Schema for information flow in communicable disease surveillance**
Task at Peripheral level:

* Diagnosis and case management
* Reporting of cases
* Simple tabulation and graphing of data.

At peripheral level in addition to public health and veterinary officers, role of NGOs, working in fields, as well as role of private sector have become increasingly important and they must be involved in surveillance plan.

Tasks at intermediate level:

* Case management which cannot be done at peripheral level.
* Analysis of data from the peripheral level for
  - Epidemiology
  - Trends
  - Achievement of control targets
* Provision of supportive laboratory data (or laboratory diagnosis if possible)
* Investigation of suspected outbreaks
* Feedback of information to the peripheral level
* Reporting of data and suspected/confirmed outbreaks to central level.

Tasks as Central level:

* Overall support to and coordination of national surveillance activities
* Provision of laboratory diagnosis data, if not available at intermediate level (Use regional or international reference laboratories, if required)
* Analysis of data from intermediate level for
  - Epidemiological links
  - Trends
  - Achievement of control targets
* Support to intermediate level for outbreak control
  - Case management
  - Laboratory
  - Epidemiology
  - Education
  - Logistics
* Field back to intermediate level and possibly to peripheral level
* Report to WHO
* Collaboration of various sectors such as agricultural, veterinary medicine and environmental sciences

Sample format for surveillance standards for various diseases:

* ICD code and disease name
* Rationale for surveillance
  - Importance of disease in short
* Recommended case definition
  - Clinical cases definition
  - Lab. Criteria for diagnosis
  - Case classification
  - Suspect, Confirm etc.
* Recommended types of surveillance
  - Routine surveillance/active/sentinel surveillance etc.
  - Immediate case reporting
  - Aggregated data reporting
  - International reporting etc.
  - Weekly, monthly reporting
* Recommended minimum data elements
  - Case base data
  - Age
  - Sex
  - Geographical information
  - Outcome
* Recommended data analysis, presentation, reports
  - Case fatality rates (Graphs)
  - Geographical location (Maps)
  - Comparison of various periods/seasons etc.
Principal uses of data for decision making

- Detection of outbreak, incidence etc.
- Timely investigation
- Spread and progress of disease assessment.
- Treatment planning.
- Effectiveness of control measures

Special aspects

- Any specific recommendation for disease

Contact information

- Regional offices (WHO)
- Headquarters etc.

Summary of South-East Asia regional plan for communicable disease surveillance:

The region has adopted an integrated approach to combat communicable diseases of public health importance.

The regional priorities include:

The eradication or elimination of diseases such as dracunculiasis (India), leprosy and poliomyelitis in Region.

Reducing the burden of malaria and tuberculosis.

Intensifying the prevention and control efforts for communicable diseases that are major public health problems in the Region, through the establishment of appropriate national and regional surveillance mechanisms.

Strengthening of surveillance and monitoring of priority communicable diseases with a potential to spread rapidly and organizing an effective response to these disease outbreaks/epidemics through planning, monitoring and evaluation of control programmes.

The following are regional strategies for the coming years towards the prevention and control of communicable diseases:

1. Strengthen epidemiological surveillance
2. Strengthen laboratory capabilities and services
3. Establish rapid response mechanisms
4. Monitor anti-microbial resistance
5. Establish international disease surveillance networking
6. Ensure advocacy and mobilization of international support.
Geographic information system for epidemiological surveillance

Special analysis and mapping in epidemiology have long history. However, recent advances in geographical information and mapping techniques and increased awareness have created new opportunities for public health administrators to enhance their planning, analysis and monitoring capabilities.

The late 1950s have seen a significant expansion in information and mapping technology including development of desktop mapping software, new programming tools and increasing connectivity to Global information superhighways such as World wide web/internet.

Use of GIS in epidemiological surveillance

Geographical information systems and maps are valuable in strengthening the whole process of epidemiological surveillance information management and analyses.

In data collection

- A GIS provides an excellent means of collecting, updating and managing epidemiological surveillance and related information. A GIS can store, handle and geographically integrate large amount of information from different sources, programmes and sectors.

In data management

- A GIS serves as a common platform for convergence of multi-disease surveillance activities. Standardized geo-referencing of epidemiological data facilities standardized approaches to data management. As such, a GIS can serve as an entry point for integrating disease surveillance activities where appropriate.

- A GIS facilitates the convergence of multi-sectoral data, including epidemiological surveillance information, population information, environmental information and health and other resources into a common platform for analyses.

In data analysis

- A GIS provides an excellent means of visualizing and analyzing epidemiological data, thus revealing trends, dependencies and interrelationship that would be more difficult to discover in other formats.

Functions of a GIS

A GIS can help answer specific questions and perform the following functions:
* generate “thematic” (ranged colour maps or proportional symbol maps to denote the intensity of a mapped variable);
* allow for overlaying of different pieces of information;
* create buffer areas around selected features (for example a radius of 10 km around a health center to denote a catchment area or 1 km around a water point or school).
Carry out specific calculations (the proportion of the population falling within a certain radius of a health facility, school, dam etc.)

Calculate distances (e.g. the distance of a community to a health facility)

permit a dynamic link between databases and maps so that data updates are automatically reflected on the maps;

permit interactive queries of information contained within the map, table or graph.

Process images such as aerial or satellite images to allow information such as temperature, rainfall, soil types, and land use to be easily integrated and spatial correlation between potential risk factors and the occurrence of disease to be determined.

provide a range of extrapolation techniques (for example, extrapolating sentinel site surveillance to unsampled areas.

**Samples GIS applications in public health**

- Determining geographical distribution and variation of disease (prevalence, incidence)
- Analyzing spatial and longitudinal trends
- Mapping populations at risk
- Stratifying risk factors
- Assessing resource allocation (health services, schools, water points)
- Planning and targeting interventions
- Forecasting epidemics
- Monitoring diseases and interventions over time.

**REFERENCES**

In the modern era, promoting the health and improving the quality of life has become an essential need. Zoonotic and food-borne diseases in particular call for a concerted attention as they account for high morbidity and mortality apart from the great economic losses in animal sector.

From time immemorial, hundreds of zoonotic infections like rabies, plague, bovine tuberculosis, anthrax, brucellosis, salmonellosis, typhus, hydatidosis etc. are known to be transmissible between vertebrate animals and man. To this list, recently Kyasanur Forest disease, monkey pox have also been added. Also several diseases are contracted from ingested food. These include staphylococcal gastroenteritis, poisoning due to Clostridium perfringens, C. botulinum and Bacillus cereus, listeriosis, salmonellosis, shigellosis, coli bacillosis, vibriosis, campylobacterial infections, Yersinia infections and food-borne viral and parasitic infections.

It is quite essential to detect infectious agents in the various sources-food or other animal sources, so that measures for effective public health programmes could be set up. With the advancement of science, various molecular approaches are becoming handy tools in the detection of zoonotic and food-borne infections.

Over the past decade, molecular biotechnology derived diagnostic methods have come up. Use of restriction enzymes that cut DNA consistently at specific nucleotide sequences, polymerase chain reaction (PCR) amplification of known DNA sequences, DNA probes with specific sequences that will bind to and identify any complementary DNA sequences and species specific monoclonal antibodies allow a confirmatory diagnosis of infections.

1. **Nucleic acid (DNA) probes**

DNA probes occupy an important niche in many diagnostic laboratories. They often decrease the time necessary to identify fastidious microorganisms.

A DNA probe consisting of labelled (isotopic/non-isotopic) specific DNA sequence of an organism of interest is used to detect the organism in the test samples. The radiolabelled DNA probe has sensitivity level to detect up to 102 organisms. DNA probes have been prepared for the detection of a number of infectious agents including Salmonella spp., Listeria spp., Staphylococcus spp., C. perfringens, enterotoxigenic Escherichia coli and Vibrio cholerae etc. Such assays could prove of particular value in investigations of food-borne diseases.

The DNA probes are used in colony hybridization, dot-blot hybridization or in-situ hybridization to detect pathogenic organisms in food and animal tissue samples. Although a number of commercial probe kits have come up, sensitivity has been the drawback to the most wide spread use of probe technology.
2. **Polymerase Chain Reaction (PCR)**

It is *in vitro* nucleic acid amplification technique involving the enzymatic duplication and amplification of specific nucleic acid sequences. It is designed to amplify a nucleic acid target sequence from a particular organism or group of organisms. Thus, only the pathogen of interest is selectively identified.

By using thermostable DNA polymerase and 5' and 3' specific primers in a cyclic reaction a single specific DNA fragment can be amplified. PCR has been employed to detect enterotoxigenic *E. coli* strains, *Listeria monocytogenes*, *Mycobacterium bovis*, *Clostridium perfringens*, *Rabies virus*, *Toxoplasma gondii* etc. PCR is fast becoming a powerful and sensitive tool in detection of infections in host tissues and disease transmitting vectors. Even when small number of host cells or food are infected PCR can specifically target and amplify a gene sequence of the targeted pathogen.

However, PCR method is subject to false positive results due to amplification product carryover. So the application of some form of amplification product inactivation will probably be necessary.

The ability to provide more definitive diagnostic information and to greatly speed up the diagnosis of slow growing or uncultured organisms ultimately will have a profound effect on understanding of infections.

### 3 Other DNA based tests

Nucleic acid sequencing provides a method of identifying microorganisms on the basis of actual sequence of their genome. When coupled with PCR, it can provide a sensitive and specific diagnostic test.

Tools like restriction fragment length polymorphism (RFLP), single strand confirmation polymorphism (SSCP) and randomly amplified polymorphic DNA (RAPD) though are basically used for typing organisms, some of the markers derived from these are quite useful in identifying specific pathogenic infections.

These nucleic acid - based typing methods have proved to be extremely useful for epidemiological investigations of diseases.

### 4 Monoclonal antibodies (Mabs)

These are epitope specific and can be authentically utilized to look for any pathogenic contamination or toxins in food samples. Mabs are being effectively utilized to identify proteins associated with specific pathogens in enzyme linked immunosorbant assay. Mabs are helpful in detection of specific antigens or antibodies in tissue samples. Recently, Mabs have been produced for specific identification of *Brucella* spp., *Mycobacterium* spp., *Leptospira* spp., *Yersinia*, *Salmonella enteriditis* etc.

Once the Mab reagents are prepared and standardised, diagnostic procedures based on Mab technology is extremely simple and can be done easily in a minimally equipped laboratory.

Diagnostic methods based on nucleic acid detection and monoclonal antibodies
have certainly increased sensitivity and specificity and even the rapidity of diagnosis for most routinely encountered pathogens. These molecular diagnostic approaches could be invaluable as confirmatory tools in diagnostics. These tools may not be ideal in present situation due to demanding expertise and cost input or applicability to large number of samples. Though initial use of these methods may be costly and limited to larger diagnostic centres, their eventual broad-based acceptance will decrease the associated costs via economies of scale, and increasing automation of sample processing will greatly decrease the associated labour.
PLAGUE

[Black death, Mad rat disease, Mahamari (in northern India), Pest]

S.V.S. Malik¹ & V.N. Bachhi²

I. DEFINITION: Plague, an acute infectious disease which principally affects wild, peridomestic and the commensal rodents, is caused by Yersinia pestis and transmitted to man by the bite of an ectoparasite of rodents, the rat flea.

According to the definition given by WHO, ‘Wild or Sylvatic plague’ is the plague existing in nature independent of human population and their activities, and ‘domestic plague’ is the plague that is intimately associated with man and rodents living with man and has a definite potential of producing epidemics’. In ‘enzootic plague’ a focus of the disease may apparently remain static for years and the bacteria keep on passing from rodent to flea and flea to rodent without variation. In ‘epidemic plague’ the disease spreads rapidly and widely causing many deaths among rodents whenever the balance between the susceptible and resistant rodents get upset.

II. ETIOLOGY: The causative agent of plague - Yersinia pestis (group 5, subgroup 1, Family - Enterobacteriaceae, Bergey’s Manual, 9th ed.) is a Gram-negative, non-motile, non-sporing, short, thick coccobacillus with rounded ends. The pathogen measuring 1.5-2x0.5-0.7 μm shows characteristic bipolar staining giving itself a safety-pin like appearance, and also produces capsule in living tissues. Alexander Yersin and Kitasato for the first time in 1894 identified the bacillus independently from plague cases in Hong Kong.

III. EPIDEMIOLOGY:

A. Distribution of disease:

1. In animals: Plague infects and kills a wide host range in animal kingdom, however, not much information is available on its status in animals except few reports including that from cats in US in 1979. Reports on seropositivity of goats, sheep and camels for plague also appeared in 1980 from Libya. The evidence is available to support the views that wild rodent plague exists in USA, Brazil, Peru, Venezuela, central and south Africa, Iran, Russia and India; and that recently it is spreading in the parts of USA (WHO, 1998).

In India, rodents from Kolar (Maharashtra) in 1979 and dogs from Beed (Maharashtra) during 1994 epidemic were reported seropositive for plague.

2. In man: The disease is worldwide, and for centuries it has caused pandemics. The first pandemic began in 542 AD at lower Egypt which spread to Asia and Europe; the second pandemic started in 1347 AD from Jaffa and spread to China, India and also to Europe; the third and last pandemic started from Yunnan in China in 1860, spread to India by 1896 and also to other countries including north and south Africa, Europe, Japan, Australia and Philippines. During 1978-92 as many as 14,856 cases of plague with 145 deaths were notified by 21 countries to the WHO, and even in 1992, 9 countries notified a total of 1582 cases of human plague including 138 deaths. Brazil, Madagascar,
Myanmar, Tanzania, Vietnam and the USA reported cases of plague nearly every year.

The global threat of plague has declined in the last four decades. However, cyclical epidemics still occur and, with a total of 290 new cases of human plague including 140 deaths notified to WHO in 1995, some countries in Africa, the Americas and Asia reported cases almost every year (WHO, 1998). Plague may also occur as per saltum infection in which a focus of plague suddenly appears several miles away from the primary focus because of the transportation of rat or rat-fleas by any means of communication, or by human beings infested with rat fleas.

In India, plague is a disease of antiquity and finds mention in Bhagwat Purana. The great epidemic of disease was recorded during the reign of Emperor Jahangir in 1612 in Agra (U.P.), and an estimated 12.5 million deaths occurred during third pandemic. The last case of human plague was reported from Kolar district in Maharashtra in 1966. But suddenly in 1994 (August to September) two epidemics were reported, one of bubonic plague in the Beed district of Maharashtra following an earthquake in September, 1993 and, the other of pneumonic plague in Surat district of Gujarat following a flood in September, 1994. A total of 876 cases of presumptive plague were diagnosed in both the episodes, and out of total 54 fatalities, 52 were in Surat. The re-emergence of plague after a gap of 28 years conforms to the cyclic nature of this disease. Outbreaks suspected to be of plague have also been reported from Himachal Pradesh and Bihar. The sylvatic foci of plague in the country are located in the Deccan plateau (Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu); in foot hills of the Himalaya (Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh and Bihar) and; in the watersheds of Vindhya, Bhanier and Maikal ranges (Madhya Pradesh).

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<th>TABLE 6: MORTALITY DUE TO PLAGUE IN INDIA</th>
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**Influencing factors:** A combination of relatively low temperature (20-25°C) and high humidity (60%) seems to favour the survival of the pathogen and, in turn, the epidemic. Recent epidemic of plague in India, which occurred during monsoon, might be attributed to the earthquake that forced rodents to come out
of burrows and invade the human habitat, and breakdown of public health measures. However, factors like increase in the tourism, greater frequency of national and international travel, explosive urbanization with overcrowding and, associated insanitary conditions attracting rats also contribute to the occurrence and fast spread of plague.

C. **Host range and reservoirs:** Rodents, mostly of genus- *Rattus* and *Bandicota* serve as the primary host, however, at least 220 species of rodents inhabiting mountains, plains, steppes, deserts, cultivated fields and forests in both temperate and tropical climates are known to be infected with *Y. pestis*. The disease has got a wide host range as more than 200 animal species have been found to be infected with the pathogen. Cats, pigs, cattle, sheep, goat, horses, camel, kangaroo, donkey, bat, baboon, deer, antelope, coyotes, ground squirrels, rock squirrels, parairie dogs, rabbits and chipmunks may be the accidental host. Guinea pigs are susceptible to experimental infection. Many carnivores rarely develop illness after ingesting the plague infected rodents or the illness may be of self-limiting nature, which results in antibody production. Dogs may become infected without showing any sign of illness, act as sentinel animals, and their seropositivity may be indicative of plague prevalence in an area. Like dogs, cats may also serve as sentinels but they differ in becoming ill.

In India, rodent fleas (*Xenopsylla* spp.) rodents and Indian gerbil (*Tatera indica*) act as reservoir of the infection.

D. **Sources and transmission:** Plague, primarily a disease of rodents, is mainly transmitted through bites of infected fleas, however, man and animals get infected accidentally through interruption of rodent-flea-rodent cycle. Once the host dies of plague, the infected flea leaves it and attacks a fresh host in search of food (blood). Meanwhile the plague bacilli multiply in the proventriculus of infected flea often blocking it completely. A hungry flea in a state of frenzy attacks new host vigorously, however, the act of sucking only leads to distention of already blocked oesophagus and, in turn, to regurgitation of blood in wound, thereby, spreading the infection.

1. **In animals:** The disease spreads from rat-to-rat only through fleas and not by contact, however, it is thought that rats may pick up the infection by feeding on their dead companions. Similarly, other animals also get infection through bite of infected fleas. The camels have been reported to get the infection through ingestion of contaminated hay. Cats may contract the disease by eating the infected rodents.

2. **In man:** The spread of human plague always starts from the plague focus and involve one of the following established routes: (i) Domestic rodent- rat flea - man. This is the basic cycle of epidemic bubonic plague, (ii) Wild rodents - rat flea/direct contact - man. This is an accidental infection, particularly in the high-risk groups, (iii) Wild rodents - rat flea - domestic rodent - rat flea - man. This is invasion of sylvatic plague to man, (iv) Man - human flea - man. The human flea, *Pulex irritans* has been incriminated in certain cases of interhuman transmission as reported in Munchuria, (v) Man - man by droplet infection. This occurs in primary pneumonic plague, (vi) Accidental transmission to man while handling infected animals, particularly the rats during skinning and trapping, (vii) Ingestion
of contaminated animal tissues, however, it is of minor importance. (viii) Use of unlaunched, dirty clothings infested with fleas, (ix) Bites of ticks, lice and bed bugs, (x) Bites and scratches of infected cats.

In India, 3 species of the rat fleas namely Xenopsylla cheopis, X. astia, X. brasiliensis and the gerbil flea - Nosopsyllus nilgeriensis act as vectors of the disease.

IV. PATHOGENESIS: The number of bacilli required to initiate an infection has been reported to be 3 to 50 cells. The virulence of the pathogen depends upon the presence of a 70-75 kb plasmid which possess genes encoding for a set of virulence and virulence accessory factors.

V. DISEASE IN ANIMALS: Most of the animals including dogs, sheep, goats do not show any clinical symptoms of plague, however, rats develop buboes. Camels suffer with mild illness along with buboes in the neck region. The infected cats characteristically show profound illness with swelling on the submandibular lymphnodes, swelling below the eyes, general lethargy, profuse sneezing with purulent exudate and rise in body temperature. During prolong illness the lymphnodes may become haemorrhagic, necrotic, oedematous and suppurative yielding creamy pus on rupture. The clinical course ends in death or improves within 7 days.

VI. DISEASE IN MAN: Clinically, various forms exist - following an incubation period of 1-7 days. The usual one is the bubonic form which occurs when plague bacilli after entering through the flea bite into subcutaneous tissue are transported via the lymphatic vessels to proximal lymphnodes where they get colonize causing swelling (bubo(-)), usually in the groin(s) as flea-bites are common on the lower extremities; less common sites are the axilla and neck. The fatality rate in untreated cases is 50-70%. The septicaemic form results from invasion of the blood stream by the bacilli, which spread to the whole body and finally localize in spleen, liver and lungs. It is associated with rapidly progressive fever, hypotension and high mortality. The pneumonic form occurs at terminal phase of septicaemic form when bacilli reach to the lungs and multiply causing cough, haemoptyses and other respiratory symptoms which facilitate spread of pathogen to other persons through droplet nuclei. This form of the disease is very severe because of (i) the absence of clinical symptoms like bubo, (ii) the extremely efficient mode of transmission, and (iii) the rapidity of fatal outcome. The fatality rate in septicaemic and pneumonic forms may reach 100% if untreated, but treated plague has a 90-95% survival rate. Unusual forms are the meningeal form in which fever and neck rigidity dominate the clinical scenario, and the cutaneous form in which pustule, eschar, carbuncle or ecthyma gangrenosum accompanies a bubo.

VII. DIAGNOSIS:

A. In animals: (i) The presumptive diagnosis of plague in rodents is made on the basis of (i) important clinico-pathological features, such as bubo, s/c and general congestion, granular liver, congested spleen and pleural effusions, (ii) demonstration of plague bacilli with characteristic safety pin appearance in smears prepared from clinical or morbid specimens, flea, or soil from burrows.
The smears are stained with Wayson stain (the choicest stain) because Giemsa stain is unsatisfactory for this purpose. FAT employing antibodies against fraction 1 (F1) antigen, which is produced by the pathogen during its growth inside the host but not in the body of flea, can also be used for examining the tissues, (iii) isolation of the pathogen from clinical or morbid specimens (bubo aspirate, blood, tissues from spleen or liver), flea or soil on selective medium like cefsulodin - irgasan - novobiocin (CIN) agar following an incubation of plates at 25-28°C (because 37°C is growth inhibitory) for 48 h. The clinical specimens for this purpose should be collected in Cary Blair medium (a transport medium in which Y. pestis can survive up to 72 h) whereas fleas are collected in 2.5% sterile saline solution, and (iv) serological examination by tests like passive haemagglutination (PHA) and passive haemagglutination inhibition (PHI) which have been recommended by WHO for screening the sera for antibodies against F1 antigen.

Some of the other available tests include complement fixation test (CFT) and ELISA for anti-fraction 1 antibodies in sera as well as for F1 antigen in host tissues. The animal plague cases can also be diagnosed on similar lines.

(ii) The confirmatory diagnosis of plague includes examining the test isolate for the characteristic strain morphology and biochemical properties, lysis by specific bacteriophage, staining with fluorescent antibody employing conjugate for Y. pestis F1 antigen and, characteristic lesions in inoculated laboratory animals such as white mice, guinea pigs, white rats and rabbits.

B. In man: The diagnosis of human plague is made on the similar lines as outlined in case of animals, and includes (i) clinical features suggestive of plague - fever, adenitis, buboes, besides rusty (blood stained) sputum with respiratory distress seen in pneumonic plague, (ii) demonstration of the pathogen in clinico-pathological material including sputum, CSF or throat specimens besides those mentioned earlier in case of animals, (iii) isolation of the etiological agent on CIN agar, (iv) laboratory animal inoculation with clinico-pathological material. The guinea pigs which are the animals of choice for inoculation, in positive cases, become very ill in 24-48 h and die within 7 days whereas the rats and white mice survives for only 48 h. On post-mortem, intense congestion, haemorrhages and exudates are seen in various organs which reveal characteristic coccobacilli on microscopic examination, (v) serodiagnosis for detecting antibodies against F1 antigen of the pathogen in serum samples (single or paired) from suspected patients by using tests such as PHA, CFT and IgM capture ELISA. A rise of four-fold or more in antibody titre in case of paired sera and a cut off titre of 1:10 in case of PHA are considered as criteria for the positivity, (vi) bacteriophage analysis for examining the lysis of Y. pestis organisms by specific bacteriophages like pesticin both at 25°C as well as 37°C. It is a rapid and confirmatory way of diagnosing plague, and (vii) PCR for detection of F1 gene of Y. pestis from suspected specimen, particularly for rapid, reliable diagnosis of plague besides epidemiological surveillance.
WHO Classification of Plague Cases: According to the criteria laid down by WHO, a case of plague can be categorized in any of the three categories:

1. **Suspected plague**: If one of the following conditions is met:
   - (i) Clinical symptoms are suggestive of plague, (ii) if a Gram-negative, bipolar coccobacillus is identified.

2. **Presumptive plague**: If one or both of the following conditions are met:
   - (i) if the organism is immunofluorescence stain positive for the presence of *Y. pestis* F1 antigen, or if it is both immunofluorescence and Wayson/Wright - Giemsa stain positive, (ii) if a single serum is detected to have antibodies against the F1 fraction of *Y. pestis*.

3. **Confirmed plague**: If one or both of the following conditions are fulfilled:
   - (i) in addition to Wayson/Wright - Giemsa and immunofluorescence positivity, the organism is grown in culture and is positive by both bacteriophage and biochemical reactions, (ii) two serum samples, taken at appropriate time intervals (10-14 days or longer), demonstrate a four-fold difference in end point titre of plague specific antibodies.

C. **Differential diagnosis**: The human cases of plague should be differentiated from other mimicking conditions such as (i) acute lymphadenitis due to lymphogranuloma venereum (LGV), filariasis, infectious mononucleosis, cytomegalovirus, toxoplasmosis, (ii) cervical lymphadenopathy or pharyngitis due to *Strept. pyogenes* group A, diphtheria, botulism, viral and gonococcal pharyngitis, (iii) pneumonic cases due to bacterial and viral pneumonia, TB, influenza, melioidosis, tularemia, psittacosis, Q-fever, mycoplasma, fungal infections, legionnaire’s disease, and (iv) septicaemia due to meningococci, typhus, malaria, haemorrhagic fever.

In case of rodents diseases like pasteurellosis, pseudotuberculosis; buboes resulting from streptococci, staphylococci, coliforms and other pyogenic organisms; and lesions caused by *Trypanosoma lewisi* may be easily confused with the plague.

VIII. **PREVENTION AND CONTROL:**

A. **In animals**: The spread of plague among animals can be effectively prevented by a comprehensive approach which includes (i) prompt diagnosis and treatment of diseased animals, (ii) effective flea and rodent control in animal sheds, (iii) quarantine of infected animals, and (iv) avoidance of feeding such meat which has originated from infected/dead animals.

B. **In man**: The following strategies are suggested for effective control:

1. **Prompt diagnosis and medical care**: Once a prompt diagnosis is made on the basis of clinical picture, serological testing and/or culture, the treatment must be initiated at the earliest especially in pneumonic plague cases which must be kept in strict isolation and under strict supervision.
2. **Chemoprophylaxis:** It is recommended that persons who are exposed to the infection in the field or in the laboratory by infected rodents, or in hospitals to pneumonic plague cases should be given tetracycline (250 mg six hourly for 1 week) or sulphadiazine (6 g daily for one week) as Chemoprophylaxis.

3. **Surveillance:** A continuous and effective surveillance programme for monitoring the plague situation in rodents, sentinels and fleas is a must for assessing any likelihood of an outbreak as well as for adjudging the efficacy of control measures. It includes four useful approaches namely (i) measurement of antibodies against *Y. pestis* in the sera of sentinel animals like dogs and cats, (ii) examination of rodent sera for plague antibodies, however, it is more expensive and time consuming than the use of sentinel animals as it involves trapping and bleeding of large number of rodents from different places and at different times, (iii) calculation of specific flea index. Experience has shown that the specific index of over one for *X. cheopis* must be regarded as indicative of a dangerous situation, especially in the beginning of a normal plague season in an endemic area, and (iv) monitoring the presence of a 9.5 kb plasmid unique to *Y. pestis* as it is useful for distinguishing the pathogen from *Y. pseudotuberculosis* and can be used as specific DNA probe for plague surveillance.

4. **Rodent control:** It is the most effective way to control the disease, and includes following measures.

   (a) **Sanitation:** It aims at (a) proper storage, collection and disposal of the garbage, (b) proper storage of food stuffs, (c) construction of rat-proof buildings, godowns and warehouses and (d) elimination of rat burrows by blocking them with concrete.

   (b) **Trapping:** The number of traps laid down should be atleast 5% of the human population. The wonder trap designed by Haffkin institute, Bombay can trap as many as 25 rodents at a time.

   (c) **Use of rodenticide:** The poisonous baits for killing rats are prepared by incorporating rodenticides. The single dose (acute) rodenticides such as barium carbonate (1:4), zinc phosphide (1:10) and arsenic acid are mixed with wheat flour or rice flour, made into balls and kept at the rat burrows which, when taken by rat cause death within 10-48 h. The multiple dose (cumulative) poisons such as diphacinone, warfarin, coumafuryl etc. are anticoagulants which cause internal haemorrhages leading to slow death in 4-10 days.

   (d) **Fumigation:** The rats and fleas, especially those hiding in burrows and ships can be effectively destroyed by fumigants such as calcium cyanide (cynogas or cymag), carbon disulfide, sulphur dioxide, methyl bromide etc. Cynogas must be fumigated with utmost care by using cynogas pump. It is noteworthy that rodent control should always be preceded by flea control as in absence of rodents the fleas may invade human population.
5. **Vector control**: The rat fleas can be destroyed by using BHC as 3% dust or 2% diazenon or 3% malathion. During an outbreak, control measures should aim at flea control rather than the rodent control.

6. **Quarantine**: It has little value in view of short incubation period and high efficacy of antibiotic therapy in plague. Moreover, international quarantine is practically irrelevant and no long term human carriers of infection exist. However, pneumonic cases must be kept in strict isolation.

7. **Vaccination of high risk groups**: Formalized killed vaccine produced by Haffkine institute, Bombay is being used in the country. Live vaccines are also available and being used in countries like USSR, however, these have more severe side effects. These vaccines provide protection against bubonic plague only and not against pneumonic plague. Therefore, persons exposed to pneumonic plague patients should receive antibiotic therapy irrespective of immunization. Recently, a recombinant capsular F1-V antigen fusion vaccine has been suggested to provide the basis for improved human plague vaccine as the vaccine was found to protect mice against pneumonic as well as bubonic plague.

8. **Health education**: People should be educated about the disease and the preventive measures to be adopted including the importance of restriction on the movement of pneumonic plague case.

**IX. TREATMENT:**

A. **In animals**: The line of treatment is same as has been outlined below for human cases.

   **In man**: *Y. pestis* remains a highly sensitive organism, which does not develop resistance to the antibiotic challenge. The drug of choice is tetracycline (4-6 g daily during first 48 h). In meningeal plague, chloramphenicol (a total of 20-25g at the rate of 50-75 mg/kg body weight) is the choicest drug as it is capable of crossing the blood-brain barrier. Streptomycin is very effective for plague therapy but large number of plague bacilli lysed by it, especially when used alone, may lead to severe intoxication. Sulphonamides alone are effective against bubonic plague but not against pneumonic plague. Penicillin is totally ineffective. However, some other drugs, which are available for the therapy, include kanamycin and ampicillin.

Further readings suggested:


* ***
Brucellosis: Diagnosis and Control

D.K. Singh,* D.K. Sinha,1 & V.I. Bishor2

Brucellosis is primarily a disease of domestic animals and man gets infection indirectly from animals. The etiological agents are the five species of Brucella, viz., B. abortus, B. melitensis, B. suis, B. ovis, and B. canis. B. neotomae, isolated from desert wood rat, has not been reported so far to cause infection in any other species of animals including man (Table-1). Many animal species, both domestic and wild, are susceptible to Brucella. Cattle, sheep, goat, pig and dog are the main reservoirs for maintenance of brucellae. Human may get infected to brucellosis through percutaneous, conjunctival or nasal mucous membrane. The disease in cattle, sheep and goats is usually characterised by abortion, retention of placenta, orchitis and epididymitis leading to impaired or reduced fertility and economic losses consequent to loss of progeny, protein/meat and milk yield.

Schwabe estimated the economic losses in India due to brucellosis in cattle and buffaloes was to the tune of Rs. 240 million every year. This amounts to more than half percent of total value of all meat and milk products produced in the country. He put the annual loss due to human brucellosis to be 30 million man days. A decade later, Kunen7 estimated that the losses due to brucellosis cost India at least Rs. 350 million annually in terms of food animals and man days of labour lost. Earlier to this, the economic loss due to brucellosis among bovines was estimated at Rs. 311.47 millions.

Polding was probably the first to make a nation-wide sera-survey of brucellosis in selected herd, area and slaughter houses in India. Serologically, brucellosis has been reported from almost all states of India by various group of workers. The disease has been reported to be common particularly in areas with high rainfall and humidity. Recently, an increasing trend in prevalence of both human and animal brucellosis over the last three decades has been recorded in tarai regions of UP. Its prevalence has been reported to vary from as low as 0.13 per cent to as high as 44.0 per cent in animals. The role of wild and feral animals in the epidemiology of brucellosis still remains debatable. These serological evidences have further been substantiated by isolation of Brucella from different species of animals except camel and poultry. Brucella agglutinins have been demonstrated in poultry. The isolation of various Brucella species from different species of domestic animals and human beings in India has been listed in Table-2.

Biotype I has been found to predominate in cattle in organised farms, biotype III has been usually reported to infect the cattle in the village population. Occasionallly, B. melitensis biotype I has also been isolated from cattle110. B. melitensis biotypes I, II and III have been isolated from sheep and goats besides B. abortus biotype I.

The prevalence of brucellosis in animals gives an indication of extent of infection in human population. B. abortus and B. suis usually infect the professional groups while B. melitensis infections are observed more frequently among the general population. B. suis biotype II, B. ovis and B. neotomae are not recognised human pathogens. Brucellosis in man has been prevalent in various parts of India. The prevalence of brucellosis have been found to vary from as low as 0.45 per cent to as

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high as 41.25 per cent in human population. Majority of human brucellosis cases have been attributed to B. melitensis from areas where caprine and ovine brucellosis is endemic.

Diagnosis

Diagnosis of any infectious disease is made on the basis of certain clinical symptoms unique to the disease and can be confirmed by detection of antibodies (Ab) produced, isolation of the organism or detection of the organism or its components in the clinical samples. In the absence of any pathognomonic symptom of brucellosis in animals, serological tests are used for its diagnosis. But the most incontrovertible diagnosis is based on isolation and identification of the Brucella. Detection of Ab is quick but all infected animals do not produce detectable level of circulating Ab. Also, diagnosis may be impaired by presence of Abs to cross-reacting organisms (Corbel, 1985). Isolation of Brucella from clinical samples is time consuming and tedious process. Quite often it is impeded by the presence of contaminating microflora. Apart from these, at times the isolation is not successful even from known positive cases. As an alternative, attempts have been made to detect the brucellae or its components in the clinical samples such as blood, milk, tissues etc. In this direction, though still a long way to go, detection of nucleic acid sequences specific to brucellae hold great promise. However, the attempts on this line has been far and between with respect of Brucella. The diagnosis could be based on isolation of organism, demonstration of brucellae, its component including nucleic acids or determine of antibodies produced against it.

Isolation of Brucella

Bacteriological isolation of brucellae from the clinical samples has always been accepted as the most accurate method for confirmation of brucellosis. A number of media (such as TSA, SDA) incorporating serum and selective agents (viz., bacitracin, polymyxin B, ethyl violet) have been used with success. The clinical samples may be grossly contaminated. Brucellae may be present in low number. Therefore, it is advisable to use more than one media.

Antigen detection: Abs raised against whole Brucella cells or its different cell fractions could be used to demonstrate the antigen(s) in the clinical samples. Within the genus majority of precipitations observed in immunoelectrophoresis were found to be common. Verstreate and Winter, too, reported that outer membrane proteins (OMP) of 49 strains were identical with minor differences. Recently, some of the conserved Brucella proteins have been isolated and genes cloned. It is expected that with recent developments in the area of molecular biology and biotechnology, increasing number of Brucella surface, periplasmic and cytoplasmic protein genes will be cloned and expressed paving way to the development of highly specific probes for detection of Brucella cells or cell fragments.

Histological Techniques

Brucellae could be demonstrated in different clinical materials by use of a variety of specific and non-specific staining techniques. Gram’s stain, Modified Kostar and Modified Ziehl-Nelson staining techniques are used to stain the organisms in various histological smears. The staining is non-specific and it is difficult to differentiate between the similar looking micro-organisms.
Alternatively, antibodies against the whole *Brucella* or its cell components could be used to bind with the antigen in histological specimens. This primary antibody is detected by use of species antibody conjugated to fluorochrome or enzyme. The specificity of the tests are dependent on the specificity of the primary antibodies.

Immunofluorescent labeling of brucellae on tissue, cytological smears and cultured cells have been used. Such histological examination requires use of fresh or fresh frozen tissues. The FAT may be highly specific and can be rapidly and easily performed.

Immuno-enzymatic staining for demonstration of brucellae in the morbid materials is another useful tool with many advantages. Evaluation of the staining requires no special microscopic equipment. Horse radish peroxidase (HRP) is the commonly used enzyme but others like alkaline phosphatase and glucose oxidase can also be used.

**Serological tests**

Different conventional tests used to diagnose brucellosis include Rose Bengal Plate Test (RBPT), standard tube agglutination test (STAT), milk ring test (MRT), 2-mercaptoethanol test (2-MET), heat inactivation test (HIT), complement fixation test (CFT). Of these, MRT could be used to locate an infected herd. Major drawbacks of the test are i) it can’t be used on dry cows and ii) its efficacy in case of sheep and goat is poor. RBPT is a cheap and easy test to be performed. The lower pH of the buffer used imparts specificity to the test, but the test said to be over sensitive especially in vaccinated animals. Its suitability in diagnosis of human, sheep & goat brucellosis is debatable. STAT is the most widely used test for diagnosis of brucellosis even today though evidences are there to indicate that it is not the most effective test for diagnosing individual animal. Its limitations are well accepted. All individuals do not develop agglutinins. Hetero-specific agglutinin produced because of cross-reacting organisms also interfere. And above all, prozone is observed because of excess of non-agglutination IgG1 which blocks IgG2. Heat inactivation or 2-MET or Rivanol tests inactivate IgM and detect only IgG2 which gives better indication of infection. CFT is by far the most efficacious of conventional tests. However, the test is cumbersome to perform, requires careful titration and standardization of reagents and suffer with prozone phenomena. There have been reports of anti-complementary activity, too.

Development of primary binding assays opened a new era of development in the diagnosis of infectious diseases including brucellosis. Radio immunoassay (RIA), application in diagnosis of brucellosis, has been reported to be highly sensitive with reasonable specificity. However, it requires costly equipment, trained manpower, shelf-life of reagents are very short and there is always a problem of safe disposal of isotopes. The FAT suffers with the biasness because of individual variation in fluorescent assessment and requires costly equipment and trained personnel. The shelf-life of the conjugate is short. With the development of EIA, the sensitivity of RIA was combined with ease of operation. The reagents had comparatively long shelf-life and problem of radioactive reagent handling, too, was eliminated.

A wide variety of LPS and protein preparations have been used as antigen in ELISA. While the LPS has been found to be a better antigen, the use of protein antigen to coat the ELISA plates seems to be the means to obviate the cross-reactivity with
certain organisms. There are a number of reviews on the different *Brucella* antigens of diagnostic significance (Corbel, 1985) and use of ELISA to the diagnosis of brucellosis. With the development of dot-ELISA it has become an on the spot test for use in field. The test can be read by anybody.

To improve upon the shortcomings of CFT, an indirect hemolysis test (IHLT) using alkali treated LPS has been developed. The conditions for its optimum performance has been described and the test is said to be free from anti-complementary activity and prozone phenomena.

To overcome the problem of differentiating vaccinal and infection antibodies, a gel diffusion with poly-B antigen has been described. Its performance under experimental condition has been good, but its use in field diagnosis of individual cases has certain drawbacks. The use of ploy-B in ELISA has been hampered by its inability to bind effectively to the polystyrene surfaces.

A delayed type hyper-sensitivity (DTH) reactivity on the skin has been developed. The test uses a protein antigen brucellin, isolated from *B. melitensis* B115. It has been found to be an excellent test with good specificity for use as a herd screening test especially in small ruminants. It is based on the detection of cellular immune response. However, its efficacy when used to diagnose individual cases is poor. A lymphoblastogenesis test for diagnosis of brucellosis has been also used but its applicability for large scale screening has been debated.

Of late, the role of cytokines, particularly interferon-gamma (IFN-Y), for its possible use to diagnose infectious diseases caused by intracellular pathogens such as *Brucella* has been advocated. A test based on IFN-Y to diagnose bovine tuberculosis is already being used in Australia. Weynats and his co-workers in 1995 reported a test using the IFN-detection for bovine brucellosis with brucellergen as antigen. However, a subsequent report indicated that this also can not rule out the infection caused by *Yersinia enterocolitica* 0:9 (Kittelberger *et al.*, 1997). It made them to remark that the 'options to develop a specific test for diagnosis of brucellosis are becoming increasingly limited'.

It is quite clear from the preceding discussion that brucellosis is wide-spread in many parts of world including India. The prevalence varies greatly from one part to another. Its diagnosis is difficult to make on the basis of clinical signs. The laboratory testing, viz., demonstration of organism and antibodies are used. The serological tests available do have a number of drawbacks.

**Prevention and control:**

Detection of truly infected animals assumes paramount importance in any brucellosis control programme. The campaign is easy to operate with high success where the farms are small with close control on the movement of animals. A test and slaughter of infected animals or extensive vaccination with approved vaccine, depending on the situation, may be used. This requires a quick, cheap and reasonably sensitive screening test besides a good confirmatory test. In case of cattle, Rose Bengal plate test (RBPT) is used as screen test followed by testing positive sera with complement fixation test (CFT) for confirmation. In case of cattle, milk ring test (MRT) could also be used for identifying infected herd with good results followed by sero-testing individual
animals. Its major drawbacks are that this cannot be used on dry animals and its
efficacy is doubtful in sheep and goats.

At present, mass immunization with recommended doses of approved vaccines
is the only way to bring down the incidence of brucellosis in areas with high prevalence.
For cattle, *B. abortus* S-19 while in case of sheep and goats *B. melitensis* Rev-1, given
at the age of 3 to 6 months (in certain cases up to 8 months) has been in use. Both are
live attenuated vaccine. In spite of certain drawbacks, these have been used with
satisfactory results. When used routinely to attain a coverage of ≥ 80% of population, a
gradual reduction in incidence is seen.

The epidemiology and planning for prevention and control of brucellosis in
sheep and goats are similar to that of cattle with minor adjustments. The probability
of success in flocks where contact with other flocks is frequent is not good. Under
such circumstances, it is advisable to test all the animals coming in contact. Allergic
test could be used to identify infected herd followed by sero-testing of individual
animals.

In case of swine, control of brucellosis poses difficulty because there is no
satisfactory test to identify individual animal nor a satisfactory vaccine. It is therefore,
advisable to slaughter the herd.

**Future need for research**:

Tremendous progress has been made since identification of brucellosis as
disease and its causative agent in every sphere. A battery of tests to diagnose brucellosis
has been developed. Different regimens to treat acute as well as chronic brucellosis
in man using combination of antibiotics and other chemotherapeutic agents have been
advocated with varying degree of success. Vaccines - both live-attenuated and killed,
have been developed. The necessary technical knowhow for the formulation and
execution of control and eradication programmes under different epidemiological
situations have been developed. However, notwithstanding all these developments, a
lot more needs to be explained. The vaccines available have certain shortcomings.
Vaccine for all species is not available. The diagnostic tests present many difficulties.
All antigens are not well characterised. The pathogenicity of brucellosis is not clearly
elucidated. The mechanism of intracellular survival and elimination of brucellae are
yet to be explained.

**Diagnosis**:

The diagnosis of brucellosis presents difficulties in spite of the fact that there is
no other disease for which such a large number of tests are available as for brucellosis.
There is cross-reaction with many bacteria, the most significant being that because of
*Yersinia enterocolitica* serotype O:9. The vaccinal antibodies are difficult to differentiate
from that of infection. Thus, there is need to separate and characterizes the *Brucella*
antigens, especially the cell-wall antigens, which have shown potentiality to differentiate
not only cross-reacting antibodies but also antibodies produced on account of
vaccination and infection. In this regard, enzyme immunoassays have shown
encouraging results. However, its utility in diagnosis of brucellosis in sheep, goat and
pigs need special attention. The role of isotypes of immunoglobulins and subsets of
lymphocytes in protection from brucellosis is an area that requires particular attention.
The efficacy and suitability of tests based on cell mediated immune (CMI) response should be explored. In this regard, delayed type hypersensitivity (DTH) test holds great promise.

**Vaccine:**

Brucellae are intracellularly facultative organism. Antibodies produced against lipopolysaccharide (LPS) - the immunodominant antigen of *Brucella*, causes only temporary reduction during early phase of infection. Protection against infection and elimination of brucellae require CMI response mediated through activated macrophage and cytotoxic lymphocytes. These are induced by the protein antigens. Many protein fractions have been analysed, but only L7/L12 ribosomal proteins have been found to be protective. There is a need to study other proteins as well as other components of *Brucella* for its possible use as vaccine.

It has been observed that the available vaccines do not have the same degree of protection against isolates from different geographical regions. At times, cross-infection of other than natural host species is not protected. It is, therefore, required to study the efficacy of available vaccines to alleviate *Brucella* infection in animals. In China, large scale vaccination with *B. suis* strain 2 (a live attenuated strain) and *B. melitensis* M5 has been used with good results. However, experiments on laboratory animals in Europe has not been encouraging. In spite of this, its potential as oral vaccine deserves detailed study. A major achievement in this regard is development of a live attenuated *B. abortus* RB51 strain to vaccinate cattle. It has been found to be stable and avirulent and does not induce antibody against LPS of smooth strains. It has been accorded status of official vaccine in the USA.

Experiments to develop a sub-unit vaccine has shown that ribosomal protein L7/L12 and some heat shock proteins have the potentiality for use as subunit vaccine. Research to identify and characterize other such proteins besides further characterization of the ribosomal proteins should be pursued. At present, there is no satisfactory vaccine available for use in human and swine against brucellosis. Concerted efforts in this direction is the need of time. The WHO consultation on the development of new/improved brucellosis vaccine held in Dec. 1997 discussed in detail on future research needs in the area of vaccine development and made similar recommendations. It also emphasised on the need to develop vaccine against rough strains of *Brucella*. No surprise that Dr. M. Kaplan, consultant, WHO, observed that brucellosis was as important now as when it first received attention of WHO some 50 years ago. Indeed, brucellosis remains as one of the most significant zoonosis on a world-wide scale.
Table 1: Species and biotypes of *Brucella* with preferred host and pathogenicity to man

<table>
<thead>
<tr>
<th>Species</th>
<th>Preferred host</th>
<th>Pathogenicity to human</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. abortus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>biotype 1-7,9</td>
<td>Cattle</td>
<td>Moderate, usually sporadic</td>
</tr>
<tr>
<td><em>B. melitensis</em></td>
<td>Sheep &amp; goat</td>
<td>High, may lead to epidemic</td>
</tr>
<tr>
<td>biotype 1-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>B. suis</em> biotype</td>
<td>1</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Not reported</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Moderate</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Not reported</td>
</tr>
<tr>
<td></td>
<td>(dessert wood-rat)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Prevalence of brucellosis in man and animals in India

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Serological positivity</th>
<th>Isolation of brucellae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Buffalo</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sheep</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Goat</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Swine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Equine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Camel</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dog</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Poultry</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Wild animal</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Man</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
RECENT ADVANCES IN DIAGNOSIS AND CONTROL OF SALMONELLOSIS.

V.D. Sharma*, Subodh Kumar and T.K. Barman

Salmonellosis is a collective description of a group of diseases caused by the members of the genus Salmonella. Ligniers in 1900 named this genus in the honour of Dr. D. E. Salmon, a Veterinary Microbiologist, who characterized this bacillus in 1885. The disease has a global distribution occurring in developed as well as developing countries. It affects both man and animals alike with varying symptoms.

The genus Salmonella is classified under the family Enterobacteriaceae. Salmonellae are Gram-negative facultative anaerobic rods. Being peritrichously flagellated, they are motile except S. gallinarum. Salmonellae do not ferment lactose, but do produce acid and gas from glucose, utilize citrate as their sole source of carbon, generally produce hydrogen sulfide gas, decarboxylate lysin and ornithine but are urease and V.P. negative and do not produce indole. Besides, biochemical tests are also used for biotyping of Salmonellae.

The Kauffmann-White antigenic scheme is mainly used for the identification of more than 2400 Salmonella serovars. It is based on somatic (O), flagellar (H), and capsular (Vi) antigenic profiles derived from serological reaction with group and factor sera specific to the antigens. Salmonellae usually harbor two genotypic forms of flagellar antigens (phase I and II).

According to the WHO Collaborating Centre for Reference and Research on Salmonella, the genus Salmonella consists of two species: (1) S. enterica which is divided into six subspecies: S. enterica subsp. enterica, S. enterica subsp. salamae, S. enterica subsp. arizonae, S. enterica sbsp. diarizonae, S. enterica subsp. houtenae and S. enterica subsp. indica; and (2) S. bongori (formerly called S. enterica subsp. bongori). These species and subspecies can be further distinguished on the basis of differential biochemical characters. In practice the subspecies name does not need to be indicated as only serovars of subspecies enterica bear name: Typhimurium is a serovar of subspecies enterica. The name Salmonella ser. Typhimurium or Salmonella Typhimurium may be used for routine.

The names of serovars must no longer be italicized and the first letter is a capital letter, as they do not have the status of species. The names of serovars were maintained only for subspecies enterica serovars which account for more than 99.5% of isolated Salmonella strains.

Some of the serovars are host-adapted and produce disease in particular or limited hosts, like S. Gallinarum is in poultry, while most of the serovars infect a wide variety of hosts including human beings. It is a global problem occurring in both developed and developing countries and affecting both man and animals alike.

Though much work has been done on different aspects of salmonellosis, still it looms large and causes substantial economic losses both directly and indirectly. It accounts for over one-half of all food-borne diseases. In 1986, losses incurred in the U.S.A. as a result of salmonellosis in man were more than $1.2 billion. In one out break involving S. Napoli infection in contaminated chocolates, the estimated value for claims due to loss of life ranged form $190,000 to $15 million.

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In poultry, salmonellosis still remains a problem of high magnitude. According to Nigel Horrox, as mentioned in the bulletin produced by International Poultry Production and International Hatchery Practice, *Salmonella* is probably the word that most poultry producers wish had never been invented and when it surfaces in media, poultrymen quiver with the feeling that their doom is imminent. Although he attributed it greatly to the fear of unknown, it is a fact that poultry industry does incur a heavy loss on account of salmonellosis and that poultry does serve as an important source of infection to man. Similarly, in other animals, particularly in pigs, *Salmonella* causes morbidity and mortality. Therefore, food of animal origin serve as a potent source of infection to man. Besides, a large variety of house pests (rodents, cockroaches, lizards, etc.), wild animals, zoo animals and free flying avifauna harbour *Salmonella* and act as reservoir thereof and contribute significantly to the transmission of *Salmonella* between man and animals.

**Diagnosis:**

The disease can be tentatively diagnosed on the basis of clinical picture, post-mortem lesions and serological findings. However, for confirmation isolation and identification of the causal agent is required. Liver, spleen and ceca are cultured for isolation of the organism.

For isolation of *Salmonella*, a three-stage cultural procedure of pre-enrichment (resuscitation), enrichment and selective plating is used. Non-selective media such as buffered peptone water is used for pre-enrichment to resuscitate the injured organism, particularly those present in foods, litter etc. For enrichment, media such as tetrathionate broth, selenite broth, Rappaport Vassiliadies (RV) broth, which favour the growth of *Salmonella* while inhibiting other bacteria are used. After enrichment, a loopful of growth is streaked on selective agar which support the growth of *Salmonella* but checks the growth of other bacteria. Additionally, many of these selective media are designed so that if the colonies produced on agar are *Salmonella*, they assume a distinctive colour. Brilliant green agar is commonly used as selective agar on which *Salmonella* colonies are of red colour. Three suspected colonies are picked up and the isolates are subjected to further identification and confirmation using biochemical and serological tests.

**Immunodiagnosis:**

For diagnosis of salmonellosis, rapid slide test (RST), whole blood test (WBT), tube agglutination test, rapid haemagglutination test, microagglutination test (MT), microantiglobulin test (MAG), cutaneous hypersensitivity test and enzyme linked immunosorbent assay (ELISA) have been employed. Of these, whole blood test is routinely used for screening flock/ herd for salmonellosis.

The whole blood test/ rapid slide test is a cheap one to perform and requires little equipment. Stained serovar specific antigen is required. For diagnosis of pullorum/ fowl typhoid, stained pullorum antigen is commercially available. On mixing the blood and antigen, agglutination reaction (clump formation) is completed within 30 seconds. Widal test in humans is widely used to detect agglutinins in sera. In widal test poly O and poly H antigens are used to detect agglutinins against *S. Typhi* and *S. Paratyphi*. Tube agglutination test is performed with the plain antigen and serially double fold diluted serum. The reactions at or more than 1:40 are considered positive. In cattle,
detection of serovar (eg. S. Dublin) specific agglutinins in the serum are used for diagnosis of salmonellosis. The test has some limitations; antibodies do not appear in the serum until about 2 weeks post infection and many non-infected animals may carry moderate titres against some Salmonella serovars. Cutaneous delayed hypersensitivity test can be used for the diagnosis of salmonellosis. However, it fails to differentiate infected from recovered animals. Specific Salmonella serovars can also be used to detect antibodies in serum of other animals but this practice is not widely carried out due to ever-changing Salmonella serovar infection.

Recently, of the 4 cytotoxins isolated from a single strain of S. Weltevreden, antiserum to the cytotoxin designated Salmonella cytotoxin-I (SCT-I) agglutinated more than 100 isolates of the organism belonging to its 30 different serovars. The strains of other genera of the family Enterobacteriaceae did not react with the antiserum revealing genus specificity of the antigen (Singh & Sharma, 1999). This indicated the possibility of using anti-SCT-I for rapid identification and eventually detection of Salmonella. Presently, attempts are being made to exploit SCT-1 for development of an ELISA test for Salmonella diagnosis.

Antigen-trap ELISA may be used to identify Salmonella. Organisms are trapped by a surface-associated monoclonal antibody specific for an antigen found on most Salmonella serovars. The trapped organisms are then positively identified as Salmonella by a second labeled antibody. Indirect ELISA is also increasingly employed to screen sera from animals infected with serovars such as S. Enteritidis, S. Typhimurium and S. Gallinarum. Lipo-polysaccharide (LPS), heat-extracted, and fimbrial antigens have been used for ELISA. LPS is the most frequently used discriminating antigen. Sera infected either with S. Gallinarum or with S. Typhimurium yielded much higher titres with homologous rather than heterologous antigen. However, varying degrees of cross reactions between group B and D LPS have been demonstrated with sera from chickens infected with S. Typhimurium. This cross reaction has also been demonstrated by immunoblotting because of O-12 antigen (Hassan et al., 1990, Barrow, 1992).

ELISA is also used for the detection of S. Dublin mammary gland infection in carrier cows. The test can differentiate between uninfected, recently infected, recovered and milk shedding carrier cows.

Dot-ELISA is used by applying antigen on nitrocellulose dipsticks, incubating in different dilutions of the test serum and then in anti IgG-HRPO conjugate, and finally dipping in the substrate. Brown coloured dot indicates positive reaction.

Several comparisons between the performance of the ELISA and other serological tests have been made. Of all the tests, ELISA was found to be more sensitive and identified more infected individual birds than did the agglutination tests, including slide agglutination.

On the basis of the results obtained, ELISA appears to satisfy most of the requirements for its adoption as a serological test to detect infections produced by S. Typhimurium, S. Enteritidis, S. Gallinarum and its biovar pullorum and other invasive serotypes. It appears to be among the most sensitive of the currently available tests and is easier to carry out than many tests. It allows for quantitation of antibody and allows discrimination between infections caused by Salmonella and by other bacteria.
Fluorescent antibody techniques - To perform the test, bacterial culture smears is made on a slides and fixed with acetone. On the fixed smear a drop of antiserum is spread and kept at 37°C for 30 min in a humid chamber. Then the slides are washed with PBS and thereafter a drop of 1:2000 diluted FITC conjugated anti-rabbit antibodies (Sigma, USA) are spread and incubated as above. Then the slide is washed and examined under fluorescent microscope in dark field for greenish yellow fluorescence.

Plasmid profile:

Molecular fingerprinting of Salmonella is a recent approach being used for the identification of Salmonella. It is sensitive and specific technique, which can also be used, in the molecular epidemiology of Salmonella. It helps to study association of one case with other and association of case with food or other vehicle, animal, geographical area or time period. It is base on the DNA content of the organism rather than the phenotypic characters as biotype or phagetype. It includes plasmid fingerprinting, restriction endonuclease analysis of DNA and Southern blot hybridization using labeled DNA probes. It also helps in studies related to virulence of the organism.

Prevention and control:

Following measures help in minimizing the incidence of salmonellosis:

1. Diagnose the disease early and hit it hard with proper treatment.
2. Detect the carriers by cultural /serological tests and eliminate the reactors.
3. Vaccinations with a specific bacterin or live modified vaccines such as auxotrophic variant have been used with variable success. Experimentally, subunit vaccines using outer membrane proteins and other surface antigens have also been attempted with varying results. Recently, a formalized toxoid prepared from Salmonella Weltevreden toxins, which have been reported to be main virulence factors, afforded solid immunity not only against the homologous serovar but also against S. Gallinarum and S. Typhimurium challenges. Besides, the vaccine also checked shedding of the organism by the experimentally infected birds. This is significant in that it will check the spread of the organism on the farm.

Killed vaccines

Initial work on development of Salmonella vaccine started with the development of killed vaccines. These vaccines, though found safe, could prove successful to a limited extent (Timms et al., 1990). Mendel et al. (1972) demonstrated that heat killed vaccines induced effective degree of immunity in mice with 100% protection and prevented extensive multiplication of the organism in the liver and spleen on i/v or i/p challenge with S. Typhimurium. Efficacy of killed vaccine was also demonstrated by other workers (Eisentein and Angerman, 1978 and Aitken et al., 1982). Ghosh (1989) found formal killed oil adjuvanted S. Virchow vaccine more effective than gel and FCA adjuvanted vaccine.

Although, many workers found killed vaccine useful contradictory reports are on record. Gaidamark (1990) failed to protect piglets after vaccination with formalin inactivated S. Choleraesuis vaccine. The same observations were also reported by Tunkl et al. (1978).
Killed vaccines have been found very ineffective because of the following three reasons. Firstly, they only contain surface antigens, which give an incomplete antibody response. Secondly, they fail to elicit cell-mediated immune response, which is important for long-term protection. Thirdly, they fail to elicit secretory IgA responses, which are potentially important in protecting mucosal surfaces (Lax et al., 1995).

**Live attenuated vaccines**

Attenuated avirulent live *Salmonella* vaccines have received considerable attention as mucosal vaccines. Following oral vaccination, *Salmonella* replicates directly in the mucosa associated tissue (e.g. Peyer's patches) and thereafter disseminates via the mesenteric lymph nodes to systemic sites (e.g. spleen). This characteristic dissemination pattern allows *Salmonella* to induce wide spectrum immune response, including cell-mediated, humoral and secretory antibody-antigen responses.

Common mutations that render Salmonella both avirulent and immunogenic in mice and other animals are:

*Salmonella* strain with a gal E mutation is reversibly rough and sensitive to galactose. Although gal E mutants are avirulent and immunogenic, gal E mutants of S. Choleraesuis (Nnalue, 1986) and S. Typhi (Hone, 1988) retain some virulence.

*Salmonella* mutants blocked in the synthesis of aromatic amino acids (aro A) which also have a requirement of PABA needed for folic acid synthesis (Hoiseth, 1981) have received greater attention and have been widely used to attenuate S. Typhimurium, S. Enteritidis, S. Dublin and S. Choleraesuis. The immunogenicity of these mutants has been found excellent (Cooper et al., 1992).

Strains with (cyr and (crp mutations which lack adenylate cyclase and the cAMP receptor proteins are avirulent and immunogenic (Curtiss, 1987, Tacket, 1992). These mutants grow slowly and display diminished resistance to nonspecific host defence mechanisms.

The phoP phoQ two component regulatory system (Miller, 1989) regulates genes for acid phosphatases and for the ability of *Salmonella* to survive in macrophages. Although P mutants have been found immunogenic, their frequency of reversion to virulent forms is relatively high rendering them unsafe (Miller, 1990).

*Salmonella* strains with htr A (Johnson, 1990) and Omp R (Dorman, 1989) mutations regulate heat shock proteins and outer membrane protein expression respectively.

Elimination of virulence plasmid yields the strain vPla- that colonizes intestine and GALT on oral administration but fails to colonize lymphoreticular organs (Gull, 1987). Though these strains are highly immunogenic and avirulent, some vaccinated animals display some disease symptoms.

A chromosomal mutation designated as cdt and (cdt renders orally administered *Salmonella* unable to colonize lymphoreticular organs. Such strains are totally avirulent.

Though a large of number of mutants have been tried to develop live oral *Salmonella* vaccines, only the aroA, Δcya, Δcrp, and Δcdt mutations have been
introduced as useful vaccines demonstrating avirulence, immunogenicity and safety not only in mice but also in animals and man.

In poultry, vaccination with two live attenuated vaccine strains of *S. Gallinarum* (smooth) and 9R (rough) afforded protection against virulent parent strain 9R, whereas the killed strain failed to induce immunity (Smith, 1956). Puka (1987) reported that a live vaccine of *S. Gallinarum* of turkey origin attenuated by 300 passages in a medium containing 0.016% acriflavin was harmless for chicks aged 5-10 days and had no adverse effect on egg production and protected fowls against challenge infection.

Cooper (1994) reviewed the applications of live vaccines against *Salmonella* and concluded that live vaccines are more effective in controlling *Salmonella*.

Though live attenuated vaccines are being worked upon most seriously and are being claimed as the most effective measures of immunoprophylaxis against *Salmonella*, they too have some drawbacks.

Firstly, they cause shedding of the vaccine strain on vaccination making it difficult to differentiate vaccinated and infected animals (Bernardo et al., 1998).

Secondly, the possibility of reversal of attenuated forms to virulent forms cannot be ruled out (Miller, 1990).

Thirdly, these vaccines give protection against the homologues *Salmonella* strains from which the vaccine has been prepared thus leaving the animal exposed to the rest Salmonella serovars (Curtiss et al., 1993).

Lastly, it has been found that live attenuated *Salmonella* vaccines while protecting against virulent *Salmonella*, paradoxically induced profound immunosuppression against non-*Salmonella* antigens and suppress lymphoproliferative response to mitogen (Al-Rama di et al., 1992).

**Subunit/Toxoid Vaccines**

Protein extracts of *Salmonella* have also been used for prevention of salmonellosis. Bouzoubaa et al. (1978) used *S. Gallinarum* outer membrane protein with and without mineral oil and compared it with killed vaccine for protection against salmonellosis. The birds, which received *Salmonella* protein with mineral oil, showed 100% protection and proved more effective than killed vaccine. Charles et al. (1993) observed that incorporation of outer membrane protein from *S. Heidelberg* into ISCOMs resulted in greater immune response in turkey.

Chaturvedi et al. (1994) reported that Porins complexed with polysaccharides of *Salmonella* Dublin 51 used for immunization of rabbits proved non-toxic and antiporins in the serum were detected at 5-6 weeks post-challange. However cell-mediated immune response was insignificant. The percentage of protection of the immunized rabbits was 66.66% against oral challenge. Miller et al. (1991) reported that *S. Typhimurium* mutant bacterin-toxoid induced higher antibody titre in vaccinated cows. Sprouse et al. (1990) found *S. Typhimurium* toxoid very effective against *S. Typhimurium* and *E. coli* endotoxin in ponies and calves.

Recently, Mishra and Sharma (1999) reported an efficient toxoid against salmonellosis in poultry. This toxoid from *S. Weltevreden* was developed after polymyxin-
B extraction, salt precipitation, dialysis, gel filtration and formalin inactivation. Formalized toxoid was found better than carbonated toxoid. FAC adjuvanated formalized toxoid gave 100% protection in vaccinated birds while toxoid alone protected 60-70% of the birds. The vaccine was found effective both on s/c and oral vaccination. Presence of antibodies in eggs from vaccinated birds was also observed.

Protective role of passive immunity against salmonellosis has also been demonstrated. Staak et al. (1989) found protection in calves from cows vaccinated through intramammary route. Pregnant mares vaccinated with bacterin-toxoid prepared from rough *Salmonella* mutant had significantly high antibody titre in serum and colostrum (Green et al., 1990). Miller et al. (1991) reported that vaccinated pregnant cows had high antibody titres in colostrum.

4. Hygienic practices, such as regular cleaning and disinfecting of used poultry litter, contribute significantly in lowering the prevalence of salmonellosis. Pests, particularly rodents play important role in the transmission of salmonellosis on the farm. Therefore, pest and environment control programs are necessary measures to check salmonellosis.

5. Avoidance of stressful conditions, particularly during transportation, minimizes the prevalence of salmonellosis.

6. Needless to mention that like other infections good husbandry practices help minimise the spread of the disease. For example, good husbandry practices with egg laying and broiler flocks, like frequent collection and dry cleaning of freshly laid eggs, fumigation of hatching eggs with formaldehyde or dipping in gentamicin, chlorine or quarternary ammonium compounds help in reducing contamination of eggs and poultry products.

7. Of late role of probiotics in prophylaxis against *Salmonella* is being increasingly being recognized. In poultry, Nurmi cultures, i.e. bacterial mixtures prepared from the intestinal flora of salmonella free adult chickens, have been used to inhibit colonization of salmonellae in the intestinal tract of day-old chicks. The nature of the protective mechanism is uncertain but may represent a phenomenon of competitive exclusion. The microbial flora present in Nurmi cultures inhibits the subsequent colonization of the intestinal tract by salmonellae. Efforts have been focused on the identification of essential bacterial components in protective mixtures with a view to incorporate the same in the oral preparations so as to improve levels of protection afforded by undefined Nurmi cultures. Some commercial organizations have started marketing preparations based on competitive exclusion. These preparations are known as probiotics.

8. From public health point of view, it is important to identify critical control points in the manufacturing process and apply suitable control mechanisms.
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LEPTOSPIROSIS: DIAGNOSIS AND CONTROL IN MAN AND ANIMALS

A. A. KUMAR

Leptospirosis is a general term that describes all infections of human and animals by spirochaetes of the genus *Leptospira*. There are two species of leptospira namely *L. interrogans* which include pathogenic strains and *L. biflexa*, the saprophytic non-pathogenic strains. *Leptospira interrogans* has 23 serogroups and more than 200 serovars. *Leptospira* has been known as an important zoonotic disease capable of causing human infection for almost a century. However, its significance as a public health problem was not recognized earlier but is emerging as an important public health problem during the last decade or so due to sudden increase in the number of reported cases and several outbreaks. The fact that the disease has a large number of animal carriers and the environment in which we live are ideal factors for the transmission of infection to man (Singhal and Sood, 1993). The carriers among animals excrete leptospira in urine thereby contaminating the environment. The persons living in such contaminated environments for a long period and repeatedly, are more prone to this infection, though all individuals are susceptible to disease (Singhal and Sood, 1998). Leptospirosis is generally considered as an occupational hazard in agriculture and farm workers, sewage workers, animal handlers etc. The disease is more common during post monsoon period and may assume an epidemic potential. It is more common in tropical areas with high rainfall than temperate parts of the world, though leptospire have been found in all parts of the world wherever it has been looked for (Singhal and Sood, 1998).

Human beings contract the infection by direct exposure to the infected animal or to an environment contaminated with urine, blood or tissue of infected animals. It is also transmitted through inflamed or broken skin, intact mucous membrane (buccal cavity, nose) or conjunctiva (Singh and Sokhey, 1998). Direct transmission from person to person is extremely rare. Rare cases of congenital infection have been reported.

The most common clinical symptoms in men are fever, body aches, headache, jaundice, oliguria, cough, haemoptysis and breathlessness, haemorrhagic tendencies including haematemesis bleeding gums and subconjunctival hemorrhage, sign of meningeal irritation and convulsions (Bharadwaj, 1998).

In animals the following symptoms are usually observed - fever, malaise, depression, loss of appetite, weakness, anemia, diarrhea, haemoglobinuria, jaundice, encephalitis, mastitis, abortion, infertility; in dogs vomition, dehydration, blood stained faces, nephritis and shivering, while in case of horses fever, anorexia, conjunctivitis, haemoglobinuria, jaundice, abortion and periodic ophthalmia.

Diagnosis:

The diagnosis of leptospirosis is based on clinical and laboratory tests. If the clinical symptoms are suggestive of leptospirosis then routine urine analysis, blood examination (ESR, TLC, DLC) platelet count, serum bilirubin, blood urea and serum amylase are carried out.
**Microscopic demonstration:**

The demonstration of leptospira under dark ground illumination from the clinical samples may aid an early diagnosis.

**Culture of Leptospira:**

Isolation of leptospira from clinical samples gives a definite diagnosis and also aids in identifying the prevalent serovar. Isolation of the organism can be attempted either in liquid medium or by animal inoculation.

**Serological Tests.**

**Microscopic agglutination test (MAT):**

This test uses multiple live antigens and is a standard test for the diagnosis of leptospirosis. Other serological tests like IHA and ELISA are still being evaluated (Bharadwaj, 1998).

**Prevention and control:**

(a) **Rodent control:**

Denying access to food by proper disposal of garbage and storage of food in rat-proof containers and houses is the most effective way to remove the rodents from domestic environment (Singhal and Sokhey, 1998).

(b) **Control measures in animals:**

Maintaining good hygiene in the animal sheds/houses is the most effective way to control the spread of infection among animals and from animals to man. Effluents from the animal houses should be properly disposed off. Identification and isolation of infected/diseased animals and treating them separately.

(c) **Control of infection in man:**

People should be made aware about the transmission of infection and taking precautions to avoid contact with environment, or blood of rodents or carrier animals. Scientists and other workers engaged in such work/high risk activities should wear protective clothing and observe standard hygiene. There is an urgent need for carrying out epidemiological study of leptospirosis in man and animals so that a proper control program can be worked out.

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ZOONOTIC ANTHRAX: STATUS, DIAGNOSIS AND CONTROL

Dr. V.N. Bachhil

Introduction:

Anthrax is a rapidly fatal infectious disease known from ancient times. This is primarily a disease of herbivorous animals like cattle, sheep and goats, but it is not uncommon among pigs, equines & dogs. Man becomes infected incidentally and develops different forms of anthrax through contact with the diseased animal carcasses or by products viz. hides, hair, wool, bone meal, blood meal etc. In human beings it may occur through ingestion of infected meat. Anthrax is enzootic among animals & endemic in certain areas of India. Among animals it is transmitted through contaminated pastures, hay, straw, bone meal, blood meat etc. having spores of anthrax. Anthrax is an important zoonosis and the etiological agent has great historical importance in development of modern day bacteriology, immunology and infectious diseases. Robert Koch for the first time identified this *Bacillus* and established the well known Koch’s postulate.

*Bacillus anthracis* is a large encapsulated gram positive nonmotile spore forming bacilli and grows vegetatively within the infected host. These can be seen as single cell or short chains in blood smears. The main feature of this agent is that it sporulates when it is exposed to atmospheric oxygen outside the host under unfavourable conditions where multiplication is not possible. These spores are resistant to heat & chemicals and can persist for years preferably in alluvial soil having pH 7.6. It can also be transmitted through rain water, flood and soil erosion. Alkaline pH of soil and presence of organic matter etc. are favorable to perpetuate & maintain the infection. Outbreaks generally occur during summer and rainy seasons when grazing is at its peak & the grass lands become contaminated with spores. Natural water sources also become contaminated due to improper disposal of effluents from meat plants, leather industries, tanneries and wool etc. The body fluids & blood coming out from infected dead animal contaminate surrounding environment and the vultures, dogs, carnivores and wild animals open these carcasses & consume infected meat. As a result not only the animals get infected but the exposed cells sporulate and get contaminated over a wide area particularly during severe summer wind wave conditions.

**STATUS IN ANIMALS:**

In India there is close relationship between man and animals. India being an agricultural country, the rural people are highly dependent on animals and even sometimes share the same roof. It has been reported from equines also. Anthrax in equines was recognised & described at Remount Veterinary Depot at MHOW, in 1889. It has also been reported from wild life. Animal anthrax has been reported from most of the states of India. However, human anthrax has been reported from certain geographic areas only mainly from the southern states of India like Andhra Pradesh, Karnataka & Tamil Nadu.

In India the status of anthrax based on animal disease surveillance reports has been worked out. The disease has been reported from many of the states. However, in Tamil Nadu, Andhra Pradesh, Karnataka, Madhya Pradesh and Maharashtra, it has been reported frequently during past ten years (1984-1993).
In Tamil Nadu & Maharastra, disease is more or less wide spread and uniformly reported from many districts. But in other states, it has restricted to certain endemic areas only. In some districts it occurred after remaining free for several years while in few other states like Assam, Goa, Sikkim, Tripura and Jammu & Kashmir, the anthrax was reported after a gap of 2-3 years.

The incidence rate of anthrax in the last twelve years (1981-94) ranged between 1.5 to 9.4 per million of population among cattle, sheep, goat and buffaloes. Higher incidence rate was reported in 1983 i.e. 9.4 per million of animal population. Year wise incidence rates of anthrax in cattle, sheep, goat & buffaloes are given in parenthesis as follows:

- 1981 —— (2.0), 1982 —— (2.6), 1983 —— (9.4),
- 1984 —— (2.2), 1985 —— (2.8), 1986 —— (1.5), 1987 —— (5.5),
- 1988 —— (5.0), 1989 —— (2.7), 1990 —— (4.9),

**States & union territories which are not reporting Anthrax during last 3-4 years:**


**Number of anthrax cases reported (1975-1995):**

**Year wise Anthrax cases in cattle, buffalo, sheep & goats are given in brackets.**

- 1975 —— (3128), 1976 —— (2455), 1977 —— (1574),
- 1978 —— (1337), 1979 —— (1605), 1980 —— (1695),
- 1981 —— (1074), 1982 —— (937), 1983 —— (3457),
- 1984 —— (732), 1985 —— (711), 1986 —— (1034),
- 1987 —— (539), 1988 —— (915), 1989 —— (2024),
- 1990 —— (1113), 1991 —— (2003), 1992 —— (413),

**Status of Anthrax in various states of India & union territories which never reported anthrax since last twelve.**

1. Uttar Pradesh
2. Punjab
3. Haryana
4. Nagaland
5. Arunachal Pradesh
6. Chandigarh
7. Delhi
8. Dadra nagar Haveli

**States and Union Territories reporting anthrax regularly since last 12 years:**

1. Tamil Nadu - (all districts ) endemic
2. Andhra Pradesh - (Mainly Nellore, Cuddapah, Chittoor and Warangal districts.)
3. Karnataka - Bangalore (urban & rural), Mandya, Kolar, Bijapur & Coorg districts.


5. Maharashtra - All districts.

States & union territories which are not reporting anthrax during last 5 years:

States & Union territories reporting anthrax after a gap of 2 years or more:
Assam, Goa, Kerala, Manipur, West Bengal, Bihar, Gujarat, Jammu & Kashmir, Orissa, Rajasthan and Tripura.

In 1994 anthrax was reported from 15 states of India involving various species of animals and overall incidence among bovines was reported as 2.29 per million. It was three times more in ovine & caprines i.e. 7.45 per million as compared to bovines

a) West Bengal  Midnapore, Cooch Behar, Jalpaiguri, 24 Paragna & Purulia districts (except 1984).
b) Orissa  Sundargarh, Sambalpur Jagatsinghpur.
c) Kerala  Except 1984, 86, 88, 90, and 1993 reported during remaining six years from Trivandrum, Wayanad, Kottayam.
d) Bihar  Except 1986, 87, 89 & 1992 Reported during remaining 7 years mainly from West Champaran districts.

States & Union Territories which started reporting since 3-4 years Gujarat, Pondicherry and Meghalaya.

Most of the states in northern Zone Uttar Pradesh, Haryana, Punjab, Delhi, Chandigarh, manipur, Arunachal Pradesh had not reported anthrax for the last 5-10 years but Tamil Nadu, Maharashtra, Andhra Pradesh, Karnataka & Madhya Pradesh reported all the time. States like West Bengal, Orissa, Bihar, Kerala are reporting frequently indicating endemic areas.

Transmission in animals:

In animals the organisms are transmitted through ingestion of contaminated hay, straw, grass pastures, inadequately processed bone meal, blood meal, feed & fodder. Transmission through tabanid flies (blood sucking) is not common.

Among Humans, exposure of persons occur through contact with infected carcasses, hides, hairs, industrial aerosols, wool, bone meal, feed & fodder. Anthrax has high mortality rate & causes great economic losses particularly to farmers because
in India, agriculture & animal husbandry practices are the back bone of rural folk who mainly depend on animals for their agricultural operations. Veterinary & medical lab. facilities are poor in rural areas and the acute & per acute cases of animal anthrax go either unreported or some time under diagnosed or undiagnosed. Hence it becomes very difficult to assess the real magnitude of the problem. cutaneous form of anthrax is most common in India and results due to ingestion of inadequately cooked meat from infected animal.

Anthrax has been sporadically reported from all over India but in some states, it has been reported frequently where it is endemic. Heavy rains, flood, soil erosion, irrigation systems can enhance the enzootics in animals. All the environmental conditions are suitable in India for its perpetuation. Recycling of anthrax bacilli may occur showing possible correlation between climatic zones & incidence of anthrax.

Wild animals do not play major role in dissemination of infection like other developing countries as Tanzania, where wild animals have also been reported to play an important role in its transmission. Occurrence of all the three forms of anthrax viz. Per-acute, acute & sub-acute or chronic form are on record. Per-acute form is present mainly among ruminants while the acute form has been reported in ruminants & horses. Chronic or sub-acute form is prevalent in pigs, cats & dogs. In per-acute form, death occurs in very short time & goes mostly unnoticed as sudden death. Bleeding (Dark tarry color blood) occurs from natural orifices and the blood does not coagulate. In chronic form among dogs & cats, the lymph nodes are swollen & enteritis occur in intestinal form of anthrax. Pigs show throat edema and gastro enteritis. Carban­culous lesions on tongue & jaw reported with severe acute gastroenteritis. It has been reported from Rajasthan state for the first time in India.

Anthrax in Humans:

It has been reported mainly from Tri-junctional zone of south west Andhra Pradesh, South East Karnataka & North Tamil Nadu. Tamil Nadu is the major region affected. First detection of human anthrax was reported in 1973 and 1977 from Kashmir and Bihar respectively. Former was a case of anthrax meningitis and the latter was anthrax of palate. Since then many sporadic cases have been reported in Nov. 1989 & 1990 and 1991, involving 30 personsd in 1989 (both in Chittoor Dist. of Andhra Pradesh).

Forty cases of human Anthrax had been recorded at Christian Medical College, Vellore, from 1977 to 1995. The number of cases recorded in each year are given in brackets.


The information suggests that among human beings also it is increasing & now it is becoming an emerging zoonosis. Mening-o-encephalitic form of anthrax has also been reported in Karnataka state where it occurred due to opening of carcass that had died suddenly (as a result of anthrax) & those who ingested inadequately cooked infected predominated.

There are three modes of transmission of anthrax to man viz. Contact, ingestion or inhalation.
**Contact:** Contact with infected animals, carcasses, hides and skins, transmit anthrax to butchers, livestock handlers, carcass handlers & farmers etc. This is also known as hide & potters disease which causes the lesions known as "malignant pustule". This occurs due to cuts, abrasions or scratches on their skin. It was reported that the site of malignant pustule (lesions) vary with the occupational variations eg., persons who carry hides, skins of dead animals in meat plants & butcheries etc. lesions are found on back & neck, but the lesions are mainly found on hands, fingers, legs and wrists in case of butchers. Pustules develop with in 2-5 days of infection filled with fluid. Mortality rate is very low except when it changes to systemic infection. Finally black eschar with edema remains at the site of pustule. However, local edema & secondary infection can create complications.

**Through ingestion of infected meat (gastro intestinal form):**

This type of anthrax has been reported in India not only in sporadic form but outbreaks have been recognized in anthrax endemic areas also. It results due to ingestion of inadequately cooked meat of animals died due to anthrax and produces violent enteritis, fever, vomiting and bloody stool. Death can occur due to shock.

**Through inhalation: (Pulmonary form or wool sorter's diseases).**

This form of human anthrax is acquired through the inhalation of spore contaminated dust. After reaching into the lungs, spores develop to vegetative form of *Bacillus anthracis* & travel to pleura leasing to fatal peritonitis and pneumonitis. Bacilli can be demonstrated in sputum before death. It is prevalent in persons who are exposed occupationally in wool sorting process, fertilizer plants & carpet Industries etc. Meningo-encephalitic form has also been recognized in India. Pulmonary & gastrointestinal forms of anthrax in human beings can produce septicemia with high mortality rate.

**Occupational Anthrax:**

**Industrial anthrax:** Human acquire infection incidentally through cutaneous inoculation via abrasions, cuts and wounds during handling of infected carcasses, wool, hair & bristles, hides & skins, borne & blood meat etc.

**Non industrial or agricultural anthrax:** In India, the contamination of agricultural lands, grasslands, pastures, feeds & fodder & straw, etc. occur through waste materials & effluents coming from industries like leather, meat and tanneries etc. Agricultural workers may come in contact with contaminated carcasses, fluids & blood of animals who have died due to anthrax. In Tamil Nadu, 15 outbreaks in bovines with attack rate of 3.27 & case fatality rate 89.80 have been recorded while among ovines & caprines (Sheep and goats), 16 outbreaks with attack rate of 3.16 & case fatality rate 96.49 were reported.

Outbreak of human anthrax has also occurred in Andhra Pradesh where the main occupation is agriculture & animal husbandry. An epizootic was reported in cattle and sheep. Men opened these carcasses and consumed infected meat. Most of them developed cutaneous form of anthrax with fever & headache. No mortality was found in cutaneous form but it was higher among intestinal, septaeamic, pulmonary & meningo encephalitic form having anorexia, abdominal pain, vomiting etc. Cutaneous lesions in dog was also reported. The person who opened carcass showed cutaneous lesions.
Earlier there was no record of human cases in this area except sporadic cases of animal anthrax. One important feature was that meat was dried under the sun. This lead to sporulation. There is custom to open pregnant dead cows & bury the cow & the calf separately.

**Diagnosis:**

**Staining:** Staining the fluid from the unbroken vesicle (pustule) is thought to be the best specimen. The films made from the exudate stained by giemsa's or gram's stain gives charastically a long tangled gram +ve chain of bacilli, several showing central spores in case of animals films of blood are made from the ear veins of stained with gram's method or by McFadyeans methylene blue method. Fluorescent Ab technique is helpful in evaluation of smears obtained from lesion of patients after initiation of antibiotic therapy.

**Culture examination:**

Culture on blood agar and nutrient agar after overnight inoculation shows typical medusa head colonies with little or no haemolysis on blood agar.

**Serological diagnosis:**

Some times it is not possible to isolate the organism (*B.antracis*) from patients who are clinically suffering from cutaneaus anthrax. In such cases serological assays to demonstrate antibodies to the anthrax toxin can be applied. Precipitin test was firstly used by Ascoli, wherein the extract of the tissue is made in slightly acidified saline with heat. The fluid is cooled and then filtered through filter paper 0.5ml of the sèrum is placed in a narrow tube and the filtrate is carefully run on the top. The development within 15 min. of a white ring of precipitate at the junction of two fluids denotes a positive results.

Several specific and sensitive enzyme immunoassays based on purified toxin antigens are also available.

**Inoculation of laboratory animals:**

Inoculation of clinical material (0.5ml) in laboratory animals as guinea pigs (s/c) and mice (i/p) for ascertaining Koch's postulate and pathogenicity of bacilli i.e. death of inoculated animal within 30-40 hrs. of inoculation.

**PCR:** PCR has been reported to detect even a single cell of *B.antracis* and these can be used in diagnosis of the infection.

**Prevention and control:**

Surveillance to work out the occurrence and distribution of the disease.

Report disease to a control point.

Institute epidemiological investigation in case of new diseases.

General hygienic sanitary and quarantine measures.

Site management.
Site management/prevention:

Carcasses should not be allowed to be thrown in open areas, only burial or burning is advisable. However, burial also leaves long term environmental contamination therefore, the best is, if possible to incinerate the carcasses died due to anthrax along with animal excreta. Straw etc. collected in hygienic manner should be disposed off by incineration.

In burial process, anthrax infected carcasses must be buried 2m deep with quick lime or chlorinated lime (30%) of surroundings must be disinfected. Infected pasture is treated with hot lime (NaOH). Hides, wool, bone meals can be sterilized by gamma radiation or by subjecting to suitable heat process.

The natural orifices of the animals died due to anthrax should be plugged with cotton soaked in 5% cresol. Lime water & bleaching powder are used to disinfect surroundings, a thin lynne over the dead animal of the chlorinated lime is spread at the place of burial (2m deep with quick lime).

In abattoirs, if a case of Anthrax is confirmed at the time of ante-mortem or postmortem, the entry of persons is stopped. Blood, manure, excreta, straw etc. are burnt & surroundings are disinfected. Carcass is condemned along with its offal, hides, skin etc. & burnt. Hands, feet and arms of abattoir workers are washed in 3% cresol solution. Masks, gloves, aprons, boots, instruments, equipment's belts are scrubbed with hot saline or boiled with washing soda for 30 minutes & finally soaked in 2% cresol solution for 30 minutes. Byres, stables. Sty or Sheep holds are disinfected with 5% cresol by using long handle brushes. Vehicles or Bullock carts used for carrying carcass must be disinfected. Blood smears are taken from all animals which die suddenly before slaughtering. Animals are isolated & those having temperature of 103°F or above shall be segregated in the same pen and blood films examined. Reopening of slaughter house is allowed when meat inspector/in-charge is satisfied that dis-infection has been completed satisfactorily. There are large number of vaccines which have been developed for immunizing animals and human beings like spore vaccine, Pasteur vaccine, Sterne vaccine and USSR, UK and US vaccines for human beings respectively.

Anthrax spore vaccine is extensively used in India and other countries in the world. The disadvantage of Pasteur vaccine could be mitigated through production of spore vaccine.

The organisms are seeded in Roux flasks containing Nutrient Agar and incubated. Sporulation is allowed to complete. Growth is washed off in normal saline solution and suspended in 50% glycerin saline.

Dose = 1 Million spores/ml (1ml cattle, horse, sheep and goats)

Immunity is established within a week and solid immunity lasts for about 9-12 months.

Education/surveillance:

Surveys of animals & human beings, in the high risk groups like farmers, butchers and those working in meat, leather, hides, skin, wool, hair carpet, blood and bone meal, tanneries and fertilizer industries including population of high endemic areas
are necessary to be screened for prevalence of anthrax. These groups must be made aware about hazards & socioeconomic impact of anthrax through mass and electronic media. Persons must be advised to take adequate sanitary measures and disinfect the site. Persons should be made aware of the symptoms of anthrax and of the animal dying suddenly. They must send information to local veterinary officer for proper carcass disposal. Establishment of field surveillance stations/units, headed by qualified veterinary professionals and equipped with raped sensitive diagnostic facilities is necessary to carry out proper investigation & confirmation of sudden death cases (suspected to anthrax). This will facilitate proper diagnosis reporting and data collection. Strict enforcement of rules & regulations to prohibit slaughtering & sale of meat of dead animals. Last but not the least the health education training is essential for the veterinary professionals to achieve the goal of controlling & eradicating the anthrax from India.

Acknowledgements:

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RECENT TRENDS IN DIAGNOSIS AND CONTROL OF 
ZOONOTIC TUBERCULOSIS

G.C. Ram* and T.K. Goswami**

*Mycobacterium tuberculosis* accounts for more death worldwide than any other single human pathogen. The incidence of tuberculosis is increasing worldwide. Growing incidence of drug-resistant strains have been observed in several countries. In developing countries TB has remained a serious public health problem with extensive infectious loads and no sign of decline comparable to that experienced in industrialized countries. Approximately a third of the world population has been infected with *M. tuberculosis* and is at risk of developing disease. The WHO estimate is about 3 million deaths per annum due to TB, of which more than 95% occur in developing countries. The re-emergence of tuberculosis is attributed to HIV infection, deteriorating social conditions, dislocations, poverty, overcrowding etc.

**Diagnosis of tuberculosis:**

The timely identification of persons with active *M. tuberculosis* infection is important, because only 10% of infected immunocompetant persons will develop active tuberculosis during their life times, and overt disease symptoms appear fairly late in the infection. Appropriate preventive chemotherapy for persons whose infection is progressing toward overt disease can dramatically reduce the development of infectious tuberculosis.

The detection of tuberculosis to detect cell mediated immune response using skin testing with tuberculin or PPD has limitation for its lack of specificity and its inability to distinguish between active disease, prior sensitization, BCG vaccination and cross sensitization by other *Mycobacterium* species. In population with high rates of tuberculosis, antibody detection tests are reasonably specific and have predictive value similar to that of the acid-fast smear. However, there is poor specificity of this test in populations with low incidence of tuberculosis because of a large number of false-positive results due to exposure to environmental mycobacteria.

**Laboratory confirmation of infections:**

A definite diagnosis of tuberculosis requires the identification of *M. tuberculosis* bacilli. Conventional procedures for detecting *M. tuberculosis* in specimens start with microscopic examinations of smears for the presence of acid-fast bacilli and culture of the organisms and then biochemical tests to identify the species of mycobacteria. The entire process often requires 4-6 weeks.

**Species identification of cultured organisms:**

Several methods for identification of species of mycobacteria have been developed recently. These methods include detection of species specific mycolic acids by chromatography, nucleic acid hybridization using species specific nucleic acid probes, gene amplification, PCR, transcription mediated amplification etc. The combination of a nucleic acid hybridization with a culture grown in BACTEC 7H 12 broth or a _-nitro-(-acetyl amino-(-hydroxypropiophenone (NAP) BACTEC 7H 12 broth can help in the detection and identification of mycobacterial species with in 4-7 days. NAP inhibits the growth of *M. tuberculosis* complex but does not inhibit growth of

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nontuberculous mycobacteria. These tests show a high level of sensitivity and specificity for *M. tuberculosis* complex, which includes *M. tuberculosis*, *M. bovis*, *M. africanum* and *M. microti*.

Mycolic acids extracted from saponified mycobacterial cells are converted to the-bromophenacyl esters, and unique mycolic acid patterns associated with each species is detected by HPLC separation of the esters. The method has been used for accurate (98.6%) identification of slowly growing mycobacterial species. The analysis is based on a comparison of retention time of the peaks and their height ratio. However, this requires a high number of bacteria to perform this test.

One easily detected component of *M. tuberculosis* is tuberculostearic acid in cerebrospinal fluid is thought to be diagnostic for tuberculous meningitis and pulmonary tuberculosis. However, this is of limited value as some nontuberculous mycobacteria may produce components that will generate a false-positive signal.

**Gene Amplification:**

Several procedures used for detection of *M. tuberculosis* include strand displacement amplification (SDA), PCR amplification, transcription mediated amplification (TMA) and reporter phage system. The *M. tuberculosis*-specific amplification assays shows excellent specificity and sensitivity. In general, each amplification system can i) produce a clear positive signal from specimens containing as few as 1 to 10 bacilli, ii) clearly distinguish *M. tuberculosis* from other Mycobacterium species, iii) detect *M. tuberculosis* in specimens containing a large excess nucleic acid from other source and iv) takes less than one day.

Various repetitive DNA elements, viz., insertion sequences (IS) short repetitive DNA sequences of *M. tuberculosis* have been used for the molecular epidemiology of tuberculosis. IS6110 a 1,355-bp sequence found in *M. tuberculosis* complex of mycobacteria. In general, 5-20 copies are present in the genome of clinical isolates of *M. tuberculosis* with nil in some of the strains. IS 1081 thought to be *M. bovis* specific DNA sequences also found in the other member of tuberculosis complex. IS6110 DNA fingerprinting has been extensively used to identify TB outbreaks.

**Current vaccination and future prospects:**

*M. bovis* BCG is the only currently available vaccine against tuberculosis. The most striking feature of the BCG vaccination trials is variation in efficacy from 80% to no detectable protection in human. The failure in protection is attributed to background sensitization of population. BCG vaccination protects against development of serious disease after primary infection. However it does not protect against reactivation of disease in adults, which is responsible for most cases of pulmonary TB and thus for most infectious load in the society.

The basis for the attenuation of the virulent *M. bovis* to BCG has not been established but esat-6 gene present in the virulent has been shown to be absent in BCG while the mpb64 gene was absent in 4 of the 8 substrains. DNA restriction fragment patterns revealed extensive genotype differences between and within BCG substrains. The BCG vaccine has been shown to induce powerful immune responses and provide cattle with significant level of protection against intratracheal challenge with virulent *M. bovis*. 
Subunit vaccine:

Recent studies have demonstrated that culture filtrate antigens from late log culture of *M. tuberculosis* along with adjuvant give significant protection to infection in mice. Short-term culture filtrate and antigen 85 complex have been shown to give protection. However, it has been demonstrated that the protection conferred by the killed or subunit vaccine is short whereas live vaccine provides significant and prolonged protection.

DNA vaccine:

Experiments on immunization of mice with DNA encoding mycobacterial proteins have indicated that it could be effective approach to generate protective immune response. Vaccination of mice with DNA coding for the secreted 85A and 85B antigens of *M. tuberculosis* has provided protection equal to live BCG. Very recently vaccination using DNA coding other mycobacterial antigens like HSP 65 and HSP 71 has been tried and have shown promising result in the experimental animals. There is a reasonable prospect that a nucleic acid vaccine could be superior to BCG as it contains a few antigens and, therefore, need not interfere in the diagnostic and epidemiological investigation. Therefore, for the control of tuberculosis immunotherapy along with chemotherapy may be helpful.

REFERENCES


L. monocytogenes: Detection and Control

K.N. Bhilegaonkar*

Listeriosis is an important zoonotic disease caused by Listeria monocytogenes. During recent years the organism has emerged as an important food borne pathogen responsible for many outbreaks in human being.

Characteristics of organism

L. monocytogenes is a gram-positive, non-spore forming, acapsular, facultative anaerobic bacillus. The organism possesses peritrichous flagella and shows a characteristic tumbling motility at 20-30oC. The organism grows well between temperature range of 4-50oC. It is catalase positive, oxidase negative and produces haemolysin. L. monocytogenes is an ubiquitous organism, it survives and can multiply under diverse environmental conditions. It has been recovered from dust, soil, water, sewage and decaying vegetation including silage and other fields. The organism capable of growing at moderately low pH and under high salt concentration.

Listeriosis in animals

L. monocytogenes has been isolated from at least 42 species of mammals and 22 species of birds, as well as fish, crustaceans and insect. There is evidence that many animals may be healthy carriers of L. monocytogenes with organism lying dormant in various locations until stress triggers release and multiplication.

Of farm animals sheep and cattle are most commonly affected with listeriosis. The different forms of listeriosis occurring in sheep, goat and cattle are:

1. Encephalitis- circling disease.
3. Septicaemia in unweaned animals.
5. Diarrhoea and septicaemia in ewes.
6. Purulent keratoconjunctivitis.

In other animals disease conditions seen are

Swine - Meningo-encephalitis, abortion.
Horses - Encephalitis, septicaemia.
Fowl - Common findings at necropsy are multiple degeneration in heart muscle with pericardial effusion, focal necrosis in liver and kidney.

Listeriosis in man

Listeriosis can occur in healthy adults and children, however, the most vulnerable groups include pregnant women, infants, elderly and immuno suppressed persons. Symptoms are variable and depend on the individual susceptibility. Disorders
associated with *L. monocytogenes* are meningo-encephalitis, influenza like low grade septicemia in prenatal period, infectious mononucleosis like syndrome, septicemia in adults, pneumonia, endocarditis, localized abscesses, papular or pustular cutaneous lesions, conjunctivitis, urethritis and abortion.

**Incidence in foods**

*L. monocytogenes* survives well in wide range of food and food products during various stages of production processing and storage. Due to its wide spread occurrence in nature, it often readily establishes in foods. The organism has been reported to occur in various dairy products such as milk, butter and cheese, later being one of the most common items incriminated in food borne listeriosis. The incidence in dairy products has been reported to be from less than 10% to as high as 87%. The presence of organism has also been reported in various other food commodities such as vegetables, seafood, poultry products and meat/meat products. Various processes such as freezing, surface dehydration and simulated spray chilling does not adversely affect its survival. In India also the presence of organism has been reported from cow milk, buffalo milk, sheep meat, goat meat, buffalo meat and vegetables.

**Mode of transmission**

Silage is primary vehicle for *L. monocytogenes* transmission in animal species. For human listeriosis the apparent source of infection is ingestion of foods of animal origin which became contaminated by *L. monocytogenes* from various environmental sources. However, there is evidence that *L. monocytogenes* may infect humans and animals by the oral, ocular, cutaneous respiratory or urogenital routes.

**Fig. 1: Transmission cycle of *L. monocytogenes***

(Source, Smith et al., 1990)
Virulence factors

Virulence of various serotypes of *L. monocytogenes* are multi factorial. These include haemolysin, phosphatidyl inositol specific phospholipase (PI-PLC), catalase activity, super oxide dismutase and iron transport system. Some surface antigens, secreted proteins and monocytes stimulating activity are also involved.

Detection of *L. monocytogenes* from foods

Detection of *L. monocytogenes* from the foods heavily contaminated with the other organism is a challenging proposition to researchers. Conventional methods of isolation and identification of organism are the most reliable and authentic but are time consuming and laborious. Now a days several newly developed methods such as nucleic acid probes, polymerase chain reaction, enzyme immuno-assays are used for detection of *L. monocytogenes*.

Conventional isolation method

Suitability of any specific method or medium is influenced by the purpose of analysis and the type of sample being analyzed. Thus media devised for isolation of organism from relatively uncontaminated clinical samples (e.g. CSF) are unsuitable for foods/feces which are often contaminated with other microflora. Among earliest methods used for recovery of *L. monocytogenes* from foods and environmental samples was use of cold enrichment. However, back of this method was long duration (upto 3 months) required for identification of the organism. More recently incorporation of certain specific selective agents such as nalidixic acid, acriflavine, Lithium chloride, phenyl ethanol, moxalactum etc. into enrichment and plating media has shown to reduce time required for isolation.

Various selective broths used for enrichment of *L. monocytogenes* are University of Vermont (UVM) broth, Fraser broth, polymyxin acriflavine lithium chloride ceftazidine aesculin mannitol egg yolk (L-PALCAM) broth, Thiocyanate nalidixic acid (TNA) broth,

Various plating media used for isolation of organism are Lithium chloride phenyl ethanol moxalactum (LPM) agar, Domínguez-Rodríguez Agar (DRA), Polymyxin acriflavine lithium chloride ceftazidine aesculin mannitol (PALCAM) agar, oxford agar, modified oxford agar, modified Vogel Johnson (MVJ) agar, Al-zoreky sandine *Listeria* medium (ASLM).

To facilitate rapid identification by conventional cultural methods various commercial firms have recently introduced rapid identification systems which are based on analysis of enzymes and carbohydrate fermentation pattern, viz., API-20, STREP, API-50CH, MAST-ID, API-*Listeria* etc.

Newer methods

In recent years several rapid immunological, nucleic acid based or other detection methods have been developed to monitor the incidence of *L. monocytogenes* in foods. These methods include enzyme linked immunosorbent assays (ELISA), Fluorescent antibody test (FAT), nucleic acid probes, polymerase chain reaction (PCR) and flow cytometry.
**Immunological methods**

Several workers have developed enzyme immunoassays and immuno-fluorescent assays for rapid detection of *Listeria*, these assays either use polyclonal antibodies or monoclonal antibodies (McLaughlin et al., 1989, Beumer and Brinkman, 1989, Oladepo et al., 1992). Two commercial ELISA kits viz. *Listeria*-tek assay and Tekra ELISA are also available for detection of *Listeria* in foods. However, all these immunological methods are useful for detection of genus *Listeria* but not *L.monocytogenes*. A more recent approach in immunological detection methods has been use of antibodies directed against specific and virulence related cell surface proteins/outer membrane proteins of *L.monocytogenes* (Chen and Chang, 1996, Bhilegaonkar, 1998).

**Nucleic acid probes**

First DNA probe for *L.monocytogenes* detection was reported in 1987 (Datta et al., 1987). Later several probes were developed based on the virulence genes of the organism. The virulence factors that have formed the basis for DNA probes are a) Haemolysin (*hly*) gene fragment b) Major secreted polypeptide (*msp*), which is identical to invasion associated gene (*iap*) c) A fragment containing the gene encoding for delayed hypersensitivity inducing protein (*DTH-IP*). Non virulence gene based probes have also been developed from rRNA sequence of *L.monocytogenes*. One currently available commercial hybridization assay viz. gene trak-*Listeria* hybridization assay uses hybridization to rRNA molecules.

**Polymerase chain reaction**

In first reported PCR for identification of *L.monocytogenes* the *hly* gene sequence was used (Bessesen et al., 1990). Numerous other PCR assays have been described for detection of *L.monocytogenes* and other *Listeria* spp. Multiplex PCR assays where parts of two different genes are amplified simultaneously, have also been evolved to detect *L.monocytogenes*.

**Other methods**

Other rapid methods that have been reported for detection of *L.monocytogenes* are use of flow cytometry, electrical impedance and immuno-magnetic separation techniques.

**Diagnosis of Listeriosis in man and animals**

Tentative diagnosis of listeriosis can be done on the basis of 1) clinical symptoms 2) demonstration of organism in smear by grams staining, peroxide-antiperoxide method or FAT. Confirmation is done by isolation of pathogen from clinical specimens such as blood, CSF, meconium of newborn or fetuses in abortion etc.

Other methods for diagnosis of Listeriosis include 1) Detection of organism by PCR, nucleic acid probes in clinical specimen. 2) Sero-diagnostic methods such as ELISA, CFT, agglutination, precipitation test etc. However, using crude antigens these tests give cross-reactions and are not useful for diagnosis. Recently the detection of antibodies against Listeriolysin-O by ELISA has been reported to be useful for diagnosis of listeriosis (Barbuddhe et al., 1998).
Control/reduction of *L. monocytogenes* in food

*L. monocytogenes* is a difficult microorganism to control in food processing establishments. The organism has ability to survive and grow in refrigerated foods, moreover, the organism can adhere to food contact surfaces and form a "biofilm" or coating which impedes the effectiveness of sanitation procedures. Thus refrigerated, moist environment coupled with organic soil deposition allows *L. monocytogenes* to survive and grow, in addition to this, there is constant reintroduction of organism into plant environment. Therefore to control organism every potential avenue of entry and cross contamination must be controlled.

HACCP based approaches and use of quantitative risk assessment have helped to identify critical control points and have increased effectiveness of control systems. To effectively control *L. monocytogenes* from foods requires total commitment to these HACCP principles.

NACMCF (1991) has recommended many procedures and practices to prevent *L. monocytogenes* contamination of food in five areas of food establishments viz. plant design and layout, equipment design, process control, employee practices and cleaning, sanitation practices and procedures.

Consumers can also follow certain simple rules of safe food production for the prevention of listeriosis these are:

* Wash hands and utensils before handling foods and especially handling raw foods.
* Reheat all foods thoroughly (above internal temperature of 165°F, 74°C).
* Keep hot food hot (above 63°C, 145°F).
* Keep cold food cold (below 4°C, 40°F).
* Thoroughly cook meat, poultry and seafood and adequately heat frozen or refrigerate foods.
* Chill foods rapidly in shallow containers.
* Keep raw and cooked foods separate.
* Wrap and cover food in your refrigerator.
* Avoid raw milk and cheese made from unpasteurized milk.

ICMSF (1994) has recommended sampling plan and microbiological guidelines for *L. monocytogenes* in foods.
Table 1: A recommended (ICMSF1994) sampling plan for *Listeria monocytogenes* in foods.

<table>
<thead>
<tr>
<th>Intended consumer</th>
<th>Health hazard</th>
<th>Conditions in which food is expected to be handled and consumed after sampling in the usual course of events</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Reduce degree of hazard</td>
</tr>
<tr>
<td>Normal individuals</td>
<td>Moderate, direct, potentially extensive spread</td>
<td>Case 10 (n=5) (c=0) (m=&lt;100/g)</td>
</tr>
<tr>
<td>Highly susceptible individuals</td>
<td>Severe, direct</td>
<td>Case 13 (n=15) (c=0) (m=0^a)</td>
</tr>
</tbody>
</table>

\(^a\) Minimum sample size = 25 g.

Prevention and control of listeriosis in animal and human

For prevention and control of listeriosis in animals various measures that should be adapted are:

1. Culling of infected animals.
2. Care in use and preparation of silage.
3. Immunization — no satisfactory vaccine is available. However, there is live attenuated vaccine developed in Bulgaria which is based upon serovars 1/2a and 4b of *L. monocytogenes*. This vaccine is available in some European countries and claimed to be effective in sheep but field trials are equivocal.

Listeriosis can be prevented in man by 1) taking care during handling of abortion cases 2) avoiding consumption of contaminated foodstuffs and 3) by avoiding cross infection especially in hospitals among infants.

REFERENCES


Rabies

M.L. Mehrotra*

Rabies is a most ancient disease with its distressing cause and reputedly invariably fatal outcome. It is usually fatal viral encephalitis of warm-blooded animals (wild and domestic), characterized by a period of long variable incubation period. It is endemic in all continents except Australia and Antarctica. Many islands viz. Great Britain, Ireland, Cyprus, Japan, New Zealand including Andaman of India are free from urban rabies. Outbreaks of rabies have been recorded among cattle and horses.

The disease is zoonotic and is great problem in all Asian and African countries. The disease has great economic impact due to heavy expenditure on vaccination and involvement of public health.

Etiology

The causative agent of the disease is virus belonging to the family Rhabdoviridae. This virus is resistant and survives long time in carcasses. But virus is very susceptible to soap and fat solvents viz. ether, alcohol.

Reservoir of virus

Both domestic and wild animals viz. dog, cat, foxes, wolf, jackals, skunks, and blood sucking bats are reservoir and transmit the virus to urban population of livestock and dogs.

Diagnosis

Diagnosis of rabies is very important. A timely and correct diagnosis will eliminate a long costly and painful course of vaccination among animal lovers and persons accidentally exposed to rabies.

Clinical Diagnosis

Usually incubation period is 38 weeks. It may be as short as 10 days and as long as 9 months in dogs. An early sign usually is change in behavior of dogs (friendly to unfriendly) last 2-5 days and in 75 percent of cases them progress to dumb or paralysis and to furious form in rest. Two syndromes occur in natural cases viz. Furious and dumb rabies. But some time both types are seen alternately. This needs confirmation through laboratories. Dogs do not exhibit hydrophobia, but paralysis and death occurs in both dumb and furious forms. Foreign bodies are seen in stomach of dogs on postmortem examination.

Laboratory Diagnosis

All cases suspected for rabies be autopsied. A systematic postmortem examination will help in diagnosis and elimination of rabies. Most of the time foreign objects are found in side stomach which give some indication. No distinct postmortem lesions are seen in dogs. While conducting postmortem, all internal organs including brain be collected in 10% formalin. The spleen and lymph nodes be collected in sterile containers. From brain small pieces of medulla, Cerebrum, cerebellum and hippocampus be collected in sterile 50% buffered glycerin and 10% formalin separately.

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All bottles should be watertight. The bottles be pack in double polythene bags. They are packed in hard boxes and dispatched to laboratories by post or courier. Few smears from brain be prepared and stained with seller's stain. Material suspected for rabies be indicated on boxes.

Tests commonly used are direct examination of impression smears, for Negri body, fluorescent antibody technique (FAT), histopathology and biological test in mice. All tests be carried out where facilities are available (Annexure-I).

In recent years enzyme coupled antibody has been claimed to be specific but less sensitive. ELISA modified as "Rapid Rabies Immuno-enzymatic diagnosis (RRIED)" in which microscopy and FAT are necessary. Further advancement into the molecular field and dot-hybridization has been used in laboratory animals. The virus isolation in BHK 21 cell lines has been attempted in laboratory trials by some workers. More recently polymerase chain reaction (PCR) has been claimed as rapid method of identification and diagnosis of virus.

Control

Regular elimination of stray dogs help in control of rabies. Dogs may be castrated if public opinion does not permit elimination. Live attenuated and inactivated vaccines are available in the market. Instructions given by manufacturers be followed for vaccination of dogs and other pets.
Japanese encephalitis (JE) has emerged as a major public health problem inflicting heavy losses of human lives in most of the countries of East and South Asia including India. On the basis of transmission cycle and antigenic relatedness, JE virus was classified as a member of Group B arboviruses belonging to genus Flavivirus of family Togaviridae. The genus Flavivirus now has been elevated to an independent family Flaviviridae.

JE virion is an enveloped spherical particle of 38-50 nm. It contains single stranded positive sense RNA genome. There are 3 structural proteins associated with JE virus. These are membrane protein (M), Core protein (C) and envelope glycoprotein (E). The virus encodes seven nonstructural proteins viz. NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. Immunologically, it cross reacts with other members of flaviviruses particularly Murray valley encephalitis, St. Luis encephalitis, yellow fever, West Nile and various types of dengue viruses.

Geographic distribution:

Encephalitis due to JE virus has occurred in Eastern Siberia, China, Korea, Japan, Taiwan, Singapore, Malaysia, Thailand, Vietnam, Indonesia, Sri Lanka, India, Nepal, Burma and Bangladesh.

Outbreaks in different parts of India occur in a rather unpredictable manner. Cases of JE were first recognized in 1955 in Tamil Nadu. Subsequently, epidemics have been reported from various parts of the country viz. Bihar, Uttar Pradesh, West Bengal, Assam, Nagaland, Manipur, Andhra Pradesh, Madhya Pradesh, Karnataka, Tamil Nadu, Kerala, Goa and Pondicherry (Banerjee, 1996).

Animal Reservoirs:

Pigs and ardeid birds such as egrets and pond herons have been incriminated as the most important hosts for maintenance of JE virus. The infection in pigs is generally inapparent except the cases of still birth or abortion in pregnant sow and aspermia in boar. About 0-50 percent of pigs have been shown to be positive for JE antibodies in serological studies in different areas of the country. Sentinel pig studies carried out in different parts have also shown very good correlation with actual JE cases. The antibodies against JE virus have been documented in horses, cattle, sheep, goats, dogs, monkey and reptiles. Horses suffer from fatal encephalitis.

Vectors:

At least 12 species of mosquitoes belonging to 3 genera viz. Culex (6 spp.), Anopheles (3 spp.) and Mansonia (1 spp.) have so far been implicated as vectors of JE in different regions of the country. Of these, Culex tritaeniorhynchus, C. vishuni, C. pseudovishuni and C. whitmorei may be the main vectors involved. In addition, Anopheles barbirostris, An. hyrcanus and An. subpictus have been reported in different outbreaks. Transovarial transmission of the virus in different species of mosquitoes is also possible.

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Seasonality:

The epidemics coincides with rainy season i.e. the period of high mosquito prevalence. However, sporadic occurrence of the disease has been reported practically through out the year in endemic areas.

Clinical features in man:

Man is considered as dead end host for JE. Incubation period is 1 to 2 weeks. The disease is characterized by acute encephalitis with sudden onset of fever and headache. After a prodromal stage of few days, complete symptoms of encephalitis appear. Nuchal rigidity is present in all cases. Other symptoms like chills, vomiting, mailase, photophobia, peruses and tremors are accompanied. The case fatality rate is around 20-25%.

Pathogenesis:

Mechanisms of penetration of the blood brain barrier are not well known. The virus may replicate in blood vessels, facilitating early replication in the brain. Grossly, the brain appears to have vascular congestion, mild oedema, and minimal overlying cellular exudate. The infection and destruction of neurons in brain stem explains the profound coma and respiratory failure. In patients dying many years after illness from JE virus lesions in brain are generally localized to the thalamus, substantia nigra, and ammon’s horn, with relatively mild lesions in the cerebral cortices.

Diagnosis:

JE infection can be detected by:

Detection or isolation of virus or viral antigen.
Detection of antibodies.

Detection or isolation of virus or viral antigen:

Virus can be isolated from cerebrospinal fluid collected during early acute stage or brain in fatal cases. The specimen should be transported and stored at 4_C or on ice. Brain tissue can be transported in glycerol saline or in nutrient broth added with antibiotics.

Viral antigen can be demonstrated in brain tissue employing the fluorescent antibody assay or polymerase chain reaction.

Isolation is achieved either by intracerebral inoculation in infant mice or by infecting the cell culture. Vero, porcine kidney or mosquito hamster kidney cell lines are used for the purpose.

The virus may be serologically identified in saline suspension of infected mouse brain or cell culture supernatant by complement fixation test. Since JE shows cross reactions with WN and dengue viruses, it is tested against known sera of these viruses. Monospecific antisera obtained by absorption of cross reacting antibodies are also used. Other tests to detect or confirm the viral antigen are agar gel diffusion (AGD), neutralization test, immunofluorescence and reverse passive haemagglutination test.
Detection of antibodies:

The best results are obtained by testing the 'paired sera' samples. The first sample should be collected in acute phase and second during the convalescence i.e. at the interval of 3-4 weeks. The samples should be absorbed with Kaolin to check the non-specific reactions.

A variety of tests have been employed for serodiagnosis, but the conventional haemagglutination inhibition, compliment fixation and virus neutralization techniques are most widely employed. However, the most reliable method is enzyme immunosorbent assay. Other tests namely indirect haemagglutination, indirect immunofluorescence, staphylococcal coagglutination, single radial haemolysis in gel and antibody capture radio immunoassay have also been developed and used for diagnosis of JE.

Prevention and control:

JE can be controlled by interruption of transmission cycle between reservoir/amplifier hosts, biological vectors and susceptible population.

Preventive measures include:

- Studies on surveillance on density of vector species and their susceptibility to various insecticides are to be carried out.
- An early warning system must be developed by surveillance and monitoring the virus or seroconversion in pigs and other animals.
- More research is required to clarify the overwintering mechanism of virus.
- Mosquitogenic conditions should be reduced by proper water management.
- Aerial or ground fogging with ultralow volume insecticides should be done.
- Immunization of known amplified host (pigs) and human population at risk.

The indigenous production of inactivated mouse brain JE vaccine has already been started at Kasauli. A formalin inactivated vaccine prepared in mice infected with the Nakayama strain is being widely used in Japan, Korea and Taiwan. In China, inactivated vaccine prepared in hamster kidney cell cultures has been given to millions of children. A live attenuated vaccine prepared from SA 14-2 strain has given very promising results in children in China (Porterfield, 1990, Haolin et al., 1995).

The people should be encouraged to avoid the exposure to mosquitoes by using mosquito net and mosquito repellent etc.

REFERENCES


CHLAMYDIOSIS & RICKETTSIOSES

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(A) CHLAMYDIOSIS:

Chlamydiosis is a zoonotic disease caused by *Chlamydia psittaci*. The Chlamydiaceae are obligate intracellular pathogens that can infect many species of birds, animals as well as man. Most, but not all, human infections result from exposure to psittacine birds. In 1893, transmission of a "flu-like" disease from parrot to man was reported in Paris and named as psittacosis after a Latin word-"psittacus" meaning parrot. Since then, cases of chlamydial infections have been reported from world over. During 1982-91, a total of 1,344 human cases of chlamydiosis, with 6 deaths, were reported from USA. In these birds, caged pet bird exposure accounted for 70% in human cases for whom source of infection was known. The largest group of affected people was pet bird owners or fanciers (43%). Pet shop employees accounted for an additional 10% of cases. Other people at risk include pigeon fanciers, poultry processing plant employees, veterinarians, veterinary technicians, laboratory workers, avian quarantine station workers, farmers, zoo workers, custodians, and construction and demolition workers.

Human infection usually develops through the inhalation of the organism aerosolized from urine, respiratory tract secretions, and feces. Other sources of exposure can include bird bites, mouth-to-beak contact, and handling plumage and tissues. Because human infection can result from transient exposure to infected birds, or their contaminated droppings, many individuals with no identified avocational or occupational risk may become infected. Moreover, because many people with confirmed and presumptive disease require hospitalization, chlamydiosis is a public health problem.

In pregnant women the infection can be life threatening. Sheep are a recognized source of human infection, the most common route of transmission being direct contact with aborted fetuses or infected ewes during lambing time. Ewes usually abort only once as a result of *C. psittaci* infection, thereafter developing a life-long immunity. Sterile immunity does not develop in non-pregnant sheep, after a natural primary infection. Instead, the organism appears to enter a 'latent' phase from which it can reactivate and cause abortion in a subsequent pregnancy. The enzootic abortion of ewes (EAE) strain of *C. psittaci* is a zoonotic pathogen that has severe repercussions if primary infection occurs during pregnancy. Infection in non-pregnant individuals is less severe and may not always be diagnosed. A group of eight laboratory workers were found to be infected with the EAE strain of *C. psittaci* while producing a vaccine. Their illnesses ranged from minor upper respiratory tract infections to influenza-like symptoms. There are instances where pregnant women have become infected after direct contact with lambing ewes from infected flocks. In one report, three women who became infected in the latter stages of pregnancy (25-28 weeks gestation) presented severe clinical symptoms, including fever, headache, vomiting and myocarditis. They all went into spontaneous labour within a few days of the onset of the symptoms. The women recovered but none of the infants survived. There is also a report of infection of a pregnant abattoir worker in the 19th week of pregnancy, which resulted in spontaneous abortion of the fetus. The women's condition was
life threatening. There is no evidence to suggest that the transmission of EAE strain of \emph{C. psittaci} occurs from human-to-human.

Although \emph{C. psittaci} can cause ovine and human abortion, there are marked differences in the pathologies of infection. The severe clinical symptoms described for pregnant women at the time of abortion are not seen in ewes. In addition, there is nothing to indicate that the organism will infect non-pregnant women, then cause abortion in a subsequent pregnancy, analogous to the situation in sheep. There may be fundamental differences in the human and ovine immune response to \emph{C. psittaci}. These remain to be elucidated. Therefore, it can be concluded that abortion as a result of infection with the EAE strain of \emph{C. psittaci} affects several ruminant species and is a rare, but severe, zoonosis. In India chlamydiosis in sheep has been identified as one of the important emerging diseases since last one decade.

**Taxonomy, Host range and Zoonotic potential:**

The bacterium has been isolated from 129 avian species and is most commonly identified in psittacine birds (e.g., parakeets, parrots, macaws, and cockatiels). Among nonpsittacine caged birds, infection develops most frequently in pigeons, doves, and mynah birds. Because several human diseases can be caused by other species of \emph{Chlamydia}, infection in human beings with \emph{C. psittaci} frequently is referred to as psittacosis. In nonpsittacine birds, the disease is referred to as ornithosis.

Within the genus- \emph{Chlamydia}, there are four species: \emph{C. trachomatis}, \emph{C. psittaci}, \emph{C. pneumoniae} and \emph{C. pecorum}, which have been classified according to their antigenic, pathological and molecular properties. \emph{C. trachomatis} and \emph{C. psittaci} were recognized as distinct species in 1968 on the basis of sulphadiazine sensitivity and iodine staining. \emph{C. trachomatis} is primarily a human pathogen (also found in pigs and one mouse biovar has been identified), which causes disease of the eye and reproductive tract. \emph{C. trachomatis} is not thought to have an animal reservoir for human infection and is not considered to be zoonosis.

The strains grouped as \emph{C. psittaci} are much more diverse in their host range and the pathology of the diseases that they cause. This has led to a re-classification of \emph{C. psittaci} strains and two other species have been recognized: \emph{C. pneumoniae} is predominantly a human pathogen causing pneumonitis (also found in horses and koalas) and \emph{C. pecorum}, which is a pathogen of ruminants and pigs, and includes a number of strains associated with symptoms of enteritis, polyarthritis, encephalomyelitis and pneumonia. One porcine abortion strain isolated in the USA has been classified as \emph{C. pecorum} but the ruminant abortion strains remain unclassified within the heterogeneous \emph{C. psittaci} species along with avian, feline, guinea-pig and koala strains, which cause pulmonary, ocular and urogenital infections.

Representations of all the four \emph{Chlamydia} species occur in animals. \emph{C. psittaci} now comprises, \emph{inter alia}, the avian subtypes (five serovars are recognized, including pneumotropic and synvotropic forms; and the agents of enzootic abortion of ewes (EAE) and feline pneumonitis. \emph{C. pecorum} has so far been found only in ruminants and pigs (\emph{pecorum} means of 'of flocks or herded animals'). Several \emph{C. pecorum} subtypes are recognized, associated variously with an arthritis/conjunctivitis syndrome (sheep and goats), encephalomyelitis, pneumonia and metritis/infertility (all cattle) and intestinal infections (ruminants and pigs). \emph{Chlamydia} isolates closely related to
C. trachomatis have been found not only in mice but also pigs. Finally, koalas may be infected with subtype of both C. pecorum and the human respiratory pathogen C. pneumoniae.

The notable zoonotic diseases caused by C. psittaci are ornithosis/psittacosis associated with avian strains and, abortions resulting from infection of pregnant women with the strain that causes enzootic abortion of ewes (EAE). Chlamydia from sheep can be readily divided into two main types on cultural characteristics, i.e., abortion type, which have compact inclusion morphology and in which the inclusions appear within 48h; and enteric type, which have diffuse inclusion morphology and the inclusions appear in 24h. In addition, the abortion type grows well in a sheep fibroblast culture whereas enteric types do not grow in this cell culture. These characteristics enable the two types to be distinguished on primary culture or early passage. Whilst there is evidence that the intestine is a reservoir for some pathogenic Chlamydia, this evidence is contradictory and other Chlamydia found in faeces may be harmless commensals.

Life cycle and Transmission:

The infectious stage is the Chlamydial elementary bodies (EBs) which infect host cells and then develop into reticulate bodies (RBs). The RBs replicate for 48 to 72 hr and mature back to EBs, which lyse the host cell and infect new cells. The incubation period in caged birds varies from days to weeks, most commonly being 5-14 days. Latent infections are common, however, an active disease may develop years after exposure. Shipping, crowding, chilling, breeding and other stressful factors may activate shedding of the infectious agent. Normal appearing birds may be carriers and intermittent shedders of C. psittaci. The organism is excreted in the feces and nasal discharges of infected birds, and also in the placental discharges of aborting ewes and does. The agent is resistant to drying and can remain infective to other hosts for several months.

Clinical Signs:

In birds:

Signs of the Chlamydial infection vary with the species of bird affected, virulence of the strain, stresses on the bird, and route of exposure. In generalized form of the disease, birds are lethargic, anorexic, and have ruffled feathers. Other signs include serous or mucopurulent ocular or nasal discharge and diarrhoea, with urates stained green to yellow-green. Anorectic birds may have sparse, dark green droppings. As the disease progress the bird may become emaciated, dehydrated, and may die.

In man:

Infected human beings typically abruptly develop fever, chills, headache, malaise, and myalgia, with or without symptoms referable to the respiratory tract. A nonproductive cough usually develops, and a pulse-temperature dissociation is not uncommon. Pneumonia frequently develops. Extrapulmonary involvement of chlamydiosis may include endocarditis, myocarditis, hepatitis, arthritis, and encephalitis. Fatality rate is less than 1% in properly treated patients. Human beings may become re-infected with antigenically distinct strains. Person-to-person transmission is rare.
Diagnosis:

In birds: **Clinical signs**- Chlamydiosis is presumptively diagnosed on the basis of clinical signs in live birds and, gross findings like cloudy air sacs, hepatomegaly and spleenomegaly in dead birds. **Culture**- The most specific diagnostic procedure is isolation of *Chlamydia* in tissue culture, mice, or chick embryos. Sample should be refrigerated, transported with wet ice and, processed in Bovernick's buffer added with antibiotic supplement. In live birds, cloacal swab specimens should be collected for 3 to 5 consecutive days because of intermittent shedding of the agent in faeces. Liver and spleen are the preferred necropsy specimens. The processed material is inoculated either in 6-7 day old embryonated chicken eggs through yolk sac route or in guinea pigs through intraperitoneal route. Appearance of red coloured intracytoplasmic inclusions resembling pin-head dots against green coloured cell cytoplasm by Gimenez staining is most characteristic histological feature in smears prepared from yolk sac membrane or peritonial fluid. **Serological tests**- The major problem with serological testing is the interpretation of results. A positive result is evidence that the bird was exposed to *C. psittaci* in the past, but does not prove the bird is free of infection. It is prudent to use direct complement fixation (CF), modified-direct (CF), a latex agglutination or ELISA in combination with antigen detection methods. Either a four-fold or greater increase in titer or a combination of a titer and antigen detection are needed to confirm a diagnosis of chlamydiosis. **Tests for antigen**- Immunofluorescent staining of cloacal or fecal smears from live birds, or impression smears from dead birds is the most useful method if the bird is shedding in large amounts of antigen. The results of ELISA for detecting *C. psittaci* must be evaluated in conjunction with clinical signs. Additional tests for antigen detection include elementary body agglutination test, microagglutination test, and micro-immunofluorescence.

In man: **Clinical signs**- The disease manifest in flu-like illness, and is commonly associated with pneumonia. Most of the persons have the history of contact with birds or, animals that have aborted/parturated recently. **Isolation**- A person is confirmed to have chlamydiosis if clinical specimens are culture positive for *C. psittaci* or there is a four fold or greater increase in *Chlamydia* CF antibody titer to 32 or greater in paired sera obtained at least 2 weeks apart. The infective agent may be isolated from sputum, pleural fluid, and clotted blood during acute illness prior to treatment with antibiotic. Lung spleen and liver are appropriate specimens for histologic analysis. **Serological tests**- Diagnosis is almost always established by testing paired sera for *Chlamydia* CF antibodies. However, these antibodies are not species-specific and may also result from *C. pneumoniae* and *C. trachomatis* infection. Microimmunofluorescence assay may distinguish *C. psittaci* infection from infection with other chlamydial species.

Control:

surgical cap and mask when cleaning cages or handling infected birds. Also by wetting the carcass to prevent aerosolization of infectious particles and working under an exhaust fan examining hood, [7] epidemiological investigations within 60 days of the onset of signs or if a bird comes in contact with a human being with confirmed chlamydirosis.

In man: Personal protective measures as explained above should be undertaken and, the disease should be reported to the health authorities for record and epidemiological investigations.

Treatment:

In birds: All affected birds should be placed in isolation and treated with chlortetracycline and vitamins in feed. During treatment the bird should be kept in isolation to avoid infection of untreated birds and environmental sources. There is no vaccine against avian chlamydirosis. Thorough cleaning and sanitizing is necessary to prevent re-infection. Stress, poor husbandry, and malnutrition will reduce the effectiveness of treatment.

In man: Oral tetracyclines are the drug of choice for treatment. Remission of symptoms usually is evident within 48 to 72 hrs, however, relapse is common, and treatment must continue for 10 to 15 days. Erythromycin is probably the best alternative drug.

(B) RICKETTSIOSES:

Rickettsial agents are associated with various forms of typhus and spotted fevers in man. Many species of *Rickettsia* are transmitted by arthropod vectors. Rickettsiae multiply within the macrophages and vascular endothelium of the host, and cause fever, headache, and the development of eschar and rashes on the skin. Rickettsias are named after Howard T. Ricketts who in 1909 for the first time identified them as the causative agent of typhus and Rocky Mountain Spotted Fever. In 1913, Stanislas Von Prowazek isolated the organism from a case of typhus. Both of these workers died of laboratory infections from these highly infectious organisms in early 1900s. *Rickettsia prowazekii*, the causal agent of epidemic typhus, was named in the honour of their pioneering work.

Among rickettsial infections, the typhus groups are named variously: (1) *Epidemic typhus*- classical typhus, louseborne typhus, war fever or jail fever, (2) *Endemic typhus*- murine typhus, rat typhus, flea typhus, fleaborne typhus or urban typhus, (3) *Indian tick typhus*- boutonneuse fever and, (4) *Scrub typhus*- tropical typhus, tsutsugamushit disease, mite (chigger) borne typhus or rural typhus.

**Etiology and host-range:** For many years, the rickettsiae were thought to be related to viruses because they multiplied only within host cells. It eventually became evident, however, that they are indeed bacteria. These organisms are known commonly as rickettsiae, or rickettsias and the diseases they cause are called rickettsioses. Rickettsiae are aerobic, Gram-negative, short rods measuring 0.3-0.5 um in diameter and 0.8-2 um in length. The organism has a cell wall and propagates well in guinea pig, yolk sac of developing chick embryo and, tissue culture.

The family Rickettsiaceae contains two genera pathogenic to humans:
*Rickettsia* and *Coxiella*. Member of another genus, formerly known as *Rochalimaeae* has now been identified as bacteria and accordingly ascribed to genus - *Bartonellav* (family- *Bartonellaceae*). Rickettsiae are the agents of diverse morphology and behaviour. On the basis of their reservoirs and vectors, these have been classified into 3 groups: (1) *Typhus group*: It includes louseborne-*R. prowazekii*, fleaborne-*R. typhi* (*R. mooseri*), mite-borne-*R. tsutsugamushi* and, tick-borne-*R. canadensis*; (2) *Spotted fever group*: It includes tick-borne-*R. rickettsii*, *R. rhipicephali*; and mite-borne-*R. akari*; and (3) *Q fever*: The causal agent of *Q fever*-*C. burnetii*; which is transmitted primarily by nonvector means such as food, fomites, and, animal products, and less commonly, by ticks; exists in two forms: (1) phase I (virulent form which exists in the nature) and phase II (avirulent form which exists in yolk sac of chick embryo).

A large number of rodent species act as reservoirs for rickettsial infections. Dog, cat, rabbit and a number of other warm-blooded animals carry the rickettsial agents. The host-range of scrub typhus is particularly broad. Numerous species of mammals and mites are involved in the maintenance and transmission of the etiological agent.

**Epidemiology:**

Rickettsial infections cause considerable morbidity worldwide. The epidemiological patterns, prevalence and severity of rickettsial diseases vary from region-to-region on account of differences in the ecological and environmental influences. These influences are intimately associated with the dynamics of vector propagation and activity in the respective regions. Rickettsial infections tend to spread rapidly during natural calamities and periods of massive ecological disturbances. Alarming morbidities are reported from overcrowded places and areas affected by repeated military operations and floods. Outbreaks are also found to occur in famine relief camps. Humans, which mostly serve as incidental or “dead end hosts”, accidentally enter the zoonotic life cycles through occupational contact with animals except in the cases of louse-borne typhus and trench fever where man serve as the reservoir.

Most vectors apparently harbour rickettsiae with no ill effect, but others, like the human body louse, die from typhus infection and do not continuously harbour the pathogen. In certain vectors such as the tick of Rocky Mountain Spotted Fever (RMSF), rickettsiae are transmitted transovarially, from infected female to her egg, creating a long-standing reservoir. Ticks and mites (technically classified as arachids) directly inoculate the skin lesion when they feed on host, however, fleas and lice harbour the infectious agent in their intestine and excrete them in their faeces. These latter insects usually transmit rickettsiae when their faeces or body is crushed into the skin of host during scratching.

The first report of a rickettsial disease in India came from Gooty in 1808 where 15 cases of epidemic typhus were admitted to the hospital. In a recent meeting of WHO, it was indicated that rickettsial infections continue to threaten human life worldwide. Serological studies reveal that typhus and spotted fever have prevalence rates of 7.6% and 5.7% respectively in India.
TYPHUS:

[1] Louse-borne epidemic typhus: Epidemic typhus is found to spread efficiently under poor socio-economic conditions. It is a disease of highland areas in Africa, Asia and South America. In India, the disease has been shown to be prevalent in a continuous endemic belt running from Baluchistan across Himalayan hills to the Kullu valley and, of late, this infection has been demonstrated in Rajasthan also.

Humans are the sole hosts of human body lice and the only reservoir of R. prowazkii. Epidemic typhus is maintained in a man-to-man cycle by a louse vector-Pediculus humanus humanus, which spreads infection, by defecating into its bite wound or other breaks in skin. Infection of the eye or respiratory tract rarely can also take place by direct contact or inhalation or dust containing dried louse faeces.

The infected lice die from the infection in 1-3 weeks after laying the eggs but without transmitting the rickettsiae to their eggs. The new generation of lice metamorphosed from eggs become infected by feeding onto infected host and, on their transfer, subsequently infects healthy persons. After an intracellular incubation of 10-14 days, rickettsiae cause high fever, chills, frontal headache, and muscular pain. Within seven days, a generalized rash appears, initially on the trunk and then spreads to extremities. In this form of typhus, neurological disturbances, which develop on account of thrombosis in blood vessels supplying to, are common. The patient recovers from the illness in three weeks time or death, which is as high as 40-60% in patients over 50 years of age, occurs due to oligourea, hypotension and gangrene. On recovery from infection, patients usually become resistant to typhus, however in some cases, the pathogen is not completely removed from the system and enters the latency. Therefore, a milder recurring form of the disease known as Brill-Zinsser disease or Recrudescent typhus may occur several years later due to reactivation of latent organism harboured in the lymph nodes. However, this form of typhus has milder symptoms, shorter duration and, often lacks a skin rash.

[2] Flea-borne endemic typhus: This disease which is similar to R. prowazekii infection except for pronounced virulence, remains widely prevalent in Asian countries including India, Burma and Kuwait. The disease, which is usually mild and occurs sporadically under a variety of environmental settings, may go unnoticed. It is reported from the hot and humid to cold and semiarid areas across the world. Murine typhus is generally associated with high morbidity in the military personnel as noticed during the Vietnam war. Human involvement is accidental. The causal agent is maintained through a rodent-to-flea cycle in nature. Rat flea (Xenopsylla cheopis) is the major vector, although other species of flea can also be involved in the transmission. The infection is transmitted through the contamination of fleabite site or skin abrasions by the infected fleas. The symptoms of illness, which is self-limiting, resemble with that of epidemic typhus and, are resolved completely in about two weeks time.

In India, R. mooseri has been isolated from rats, fleas as well as human beings from different places including Bombay, Himachal Pradesh, Jabalpur, Kashmir, Lucknow and Pune. Maximum isolations have been made from Bangalore, Mysore region of Karnataka state. Haryana and West Bengal have also recently reported cases of murine typhus.
Mite-borne scrub typhus: This form of typhus, which is caused by *R. tsutsugamushi*, constitutes an important rickettsial infection in Asia. The disease was first described nearly two centuries ago in Japan. Chigger mites while passing through forests and parkland attack humans. The pathogen lives naturally in the bodies of mites. Many species of mammalian and avian hosts help perpetuate the reservoir. Mortality in untreated disease reaches 50%, but with prompt diagnosis and therapy, fatalities can be reduced greatly.

The Himalayan regions in India and highlands of Pakistan and Afghanistan are enzootic for the disease. In India, scrub typhus came into prominence as a war disease, especially in eastern parts of the country during the second Great war. The outbreaks had been reported in the places where troops were stationed even during 1965 war with Pakistan. Cities like Madras, Mumbai and Bangalore are not free from the infection as the disease has been reported from these places in the past and, the activity of this pathogen has also been recently suspected in Andaman & Nicobar islands. The infection is maintained through a rodent-to-mite cycle. Infection in man occurs accidentally through infected *Leptotrombidium* mites.

Tick-borne typhus: This *R. conorii* induced typhus spreads through ixodid ticks. The infection in India has been reported from Karnataka, Kerala, Maharashtra, U.P. and other regions. The infection is maintained in nature in a rodent-to-tick cycle. The infection is transmitted to man through the tick-bite.

Disease in animals: Only scanty information is available on the clinical nature of the infection in rodents and other mammals. In monkey, typhus resembles human disease in many aspects. Blood from human cases of typhus on inoculation in guinea pig causes an elevation in the body temperature of the experimental animal. Mice may develop pneumonia.

Disease in man: Typhus is a disease of blood vascular system. The agent is localized in the endothelial cells of small blood vessels, blood cells and skin. Human rickettsial infections have an incubation period of 6-14 days and are characterized by fever, headache, malaise, bodyache, anorexia, chill, eschar and skin rashes. Neurological symptoms can be observed in some patients. Skin eruptions appear between 4 and 7 days of the infection. Severe cases of the disease exhibit stupor and delirium.

SPOTTED FEVERS:

Rocky Mountain Spotted Fever (RMSF): The disease was first seen in the Rocky mountains of Montana and Idaho, and therefore, so name. It has great impact on people living in north and western America, where prevalence of RMSF is approximately 500 cases per year especially among children in rural and mountain areas. The disease is common in summer and spring months. The principal reservoirs and vectors of *Rickettsia rickettsia* are hard ticks, especially the dog tick-*Dermacentor variabilis*. The tick passes the pathogen to its offspring, to various domestic wild mammals, and to humans. After 2 to 4 days of incubation the symptoms of fever, chills, headache and muscular pains are noticed. The distinctive spotted rash which starts 2 to 4 days later appears on the wrist and ankles, moves to arms and legs, converges towards the chest and eventually covers the entire body. Initial lesions are like measles, later on become macular, maculopapular and even petechial. Fatalities occur in about 20% of untreated cases and 5-10% of treated cases.
Q FEVER:

Q fever or 'query fever' which has a worldwide distribution, was first recognized as a new clinical entity by Derrick in 1935 while investigating an outbreak of febrile illness amongst abattoir workers in Brisbane, Australia. Davis and Cox in 1938 suggested that ticks were involved in the transmission of the disease. The name Rickettsia burnetii was proposed to describe the agent of Q fever by Derrick in 1939. Its origin was mysterious for a time, until Harold Cox working in Montana and Frank Burnet in Australia discovered the causal agent later named Coxiella burnetii. Q fever is now known by different names like rickettsiosis, Burnet's rickettsiosis, queries fever, abattoir fever, Nine-mile fever and coxiellosis. In man, the pathogen causes a disease with flu-like symptoms and pneumonia and the chronic disease is associated with extra-pulmonary localization of the agent. Human infection usually occurs in the countries where the disease is endemic among domestic animals. In animals, the infection usually remains inapparent. However, the excretion of C. burnetii in milk of domestic animals is well known. The isolation of Q-fever agent as well as the demonstration of Q-fever antibodies from human milk and aborted human placenta has also been reported from many countries. Human infection occurs either through gut by breast feeding, consuming raw milk, dairy products or the under cooked meat; or through inhalation of infectious aerosol which are formed in great numbers from foetal discharges during parturition-abortion in case of infected man and animals. The infection also spreads through tick bite and, at least 10 genera of ticks have been found to carry the infection. C. burnetii is also excreted in the urine and faeces of infected subjects. It has been shown that a single virulent cell has a potential of causing coxiellosis.

In India, the first confirmed human case has been reported way back in 1954. The serological investigations have indicated that C. burnetii infection is endemic among man (2.7-26%) and domestic animals (sheep (6.2-14.41%), goats (14.26-17.5%), cattle (14.98%), buffalo (14.5-15.22%), equines (20.1%), swine (14.67%), dog (16%) and poultry (11.87%). The antibodies against the agent has also been detected in the milk of humans (3.09% & 14.37%) and animals (cow (8.54%), buffalo (5.68%), and goat (10.16%). The isolation of C. burnetii has also been reported from aborted placenta of humans (6.25%) and animals as well as from infected ticks.

Diagnosis:

(1) Clinical Symptoms: Rickettsial infections do not always produce the true clinical picture. Therefore, typhus is often confused with many other undifferentiated pyrexias and hepatic conditions. Diagnosis of Rickettsial infections of merely clinical basis can be misleading. Development of characteristic eschar and rash may, however, provide clue to diagnosis.(2) Isolation: The rickettsial agents except C. burnetii in foetal discharges and R. typhi in dried flea faeces, do not survive out side the host cell. Therefore, clinical material for isolation should be collected in Bovernick's buffer (without addition of antibiotic supplement) and processed either in 6-7 day old embryonated eggs through Yolk sac inoculation or in guinea pigs through intraperitoneal inoculation. (3) Serological methods: These are considered valuable in the diagnosis of rickettsioses. Weil-Felix agglutination test using the O-antigen of proteus-OX strain
against typhus antibodies has been used for a long time to being replaced by more efficient serological tests. Indirect IF, ELISA, Dot-ELISA, CFT, HA and microagglutination tests are finding favour for the detection of antigens or antibodies. Diagnosis of Q fever is frequently done by capillary agglutination test (CAT) employing haematoxyline coloured antigen, and a serum titre of 1:8 or more is taken as positive.

Prevention and Treatment:

Tetracyclines and chloramphenicol provide promising results in the treatment of rickettsial infections. However, prognosis can be poor in patients with advanced circulatory or renal complications. Vector control by means of insecticide spray helps immensely in preventing the transmission of Rickettsiae. Individual treatment with an anti-louse shampoo or ointment is also effective. Clearing unwanted vegetation and rodent control go a long way in eliminating the reservoirs of rickettsial infections and controlling the vector densities. Control of Q fever calls for reduction in the generation of infected aerosols and dust particle by proper management and sanitation, pasteurization of milk and vector control.

Vaccination against the rickettsial agents provides yet another effective method of control. Vaccine against RMSF is available but not completely effective. However, live attenuated E strain vaccine against epidemic typhus has shown some promise. Similarly, a vaccine called Q-vax, which is prepared from formalin inactivated phase I of C. burnetii and given @ 30g subcutaneously, has proved promising as it protected abattoir personnel against Q fever for 5 years.

Further readings suggested:

Chlamydiosis:


Rickettsiosis:


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<td>Replication Inside Host cell</td>
<td>*Binary fission of Reticulate bodies</td>
<td>*Binary fission of Initial bodies</td>
<td>*Binary fission only in Eukaryotic cells</td>
<td>*Host machinery used for synthesis</td>
<td></td>
</tr>
<tr>
<td>Obligate Parasitism</td>
<td>*Yes (No AMP synthesis, Lack cytochrome system,</td>
<td>*Yes</td>
<td>*Yes(No AMP synthesis) while</td>
<td>Yes.</td>
<td></td>
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<td></td>
<td>called Energy parasite)</td>
<td>*Not necessarily</td>
<td>Bartonella(formerly Rochalimaea) can grow on</td>
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<td></td>
<td></td>
<td></td>
<td>sheep/rabbit blood agar in 5 weeks</td>
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<tr>
<td>Nucleic Material</td>
<td>RNA &amp; DNA</td>
<td>RNA &amp; DNA</td>
<td>RNA &amp; DNA</td>
<td>RNA &amp; DNA</td>
<td>RNA/DNA</td>
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<tr>
<td>Susceptibility Material</td>
<td></td>
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</tr>
<tr>
<td>A. Environment</td>
<td>*Survives for months in animal secretions In</td>
<td>*Resistant</td>
<td>*Resistant</td>
<td>*Highly susceptible except R. typh</td>
<td>*Generally susceptible</td>
</tr>
<tr>
<td></td>
<td>vitro in Bovernick's buffer(with antibiotics)</td>
<td></td>
<td></td>
<td>typh in dried film faces</td>
<td></td>
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<td></td>
<td>at 4°C</td>
<td></td>
<td></td>
<td>*C. burnetii which forms endospore- like</td>
<td></td>
</tr>
<tr>
<td>B. Antibiotics</td>
<td>*Susceptible to tetracyclines</td>
<td>*Susceptible</td>
<td>*Susceptible</td>
<td>structure inside cell</td>
<td>*Not susceptible</td>
</tr>
<tr>
<td>Host preference</td>
<td>*No for some, Yes for some</td>
<td>*No for some, Yes for some</td>
<td>*Primarily diseases of vectors and usually don’t</td>
<td>Generally host- specific</td>
<td></td>
</tr>
<tr>
<td></td>
<td>*Well known Vety. pathogens</td>
<td></td>
<td>kill them except infected lice in Epidemic typh</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>*Vertical transmission of R. rickettsii in</td>
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<td></td>
<td></td>
<td></td>
<td>infected ticks in RMSF.</td>
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<td></td>
<td></td>
<td></td>
<td>*Human is an accidental, dead end host (but</td>
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<td></td>
<td></td>
<td></td>
<td>serves as reservoir in case of epidemic typhus</td>
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<td></td>
<td></td>
<td></td>
<td>&amp; trench fever)</td>
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<tr>
<td>Mode of transmission</td>
<td>Aerosol (EB)</td>
<td>*All are tickborne</td>
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<td></td>
<td></td>
<td>*Rhipicephalus-</td>
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<td></td>
<td></td>
<td>xangulus*(Dogtick)</td>
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<td></td>
<td></td>
<td>larvae &amp; nymph</td>
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<td>*Dog is known</td>
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<td></td>
<td></td>
<td>reservoir for E. canis</td>
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<td></td>
<td></td>
<td>*Contact</td>
<td></td>
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<td></td>
<td></td>
<td>*faecal-oral</td>
<td></td>
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<td></td>
<td></td>
<td>*Rubing of faeces of</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>infected vector in skin or</td>
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<td></td>
<td></td>
<td>wound during scratch.</td>
<td></td>
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<td></td>
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<td>*Crushing of infected</td>
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<td></td>
<td></td>
<td>vector in wound.</td>
<td></td>
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<td></td>
<td></td>
<td>*Aerosol (of dried</td>
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<td></td>
<td></td>
<td>faeces) inhalation.</td>
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<td></td>
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<td>*Conjunctival contact (of</td>
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<td></td>
<td></td>
<td>vector’s faeces)</td>
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<td>*Direct bite (of infected</td>
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<td></td>
<td></td>
<td>vector)</td>
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<td></td>
<td>*in Pharynx (of mite)-Inf</td>
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<td></td>
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<td>tribes on rat eating</td>
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<td>*in skin, through saliva</td>
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<td>of infected Argasidae (tick</td>
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<td>&amp;mite) only but not of</td>
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<td></td>
<td></td>
<td>insects (flea &amp;lice)</td>
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<td>Clinical signs and symptoms</td>
<td>In Man:</td>
<td>*Flu-like</td>
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<td></td>
<td></td>
<td>symptoms, also</td>
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<td></td>
<td></td>
<td>absorge, arthritis,</td>
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<td></td>
<td></td>
<td>diarrhoea,</td>
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<td></td>
<td></td>
<td>pneumonia,</td>
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<td></td>
<td></td>
<td>encephalitis.</td>
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<td></td>
<td>*Diarrhoea with</td>
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<td>yellow / green</td>
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<td>bile salts; fever;</td>
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<td>anorexia,dullness,</td>
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<td>and ocuclusal</td>
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<td></td>
<td>discharge</td>
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<td></td>
<td>In Man:</td>
<td>*Sennetsu</td>
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<td></td>
<td></td>
<td>fever(by R. sennetsu,</td>
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<td></td>
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<td>reclassified as</td>
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<td>Symptoms resembles</td>
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<td></td>
<td></td>
<td>with RMSF (where</td>
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<td></td>
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<td>80% of patients have</td>
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<td></td>
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<td>rash on extremities)</td>
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<td>except that rash is</td>
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<td>observed only in</td>
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<td>about 20%cases</td>
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<td>&amp;&lt;5% on their</td>
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<td>palms &amp; soles.</td>
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<td>-Fever, headache,</td>
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<td></td>
<td></td>
<td>Anorexia, Myalgia,</td>
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<td></td>
<td>nausea/vomiting,wt.</td>
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<td></td>
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<td>Loss</td>
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<td></td>
<td></td>
<td>*Thrombopenia</td>
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<td></td>
<td></td>
<td>*Leakopenia</td>
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<td>because of</td>
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<td>haemorrhage and</td>
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<td>leads to anaemia,</td>
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<td></td>
<td></td>
<td>jaundice, oedema,</td>
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<td></td>
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<td>oligourea</td>
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<td>In dogs TCP due to</td>
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<td></td>
<td></td>
<td>(R. canis reclassified</td>
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<td></td>
<td></td>
<td>as E. canis in 1945)</td>
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<tr>
<td></td>
<td>In equines:</td>
<td>PHF &amp;</td>
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<td></td>
<td></td>
<td>diarrhoea caused by</td>
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<td></td>
<td></td>
<td>(E. risticii)</td>
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<td></td>
<td></td>
<td>In equines &amp; other</td>
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<td></td>
<td></td>
<td>hosts: Granulocytic</td>
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<td></td>
<td></td>
<td>choriomeningeae (E. equi)</td>
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<td></td>
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<td>In sheep, cattle &amp;</td>
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<td>hogs:</td>
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<td>E. phagocytophilus</td>
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<tr>
<td>Control</td>
<td>*Novaccine</td>
<td>*Tick control</td>
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<tr>
<td></td>
<td>*Avoid contact</td>
<td>*No vaccine</td>
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<td></td>
<td>with sick</td>
<td>*Vaccines are</td>
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<td></td>
<td>pet/aborting</td>
<td>available against</td>
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<td></td>
<td>animals</td>
<td>many.</td>
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<td>*Vaccines available:</td>
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<td></td>
<td></td>
<td>-Yes for epidemic typhus</td>
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<td>-No for scrub typhus</td>
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<td>-Yes for RMSF in case</td>
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<td></td>
<td></td>
<td>of dogs, Not for man.</td>
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<td></td>
<td></td>
<td>*Vector control</td>
<td></td>
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*Arthropods, +
*inhalation, +
*ingestion and +
*contact. +

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HYDATIDOSIS, ITS DIAGNOSIS AND CONTROL

B.P. Singh*

Echinococcosis is one of the most important zoonoses in the world. The disease is widely spread geographically with endemic foci on every inhabited continent (Matossian et al., 1977). Despite great efforts to educate the people at risk and successful control programmes in few countries, cystic echinococcosis still constitutes a substantial economic loss on account of reduced productivity, condemnation of various organs and carcasses and health hazards in most livestock raising areas of the world (Matossian et al., 1977; Williams et al., 1971). The possible strain variation in *Echinococcus granulosus* in India has been reported (Singh et al., 1987; Singh and Chattopadhyay, 1988). Systematic studies on cross infections in various animals in order to find out the *Echinococcus* strains infecting human being in India is not reported. A preliminary work on the serodiagnosis of experimental echinococcosis in pups has been attempted (Singh and Dhar, 1988; Singh, 1982). Efforts have also been made for immunodiagnosis of hydatidosis in buffaloes (Rao and Mittal, 1973) using indirect haemagglutination (IHA) and in goats (Pal and Singh, 1995) using enzyme-linked immunosorbent assay (ELISA).

There is a need to characterize hydatid antigen(s) and to standardize serological and immunological methods for an early diagnosis of hydatid disease and its control in domesticated animals.

Diagnosis

Serological diagnosis in dogs, experimentally infected with *E. granulosus*, is reported without showing any cross reactions (Jenkins and Richard, 1985; Singh and Dhar, 1987, 1988a), but attempts to detect *Echinococcus* antibodies in intermediate hosts like pigs and goats (Martinez-Gomez et al., 1980), cattle (Alencar Filho, 1978), buffaloes (Rao and Mittal, 1973) and camels (Doda et al., 1981) remained inconclusive.

Cross reactions with other parasites showing false positive results is a problem in the serodiagnosis of helminthic infections, which can be reduced to some extent by characterization and purification of antigen(s) (Gottstein et al., 1983). The weak antibody response in naturally occurring hydatid infected animals, particularly in food animals has been a major hindrance to the progress of serodiagnosis of hydatidosis (Thompson, 1986). ELISA antibodies against hydatid antigen in experimental sheep could be detected after 4-6 wks post-infection (Craig, 1981) and persisted up to 4 years (Lightowlers et al., 1984) but showed cross reaction with other cestodes and prohibit the specific diagnosis of hydatid infection (Craig, 1981; Lightowlers et al., 1984).

Attempts to develop competitive assays using monoclonal antibodies to hydatid antigen (Craig et al., 1980) have not been successful for the diagnosis of hydatidosis in sheep. Purification of crude antigens using affinity chromatography with antibodies from animals immunized with homologous antigen (Craig and Richard, 1981) or affinity depletion of cross reactive antigens with monoclonal antibody (Craig et al., 1981; Lightowlers et al., 1984) have only been partially successful in reducing cross reactions. Monoclonal antibody methods may be useful in the development of specific immunological probes to detect antigen(s) against larval as well as strobilar phase of *E. granulosus* in animals (Dhar et al., 1996).

*Head, Division of Parasitology, IVRI, Izatnagar-243 122 (U.P.)
Control

Echinococcosis caused by *E. granulosus* in dogs is of great epidemiological significance for hydatidosis in man and animals. The eggs liberated by the parasite in dog’s faeces or dispersed from autolysed gravid segments of the worms which are being picked up by man and its domesticated animals through contaminated food and water are responsible for cystic echinococcosis in liver, lungs and over vital organs of the body. Therefore, there is great need for suitable cestodicidal compounds against *E. granulosus* which may interfere in the development and propagation of unilocular and alveolar hydatidosis. The efficacy of bunamidine hydrochloride, mebendazole, nitroscanatel and various other compounds at various doses and intervals have been tested against *Echinococcus* and its larval stages in man and animals starting from short-term to long-term treatment. Promising results have been achieved particularly with praziquantel against adult stages of *E. granulosus* at 1-3 mg/kg body wt. In dogs for the elimination of the parasites and as a control measure of hydatid disease in general. Two treatments of bunamidine hydrochloride at 40mg/kg is effective against cystic echinococcosis when treated for a long duration and results in degeneration of hydatid cyst in the host.

‘Prevention is better than cure’ and it is advisable to produce vaccine against echinococcosis. Efforts in this direction have already been made and a significant protection has been achieved against echinococcosis in experimental pups and laboratory animals using irradiation attenuated protoscolices against challenge infection of *E. granulosus*. A significantly progressive decline in worm establishment was observed in pups given an infection of *E. granulosus* protoscolices exposed to increasing level of gamma irradiation from 100 to 600 Gy. No worms established in pups infected with protoscolices irradiated at 400 and 600 Gy, respectively. Worms developing from irradiated infections in pups were stunted and showed developmental abnormalities.

Movassijan *et al.* (1968) showed that 500-3000 infective worms of *E. granulosus* irradiated at 200, 250 and 300 Gy or administered in one or two immunizing doses stimulated a high degree of protection in dogs (58-02%) to subsequent challenge infections with 2500-5000 normal protoscolices. More studies are, however, needed to determine the precise dose of radiation required for attenuation of *E. granulosus* protoscolices and the mode of manner of administration of radiation attenuated protoscolices in the host for stimulating a strong protective immune response to *E. granulosus* in dogs.
LEISHMANIASIS

S. Samanta*

Leishmaniasis is one of the most important vector borne zoonoses caused by the obligate intracellular protozoan parasite of genus *Leishmania* and is distributed throughout the world. There are three main clinical forms occurs in man, viz., visceral, cutaneous and mucocutaneous Leishmaniasis by different species of *Leishmania*, however, the distinction is not absolute. The wide diversity of the clinical manifestation and epidemiological complexity coupled with increasing spread and occurrence of the disease in the world leads to recognition as problem of great public health importance. The majority of the leishmaniasis are recognized as zoonoses involving wild and domestic mammals (rodent, marsupials, edentates and canines) as reservoir hosts. Sandfly of the genus *Phlebotomus* and *Lutzomyia* are the vector known to transmit the disease. The resurgence of Leishmaniasis in man in different regions of the world was noticed with the discontinuation of malaria control programme which also reduces the sand fly population simultaneously. In the past few years, developments in the typing of strains by isoenzyme analysis and DNA buoyant density have helped in the better understanding of the epidemiology of Leishmaniasis.

Causative Agent

All the *Leishmania* exist in two developmental stages:

*Promastigotes*- are long, slender, spindle shaped measuring 15-25 μm in length and 1.5-3.5 μm in breadth with a delicate single flagellum. They are present in the digestive tract of vectors and grows in cultures. The promastigate is the infective stage which is injected into the skin through bite of the infected vector.

*Amastigotes*- are aflagellar, round or oval body (2-4 μm) found intracellularly in the neticuloendothelial cells of human and other vertebrate reservoir hosts.

Visceral Leishmaniasis

Visceral Leishmaniasis also known as Kala-azar, Dum-Dum fever, Asian fever and infantile splenomegaly etc., is caused by *Leishmania donovani* and is distributed worldwide mostly in Asia, Africa, Europe and western hemisphere. Indian Kala-azar, African Kala-azar, Chinese kala-azar, Russian Kala-azar and American Kala-azar are the different ecological forms caused by different subspecies, namely, *L. donovani donovani*, *L. donovani infantum*, *L. donovani chagasi*, *L. donovani sinensis*.

Visceral Leishmaniasis is frequently a fatal disease in man initiated through inoculation of promastigotes into the skin of vertebrate during bite of sand fly. Promastigotes enter the fixed macrophages at the local site and metamorphosed into amastigotes. After a period of weeks or months, the parasitized macrophages carried out through blood stream and invade the reticulo-endothelial cells of nearly all inner organs mostly in liver, spleen, bone marrow, lymph node and intestine and multiply there. In this process of destroying macrophages, massive enlargement of the organs occurs, particularly in spleen where the splenic pulp is completely replaced by parasitized cells. Depressed haematopoiesis leads granulocytopenia and anaemia. In advanced stages, involvement of digestive tract causes severe haemorrhages in the intestinal mucosa resulted by thrombocytopenia and depletion of prothrombin.

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Pneumonia, dysentery, tuberculosis are the common intercurrent infection seen in kala-azar. Mortality may reach upto 90% in untreated cases and death occurs from a few weeks to few years after infection.

**Clinical signs**

Clinical manifestation varies in endemic, epidemic and sporadic areas for visceral Leishmaniasis. In endemic area, children of 1 to 5 year age are most commonly affected groups, however, in India it is the age between 5 to 10 years. In epidemic forms, persons of all ages may be affected. Incubation period varies greatly ranging from 10days to as long as two years. First noticeable sign is fever which is chronic and irregular with 2 to 3 peaks in a day accompanied by chill and sweating. The patient become emaciated and anaemic alongwith distended abdomen and the skin loosens its elasticity with alopecia. The hair and nail become dry and brittle. The darkening of skin of face, especially around the mouth and forehead, hands, feet and abdomen named the disease as kala-azar (black fever).

Post Kala-azar dermal leishmaniasis (PKDL) is the characteristic features of the disease in Indian subcontinent, which appears after two years of recovery from acute illness. Skin is commonly affected and the chronic lesions comprising of multiple modular infiltration of the skin without ulceration.

**Kala-azar in India**

In India, visceral Leishmaniasis has been showing increasing trend and spreading to hitherto non endemic areas. The disease has been reported to exist more than a century. Epidemics of kala-azar have been reported from Bengal in 1824, 1832, 1833 and 1857. Burdwan fever which was suspected to be took a toll of 75000 lives during 1862-1872. Subsequently, epidemics have appeared in Assam, Bihar, and entire eastern part of India and some pockets of southern states also. Sporadic reports have come from almost all states in India. The resurgence of kala-azar in late 60s and onwards is associated with discontinuation of malaria control programme which cause transient disappearance of this disease. The number of kala-azar cases in worst affected state of Bihar as been showing on alarming increasing trend since 1985 (Table). The sandfly Phlebotomus argentipes is responsible for visceral Leishmaniasis in India.

<table>
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<td>75523</td>
</tr>
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</table>
Old world cutaneous leishmaniasis

Old world cutaneous Leishmaniasis is caused by three species of *Leishmania tropica*, *L. major* and *L. aethiopica* which have distinct biochemical and ecological characters. The disease is also known as oriental sore, aleppo button, Delhi boil or Bagdad boil etc. The life cycle of *Leishmania* causing cutaneous leishmaniasis (CL) is similar to *L. donovani* except that the intracellular amastigotes are restricted in the reticuloendothelial cells and lymphoid tissue of the skin and not in the visceral organs. Various forms of cutaneous leishmaniasis have been reported from different parts of the world depending on the susceptible population, reservoir hosts and sandfly vectors. The development of ulcer at the site of the bite is the primary lesions of all types of cutaneous leishmaniasis and usually found on the exposed parts of the body. The ulcer may be single or multiple which begins as macule, then it develops as papule which breaks down finally resulting into formation of ulcer depressed at the centre with well demarcated raised margins. Dogs and rodents particularly the gerlibs, suslik and hedgehogs are the most important reservoir host.

Cutaneous Leishmaniasis is prevalent in many parts of world including India. The disease have been mostly reported from eastern Mediterranean countries, southern parts of USA to Agrentina and Mediterranean European countries. In India, unlike wide distribution of Kala-azar, cutaneous Leishmaniasis is restricted to dry western part from Amritsar in Punjab to Bhuj in Gujarat through Haryana, Delhi, Rajasthan along the India-Pakistan border (Sharma et al., 1973). A large scale outbreaks of Delhi boil was recorded in pre-DDT era during 1940 where at least 20,000 people had suffered. After transient disappearance of the disease alongwith the launching of National Malaria Eradication Programme (NMEP) in 1958, resurgence of the disease occurs in endemic areas. Zoonotic foci has been discovered in Rajasthan area where about 628 case of zoonotic cutaneous Leishmaniasis have been reported from the rural areas in peak year of 1971. Studies revealed that *Meriones hurrianae*, the common Indian gerbil is the reservoir host and the sandfly responsible to transmit the disease for man were *Phlebotomus salehi* and *P. papatasi*. The disease is found more prominent in hamlets closer to the gerbil burrows and the community affected by the disease as occupational hazards.

New World Cutaneous and Mucocutaneous leishmaniasis

New World Cutaneous and Mucocutaneous leishmaniasis is caused by different subspecies of *L. mexicana* complex and *L. braziliensis* complex (Lainson and Shaw, 1978). *L. mexicana* comp. are fast growers in NNN medium where as *L. braziliensis* comp. are slow growers in the same medium.

*Leishmania mexicana* complex:

*L. mexicana*: caused chiclero ulcers and occurs in Mexico, Guatemala and British Handuras.

*L. m. amazonensis*: occurs in American basin, Brazil and Trinidad; rarely infects man.

*L. m. pifana*: causes a rare form of chronic cutaneous Leishmaniasis in Venezuela.
Leishmania braziliensis complex:

*L. braziliensis braziliensis*: causes the classical espundia and occurs in Brazil and adjacent forest areas of Andes.

*L. b. guyanensis*: causes pian bois and occurs in the Guyanas and other parts of northern South America.

*L. b. panamensis*: nasopharyngeal involvement is rare and occurs in Panama, Costa Rica and Honduras.

*L. b. peruviana*: causes uta and occurs in Peru and western slope of Andes.

The primary lesions are similar to other types of cutaneous Leishmaniasis as the lesions are typically found in skin. But subsequently the mucosa of mouth, nose, pharynx and larynx are involved specially in espundia. Metastasis spread to the mucosa of oropharynx or nasopharynx which may occur during the primary lesion or up-to 30 years later. The soft tissue and cartilages of the oropharynx/nasopharynx and laryngeal cavity undergo progressive erosion alongwith ulceration, disfigurement and swelling may lead into Tapir nose. Unlike cutaneous Leishmaniasis, the disease may last for many years and spontaneous recovery of the lesions are rare. Suffering and mutilation are severe and death may occurs due to septicemia and bronchopneumonia.

The transmission of the disease is mainly sylvatic and is maintained forest areas by various sylvatic animals and forest sandflies of genus *Lutzomyia*. Various forest animals viz., sloth, aguitis, ant-eaters and rodents are the important reservoirs. Dogs are also serve as secondary reservoirs. The infection is mainly predominant in forest workers and habitat. This disease is not reported from India so far.

Diagnosis of leishmaniasis

Typical clinical feature of different forms of Leishmaniasis may be suggestive of the specific infection in endemic areas. Clinically, these conditions must be differentiated from other similar disease.

However, the definitive diagnoses rest on the either the demonstration of the parasite in the specimen obtained from the lesions. Aspirates of liver, spleen, bone marrow and lymph node are taken for direct demonstration of parasite in visceral leishmaniasis while slit skin smear from the edge of the ulcer or nodules or ulcer in mucous membrane are to be taken in cutaneous and mucocutaneous leishmaniasis. Giemsa or Leishman stain is generally used for demonstration of parasites in the biopsy materials.

No doubt, aspiration from spleen is most efficacious (98% positivity) however, bears increased risk of haemorrhage in advanced stages of the disease. Prothrombin time and platelet count must be taken before going for splenic aspiration. Biopsy material from liver, bone marrow and lymph nodes are safer but show lesser degree of sensitivity (50-80%). *Leishmania* amastigotes may be demonstrated within leucocytes of the peripheral blood in heavy infection.

In vitro culture in various media and in vivo cultivation in animal model are used for primary isolation of *Leishmania* from the clinical specimen. NNN media is one of the first culture media for the cultivation of *Leishmania* is still widely used which is
incubated at 24°C for 4-6 weeks. Various modification of NNN medium and tissue culture media either mammalian cell culture (HO-MEM supplemented with 10% FCS) and insect gut cell culture (Schneider's Graces etc.) are used for azenic growth of promastigotes.

Golden hamster is the most commonly used animal model for routine isolation either from man, animal or sandfly. Animal model is specially helpful where some species of Leishmania show poor growth in culture media. However, L.b. braziliensis is a slow grower and difficult parasite to be isolated in culture media or animals.

Indirect evidence of Kala-azar comprised of leucopenia with relative neutropenia seen in perisherl smear and several nonspecific test namely, aldehyde test, Napier antimony chopra and precipitation test of sia. These tests are used to demonstrate increased globulin fraction and are good for surveillance purpose.

Various serological tests have been used for demonstration of specific antibody. Immunodiagnosis are the valuable adjunct as they are safer than the parasite isolation and are sensitive also. Earlier complement fixation test (CFT) was developed using Mycobacteria as antigen, now been replaced by other sensitive and specific test like indirect haemagglutination (IHA), indirect fluorescent antibody test (IFAT) and enzyme linked immunosorbent assay (ELISA) etc. Cultured promastigotes are usually used as antigen for these tests. Direct agglutination test (DAT) and indirect immunoperoxidase assay has been developed recently and proved as a sensitive and specific test to confirm visceral Leishmaniasis. These tests are simple, economical and may be recommended for rapid diagnosis under field condition (Singla et al., 1993;; Jain et al., 1994).

Montenegro (Leishmanin) skin test has diagnostic value in all forms of leishmaniasis and is always negative in active infection but becomes positive one year after recovery from the disease indicating prior exposure to leishmania parasites.

Western blot analysis provides a highly sensitive test for PKDL patients in India (Salotra et al., 1999). Moreover, it leads to identification of 2 parasites antigen(110 and 65 Kda) that elicit an antibody response in 97-100% of PKDL patients. Thereby purified or recombinant versions of these proteins deserves consideration as potential target antigens.

Use of recombinant ORFF antigen (rORFF) from L. infantum has been proved as a sensitive reagent for the differential diagnosis of VL caused by the L. donovani complex (Raj et al., 1999).

The application of techniques based on detection of parasite deoxyribonucleic acid (DNA), such as polymerase chain reaction (PCR) for early diagnosis of leishmaniasis has been reported (Hassan et al., 1993; Adhya et al., 1995). The highly sensitive PCR technique was used to amplify kinetoplast DNA minicircles or miniexon derived ribonucleic acid (med RNA) genes (Hassan et al., 1993).

The biochemical and immunological characters have been studied by DNA sequence analysis, DNA buoyant density, restriction enzymeanalysis, isoenzyme pattern, serotyping of excreted factors and monoclonal antibodies etc. Although these techniques are essentially the research tools of laboratory at present but have potential for adoption in the diagnosis of clinical cases under field condition.
Prevention and Control

The preventive measures to control all forms of leishmaniasis largely based on following four main directions:

a) Control of vector
b) Control of parasite
c) Immunoprophylaxis
d) Health Education

a) Control of vector:

Since mass elimination of parasite in man and reservoir hosts is not feasible, the control strategies are chiefly directed to vector control which consist of measures for (i) reducing sand fly population, and (ii) preventing exposure to sand flies.

i) Reduction of sandfly population: can be achieved by chemical methods or environmental managements. The chemical methods of sandfly control include spraying of the insecticide viz. DDT, BHC dieldrin and malathion etc. As per recommendations of the group of Experts constituted by Govt. of India two rounds of DDT at the rate of 1g/sq meter should be sprayed upto a height of 6 ft in a year for the vector of Indian Kala-azar, Phlebotomus argentipes. Each spray squad should cover around 60 to 65 houses in a days work for 75 days in each of two rounds.

The vector control tools have been changing with time based on overall epidemiological features of disease as well as bionomics of the vectors. The basis of environmental management is to provide or modify conditions which are not conducive for survival of vectors. These measures include destruction of sandfly breeding places or resting site and provision of well lighted, ventilated dwelling with proper hygienic and sanitary measures through community participation.

ii) Prevention of exposure to sandflies:

These include mechanical means e.g. bed nets, screening of doors, windows with fine mesh etc. or by use of repellent in the form of lotions, cream, spray. Repellents either applied directly to the skin or impregnated into clothings, meshor screens, etc. which prevent the man-fly contact.

b) Control of parasites: rest on case detection through continuous surveillance, effective treatment of detected cases and controlling the reservoirs also.

The objective is achieved through an intelligent vigil over the situation to check the spread of disease, early detection of cases or disease carriers among the population and their immediate therapy. Among the pentavalent antimonials, sodium stibogluconate is the drug of choice for initial therapy. Pentamidine have been used as second line of treatment for relapse on unresponsive cases. In recent years, amphotericin B also been tried in unresponsive case as third line of drug with much success. Use of cytokines like Interferon-gamma (IFN-gamma) enhances the therapeutic response to pentavalent antimony in patients of kala-azar.
Control of reservoir host include treatment or elimination dogs and rodents according to the situation.

c) Immunoprophylaxis

Research is going on throughout the world to develop immunoprophylactic against leishmaniasis. However, lot of work yet to be done before using in human beings. The attempts to immunize by non virulent or dead oranisms proved to be failure, however, like virulent strains of *L. major* isolated from human has been practised as immunization against cutaneous Leishmaniasis in hyperendemic areas of Israel, Iran and USSR. In India, first generation vaccine using killed *L. major* mixed with low concentration of BCG as adjuvant have success in preventing infection with *L. donovani* (Bora, 1999). Immunization with killed *L. donovanii* with ghean as adjuvant (Halbrook et al, 1981) and the parasite derived proteins like dp 72 were proved to be immunoprotective in visceral leishmaniasis (Rachamin and Jaffe, 1993). The *Leishmania* parasite, UR6 (MHOM/IN/UR6) has the ability to act as immunoprophylactic and immunotherapeutic agent against experimental visceral leishmaniasis (Mukhopadhyay et al, 1999). Priming of hamsters with either live or sonicated UR6 in the absence of any adjuvant provides strong protection against subsequent virulent challenge and also display a greatly extended life span as compared to infected hamsters.

d) Health Education

Mass awareness using communication media like radio, TV, cinema, posters, slides should be made to the general public of the affected areas to familiar within the various aspects of the disease. Community participation is also a paramount importance in control of the disease.

As the epidemiology of Leishmaniasis is a dynamic process continuous monitoring and research are necessary to modify control strategies. The controlling agency should also take an integrated approach using most recent epidemiological, immunological and entomological tools. Training of personnel should be continued to tackle the situation in case of a setback.

REFERENCES


TOXOPLASMOSIS
S. Samanta

Toxoplasmosis is one of the important zoonotic disease caused by the obligate intracellular protozoan parasite, *Toxoplasma gondii*. Wide distribution of the infection throughout the world have been reported as it occurs in all the warm blooded animals including important domesticated animals and man. It has been estimated that about 500 millions of human beings are having the antibodies of *T. gondii*. (Dubey and Beattie, 1988). Cats play the pivotal role in the epidemiology of toxoplasmosis in man. The organism can infect virtually any cells of the body and a wide spectrum of clinical disease may be produced in man, especially in congenitally infected children and immunosupressed individuals, like AIDS and cancer patients. In animals, similar clinical spectrum of disease may occur as in man and the parasite is considered to be a major cause of abortion and neonatal mortality in sheep and goats.

The parasite and transmission

Tachyzoites, tissue cysts and oocysts are the three stages observed in the life cycle of the parasite and all the three stages are infectious. Tachyzoites are the vegetative form, crescent shaped with pointed anterior and rounded posterior and they can virtually invade any cells of the body and multiplies asexually by endodyogeny within the host cells. Tissue cyst is practically the resting stage of the parasite, where hundreds of banana shaped bradyzoites/cytozoites are enclosed within it. Cysts can be formed in any organ, however, more commonly seen in brain, liver and muscles and may remain in the host for life long (Dubey, 1998). Oocysts are excreted in faeces of the definitive host, cat. The exereted oocyst becomes sporulated one within 1 to 5 days in optimum environmental condition, which is then infective in nature. Life cycle of *T. gondii* is completed in two classes of host where cats including wild Felidae viz., mountain lion, bob cats, ocelots, margays, Bengal tigers etc. are act as definitive host. Whereas man, rodents and other non feline host (sheep, goat, pig, cattle and birds) act as intermediate host. The three principle mode of transmission of toxoplasmosis are faeco-oral (ingestion oocyst through food and drink contaminated with infected cat faeces), carnivorism (ingestion infected tissue containing cyst) and congenital (transplacental transmission from pregnant mother to neonates). Transmission of *T. gondii* through blood transfusion and organ Trans plant action are rather unusual mode, but often fatal as the organ recipients are under immunosuppressive therapy.

The cat is crucial for the perpetuation of the infection as a cat is capable of excreting millions of oocysts in its faeces. Moreover, the oocysts are very resistant and can survive in the environment upto 18 months and are remarkably resistant to most disinfectants (Dubey, 1994). Therefore, large areas are generally contaminated for long period, not only restricted to the home range, but also mechanically transmitted to wider distribution through surface water, invertebrates, and harvested feeds. Epizootiological studies suggest that cats become infected soon after they begin to hunt or share the food hunted by their mother during the preweaning period. Cats usually become infected by preying birds, rodents and small mammals having *T. gondii* infection. Aborted foetuses, faetal fluid or membrane are also a good source of infection for carnivorous birds and mammals.
Toxoplasmosis in man and animals varies greatly in different geographical areas. Environmental factors, animal fauna and socio-cultural habits etc. may determine the degree of natural spread of *T. gondii*. The rates of congenital infection vary among region and countries and it is generally between 1 and 6 per 1000 pregnancies. Small proportion of congenital infection occurs in man and the mother of congenitally infected children do not give birth of infected children in subsequent pregnancy. The relative importance of oocyst and cyst stage in transmission of *T. gondii* infection to non feline host varies according to the host concerned. In India, where majority people are vegetarian or meat is usually cooked throughly, food and drink contaminated with oocyst may be the main source of infection.

**Clinical toxoplasmosis in man**

Toxoplasmosis in man may occur as a congenital or as an acquired infection. Although majority of the infection are asymptomatic, but at times it may produce devastating disease particularly in congenital infection or in immunosuppressed patients.

Congenital infection occurs only once when a woman has a primary exposure during pregnancy. About 30% women of child bearing age in USA have the *Toxoplasma* antibody. Therefore remaining 70% are at risk of getting infection during pregnancy (Dubey, 1994). Although most of infected children do not show overt clinical manifestation at birth, but may have manifestation of the disease later. In severe congenital infection in associated with abortion, death of new born or disease involving full tetrad of signs, hydrocephalus, retinochoroiditis, convulsion and intracerebral calcification. Evidence suggested that the severity of congenital infection differs with the duration of infection of the foetus. Early infection during first trimester of pregnancy results more severe lesions.

Post nataally acquired infection is usually less severe in man, associated with lymphoandonopathy, general malaise, muscle pain, sore throat and headache. An acute generalized toxplasmosis may develop in person undergoing immunosuppressive therapy in AIDS patients where encephalitis is the predominant signs of toxoplasmosis (Luft and Remington, 1982).

In domestic animals, clinical signs are similar as in human. Fatal epidemics have been reported in swine, rabbits, mink, new world monkeys and marsupials. Abortion in sheep and goats reported from many parts of the world which causes great economic losses is a major consequence of *T. gondii* infection.

**Diagnosis of Toxoplasmosis**

The diagnosis of *Toxoplasma gondii* infection is mainly based on isolation of the parasite, histological examinatin and serodiagnostic methods. Clinical signs cannot be relied on as they are non specific and imitate many other infection.

Impression smears from lesion of tissue biopsy or at necropsy is useful for rapid diagnosis in severe infection. However, definite diagnosis should not be made unless the organisms are in typical crescent shaped structure since degenerated host cells after resemble degenerated organisms (Dubey and Beattie, 1988).
In histological sections of tissue stained with haematoxilin and eosin, tachyzoite can be differentiated easily and tissue cysts are found occasionally of which bradyzoites are strongly PAS positive. Immunohistochemistry ( Peroxidase-antiperoxidase technique, immunofluorescence, immunologioid technique) can be used for rapid diagnosis on tissues and body fluids.

Mice is commonly used as laboratory animals for diagnostic purpose by the isolation of parasites from the clinical materials through intraperitoneal route. The parasites maybe sought in mouse peritoneal exudate during first week or in the brain at 4-6 weeks after inoculation.

A variety of serological tests are in use for diagnosis of T. gondii infection. Sabin and Feldman dye test is still used in many laboratories. Due to the inherent hazards in dealing with the live parasite as antigen in the dye test, it is now being replaced by other tests in which reagents are non infective and more standardized. Among them, indirect haemagglutination (IHA) and complement fixation test (CFT) and more recently enzyme linked immunosorbent assay ELISA and modified agglutination test (MAT) are the commonly used to improve the specificity and sensitivity. In MAT, specificity and sensitivity is increased by incorporating mercaptoethanol or dithereitol and practically it becomes a new standard for sero-diagnosis of T. gondii infection in man and animals (Dubey, 1995b, Oksanen et al., 1998). Added advantage of the serological tests that can differentiate the immunoglobulin classes by which recent nature of infection or a recrudescence may be determined. Detection of IgM antibodies indicates the recent infection while the presence of IgG in serum is suggesting the host infected at sometimes in the past. Antibody titre is important to gauge the clinical form and a rising titre of sera taken at interval is suggestive of acute infection.

In the recent years, molecular biology techniques have been successfully employed for the detection of parasite DNA from a diverse assay of biological specimens with the advent of polymearase chain reaction (PCR), the threshold sensitivity of detection of T. gondii has been remarkably increased to 0.01 zoite in a crude cell lysate (Macpherson & Gajadhar, 1993). There is a renewed interest in the differential diagnosis of toxoplasmosis using molecular biology techniques after the emergence of neosporosis (due to Neospona caninum) as a major cause of disease in livestock (Dubey & Lindsay, 1996). In veterinary practice, the technique has been evaluated for its diagnostic performance for detection of Toxoplasma DNA in naturally or experimentally infected cats, dogs and sheep.

Molecular methods of diagnosis such as DNA probe hybridisation and PCR assays are emerging techniques, that offer the advantages of remarkable sensitivity, high specificity and speed. DNA hybridization or DNA probes were the earliest molecular biological methods used in the detection of T. gondii. Wiess et al.(1991) used T. gondii species CDNA clones as probes to detect toxoplasmosis in murine model. Repetitive DNA sequences were used as probes in a dot-blot hybridization assay by Blanco et al. (1992), Angel et al. (1992) used a new repetitive element (ABG Tg 7) and obtained sensitivity levels comparable to PCR.

The advent of PCR assay has provided solution to the problem of low sensitivity of DNA hybridization probes. PCR based diagnosis of T. gondii has been identified as a priority area of research in veterinary parasitology in the new millennium (Thompson, 1999).
The most commonly used assay is based on in vitro DNA amplification of sequence in the 35 fold repetitive B1 gene by PCR and detection of the amplified product by radioactive probe (Burg et al., 1989). B1 gene amplification has also been used in the detection of toxoplasmosis in animals.

Weiss et al. (1991) targeted the P30 gene, which codes for a cell membrane protein and achieved a high sensitivity of less than 10 organisms. A 110 fold repetitive rDNA sequence was amplified to detect the presence of T. gondii by Guay et al. (1993). Using SS RNA primers, Macpherson & Gajadhar (1993) obtained a high theoretical sensitivity level of 0.01 zoite and standardized the assay for detection of T. gondii in animal clinical sample.

A perusal of the literature suggests that PCR based diagnostics have not received any attention in India.

Prevention and Control

Preventive measures of Toxoplasmosis gondii may largely based on control of transmission via meats or via cats and other public health measures.

The consumption of raw or undercooked meat or meat products is probably the major source of human infection. The main meat sources for human infection should be identified for every country of community. In judging the quality of meat, freedom from Toxoplasma infection should be considered as one of the criteria. Meat should be properly cooked for human consumption, particularly the pregnant women should not eat raw meat (especially pork or mutton) not even taste sample during meat preparation Freezing is not dependable as cysts in meat can survive at 40°C upto 3 weeks and can be killed at-15°C for longer than 3 days or at -20°C for more than 2 days. (Jacobs et al., 1982) People should wear gloves during handling of meat and wash their hands and other utensils thoroughly with soap and water after meat handling.

Cat should never fed with raw or uncooked meat and prevent them for hunting. Cats should not be kept for rodent control and alternative approach should be applied for rodent populations. Responsible cat ownership should be promoted and other methods for elimination of stray cats should be explored for population management. Trash container should be covered and dead foetuses or faetal membrane should be handled cautiously and removed promptly to prevent scavenging by cats and pigs (Dubey et al. 1986).

Cat should not be allowed near to pregnant women or sheep and goats. Animal caretakers should wear mask and protective clothing during cleaning cat litters and removal of cat faeces everyday before oocyst become infective. Cat shedding oocysts should be quarantined and treated with combination of sulfonamides and pyrimethamine.

An immunophylactic vaccine for cats have been developed with live bradyzoites from a mutant strain T-263 to prevent oocyst shedding. After oral inoculation of T-263 strain bradyzoites, oocysts formation do not take place as the sexual cycle have been arrested. However, extraintestinal cycle is not affected by the mutant strain vaccine in cat (Frenkel et al. 1991; Freyre et al. 1993).
It is desirable to have a vaccine against economic losses due to abortion with this infection and to prevent contamination of environment with abandoned placenta and the food chain (sheep and goat meat). A commercial vaccine is available in Europe and New Zealand by using S48 strains of tachyzoites which does not persist in tissues of sheep. Ewes vaccinated with the S48 strain reduces fetal damage and immunity last for 18 month (Buxton,1993).

T. gondii infection can be prevented by several ways, from washing hands, fruits and vegetables before eating to reliable serological check up and effective treatment. Practically, these are not sufficiently known to the population at risk, physicians, veterinarians and public health workers, nor the public at large and thereby priority on a low cost, long term mass awareness programme is essential in prevention and control of toxoplasmosis.

REFERENCES


RECENT TRENDS IN DIAGNOSIS AND CONTROL OF MYCOTIC ZOONOSES

Dr. Mahendra Pal*

Mycotic zoonoses are important public health problems of considerable magnitude in rural and urban areas of the world, and are caused by many fungal pathogens, which are cosmopolitan in distribution. These diseases are also of great economic importance which result into huge financial losses to the animal industries. Numerous mycotic zoonoses such as aspergillosis, blastomycosis, candidiosis, chromoblastomycosis, cryptococcosis, coccidioidomycosis, dermatophytosis, histoplasmosis, maduromycosis, rhinosporidiosis, sporotrichosis and zygomycosis have been recorded in man and in a wide variety of animals. These zoonoses are not notifiable diseases, and, therefore, the exact/correct data on their prevalence and incidence is rather inadequate and fragmentary. In India, the work on the mycotic zoonoses is like the tip of an iceberg. The future research should be intensified on the pathogenesis, epidemiology, diagnosis and chemotherapy of mycotic zoonoses. The active collaboration among the mycologists, physicians, veterinarians, pathologists and surgeons is urged in order to have an early diagnosis and proper management of these fungal zoonotic diseases which have global distribution.

The etiologic agents of most of the mycotic zoonoses occur as saprobe in non-human environment. The infection is caused by inhalation of great numbers of infectious fungal cells through the respiratory tract. Traumatic implantation of the pathogen in the skin or subcutaneous tissues following the prick of a thorn, stone, wire, splinter or other objects can produce disease. The infection is also transmitted to man thorough direct contact with other diseased animals or humans.

These diseases occur in sporadic as well as epidemic form resulting into considerable morbidity and mortality among the human beings and animals. Some of the fungal zoonoses produce life-threatening infections in the immuno-compromised hosts, particularly AIDS patients. The incidence of fungal zoonoses has increased in persons whose defence mechanisms are seriously impaired by prolonged and extensive use of corticosteroids, broad-spectrum antibiotics, cytotoxic drugs and antineoplastic agents or other concurrent diseases such as tuberculosis, diabetes mellitus, leukemia, AIDS, lymphosarcoma, sarcoidosis, Hodgkin’s disease and cancer, etc. Many occupational groups like agriculture workers, gardeners, horticulturists, construction workers, florists, nurserymen, military personnel, splenekers, zoologists, archeologists, anthropologists, laboratory workers, zoo attendants, fur cleaners, wool carders, grain miller, sugarcane producers, pathologists, dairy farmers, pet owners, animal handlers and veterinarians are more at a great risks of acquiring fungal zoonoses. Men are affected more frequently than the women.

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<td>3.</td>
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<td>Worldwide</td>
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<tr>
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<td>Coccidioides immitis</td>
<td>Endemic in USA</td>
</tr>
<tr>
<td>6.</td>
<td>Cryptococcosis</td>
<td>Cryptococcus neoformans and C. gattii</td>
<td>Worldwide</td>
</tr>
<tr>
<td>8.</td>
<td>Histoplasmosis</td>
<td>Histoplasma capsulatum</td>
<td>Endemic in USA</td>
</tr>
<tr>
<td>9.</td>
<td>Maduromycosis</td>
<td>Madurella spp., Curvulasia spp. Fusarium spp. and others</td>
<td>Asia, Africa and Latin America</td>
</tr>
<tr>
<td>10.</td>
<td>Phaeohyphomycosis</td>
<td>Alternaria spp. Aureobasidium spp.; Bipolaris spp. and others</td>
<td>Mainly reported from USA</td>
</tr>
<tr>
<td>11.</td>
<td>Rhinosporidiosis</td>
<td>Rhinosporidium seeberi</td>
<td>India, Ceylon, Brazil, Argentina</td>
</tr>
<tr>
<td>12.</td>
<td>Sporotrichosis</td>
<td>Sporothrix schenckii</td>
<td>India, Australia, Japan, USA, South Africa and Latin America</td>
</tr>
</tbody>
</table>

The diagnosis of most of the fungal zoonoses pose a problem due to absence of pathognomic clinical symptoms and radiologic findings and hence, the laboratory investigations are imperative for establishing an unequivocal diagnosis. Clinical observations are helpful in cases of dermatophytosis, maduromycosis, chromoblastomycosis and rhinosporidiosis. X-ray, computed tomography (CT) and magnetic resonance imaging (MRI) may be useful to detect the extent of the disease. Microscopic examination of the sputum, pus, exudate, aspirate, CSF, BAL, skin scrapings, crusted material, biopsies, etc. by potassium hydroxide technique can reveal the presence of fungal elements. Smear prepared from peripheral blood, sternal bone marrow and stained with Wright or Giemsa helps in the demonstration of H. capsulatum. Direct immunofluorescence of specimens helps in rapid and correct diagnosis. The clinical specimens from the patients should be cultured on various
mycological media such as Sabouraud medium, Dermatophyte test medium (DTM), Pal's sunflower seed medium, brain heart infusion (BHI) agar for isolation of the causative organism. Cycloheximide should not be incorporated into the medium when the sample is suspected for *Aspergillus, Candida* and *Cryptococcus*. Czapek Dox agar, malt extract agar, corn meal agar and slide culture are used for identification of some fungi. Biochemical tests are of immense importance for the identification of yeast pathogens.

A number of immunological tests like agar gel immuno-diffusion (AGID), complement fixation, latex agglutination, counter immunoelectrophoresis (CIE), radio-immunoassay (RIA) and enzyme linked immunosorbent assay (ELISA) are employed to demonstrate antibodies as well as antigens in the serum of affected individuals. Skin test is of little diagnostic value but is found useful to identify the endemic areas of histoplasmosis.

Laboratory animals mainly the mice are employed for the recovery of organisms such as *B. dermatitidis, H. capsulatum* and *S. schenckii* from pathological and environmental materials. Very recently, DNA probes are used for the diagnosis of blastomycosis and coccidioidomycosis. Restriction fragment length polymorphism (RFLP) analysis is an effective tool in the epidemiological investigation of cryptococcosis. Use of molecular techniques in the diagnosis of mycotic zoonoses are very expensive and, hence, cannot be applied routinely in the laboratory.

Histopathological examination of the biopsy organs or autopsied materials with haematoxylin and eosin (H&E), Gomori methanamine silver (GMS), Periodic acid-Schiff (PAS), Mayer's mucicarmine staining techniques helps to detect the fungal agent in tissue sections.

The direct demonstration of fungal pathogen in clinical materials and its isolation in pure and luxuriant form are still considered the best methods to conclusively confirm the diagnosis of mycotic zoonoses. It is pertinent to mention here that wider application of Pal's sunflower medium helped the microbiologists to establish a quick and correct diagnosis of cryptococcosis both in immuno compromised and immunocompetent hosts. This selective medium also proved very useful to conduct epidemiological investigation of this important mycotic zoonosis.

Mycotic zoonoses such as dermatophytosis and cutaneous candidiasis can be treated with topical application of 2% miconazole, 2% ketoconazole, 1% clotrimazole and 1% ciclopirox olamine. Surgical excision of the affected tissues is advised in maduromycosis, zygomycosis, chromoblastomycosis, sporotrichosis and rhinosporidiosis. Oral therapy with amphotericin B (0.1 to 1.0 mg/kg IV), fluconazole (15 to 150 mg/kg PO), ketoconazole (100 to 200 mg PO), fluconazole (100 to 400 mg PO) and itraconazole (50 to 400 mg PO) is recommended in most of the fungal diseases. Despite known toxicity, amphotericin B is still considered the gold standard for chemotherapy of mycotic zoonoses.

As fungal pathogens are widely prevalent in our environment and their spores are resistant to a variety physical conditions, complete eradication of fungal zoonoses is rather difficult. However, protective clothings (apron, gum boots, face mask, gloves) to various occupational groups, early diagnosis and chemotherapy, isolation of sick patients, care during handling of diseased animals or infected materials, proper
destruction of skin crusts, scales and other clinical materials, thorough washing of hand with antiseptic solution after the examination of sick patients, immediate care of traumatic skin lesions, avoid bathing in stagnant water, decontamination of building, bat guanos, pigeon droppings, soil and other environmental materials with 3-5 per cent formalin may help in the control of mycotic zoonoses. In addition, immunocompromised patients must not visit old and abandoned buildings, historical monuments, caves, tunnels, mines, zoos, forests and other contaminated sites, and also not handle diseased animals or infected materials. The health education to the public about the source of infection, mode of transmission, severity of disease and personnel hygiene should be imparted.

Unfortunately, mycotic zoonoses did not receive much attention in India. The dramatic upsurge in the number of immunocompromised patients in recent years, have resulted in increase incidence of mycotic zoonoses particularly caused by opportunistic fungal agents. It is, therefore, recommended that good medical mycology laboratories should be established in the country. The staff should be well qualified and experienced to undertake work on diagnostic mycology. It is also imperative to create awareness among the physicians and surgeons and about the fungal zoonoses. These strategies will certainly help to combat the human sufferings caused by mycotic zoonoses.

REFERENCES

Food-borne infections and intoxications are one of the major problems of developing countries. Of all the food-borne diseases, bacteria accounts for 85.0% of the cases in countries having better surveillance and reporting system (Proceedings, 1980). Foods of animal origin are prone to a variety of endogenous and exogenous contaminations and serve as an important vehicle for transmission of a number of microorganisms which cause food poisoning in man. A number of outbreaks of meat borne infections and intoxications are reported in man every year (Bachhil,1985). A great variety of foods, cooked and uncooked, processed and unprocessed, contaminated to some degree with potentially dangerous organisms come to market and cause not only food poisoning but a wide dissemination of infective material among human and animal population (Wilson,1944).

A critical review of the researches done in India on food-borne infections and intoxiciations reveal that it remained limited to slaughter house concept till sixties. Similarly, microbiological analysis of milk, eggs, poultry and fish also revolved around total counts, coliforms and some other selected pathogen or indicator groups. However, with the opening of new avenues of production, processing, demand and quality consciousness; which has been a global phenomenon, public health veterinarians also geared up the process of microbiological evaluation of foods to assess safety and shelf life of the product.

The trend and the quantum of work done on food-borne infections and intoxications in India is encouraging. However, it is scattered and lacks entirety. This lacuna forbids the use of such voluminous data directly in food quality control programs. There is need for more concerted and systematic analysis of food-borne infections and intoxications at various stages from production to consumption.

As far as the work done in India is concerned, almost all the foods namely meat and meat products, milk and milk products, egg, poultry and their products, fishes, shrimps and prawns and canned foods have attracted the scientists with different approaches.

A brief profile of food-borne infections and intoxications research in India has been summarized as follows:-

1. Incidence and Prevalence
   i) Sea foods
   ii) Meat and Meat products
   iii) Egg and Egg products
   iv) Poultry and Poultry products
   v) Milk and Milk products
   vi) Mycotoxises
2. Food Poisoning Episodes

3. Microbiological specifications

4. Future Needs

Incidence and Prevalence:

i) Sea Foods

The fish and other sea foods carry many of the common food-borne pathogens as a result of their exposure to environmental pollution during culture, capture, post harvest processing and handling. Freshly caught sea foods may contain pathogens such as *Salmonella*, *V.cholerae*, *Pseudomonas*, *Aeromonas* and *Vibrio parahaemolyticus*. Besides, there may be other enteropathogens present in prawns due to exposure to polluted ecosystems. There are of great concern to human health and sea-food industry.

Little attention has been paid to identify their presence and the risk of health hazard due to these food-borne infections and intoxications in our country. It is important to emphasise the role of food spoilage organisms also in reducing man’s already inadequate food supplies. Hence, every possible efforts has to be directed in preventing microbial spoilage of sea foods. In order to safe-guard human health, many importing countries insist on a safety certificate from duly recognised agency of the exporting country, conforming to International standards.

Foodborne pathogens

Studies conducted in USA during 1970-78 reported that marine sources accounted for 10.9% of all food-borne disease outbreaks, out of which fish accounted for 7.4%, molluscs for 1.9%, marine crustaceans for 1.4% and sea mammals for 0.2% (Bryan,1980). Acone et al.(1984) from a ten years study on market edible molluscs, indicated that *Salmonella*, *E.coli*, *V. parahaemolyticus*, *V.cholerae* were the major enteropathogens of public health importance present in shell fishes.

Similar studies on fish and sea foods undertaken in India also revealed the presence of these pathogens in addition to *S. aureus*, *Aeromonas* spp., *Plesiomonas* spp.,*E. tarda*, *C. perfringens* and *Klebsiella* spp. (Saxena and Kulshrestha,1985)

Aquatic environment is the most common source of bacterial contamination of sea foods. The water pollution is brought about by the discharge of sewage of human and animal origin (Ogbonde and Okaeme,1985). Qualitative study of fish surface, slime and gills revealed presence of *Enterobacter*, *Aeromonas*, *Alcaligones*, *Bacillus*, *Clostridia*, *E.coli*, *Klebsiella*, *Micrococcii*, *Proteus* and *Pseudomonas* (Bachhil,1981). While working on the occurrence of entero-pathogens in fresh water fishes using recycled organic wastes in fish ponds, Bachhil (1987) isolated spoilage and pathogenic organisms viz. *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Corynebacterium bivis*, *Proteus morgani* and *E.coli* (serotypes 017 and 02) from gills, slime and intestinal contents.

*Aeromonas* are more commonly reported in fresh water animals than in marine animals. They are pathogenic to man and cause spoilage of fishes also (Mukherjee et al.,1992). Some strains may cause diarrhoea in human being due to heat labile enterotoxin (Sanyal et al.,1975).
Coliform organisms are known to be indicator of hygienic quality of foods. Among coliforms, presence of *E. coli* indicates fecal contamination of animals and/or human origin. Many of the *E. coli* strains specially those belonging to entero-pathogenic, entero-toxigenic, entero-invasive and entero-adhesive types are known to cause gastroenteritis in adult and children (Bachhil, 1983; Kapoor, 1989).

*Salmonella* reach to fishes through contaminated water bodies and find access to processing plants, handlers and markets. Large number of *Salmonella* serotypes have been reported to occur in natural waters, as a result of contamination from sewage. Out of these, *S. weltevreden*, *S. senftenberg*, *S. anatum*, *S. enteritidis* and *S. bareilly* are most common serotypes (Dewan et al., 1976). Iyer et al. (1986) isolated *S. alachua*, *S. omienberg*, *S. ohio*, *S. eastbourne* from 4.4% samples of fish from Bombay fish market. Gore et al. (1980) studied prevalence of *Salmonella* on sea beaches of Kerala and reported its presence in 22.7% samples. They possess a potential hazard for human being contaminating sea water, sea foods and fish products.

Iyer and Verma (1980) reported that water from culture ponds, coastal sea waters, sea beach sand, process water, ice, shrimp contact surfaces, floor, rodent and lizard droppings were traced to be the major sources of *Salmonella* contamination.

Iyer and Srivastava (1989) isolated 4 serotypes of *Salmonella* from frozen shrimps. *S. weltevreden* predominated in frozen shrimps. *S. roan* and *S. larochella* were isolated for the first time in India.

Not only marine environment, but fresh water and fresh water fishes also have been found to contain *V. parahaemolyticus*. It is more prevalent and easy to isolate in summer season from fresh water environment (Sarkar et al., 1985). The occurrence of *V. parahaemolyticus* from sea foods in tropical and sub-tropical countries vary according to season, temperature and salinity. The sea food samples have been reported to harbour almost 50% or more *V. parahaemolyticus* (Natarajan et al., 1980; Pradeep and Lakshman Perumalsamy, 1986).

Iyer and Srivastava (1989) opined that *E. coli* and faecal streptococci were of little value as indicators of the possible presence of *Salmonella* in frozen fishery products. In fact it should have been taken as indicator of sanitation rather than an indicator of *Salmonella* spp. *E. coli* and faecal streptococci are taken to be good indicators of faecal pollution in fresh and frozen foods respectively (WHO, 1976; Bachhil, 1983 and Bachhil, 1985).

**Spoilage pattern:**

The bacteria most often involved in spoilage of fish are part of the natural flora of the external slimes of the fishes and their intestinal contents. Normal microflora of fish and other sea foods include variety of organisms, few pathogens of public health importance and some opportunistic pathogens of fish. Most consistent organisms of normal microflora are *Vibrio*, *Aeromonas*, *Corynebacteria*, *Coliforms* and members of other *enterobacteriaeae*, *Acinetobacter*, *Micrococi* and *Pseudomonas* etc. (Chandrasekharan et al., 1985). In addition to these micro-organisms, other less common microorganisms of normal microflora are *Moraxella* (Surendran et al., 1985), *Bacillus* spp. (Durairaj et al., 1983), *Pseudococcus halophilus* and *Planococcus* spp. (Abu and Abu, 1985).
Faulty processing and defective storage can lead to early bacterial spoilage of fish, sea food and their products (Surendran et al., 1985; Saxena, 1985; Sreekumari et al., 1988). As the days of ice storage increased, the proportion of *Acinetobacter* and Moraxella also increased considerably and constituted 70-78% of flora at the time of spoilage. Spoilage by *Pseudomonas* was very insignificant in prawns (Surendran et al., 1985). Whole as well as washed fishes had refrigerated (8±1°C) life of not more than 4 days. Qualitative study of surface slime and gills revealed presence of *Aerobacter*, *Moraxella*, *Alcaligenes*, *Bacillus*, *Clostridi*, *E.coli*, *Klebsiella*, *Micrococcus*, *Proteus* and *Pseudomonas* (Bachhil, 1981).

During refrigerated storage of fish flesh, microbial growth occurs resulting into physico-chemical changes and ultimately spoilage of fishes during storage. It has been observed that extract release volume (ERV) of fish muscle decreases but the muscle pH and psychrophillic counts increase during storage. It has been suggested that ERV can be taken to assess freshness of the fish (Bachhil, 1982).

ii) **Meat and Meat Products**

Meat is prone to a variety of endogenous and exogenous contaminations. The fresh from a physiologically healthy animals is considered to be sterile. The initial invasion of microorganisms occurs during slaughter and continues throughout subsequent handling and processing of meat. The micro-flora that comes into contact with meat during production, processing, transport and storage thus present a challenging menace to meat industry and calls for a serious thought. The microbiological status of the food when finally retailed reflects the effectiveness with which the principles of food hygiene have been applied. Heavy microbial population not only hasten the spoilage but also pose problems of infections and intoxications. This assumes greater magnitude in India where temperature and humidity are ideal for microbial multiplication.

**Microbial spectrum of meat and sources of contamination:**

Meat gets contaminated during dressing and evisceration. Both intrinsic and extrinsic environment come into play of which man is the most responsible factor. The earlier work done in India is limited to studying the contamination of carcass meats from slaughter houses or the intrinsic infections already existing in animals. This too has been limited to certain selected veterinary colleges and research institutes. The microbial spectrum of fresh meat is influenced by following factors:

a) Abattoir environment and pre-slaughter treatment of animal.
b) Microbial quality of meat in raw-state
c) Sanitary status of handling and processing
d) Subsequent handling, packaging, storage and transportation.

Microflora of fresh meat has been shown to consist of *Acromobacter*, *Micrococcus*, *Flavobacterium*, *Pseudomonas*, *Aerobacter*, *Proteus*, *Streptococcus*, *Thermobacter*, *Clostridia* and *Bacillus*. But the qualitative and quantitative spectrum of microbial flora is altered during subsequent handling and processing under varying environmental conditions.
Krishna Swami and Lahiri (1962) showed the presence of staphylococci, enterococci, coliform, lactobacilli from sheep and goat meats. Bachhil and Ahluwalia (1973) reported the occurrence of *E. coli*, *Aerobacter*, *E. freundii*, *S. aureus*, *S. epidermidis* and *Salmonella* species from various organs of goat carcasses collected from different slaughterhouses. Murthy (1976) reported presence of many *E. coli* serotypes viz. 011, 015,-29,039,041, 054, 055 and 085 from goat meats. Psychrophiles, which are the principal spoilage organisms shown to be present to the tune of log 3.11g. Misra (1978) reported presence of *Pseudomonas aeruginosa*. Fresh carcass meats of sheep, goats, pork and sausages (fresh and frozen) have been shown to harbour very heavy bacterial loads and wide range of microbial flora viz. *Klebsiella*, *Enterobacter*, *Proteus*, *Staphylococcus*, *Hafnia*, *E. coli* organisms of A-D group, *Citrobacter*, *Eriwinia* and *Seratia* (Bachhil, 1980; Murthy and Bachhil,1980).

Fresh raw meat while in distribution may be a source of zoonotic infections which are insidious and go unnoticed. Randhawa and Kalra (1970) demonstrated presence of *Brucella meletensis* from fresh meat.

A variety of *Salmonella* which might be present in meat as a result of surface contamination or intrinsic in nature have been reported from almost all types of fresh meats eg. mutton, beef, pork, chevon, poultry and sea foods. Bachhil (1968) and Randhawa and Kalra (1970) reported presence of *Salmonella* spp., *S. anatum*, *S. dublin*, *S. wittevereden* and *S. virginia* from sheep and goat meats. Mandokhot et al. (1972) showed 4.4% sheep and goats to be positive for *Salmonella* belonging to 10 serotypes viz. *S. anatum*, *S. infantis*, *S. london*, *S. newport*, *S. stanley* and *S. typhimurium*. Bhatia and Pathak (1971), Das Gupta (1974), Das Gupta (1975), Manickam and Victor (1975) showed 5% beef carcasses harboured *Salmonella* organisms. They reported presence of following *Salmonella* serotypes occurring either on pork or beef: *S. paratyphi B.*, *S. anatum*, *S. bareilly*, *S. newport*, *S. kottbus*, *S. choleraesuis*, *S. butantan*, *S. typhimurium*, *S. london*, *S. dublin*, *S. virchow*, *S. kiambu* and *S. stanley*.

Many other miscellaneous products, byproducts and sea foods have been shown to contain *Salmonella* (Nath et al., 1970; Mandokhot et al., 1972; Manickam and Victor, 1975). Bone meal, meat meal and fish meal were reported to be contaminanted with some of the following *Salmonella* serotypes: *S. tennesse*, *S. typhimurium*, *S. newport*, *S. wellevereden*, *S. nchanga*, *S. poona*, *S. bareilly*, *S. virchow*, *S. enteritidis*, *S. give*, *S. anatum*, *S. infantis* and *S. paratyphi* (Rao and Nandy, 1976).

Bachhil and Ahluwalia (1974) while assessing the hygienic standards of slaughter, production and marketing of goat meat, reported staphylococcal counts of log 7.02-7.25/g from muscle and liver. Though most coagulase positive strains (with few exceptions) are able to produce enterotoxin, such high levels of contaminations in meat is alarming more so when 54.8% of strains were found to be coagulase positive. Rao (1977) reported incidence and types of staphylococci in raw meats. Buffalo meat, pork and mutton harbored 27.27%,25% and 45% coagulase positive staphylococci respectively. In all, 16 strains (out of 72) produced different types of toxins CD and A either alone or in combination.

Both bacilli and clostridia are in abundance in nature. They are present in all types of meats. Narayan (1966) described occurrence of *C. perfringens* type A and C, *C. sporogenes*, *C. bifermentans*, *C. fallax*, *C. paraputri*, *C. capitovale*, *C. foetidum*, *C.
caproccum, S. multifermentans, C. tertium, C. innominatum and C. tatenomorphum from different organs or beef.

As a category of meats, comminuted meats are more a problem when the initial contamination from slaughter house is very high. In commercial establishments, chopping blocks, the meat grinders, cutting knives and storage utensils are rarely cleaned to the desired extent and manner. This results into heavy bacterial counts of processed meats. The time lapse between slaughter of animal, production of meat, processing and consumption is too much considering the type of handling and storage practiced. Extrinsic and intrinsic factors for bacterial growth are excellent. The magnitude of the problem can be easily thought of when this meat has to go for processing and preservation. The sources of contamination of meat are many and varied.

Soil and water are primary sources of infection. Thapliyal (1972) studied the quality of waters with regard to their sanitary quality. Most of the sources revealed higher counts (SPC; coliform and enterococci) than the permissible limits. Apart from water, vegetation, utensils, intestinal tract of man and animals, handlers, additives, animal feeds, hides, dust and spices, all are good sources of contamination.

**Effect of handling and processing:**

During handling, staphylococci and the members of enterobacteriaceae are considerable importance. Presence of additives e.g. milk powder and flour in meat preparations lead to the addition of lactic acid bacteria, Bacillus and clostridia.

Good quality spices are essential to make wholesome product. Patel et al. (1976) reported contamination of various spices, viz., onion, garlic, black pepper, dry ginger, coriander, fennel, red chilies, cumin, fenugreek, turmeric, mustard and curry powder. They revealed E.coli, K. pneumoniae, K. ozaenae, Enterobacter hafniae, Entero, liqufaciens and Pectobacterium.

Curing of meat causes predominance of micrococi and inadequately cooked products show spore of thermo-resistant bacteria. In general ground meats have higher microbial counts. The total counts, coliform and staphylococcal counts on carcass meats in general were observed to be less than the processed meats. Processing adds more to the total and coliform counts as evident from Appendix 2. Low counts are attainable by adopting good manufacturing practices. The total counts of processed meats (sausages) could be recorded as log 4.09, coliform log 1.3 and staphylococci log 2.0 per gram (Bachhil,1980). Based on the survey of All India Coordinated Project also, microbial load of processed meats were observed to be as low as 1.9-6.7x104/g. Increase in microbial load can be attributed to the cutting blades, saws, cutting blocks, grinders and knives etc. in addition to the sources (Loc. cit).

Heavily contaminated piece of meat is sufficient to contaminate other portions during grinding and mixing. Bachhil (1972,1973 and 1974) showed heavy contamination of liver and kidneys from goats. They revealed different bacterial loads (total, coliform and staphylococcal counts) in the range of log 4.47-log 8.54/g.

It has been emphasized during the first All India Workshop (1980) that the meats are processed in to many products such as canned meat, sausages, smoked meats, dehydrated minced meat and pickle products. The microbiological problem
with each type requires individual care. Most of the frozen meat and some of the other products are for export especially to the middle-east countries. This urgently needs careful microbiological monitoring of the frozen meats. Preservation of meat by cold storage and canning are not practiced on large scale in this country. Studies conducted on frozen meats indicated reduction in bacterial load during frozen storage. Storage of muscle, liver and kidneys at 8°C for 14 days resulted in reduction of total counts in the range of 44.7%. Reduction also occurred when sausages were frozen at -10°C. The total counts came down to log 6.00 from 6.26 and coliform to log 1.95 from 4.7/g (Bachhil, 1980).

In India, detection of pathogens e.g. *Salmonella* has been limited mostly to the raw meat. However, Parmanik and Khanna (1976) were able to isolate *S. paratyphi A* from processed bacon. Most of the work on detection of *E.coli* also been limited to raw meats. Incidence of microbial loads from selected meat products are presented in Appendix 1.

**Spoilage:**

Hardly any work is available on the microbiological aspect of meat spoilage in India. The spectrum of flora responsible for spoilage consists of *Pseudomonas, Achromobacter, Streptococcus, Leuconostoc, Bacillus, Micrococcus* and *Lactobacillus*. These can produce slime, which is evident when counts exceeds 108-109/g. Whiskers and black spots are formed by the growth of fungi e.g. *Penicillium, Thamnidium* and *Cladosporium* in refrigerated meats. Bone taints are attributed to anaerobes and *Pseudomonas*, sometimes *Serratia* and *Sarcina* give rise to discoloration.

During spoilage physico-chemical changes and increase in microbial load occurs. Rai (1969) made certain studies on physico-chemical and microbiological characteristics of goat meat at different temperatures and periods of storage. In an attempt to delay the spoilage, studied efficacy of different antibiotics and showed suitability of tetracycline in meat preservation, Murthy and Bachhil (1980) studied extract release volume (ERV) of fresh pork stored at 7°C up to 20 days. ERV decreased with increase in microbial counts and pH. The increase in hydration of meat protein during spoilage depended on the pH of raw meat and the extent of change in pH. At the onset of off odours the total aerobic plate counts were reported to be log 7.35/g and at clear spoilage log 9.09/g. Bachhil (1968) also observed increase in counts in the range of 6.13 to 11.23/g after 7 days storage at 11°C. However, data on fundamental aspects e.g., flora spectrum, level of contaminants with reference to climate and spoilage patterns are lacking.

**iii) Poultry and quail meat:**

Rapid developments have taken place in poultry during the last 10 to 15 years. Back yard poultry comprising few birds has taken the shape of an industry. Intensive production line slaughter of the birds in processing plants, storage, transportation and packaging and spoilage organisms both at the processor and retail level. Slaughter and dressing of quails for meat purposes is also undertaken to a limited extent in few places in India.

Sources of contamination:

There are four main sources of contamination as listed by Panda (1975).
Proper bleeding and air sac contamination are important control during processing of poultry. Keeping quality has been observed to be better when bleeding was perfect and the amount of blood recovered was more than 3.5%. Scalding is another important factor contributing towards microbial quality of meat. Bacterial load on the surface of ready to cook quails depends upon time and temperature of scalding, showed that the use of sanitizing agent in the scalding tank is quite beneficial in reducing microbial load. Very little information is available about the level of bacterial contamination during cutting and packing of these birds.

Microbial spectrum of dressed chicken and quail:

The following genera of bacteria have been reported to occur in dressed chicken: Achromobacter, Aerobacter, Bacillus, Escherichia, Flavobacterium, Lactobacillus, Micrococcus, Salmonella, Staphylococcus, Streptococcus, Pseudomonas, E.coli and EEC (Panda, 1973; Panda and Basu, 1973; Panda, 1973; Rao et al., 1974; Kulshrestha and Kumar, 1976). The incidence of salmonellae in poultry has been investigated by many workers. S. brandey, S. dublin were isolated by Manickam and Victor (1975). Jankiraman and Rajendran (1974) also reported salmonellae isolations. Panda (1977) and 1973) showed poultry as reservoir of S. Chester, S. Paratyphi B, S. Wichita and S. Gallinarum. Not only these pathogenic but toxigenic strains have also been reported. Rao (1977) detected enterotoxigenic staphylococci from dressed chicken. Microbial load of dressed warm eviscerated chicken ranged from $2.5 \times 10^5$ to $5.4 \times 10^6/cm^2$ (Panda, 1971). High counts reduced shelf life and off odours become prominent when microbial count reaches by $7-10/cm^2$.

Sharma (1980) reported initial total count of $8 \times 10^4/g$ of dressed quails which increased to objectionable levels ($10^7/g$) giving off odour after 9 days of storage at 4°C. Cooked quail meat samples after storage at 40°C were shown to harbour $6.3 \times 10^3$ organisms per g of meat after 9 days. Frozen storage of fresh quail meat showed decreases in total microbial counts from $8 \times 10^4/g$ to $3.8 \times 10^3$ and $3.1 \times 10^3$ after one and two months of respective storage periods. Choudhary (1978) also reported reduction in counts during frozen storage at -150°C. Pickled quails probably do not pose much of microbiological problem as evident from the findings of Kulshrestha (1978). He showed absence of coliforms, staphylococei and anaerobic organisms. Total counts in pickled ones were in the range of 10-100/g whereas fresh quail meats yielded higher counts. Sharma et al. (1973) while attempting preservation of poultry meat by curing and smoking reported initial decrease in bacterial population of chicken. But later it increased gradually and by 5th day cured meat exhibited clear spoilage. After refrigeration, the storage period could be increased to a fortnight (16 days). They showed further reduction in microbial population by smoking and cured meat. The surface contamination of poultry birds comprises pathogenic organisms e.g., Staphylococcus, coliforms, psychrotrophs, streptococci, yeast and molds, Salmonella, S. aureus and E.coli (Yashoda et al., 1991).
In India 3384 *Salmonella* strains have been isolated and 1612 have been found associated with diarrhoea (Singh, 1985).

Bachhil (1999) studied the microbiological quality of hot and cold boned poultry meats. In pre and post-rigor meats, *C. perfringens* on zero day were 44.6 and 43.4/g. The load of *S. aureus* on zero day were log 3.36 and 2.23/g.

iv) **Egg and Egg Products:**

Surface contamination on the fresh eggs have been reported as log 8.0/egg (Savagaon, 1978) and up to log 4.25 in duck eggs (Panda, 1988). These contaminants belong to various species e.g., *Streptococcus, Micrococcus, Staphylococcus, Bacillus, Pseudomonas, Escherichia, Proteus, Aerobacter* and *Salmonella* in hen’s eggs and *C. perfringens, Pseudomonas, Sarcina*, in addition to those described above, in duck eggs (Panda and Rao, 1980).

Liquid egg products (dried and frozen) also contain large numbers and types of organisms depending upon the extent of sanitation and handling. These may contain *Alcaligenes, Pseudomonas, Flavobacterium* and *Escherichia*. Pasteurization reduced bacterial load from log 5.19 to log 2.17 (Savagaon, 1978 and Panda, 1988). The authors, however, opined that this cannot be taken as safe with certainty.

v) **Milk and Milk Products**

Milk is one of the major diet for infants as well as old, patients and for those who are in convalescent stage. There are several diseases which are transmitted through milk. Milk serves as an efficient vehicle and protective medium for a variety of infectious agents. Milk-borne infections are explosive and sporadic and localized in the population who happen to consume milk or milk products containing the infectious agents.

Several outbreaks of diseases have been recorded by several workers by ingestion of contaminated milk and milk products in India (Singh et al, 1970; Shobha et al., 1987). In general, food-borne outbreaks in India arise from consumption of food and food products in religious functions, social feasts, community dinners etc. Within 1979 to 1980 there were atleast seventy deaths and more than 5800 people were taken ill in some 35 incidences (Singh, 1985). The average size of these incidences was 179 people. Milk products and sweets figured the major source of infection.

Milk in India is generally boiled. As such, the chances of transmission of tuberculosis, brucellosis and other endogenous infections derived from udder are minimized. The use of cream, butter could pose serious problems because of many occasions they are not pasteurized. Similarly, ice cream could be source of infection. Fecal contamination of water supply may contaminate the milk and milk products with enteropathogenic and enterotoxigenic *E.coli, Staphylococcus, Salmonella* and *Shigella* etc., resulting in gastroenteritis to the consumers. National Seminar on Safety of Milk Products held at NDRI (1984) discussed several aspects of the problems in processing and production control, marketing quality of dairy products, safety aspects of milk handling, animal health in relation to safety of milk and food-borne diseases.

Milk is an ideal medium for microbial proliferation. A large number of pathogens have been isolated from milk and milk products viz., fresh milk, market milk, pasteurised and dried milk, butter, cheese, ice cream, dahi, khoa, gulab jamun, paneer, burfi and
pera and milk sausages. Microbial spectrum of such products encompasses S. aureus, E. coli (EEC and ETEC), Klebsiella, Proteus, Enterobacter, Citrobacter, Bacillus, S. epidermidis, M. tuberculosis, Clostridia, S. Enteritidis and Micrococcus etc. (Yadav and Bachhil, 1986; Satish et al., 1984; Appuswamy and Ranganathan, 1981; Kulshrestha, 1990 and Singh et al., 1970) as shown in the table.

<table>
<thead>
<tr>
<th>Products</th>
<th>Reported organism(s) and their counts etc.</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk sausage</td>
<td>APC, log 5.06; E. coli, log 1.53 and S. aureus, log 2.04 per g.</td>
<td>Yadav and Bachhil, 1986</td>
</tr>
<tr>
<td>Infant foods</td>
<td>S. aureus enterotoxin, 0.7 μg/20gm.</td>
<td>Anand and Singh (1998)</td>
</tr>
<tr>
<td>-do-</td>
<td>Clostridium spp. 65.5% of 29 samples count 150/g</td>
<td>Appuswamy and Ranganathan (1981).</td>
</tr>
<tr>
<td>Kulfi</td>
<td>E. coli</td>
<td>Singh and Ranganathan (1972).</td>
</tr>
<tr>
<td>-do-</td>
<td>Salmonella alongwith E. coli, Klebsiella, Pseudomonas, Proteus etc.</td>
<td>-do- (1972)</td>
</tr>
<tr>
<td>-do-</td>
<td>Total bacterial and enterococcal counts were very high and many were pathogenic.</td>
<td>Balish et al., (1984)</td>
</tr>
<tr>
<td>Kalakand</td>
<td>SPC 41160/g from 1 source out of 5, coliform 0-230/g, yeast and mold count 2-154/g, spore count 100-5800/g.</td>
<td>Magadum (1990)</td>
</tr>
<tr>
<td>Pedha</td>
<td>25% contained coliforms. 14.6% staph. and proteus (In case glass container), 36 In case of openly kept samples.</td>
<td>Patel (1985)</td>
</tr>
<tr>
<td>Paneer</td>
<td>Coliform 60% samples: were highly contaminated.</td>
<td>Kumar and Sinha (1989).</td>
</tr>
<tr>
<td>-do-</td>
<td>Salmonella spp. alongwith E. coli, Klebsiella Pseudomonas were isolated.</td>
<td>Sharma and Joshi (1992)</td>
</tr>
<tr>
<td>Gulab Jamun</td>
<td>Coliform 60% samples: were highly contaminated.</td>
<td>Kumar and Sinha (1989).</td>
</tr>
</tbody>
</table>

Emerging food borne pathogens:

Aeromonas hydrophila, A. sobria, A. caviae, Campylobacter jejuni, C. coli and L. monocytogenes have been extensively worked out in our laboratories (VPH Division, IVRI). These are important and group of emerging food-borne pathogens. Species were prevalence of these organisms are as follows

Aeromonas, Campylobacter, L. monocytogenes.

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishes</td>
<td>28.57</td>
<td>21.4</td>
<td></td>
</tr>
<tr>
<td>Poultry meat</td>
<td>16.67</td>
<td>14-28.5</td>
<td></td>
</tr>
<tr>
<td>Goat meat</td>
<td>12.00</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>Buffalo meat</td>
<td>7.69</td>
<td>2.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Poultry egg</td>
<td>12.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow milk</td>
<td></td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>Buffalo milk</td>
<td></td>
<td></td>
<td>5.7</td>
</tr>
<tr>
<td>Goat milk</td>
<td></td>
<td></td>
<td>2.9</td>
</tr>
<tr>
<td>Raw retail milk</td>
<td></td>
<td></td>
<td>5.0</td>
</tr>
</tbody>
</table>

References
Most commonly isolated species of these genus are *A. hydrophila*, *A. sobria*, *A. caviae*, *C. jejuni*, *C. coli*, *L. ivanovii* and *L. monocytogenes*.

**Mycotoxicoses**

The presence of mycotoxins in a wide range of food stuffs can lead to many different toxic conditions in both man and domestic animals. The major fungi responsible for producing these toxins are spp. of *Aspergillus*, *Penicillium*, *Fusarium* and *Alternaria*, although other genera are involved as well e.g. *Claviceps*, *Diplodia* and *Arthrinium*. The toxins are aflatoxin, cyclopiazic acid, trichothecenes, patulin, ochratoxin A, diplodixトイxin and diplosporia etc. Limbhe and Ranganathan (1978) examined 126 moulds which were isolated from milk, khoa, cheese, yog hurt, dried milk and reported that amongst the various isolates, Aspergilli (24.56%) were capable of synthesising aflatoxin. Bhat *et al.* (1988) reported that a food borne disease outbreak occurred in man in Bombay due to fish consumption. A total of 132 persons (80 male and 52 female) were affected on 20 November, 1987 with a disorder after consumption of fish; 4 out of those affected died. The cause of the disease outbreak was due to ingestion of algal toxins ingested through fish. Sinha and Ranjan (1991) isolated nine dominant fungi from 19 samples of cheese commonly consumed in Bhutan. Incidence of *Aspergillus ochraceus* was greatest (74%) followed by *Penicillium citrinum* (86%). 12 out of the 19 cheese samples were contaminated with different levels of mycotoxins. Citrinin was detected in the greatest number of cheese samples (six) either alone or as co-contaminant of ochratoxin A. 3 samples of cheese were also contaminated with aflatoxin.

Tiwari and Chauhan (1991) detected aflatoxin in 25 of 60 milk samples collected from buffalo, cow, sheep, goat and camel. Aflatoxin B1 and M1 were found in milk from buffalo, cow and sheep and aflatoxin M2 in goat only. Sharma *et al.* (1993) collected the samples of fermented milk product in sterilised containers under refrigeration from local markets of Ludhiana city. *Aspergillus flavus*, a potential producer of mycotoxin was detected in eight (7.5%) samples.

**Food Poisoning Episodes**

Two outbreaks seem to have occurred in Sri Lanka due to *Cl. perfringens*. In India, Hobbs observed an outbreak likely to be due to *C. perfringens*, and the food implicated was chicken curry (cited by Singh, 1979). *C. perfringens* type C does not seem to have been reported from whole of South East Asia and India. However, Kulshrestha *et al.* (1972), Harbola and Kumar (1976), Harbola *et al.* (1975 and 1975a) have reported its isolation from haemorrhagic enteritis in sheep in India. Though innumerable reports of food poisoning episodes are reported every year by Media, some cases of food poisoning as listed below have been investigated from time to time through various foods:
<table>
<thead>
<tr>
<th>Type of outbreak</th>
<th>Food Implicated</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcal</td>
<td>Curd</td>
<td>Saha and Ganguly (1957)</td>
</tr>
<tr>
<td>-do-</td>
<td>Curd</td>
<td>Ghosh and Chatterjee (1963)</td>
</tr>
<tr>
<td>-do-</td>
<td>Sweet milk</td>
<td>D'Souza et al. (1965)</td>
</tr>
<tr>
<td>-do-</td>
<td>-d-</td>
<td>Srivastava et al. (1965)</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td>Rice</td>
<td>Lakhani (1979)</td>
</tr>
<tr>
<td>Aflatoxin</td>
<td>Maize</td>
<td>Krishnamachari et al. (1975)</td>
</tr>
<tr>
<td>Unknown</td>
<td>Boar’s meat</td>
<td>News item 19 Nov., 1978</td>
</tr>
<tr>
<td>Unknown</td>
<td>Goat meat</td>
<td>News item 11 March, 1981</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>Chicken curry</td>
<td>Hobbs observation cited by Singh (1979)</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>Milk</td>
<td>Agarwal et al. (1970)</td>
</tr>
<tr>
<td><em>S. weltevreden</em></td>
<td>Yoghurt</td>
<td>Gupta et al. (1986)</td>
</tr>
<tr>
<td><em>S. cuba</em></td>
<td>Meat and cereal foods</td>
<td>Lakhani (1979)</td>
</tr>
<tr>
<td><em>S. bareilly</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Milk/Milk powder</td>
<td>Lakhani (2979)</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>Pera, Burfi</td>
<td>Mandokhot (1981)</td>
</tr>
<tr>
<td>Unknown, Bhiwandi,</td>
<td>Food</td>
<td></td>
</tr>
<tr>
<td>Maharashtra</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Microbiological specifications for meat, poultry and sea foods**

There are large number of food borne microbiological hazards related to faulty handling, processing and storage (WHO, 1976). Microbiological specifications for foods have been suggested from time to time to ensure safety to consumers, enhance the storage stability of the perishable products, check the handling and processing defects and/or assess food plant sanitation. Such limits are valuable means of ensuring microbiological quality of foods including the foods of animal origin viz., meat, fish, poultry and egg etc. It may be emphasised that in practice, it is not possible to analyses each and every sample for all the pathogenic, toxigenic and spoilage organisms present in food. Safety of a product is assessed by analyzing certain pathogenic and indicator organisms like *Salmonella, E. coli, S. aureus* and *Clostridium perfringens* etc.; whereas, keeping quality, state of freshness and handling history of the product are indicated by total microbial counts (Bachhil, 1985; 1987; Bachhil and Jaiswal, 1989). Tamhane (1978) mentioned that microbiological quality control can be exercised at three levels viz., raw material, process control and the finished product. Internal standards can be brought out on the basis of quality control. Chief purpose of microbiological specifications aim to eliminate or minimize the risk of spread of infectious diseases or for food poisoning to consumers, to ascertain that filth has not been introduced in it and that the food has not deteriorated or unduly contaminated during handling, processing, packaging and storage.
Attempts have been made to recommend microbiological specifications from time to time for various foods. There exist some microbiological standards for a few milk and milk products, poultry and egg, sea foods and meat and meat products enforced by different agencies like Bureau of Indian Standards, MPEDA, Ministry of Food and Agriculture. However, there is urgent need to work on latest lines of 2 and 3 attribute schemes.

Standards should be of two types- one for pathogens and the other based on indicator organisms. Any standard should be based on factual studies, practically attainable and applicable to hazardous foods first. Few examples of the proposed/existing standards are given in Appendix 2.

**Future Needs**

Now, the responsibility of researchers has increased on food front particularly in the field of quality control. There have been some bold, valuable and timely decisions of the Government by creating a Ministry of Food Processing, laying emphasis on food exports and fixing extreme thrust areas. The trend in our researches has also been changed to more systematic study of food-borne infections and intoxications keeping in view the health hazards, critical control points, newer preservatives and packaging methods and quicker and reliable methods of quality testing.

Some of the thrust areas that need attention are as follows:

a) **Research**

1. Rapid enumeration and isolation techniques e.g. development of kits and better culture media.
2. Methods to recover stressed cells from processed foods.
3. To work out critical control points (CCP) in food production.
   1. Sources and control of spoilage flora in foods.
   2. Role of food-borne viruses and other chronic bacterial diseases and their CCP.
   3. Proper reporting system of food-borne infections and intoxications
4. To work out microbiological specification for various foods under 2 and 3 attribute schemes.
5. Microbiology of canned foods and sea foods need immediate attention.
6. Generation of data and formulation of food standards.
7. Status of mycotoxins/insecticides and pesticides in foods of animal origin.
8. Detection of toxins/enzymes in foods- Development of tests.

b) **Training:**

1. Training and participation of veterinarians in food hygiene, food quality control and health programmes of the country.
2. Inclusion of “Food Microbiology” on Veterinary curriculum at undergraduate level.
<table>
<thead>
<tr>
<th>Product's name</th>
<th>Organisms isolated</th>
<th>Counts reported (log/g)</th>
<th>Occurrence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>Campylobacter jejuni biotype 2 (82.3%)</td>
<td>1.2x10^4 cfu/g</td>
<td>India</td>
<td>Prasad et al., (1990).</td>
</tr>
<tr>
<td>Muscle tissue &amp; beef fat</td>
<td>Putrefactive Pseudomonas</td>
<td>Spoilage of muscle tissue and fat by increase in pH</td>
<td>India</td>
<td>Bahiker et al., (1989).</td>
</tr>
<tr>
<td>Fresh meat</td>
<td>S. aureus</td>
<td>1.5x10^4</td>
<td>India</td>
<td>Bachhil (1989)</td>
</tr>
<tr>
<td>Pork (fresh)</td>
<td>S. aureus</td>
<td>1.9x10^5</td>
<td>India</td>
<td>-do-</td>
</tr>
<tr>
<td>Buff beef</td>
<td>S. aureus</td>
<td>5.2x10^4</td>
<td>India</td>
<td>-do-</td>
</tr>
<tr>
<td>Goat &amp; poultry</td>
<td>S. aureus</td>
<td>1.8x10^5</td>
<td>India</td>
<td>-do-</td>
</tr>
<tr>
<td>Pork</td>
<td>S. aureus (11/19)</td>
<td>Thermostable nuclease</td>
<td>India</td>
<td>-do-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chevon</td>
<td>S. aureus(8/17)</td>
<td>Thermostable nuclease</td>
<td>India</td>
<td>-do-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poultry</td>
<td>S. aureus (4/10)</td>
<td>Thermostable nuclease</td>
<td>India</td>
<td>-do-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meat</td>
<td>S.anatum, E.coli &amp; Kurthia 3.10:eh:1.6</td>
<td></td>
<td>India</td>
<td>Bachhil, (1988)</td>
</tr>
</tbody>
</table>
### APPENDIX-2

**Suggested microbiological specifications for meat and meat products unc 2 and 3 class sampling plan (Bachhil, 1985 and 1987).**

<table>
<thead>
<tr>
<th></th>
<th>Counts/g</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>m</td>
<td>n</td>
</tr>
<tr>
<td>Chilled and frozen raw meats (carcass, whole cuts).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPC, <em>E. coli</em>, Salmonella**, and S. aureus.*</td>
<td>$1.0 \times 10^6$, $5.0 \times 10^5$, $1.0 \times 10^3$;</td>
<td>$5.0 \times 10^5$, $10.0$, &amp; $5.0 \times 10^2$ respectively</td>
<td>10</td>
</tr>
<tr>
<td>Precooked/semi-cooked meat products</td>
<td>$10.0$, $0.0$, $1.0 \times 10^2$, $1.0 \times 10^3$, $10.0$ respectively</td>
<td>$5.0$, $0.0$, $10$, &amp; $5.0 \times 10^2$, $0.0$ respectively</td>
<td>5</td>
</tr>
<tr>
<td>Thoroughly cooked meat products of heat sterilised products.</td>
<td>Nil</td>
<td>Nil</td>
<td>5,5,00</td>
</tr>
</tbody>
</table>

** Salmonella/50g.

- **M**: Level at or above which the lot has to be rejected.
- **m**: Maximum permissible number of relevant bacteria. The values above this are marginally acceptable or unacceptable.
- **n**: Number of samples to be tested.
- **c**: Maximum allowable number of sample units having microbial counts between 'm' and 'M'.

## REFERENCES

**Sea foods:**


**Meat, Poultry and Egg**

All India Coordinated Project, Aligarh (1979).
Indian (1957). Report on marketing of meat in India. Min. of Food & Agri., Govt. of India, New Delhi.


**Milk and Milk Products**


**Microbiological Standards**
STAPHYLOCOCCUS AUREUS INTOXICATIONS WITH SPECIAL REFERENCE TO DIAGNOSIS AND CONTROL

K.N. Kapoor¹, Sandeep Ghatak² and Rabin Banerjee³

HISTORY

In 1880 the Scottish surgeon, Sir Alexander Ogston showed that a cluster-forming coccus was the cause of a number of pyogenic diseases in man and subsequently, named the organism “Staphylococcus” (Baird-Parker, 1990). Of various staphylococci, Staphylococcus aureus is considered as the type species of the genus.

S. AUREUS TOXINS IN HUMAN DISEASE:

The type species S. aureus is a recognized pathogen since inception of clinical microbiology. It possesses an impressive armoury of extracellular toxic factors (Table-1) to qualify as a successful pathogen. Nevertheless, four of the main exotoxins, alpha toxin, epidermolytic toxin (ET), toxic shock syndrome toxin (TSST) and enterotoxin are considered to be of prime importance.

<table>
<thead>
<tr>
<th>Toxins</th>
<th>Exoenzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane-demaging toxins</td>
<td></td>
</tr>
<tr>
<td>alpha-toxin</td>
<td>Coagulase</td>
</tr>
<tr>
<td>beta-toxin</td>
<td>Staphylokinase</td>
</tr>
<tr>
<td>gamma-toxin</td>
<td>Proteases</td>
</tr>
<tr>
<td>delta-toxin</td>
<td>Phospholipase</td>
</tr>
<tr>
<td>leucocidin</td>
<td>Lipase</td>
</tr>
<tr>
<td>Epidermolytic toxin</td>
<td>DNase</td>
</tr>
<tr>
<td>Toxic shock syndrome toxin</td>
<td>Hyaluronidase</td>
</tr>
<tr>
<td>Enterotoxin (7 serotypes)</td>
<td>Phosphatase</td>
</tr>
<tr>
<td>Pyrogenic exotoxin</td>
<td></td>
</tr>
</tbody>
</table>

alpha-TOXIN:

Since its identification as a separate entity by Macfarlane Bumet alpha-toxin has been considered as a prime determinant of pathogenicity. It is a lethal, necrotizing, cytolytic toxin that damages the membrane of a variety of cell types.

EPIDERMOLYTIC TOXIN (ET):

Certain strains of S. aureus cause blistering lesion in skin resembling scalding. The condition is attributable to a soluble product known as epidermolytic toxin or exfoliative toxin.

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TOXIC SHOCK SYNDROME TOXIN (TSST):

Toxic shock syndrome (TSS) is characterized by high fever, headache, confusion and development of sunburn-like rash; accompanied by sore throat, vomiting, watery diarrhoea and hypotensive shock. Most of the cases are reported in young women. Actual bacterial product responsible for the syndrome is known as toxic shock syndrome toxin (TSST).

Asymptomatic colonization

Or local infection

Skin
Nasopharynx
Eyes
Gastrointestinal tract (GIT)
Urethra vagina

Release of Extracellular Products

Bacteraemia (Asymptomatic/acute)

Metastatic sites of infection

Bones, joints: osteomyelitis, arthritis
Lungs: pneumonia
Muscle: abscesses
Heart: endocarditis
Abdominal organs: abscesses
Central nervous system: cerebritis, meningitis, brain abscesses.

Skin: carbuncles, furuncles, subcutaneous abscesses
Nasopharynx: sinusitis, transillinitis, glandular infections.
Eyes: deep orbital infection
GIT: food poisoning
Urethra: cystitis, prostatitis
Vagina: cervicitis, salpingitis pelvic infection

Contiguous infection

Fig.1: Staphylococcus aureus: its host/pathogen relationship in man. (Arbuthnott et al., 1990)

ENTEROTOXINS:

The enterotoxins of S. aureus form a group of seven serologically distinct extracellular proteins, designated A, B, C1, C2, C3, D and E. They are recognized as the cause of staphylococcal food poisoning. An estimated 14% of all foodborne outbreaks and 1 to 2 million cases of food poisoning in the United States each year are caused by the toxins produced by S. aureus (Giese, 1998).
Meat and meat products such as ham and hot dogs, prepared salads, cream filled baked goods and cheeses have all been implicated as sources of *S. aureus* contamination. Particularly vulnerable are foods processed without heat treatment such as fermented meats and dairy products. Ingestion of preformed toxin in such contaminated foods leads to the rapid development (2-6 h) of the symptoms of vomiting and diarrhoea that characterize staphylococcal food poisoning.

**DETECTION OF STAPHYLOCOCCAL INTOXICATION**

For detection of staphylococcal intoxication, standard microbiological methods as well as novel techniques are used. Following table (Table 2) lists some of them.

**Table-2. Minimum detectable levels of *S.aureus* toxins or organisms by various methods** (Modified and adapted from Jan.,1996).

<table>
<thead>
<tr>
<th>Methods</th>
<th>Sensitivity (for toxin or organism)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PHYSICAL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microcalorimetry</td>
<td>2 cells in 12-13 h</td>
<td>Measures the enthalpy change involved in the breakdown of growth substances. The heat production measured is closely related to the cells catabolic activity.</td>
</tr>
<tr>
<td><strong>CHEMICAL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermostable nuclease DNA probe</td>
<td>2.5-10ng/g</td>
<td>Measures the amount of thermostable nuclease in the food because a high correlation is found between the production of coagulase and thermostable nuclease by <em>S.aureus</em> strains, especially enterotoxin producers.</td>
</tr>
<tr>
<td><strong>IMMUNOLOGIC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorescent antibody</td>
<td>1-10 cells</td>
<td>Highly sensitive and specific</td>
</tr>
<tr>
<td>Radioimmunoassay</td>
<td>0.1-2.2ng of enterotoxin/g or ml of food</td>
<td>It is rapid and highly sensitive method</td>
</tr>
<tr>
<td>Micro-Ouchterlony gel diffusion</td>
<td>10-100ng/ml</td>
<td></td>
</tr>
<tr>
<td>Single radial immunodiffusion</td>
<td>0.3µg/ml</td>
<td></td>
</tr>
<tr>
<td>Haemagglutination inhibition</td>
<td>1.3ng/ml</td>
<td></td>
</tr>
<tr>
<td>Reverse passive haemagglutination</td>
<td>1.5ng/ml</td>
<td></td>
</tr>
<tr>
<td>Enzyme linked immunosorbent assay (ELISA)</td>
<td>0.1-1 ng/ml</td>
<td>Rapid highly sensitive and specific as well as easy to perform method.</td>
</tr>
</tbody>
</table>

Commercially available assays (*α-* toxin)
Table 3. Commercial assays for detection of *S. aureus* or its toxin

<table>
<thead>
<tr>
<th>Name of the Assay</th>
<th>Manufacturer</th>
<th>Sensitivity</th>
<th>Time requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reversed passive latex agglutination (RPLA) kit</td>
<td>Denka Seiken Co. Ltd., Tokyo</td>
<td>1-2 ng/ml</td>
<td>24 h</td>
</tr>
<tr>
<td>RPLA using high density latex particle</td>
<td></td>
<td>0.5 ng/ml</td>
<td>3 h</td>
</tr>
<tr>
<td>Sandwich ELISA</td>
<td>Labor Dr. W. Bommeli, Bern, Switzerland</td>
<td>0.01-0.1 μg/100 g of food</td>
<td></td>
</tr>
<tr>
<td>Aureus Visual Immunoassay (VIA) kit</td>
<td>TECRA Diagnostics Redmond, Washington</td>
<td>Overnight enrichment followed by 1.5 h ELISA</td>
<td></td>
</tr>
</tbody>
</table>
AEROMONAS AND PLESIOMONAS

R.K. Agarwal*

1. INTRODUCTION

Aeromonas and Plesiomonas have emerged as important food borne human pathogens. Both the organisms are Gram negative oxidase positive, motile, rod shaped bacteria belonging to family Vibrionaceae. Recently, a separate family named Aeromonadaceae has been proposed for Aeromonas spp. (Colwell et al., 1986). The genus Aeromonas includes two well separated groups of organisms, a psychrophilic, non-motile group and a mesophilic motile group. The psychrophilic non-motile aeromonads, i.e., A. salmonicida are important pathogens for fish and other aquatic animals, whereas mesophilic group is of public health importance. The mesophilic group is divided into three species, viz., A. hydrophila, A. sobria and A. caviae. DNA hybridization studies have shown there to be at least 11 species of mesophilic aeromonads seven of which have taxonomic understanding. Most human pathogenic strains fall into hybridization groups 1 A. hydrophila, 4 A. caviae and 8 A. sobria. Other species have also been associated with diarrhoeal disease including A. veronii and A. schuberitii.

On the other hand, P. shigelloides is still the only species in the Plesiomonas group. Due to oyster and other water associated outbreaks of diarrhoeal diseases in humans, P. shigelloides is now established as a human intestinal pathogen.

2. SEROTYPING

In comparison with many other enteropathogenic bacteria the serotyping of these organisms is in its infancy. Ewing et al. (1961) is credited with the preliminary work on the serology of A. hydrophila. They distinguished 12 somatic and 9 flagellar antigens. Based on O-antigen Aeromonas has been grouped into various serogroups (Sakazaki and Shimada, 1984). Another serotyping scheme based on lipopolysaccharide antigen has also been developed (Fricker, 1987), but only 16% strains are typable by this scheme.

Serotypes 03, 06, 011 and 019 are stated to be most pathogenic. However, in India serotypes 034, 016 and 011 have been found to predominant among clinical as well as environmental isolates. A typing scheme based on 50 O-serogroups and 17 H-antigens have also been described for P. shigelloides. Some of these serovars are ubiquitous, whereas others are isolated only in specific regions.

3. DISTRIBUTION

The broadest ecological niche for Aeromonas spp. in the nature is the aquatic phase of the ecosystem and considered to be the major source of infection. Large number of aeromonads have been found in all waters including free, saline and brackish water and unchlorinated drinking water, treated and untreated sewage, clean river water, domestic and industrial waste water, abattoir waste water and bottled uncarbonated mineral water. Published reports indicate that aeromonads are also able to colonize slow sand filters.

Epidemiological studies indicate that a wide variety of foods are also associated

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with *Aeromonas* implicated gastroenteritis. The foods include seafood, chicken and red meat, vegetables, raw milk and milk products. Among seafoods, aeromonads are reported in fish and fish-eggs, shrimp, prawn, finfish, shell fish.

*Aeromonas* spp. have also been found in many types of meats including ground beef, chicken meat, pork sausage, ground and unseasoned pork, modified atmosphere packaged pork.

Reports on the prevalence of *Aeromonas* spp. in foods in India are far more limited. However, the organism has been isolated from variety of foods including fish, meat, milk, egg, tortoise and snail. A study in Haryana (Khurana and Kumar, 1997), showed distribution pattern of aeromonads to be 37.5% in sheep meat, 32.6% in edible organ of poultry, 28% in poultry meat, 24.2% in edible organs of sheep and 0.9% in poultry eggs. Agarwal (1997) after investigating a wide range of food found aeromonads in 22.22% of fish, 5% of tortoise, 6.25% of snail, 4.08% of other aquatic foods, 8.88% of goat meat, 2.85% of buffalo milk and 18% of quail eggs. *Aeromonas* spp. have also been isolated from about 12.5% of ice cream samples in Chennai.

Pathak et al. (1988) recovered *Aeromonas* spp. from fish all through the year, unrelated to the density of *Aeromonas* spp. in the water and hence concluded that fish might form a reservoir of *Aeromonas* spp. Later, Ramteke et al. (1992) isolated *Aeromonas* spp. in 32.6% of drinking water samples in Northern India. Aeromonads could also be identified in domestic sewage and industrial effluents in central parts of India.

*P. shigeloides* has so far only been isolated from fish and sea food but no systematic studies have been performed on other foods. It is a point to mention that *P. shigeloides* is not psychrotropic like *Aeromonas* spp. and hence its isolation rate shows a distinct seasonal variation.

### 4. PATHOGENESIS AND VIRULENCE

*Aeromonas hydrophila* has been recognized as a pathogen of amphibians, reptiles, fish, snails, and humans, majority of clinical isolates being recovered during the spring and summer months. It has been associated with the human gastroenteritis cases and several extra intestinal human infections such as meningitis, endocarditis, peritonitis, osteomyelitis, septic arthritis, septicaemia, eye and urinary tract infections. Epidemics of nosocomial infections have also been reported. *P. shigeloides* is not a normal inhabitant of the human intestinal tract, but the organism is being increasingly associated with diarrhoea and, less commonly with extra-intestinal infections. Both these organisms are opportunistic pathogens, infections involving the very young, the aged or people with predisposing conditions. It seems most important to include Aeromonas spp. among the organisms, which should be suspected in water-borne outbreaks. The situation is different with *P. shigeloides* since there are a few well-documented fish and oyster borne outbreaks reported.

Possible virulence determinants in *Aeromonas* spp. can be summarized as follows.

1. **Toxins**
   A. **Endotoxins**
   B. **Exotoxins**
a. Cytotonic enterotoxin
b. Cytotoxic enterotoxin
- Aerolysin (B-haemolysin)
- Alpha haemolysin
C. Other extracellular enzymes
a. Proteases
b. Other enzymes-lactithinase, DNase
2. Colonization factors
A. Fimbriae
B. Non fimbrial outer membrane proteins
- Haemagglutinins

The production of virulence factors by aeromonads varies with geographic location, source of isolation and their species. Cytotoxin production is considered to be an important virulence factor but its role in pathogenesis is controversial. Cytotonic enterotoxin of *Aeromonas* produce fluid accumulation without mucosal damage in ileal loops of rabbits, rats and mice.

Surface adhesion ability is also an important factor responsible for pathogenesis of aeromonads. Haemagglutination property is used to characterize adhesion properties.

**Putative virulence factors of *P. shigelloides* are given below.**

1. Adhesive
2. Invasiveness
3. Endotoxins
4. Elastase

Relatively little is known about *Plesiomonas* toxins. Studies have indicated that *P. shigelloides* does not produce an enterotoxin or that the enterotoxin can not be demonstrated by the systems used to study *Aeromonas*, *Vibrio* or *E. coli* toxins.

5. **ISOLATION**

5.1 **Enrichment media**

Very few enrichment broths have been developed specifically for *Aeromonas* spp. Till recently trypticase soy-ampicillin broth and alkaline peptone water (APW) were in use but they are not sufficiently selective for *Aeromonas* spp. A new selective enrichment broth named cephalothin alkaline peptone water-10 (CAPW-10) has been developed and found to be superior over commonly used enrichment broths (Sachan, and Agarwal, 2000).
No enrichment media have been developed specifically for isolation of *P. shigelloides*. Though, alkaline peptone water and tetrathionate broths are used for its isolation.

5.2 Selective media

A wide range of selective plating media has been developed for *Aeromonas* spp. These include media designated for *Enterobacteriaceae*, media containing antibiotics such as ampicillin, novobiocin, bile salts, brilliant green, ethanol etc, as selective agents. Ampicillin dextrin agar (ADA) remains the most commonly used plating medium till now. For isolation of *P. shigelloides* some specific media like inositol brilliant green bile salts agar and *Plesiomonas* medium are used.

6. RAPID DIAGNOSTIC TESTS

To overcome the problems of conventional microbiological procedures, some commercial identification systems such as AP 120 E, AP/20 NE and microbact 24 E have been developed for *Aeromonas* spp.

Enzyme immunoassays, nucleic acid probes and polymerase chain reaction (PCR) have also been developed for *Aeromonas* spp. Recently, outer membrane protein (OMP) based ELISA utilizing crude as well as 25, 35 and 68 kDa peptides of OMP has been developed for the rapid detection of *Aeromonas* spp. from foods (Sachan, 1998; Ghatak, 1999).

REFERENCES


DIAGNOSTIC AND VIRULENCE RELATED ASPECTS OF
YERSINIA ENTEROCOLITICA

D. C. Thapliyal*

Yersinia enterocolitica is a remarkable infectious agent that emerged as a pathogen in 1980. The organism has been classified variously in the past. It was examined more critically as a pathogen only after its role as an agent of food-borne gastroenteritis became apparent. The fact that it can grow at lower temperature, and has in swine a very important reservoir, has provided a unique basis for probing its patho-epidemiological patterns. The organism is distributed widely in nature and various animal hosts, and has been recovered from whichever source or region of the world it has been sought. Global distribution patterns indicate that the organism ranks second only to Salmonella infections in parts of Europe. On account of its ever increasing importance as an agent of human clinical conditions worldwide and the role of animal reservoirs in its transmission, it has begun to be investigated with more objectivity. The homogeneity among human and porcine strains reflects that pigs pose a serious threat to human health.

Classification of the organism

Y. enterocolitica was first isolated in 1933 in New York state by M.B. Coleman. It is Gram-negative cocobacilli oxidase negative with ornithin decarboxylase positive.

The organism is motile below 30°C. classified the genus - Yersinia within the family enterobacteriaceae because it shared the common antigen with other enterobacteria and also shows DNA relatedness with them. The organism is one of the few enteric pathogens that possesses the ability to grow at refrigeration temperature (2-4°C) which are employed to store perishable foods.

Y. enterocolitica strains were initially subdivided into five biogroups later, the biogrouping scheme for Y. enterocolitica was revised to create 6 groups on the basis of positive pyrazinamidase and proline peptidase reactions.

Strains of Y. enterocolitica share their finer architecture and lipopolysaccharide-o-antigen with other enterobacterial species. Many investigators have contributed to serogrouping of different strains during the last two decades. There are as many as 57-0-serogroups in Y. enterocolitica.

Phage typing scheme for Y. enterocolitica strains has yet to be fully developed for use in epidemiology and diagnosis. However, in one study of 1202 strains of Y. enterocolitica examined 86.4% were classified as lysosomic when grown at 25°C, but not at 37°C.

Epidemiology

Y. enterocolitica is worldwide in distribution. More than 40 countries have reported the presence of the organism. Gastroenteritis due to Y. enterocolitica has been reported in increasing frequency across the world. In fact, lower temperature (temperate climate) is found to facilitates the growth of the organism. An increasing awareness about the role of the organism has evoked considerable interest in the epidemiology of yersiniosis in developed as well as tropical countries resulting in

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frequent isolation of the organism from various sources. The organism has also been reported from several Asian countries including India.

Changes in the geographical distribution of pathogenic serogroups have been noticed in respect of 0:3, 0:9 and 0:5, 22 which are more common causes of disease in Europe, Canada and some other regions. Strains, viz., 0:8, 0:4, 32, 13a,13, 0:18, 0:20 and 0:21 are commonly associated with the disease in USA. In India status of the disease is not well known but 0:3 and 0:9 are more prevalent. Studies carried out in Delhi revealed presence of serogroups 6, 7, 8, 10, 19, 36 and 34 in environment and pork.

Various animal species appear to serve as reservoirs of Y. enterocolitica. Pig, especially the tonsils and tongue of the animal, is considered to be the chief reservoir and important sources of the organism. Similarities observed between restriction endonuclease patterns of plasmids harboured by porcine and human isolates further suggest about the pivotal role of pigs in the epidemiology of human Y. enterocolitica infections.

Refrigerated foods not only act as vehicles of transmission, but also as suitable media for microbial proliferation. Most of the strains isolated from foods may, however, not be pathogenic to humans as most of them owe their origin to environment (mostly water).

Disease in man

Among foods, milk and milk products play an important role in the transmission of disease. The organism is mainly associated with diarrhoea and appendicities-like syndrome. Arthritis, septicaemia and mesenteric lymphadenitis can be seen though generalised infections are uncommon. Hepatic cirrhosis, aplastic anaemia, thalassemia, malignancy, malnutrition and iron overload are considered predisposing factors for the infection.

Virulence in Y. enterocolitica

Y. enterocolitica produces heat-sable enterotoxins (ST-1 &ST-2) that survive for 20 min at 100°C. Enterotoxins are chromosomal mediated and are produced at 25°C but not at 37°C. Though pathogenic strain of Y. enterocolitica produce enterotoxin but evidence suggests that enterotoxin may not be critical to virulence.

The mechanism of pathogenesis in yersiniosis is very complex and poorly understood. The pathogenicity of fully virulent strains of Y. enterocolitica depends on the presence of virulence related plasmids in molecular wt. range of 40-48MDa. These plasmids also encode for virulence associated V and W antigens, and for at least 9 outer membrane proteins.

Diagnosis

1. The organism is isolated conventionally from faeces and contaminated food by means of cold enrichment in PBS (4°C for 3 weeks) followed by selective enrichment in Rappaport medium (25°C for 2 days). MacConkeys agar with or without Tween-80, bismuth sulphite agar and Salmonella-Shigella agar have been used for plating the enriched specimens (Gilmore and Walker, 1988).
2. A range of virulence related characteristics that will be discussed in subsequent sections, are used to detect the presence of pathogenic strains in clinically suspected cases.

3. Nowadays, DNA based methods have begun to be employed for the detection of the pathogens in clinical and food specimens. Developed a multiple polymerase chain reaction has been to detect the presence of oil, st and vioF genes of Y. enterocolitica quickly and accurately. It could detect the organism from within a concentration of 5-10 CFU/ml.

Methods for virulence detection:

The recognition of genetic basis for virulence and consequent expression of certain phenotypic characteristics have led to the development of a number of in vitro tests for identifying virulent and avirulent Yersinia. In vitro methods are more convenient and yield quick results. Some such tests are given below:

1. **Calcium dependence:** Among yersiniae, only the virulent strains require calcium for growth. Virulent strains of Yersinia form pin head colonies (< 0.05 mm in diameter) or there may be complete absence of growth. In case of avirulent strains large colonies (0.5 mm or more in diameter) are produced. Calcium deficient magnesium oxalate (MOX) agar is used to differentiate between virulent and avirulent strains of Yersinia.

2. **Autoagglutination:** Autoagglutination is a simple and reliable method for screening virulent in Y. enterocolitica. Virulent strains show autoagglutinability in tissue culture medium (RPMI-1640 with 10% calf serum and mM HEPES) when grown at 36°C. Avirulent strains fail to show this plasmid mediated property. Yersinia enterocolitica protein (YeP1) which is structural component of fimbriae and probably represents, adhesine which enable the bacteria to attach to each other forming autoagglutinating mass. Congo red pigmentation: Pigmentation of Y. pestis in the presence of haemin or the congo red dye has long been known to correlate with virulence demonstrated that virulent strains of Y. enterocolitica take the dye on Congo red acid-morpholine propane sulfonic acid pigmentation agar. Pigmented variants express various virulence associated properties including autoagglutination, calcium dependence, serum resistance, lethality for iron overloaded mice and presence of 40 to 50 Mdal plasmid, whereas, non pigmented variants are negative for all these assays. All pigmented strains are not virulent, but Congo red pigmentation of Y. enterocolitica is an essential prerequisite for virulence.

3. **Differential colony morphology:** Virulent Yersinia strains dissociated into virulent and avirulent clones after cultivation. the virulent Y. enterocolitica into small and large colonies on RPMI-1640 agar can be used to discriminate between plasmid bearing and spontaneously derived plasmid free variants of Y. enterocolitica. Tryptic soy agar (TSA) can also be used to differentiate between the two types of strains.

4. **Crystal violet binding:** Virulent colonies of Y. enterocolitica grown at 37°C are able to bind crystal violet. Such strains also demonstrate various other attributes of virulence both in vitro as well as in vivo. The colonies, which fail to bind crystal violet, are consistently negative for most virulence related assays.
5. Invasion of HeLa Cells: Virulent strains isolated from fatal enteric (human and animals) infections invade the HeLa cells. HeLa cell invasive strains also demonstrate other in vitro and in vivo virulence characteristics. HeLa cell invasiveness was found to be limited to clinical strains that were sucrose positive, and salicin and esculin negative, However, there are reports that virulent as well as avirulent strain have equally invasiveness to HeLa cells.

6. Serum resistance: Resistance of virulent Y. enterocolitica strains to bactericidal effects of normal serum is temperature dependent and plasmid mediated property that occurs only at 37°C. In the presence of 10% normal serum, avirulent Y. enterocolitica are neutralized within one hour at 37°C. Virulent strains that show resistance tend to multiply.

7. Mannose resistance haemagglutinin (MRHA) production: MacLagan and Old (1980) were the first to describe two different haemagglutinins in Y. enterocolitica that were later exploited for diagnostic purposes. Later on virulence related MRHA production in Y. enterocolitica was demonstrated. The property was plasmid encoded.

8. Hydrophobicity: Hydrophobic interactions enable various pathogenic bacteria to adhere to eukaryotic cells and other surfaces. Increased surface charge and hydrophobicity in Y. enterocolitica strains bearing 40-48 Mda virulence plasmids was demonstrated. It is also expressed at 37°C, however, many strains which express this character were avirulent.

9. Hydrolysis of esculin and fermentation of salicin: Esculin hydrolysis and fermentation have been advocated for the differentiation of the environmental and pathogenic. All esculin and salicin positive strains belong to biotype I and are non-pathogenic.

10. Colony hybridization with DNA probes: An artificially synthesized oligonucleotide probe and a cloned polynucleotide probe consisting of a cloned fragment of virulence gene (YOPA) for comparative evaluation of virulent Y. enterocolitica in identification and enumeration of virulent Y. enterocolitica has been used DNA-DNA colony hybridization correctly identified the plasmid bearing variants of pathogenic serogroups.

Animal models for detection of virulence

1. Guinea pig and mouse sereny tests: The strains belonging to serogroup 0:8 of Y. enterocolitica produced severe conjunctivitis in guinea pigs. This production of lesion was found to be associated with 41 Mdal plasmid. Later positive reactions in case of other virulent Y. enterocolitica strains has also been observed.

2. Mouse lethality test: Intraperitoneal inoculation of bacterial suspension in mice helps detect virulence in Y. enterocolitica. Plasmid harbouring strains of Y. enterocolitica induce fatal infection in suckling and adult mice. But plasmidless strains show no such effect.

3. Mouse peroral test: Intragastrically challenged mice with Y. enterocolitica 028 developed initial lesions in Peyar’s patches in distal ileum with 50% mortality whereas survivors excreted the organism for 4 months.
4. **Pathogenicity in guinea pigs:** It has been reported that intraperitoneal administration of *Y. enterocolitica* 0:3 (3 x 10⁹ cells) caused death of guinea pigs within 24-48 hours.

5. **Test for enterotoxigenicity:** Many strains of *Y. enterocolitica* produce ST-Type enterotoxin. The test can be assayed in ligated rabbit gut segments. Studies, however, show that many strains found virulent through in vitro tests, could prove to be non-enterotoxic. Studies on virulence markers indicate that no one test could be relied upon for the detection of virulent strains. It is advocated that a battery of tests be applied to find the presence of virulent strains isolated from clinical cases and food specimens.

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DETECTION AND CONTROL OF ESCHERICHIA COLI

K.N. Kapoor,¹ R. Banerjee² and Sandeep Ghatak³

Although Escherichia coli were first indentified in 1885, their pathogenic potential was first discovered only in the 1940s as the causal agent of summer diarrhoea. Since then, several studies by workers worldwide established the bacteria as a potential pathogen of both medical and veterinary importance. To combat these E. coli disease problems, effective and efficient diagnosis and prophylactic measures are essentially crucial. A brief look on the classification pattern of the pathogenic E. coli could facilitate our discussion.

Classification based on virulence and disease conditions manifestation:

1. **Enteropathogenic E. coli (EEC or EPEC)**
   - They are mainly responsible for infantile diarrhoea (mostly <6 months children, rarely affect children over 1 year of age).

2. **Enterotoxigenic E. coli (ETEC)**
   - Presently, among the children less than five years, in developing countries, diarrhoeal episodes have been estimated to be 1.5 million annually, accounting for 3 million deaths. Among these ETEC are responsible for about 210 million diarrhoeal episodes and approximately 3.8 lakhs death annually (WHO, 1999).

3. **Enteroinvasive E. coli (EIEC)**
   - Watery diarrhoea followed by gross dysentry consisting of scanty stools of blood and mucous is main feature of EIEC diarrhoea.

4. **Enterohaemorrhagic E. coli (EHEC)**
   - Since their first recognition as human pathogen in 1982, EHEC has emerged as a significant public health concern. EHEC has been recognized to be the cause of a number of life-threatening human diseases like haemorrhagic colitis, haemolytic uraemic syndrome, thrombocytopenic purpura etc. In animal also, they produce bloody or non-bloody diarrhoea, swine edema disease. Domestic animals mainly cattle are their reservoir and food of animal origin is the main vehicle of human infections.

5. **Enteroaggregative E. coli (EaggEC)**
   - Diarrhoea caused by these pathogen lasts for more than 14 days.

**E. coli detection**

1. **Isolation**
   - Samples are to be enriched first in selective enrichment media. Enriched inoculum is then streaked onto selective solid media. E. coli forms pink, smooth round colony in MacConkey’s agar and metallic sheen colony on EMB agar.

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2. **Biochemical characterization**

Typical biochemical reactions should be taken into account to identify and characterize the isolated organisms to confirm as *E. coli*.

3. **Serotyping**

*E. coli* serotyping based on somatic (O), capsular (K) and flagellar (H) antigens, is mostly used for subspecies specific differentiation. Currently, more than 200 'O' serotypes, 80 'K' antigens and 55 'H' antigens have been described in various combinations. The publications which may be consulted for full description of serotyping procedures are Sojka (1968) and Ewing (1986).

4. **Identification of ETEC**

A. **Tests for heat-labile toxin (LT)**

1. **Rabbit Ligated Ileal Loop test (RLIL)**

Fluid accumulation ability of LT can be visualized in the ligated segments of small intestines of rabbits (or pigs). For details, De and Chatterjee (1953) can be referred.

2. **Tissue culture assays**

In Y-1 (mouse adrenal tumor) or CHO (Chinese hamster ovary) cells, LT causes rounding and elongation of the cells, respectively. But in Vero cell, the cells lose their elongated appearance and tend to become round or globular, sometimes with tails or tendrils. The following references could be consulted for details-Sack and Sack (1975), Guerrant et al. (1974), Konowalchuk et al. (1977).

3. **Latex particle agglutination test**

Latex particles are sensitized with the polyclonal antisera. These latex particles agglutinate in presence of LT in culture fluid. (Finkelstein and Yang, 1983).

B. **Detection of heat-stable toxins (ST)**

i) **STa**

ii) **Suckling mouse test.**

Injection of toxin preparation into stomach of suckling mice causes fluid accumulation in the intestine. If the ratio of the pooled gut weight to the combined weight of the remaining carcasses is more than >0.9, it is taken as a positive result (Giannella, 1976).

ii) **Radio-immunoassays**: For details of this test, Frantz and Robertson (1981) could be referred.

iii) **ELISA**: Methodology of the test could be obtained from Carroll et al. (1990).

2. **STb** (i.e., in porcine *E. coli* isolates)

i) **Animal inoculation**

Intestinal loops of rats or mice are injected with toxin solution. After 21/2 hrs, volume of fluid per cm of loop is determined. For details, Whip (1990) could be referred.
ii) ELISA

A synthetic peptide, analogous to STb coupled to keyhole limpet haemocyanin is used to immunize rabbits. Purified antibodies are used to develop a direct binding STb ELISA, which can detect 1-2 ng of toxin in crude culture filtrates (Urban et al. 1990).

5. Detection of verotoxic E. coli (VTEC)

A. VTEC Isolation:

i) Isolation of O157: H7 VTEC

The isolation of O157: H7 serotypes is facilitated by while more than 90% E. coli ferments sorbitol within 24h and have B-D glucuronidase enzyme, it does not do so. Based on this, sorbitol MacConkey agar (SMAC) and several other commercial media have been developed and are now being commonly used for isolation of O157: H7 E. coli. A rapid fluorometric assay for detection of E. coli has been developed. The fluorescence is due to hydrolysis of 4-methylumbelliferyl-B-D glucuronide (MUG) by the glucuronidase enzyme. As E. coli O157: H7 is glucuronidase negative they don’t show fluorescence under UV light.

ii) Isolation of non-O157 VTEC

At present there is no simple bacteriological, i.e., biochemical, serological, morphological characteristic test, available to detect VTEC belonging to non-O157 serogroup. This significantly complicates the isolation of such organisms. However, production of enterohaemolysin has been described to have a high predictive value for all VTEC strains.

B. Test for verotoxin (VT) production

i) Vero cell cytotoxicity Assay (VCA)

VCA, till now, remains as the ‘golden standard’ for the confirmation of VT-producing isolate. The VCA involves, the treatment of Vero monolayers (usually 96-well trays) with sterile extracts of filtrates (sometimes serially diluted) and examining for 48-72 hr of incubation. Neutralization by specific anti-toxin further confirms the presence of VT in the sample. The test, though laborious, is definitive for VTEC.

ii) ELISA for VT-detection

During the past decades, a number of ELISA have been developed including commercial kits. Inspite of its value in VT detection where tissue culture facilities are not available, the test is not as sensitive as VCA.

iii) Detection of VT gene(s)

The use of DNA probe synthetic oligonucleotide probes, amplification of VT genes by PCR have also been successfully evaluated for identifying VTEC, but they are cumbersome, confined only to a few reference laboratories and require highly skilled staff.
iv) Serological detection

Lipopolysaccharide (LPS) antigen of different serogroups of VTEC have been used for detection of specific antibodies in patients serum but it has limited application as antigen is serogroup specific.

6. Test to detect colonizing ability of E. coli

i) Latex particle agglutination test

For detection of F4, F5,F6 and F41 fimbrial adhesions monoclonal antibodies coupled to latex particles to form stable agglutination reagents can be used (Thoms et al., 1989)

ii) ELISA

Details of the determination of colonizing ability of E. coli by ELISA could be obtained from Mills et al. (1983) and Thorns et al. (1992).

iii) Haemagglutination

A heavy bacterial inoculum is mixed with small volume of washed RBC (with or without D-mannose) on a glass slide. The slide is rocked gently and observed for agglutination (Duguid et al., 1979).

7. Tests to identify EPEC

EPEC strains have long been defined by exclusion as those E. coli strains don’t belong to any of the other categories. But presently, they are differentiated by their localized adherence ability to Hep-2 cells (Ravito et al., 1979). However, some less common serogroups are not adherent to Hep-2 cells. For them, localized adherence test to HeLa cells can be performed (Pedroso et al., 1993).

8. Test to detect EIEC

In vasiveness of EIEC can be detected by a positive Sereny test, i.e., induction of keratoconjunctivitis in guineapig. For details of the tests Murayama et al., (1986) could be referred.

9. Tests to detect EAygEC

A 'Stacked-brick-like' aggregative pattern of adherence to Hep-2 cells in vitro, caused by formation of microcolonies on tissue culture cell surfaces is indicative of EAygEC (Vial et al., 1988). A rapid screening test for EAygEC in which positive strains produce a scum on the surface of Muller-Hinton broth incubated at 37°C have also been described (Albert et al., 1993).

Gene Probe and PCR to identify pathogenic E. coli

Applying this technique several toxin genes of E. coli viz.LT-1,LT-2, STa,STb,VT-1,VT-2 can be detected. For details, Gyles (1996) can be referred. A sensitive and specific DNA probe for EPEC adherence factor (EAF) has also been developed to detect EPEC strain (Nataro et al.,1985) For detection of EAygEC also a specific DNA probe is there. Details could be obtained from Vial et al. (1989). A good deal of PCR applications in identifying several E. coli toxin have been described by Gyles (1996).
CONTROL

Like any other foodborne diseases, *E. coli* disease outbreaks are also contributed most frequently by the following factors:-

i) Inadequate refrigeration

ii) Preparing foods for advance of planned service

iii) Infected person’s practicing poor personal hygiene

iv) Inadequate cooking or heat processing

v) Holding foods in warming devices at bacterial growth temperatures

So, in order to check *E. coli* disease outbreaks, these factors should be kept in mind.

Vaccines

1. Against ETEC diarrhoea

   Both live and killed vaccine candidates have been developed with greatest progress in killed oral vaccines. Several approach include vaccines consisting of purified colonization factors, LT only or LT-ST toxoid and edible transgenic plant vaccine that express cholera B subunit. In the live vaccine approach, strategy is to use live attenuated *Shigella* organisms as vectors for expression of ETEC fimbrial and LT antigens which might protect both *Shigella* and ETEC.

2. Against EHEC

   Immunization against O157:H7 alone or against the broader group of STEC (or VTEC) pose overlapping challenges. Approaches include-live, attenuated, killed whole-cell vaccines, polysaccharide-protein conjugate vaccines and Stx toxoid subunit vaccines.

Vaccines in animal

Presently, a number of commercial vaccines are available against neonatal diarrhoea in pigs, calves and lamb in other countries. Improved sanitation, the use of antibiotics and the assurance that neonates receive colostrum early in life are also important in the disease prevention.

A. Empirical vaccines

   Here killed or live whole cell preparations are used as vaccine, no need to know specific pathogenic mechanisms.

B. Virulence factors based

   i) Pilus-adhesins

   Antibodies that specifically neutralize the adhesive activity of the pilus-adhesin should prevent attachment, colonization and disease
ii) **Enterotoxin**

LT provides a good production again LT producing ETEC but not against ST. But neither STa nor STb is inherently antigenic. To protect swine edema disease, genetic techniques have been employed to reduce the cytotoxic activity SLT (SLT-II / VT2) (produced by mostly VETEC O138, O139, O141) while retaining its immunogenic activity.

**REFERENCES**


FOODBORNE CLOSTRIDIAL POISONING WITH REFERENCE TO C. PERFRINGENS AND C. BOTULINUM

P.C. Harbola

Clostridial organisms particularly Clostridium perfringens and Clostridium botulinum are important agents which cause food poisoning.

Clostridium perfringens Enteric Infections

C. perfringens is recognised as cause of food borne toxi-infection and is widely distributed in the environment. Meats are frequently contaminated with it. Yet, it constitutes a public health hazard only when a food is mishandled after cooking. C. perfringens is a gram positive anaerobic bacterium that is able to form spores. It is wide spread in the environment and is commonly found in the intestines of animals and human. In human it can cause gas gangrene and gastrointestinal disease (eg. Food poisoning and necrotic enteritis) C. perfringens does not invade healthy cells but produces various toxins and enzymes that are responsible for the associated lesions and symptoms. C. perfringens strains are classified into five toxinotypes ABCDE based on the production of four major toxins (Table-1). Each toxinotype is associated with a particular disease. C. perfringens type A is ubiquitous and is the most common toxinotype in the environment and is responsible for food poisoning in human besides gas gangrene. It synthesizes an enterotoxin (CPE) that is responsible for the gastrointestinal symptoms. Unlike other toxins, CPE is produced only during sporulation. However C. perfringens sporulates poorly, if at all, in the usual culture mediums, hampering the identification of enterotoxigenic strains based on CPE detection. Type A strains containing the cpe gene (5% of the global population) are mostly isolated in outbreaks of food poisoning and from livestock such as foals and fattening pigs with diarrhoea. CPE is also produced by strains of toxin types B-E.

A novel toxin (2) has recently been identified from a strain isolate from a piglet that died of necrotic enteritis.

This strain was originally classified as type C, but (2 toxin is also produced by some type A strains as identified by the mouse test. The (2 toxin is necrotizing and has 15% sequence identity and a low level of immunological cross reaction with the known ( toxin (called (1). In preliminary epidemiological investigation, (2 toxin producing C. perfringens strains seem to be involved in some cases of necrotic enterocolitis in piglets and in typhlocolitis in horses.

### Table 1. Diversity of *Clostridium perfringens* toxinotypes, genotypes and associated diseases

<table>
<thead>
<tr>
<th>Toxinotype</th>
<th>Major toxin</th>
<th>Genotype</th>
<th>Associated pathology</th>
<th>Others animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+</td>
<td>pic</td>
<td>Gangrene</td>
<td>Necrotic enteritis in fowl</td>
</tr>
<tr>
<td></td>
<td></td>
<td>plc, cpe</td>
<td>Gastrointestinal diseases</td>
<td>Diarrhea (foals, pigs...)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>plc, cpb2</td>
<td>(food poisoning, antibiotic-associated diarrhea, sporadic diarrhea, some cases of sudden infant death syndrome)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>plc, cpb2, cpe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>plc, cpb1, etx</td>
<td>Dysentery in newborn lambs</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>plc, cpb1, etx, cpe</td>
<td>Hemorrhagic enteritis in neonatal calves and foals</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>+</td>
<td>plc, cpb1</td>
<td>Necrotic enteritis (Pigbel, Darmbrand)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>plc, cpb2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>plc, cpb1, cpe</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>plc, cpb2, cpe</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>plc, cpb1, cpb2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>plc, cpb1, cpb2, cpe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>+</td>
<td>plc, etx</td>
<td>Enterotoxemia in lambs, sheep, calves and goats</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>plc, etx, cpe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>+</td>
<td>plc, iap, ibp</td>
<td>Enterotoxemia in calves</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>plc, iap, ibp, (cpe)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>plc, iap</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Toxinotypes based on mouse lethality tests.

Data taken from Refs 2-4, 7.

*Caused by enterotoxigenic strains.

Some strains containing the cpb2 gene are classified as type A by the mouse lethality test.

C. *perfringens* type C necrotic enteritis has been observed in New Guinea populations (Pigbel) and in Germany after World War II (Darmbrand).

Gene in brackets is not expressed.

Table cited from Trends in Microbiology Vol. 7 No. 3 March 1999.

### Toxin Encoding Genes

Most of the virulence genes from *C. perfringens* have been characterised. In the present discussion we are concerned with food poisoning organism, therefore, only genes pertaining to *C. perfringens* causing food poisoning will be discussed. The cpe gene is found in many strains involved in human food poisoning and on a large plasmid in isolates from non-food borne human gastrointestinal disease and cases from animals. The large plasmids carrying the toxin genes might originate from extra chromosomally located phages. When chromosomally located, the cpe gene is situated on a 6.3kb transposon that comprises two flanking copies of the insertion sequence IS 1470, a member of the IS 30 family transposases and one copy of IS 1469 (related to IS200 from Gram's negative bacteria) immediately upstream of the cpe gene. When localised on a plasmid, the cpe gene is flanked by IS1469 and in some strains, by another insertion sequence, IS1157. This suggests that cpe and etx are or have been located on mobile elements. The cpe gene is subject to transcriptional regulation, probably by sigma factors (Sig E&Sig K) and are Hpr - like transition state regulation, all of which are known to be involved in sporulation in *Bacillus subtilis* (Table-2).
Table 2. The location, mode of action and biological activity of *Clostridium perfringens* toxin-encoding genes

<table>
<thead>
<tr>
<th>Toxin/enzyme</th>
<th>Gene</th>
<th>Genetic localization</th>
<th>Mode of action</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>plc</td>
<td>Chromosome</td>
<td>Phospholipase C</td>
<td>Spingomyelinase activity</td>
</tr>
<tr>
<td>b1</td>
<td>cpb1</td>
<td>Plasmid</td>
<td>Pore-forming activity</td>
<td>Cell membrane disruption</td>
</tr>
<tr>
<td>b2</td>
<td>cpb2</td>
<td>Plasmid</td>
<td>Pore-forming activity</td>
<td>Cell membrane disruption</td>
</tr>
<tr>
<td>e</td>
<td>ctx</td>
<td>Plasmid</td>
<td>Altered cell membrane</td>
<td>Permeability (pore-forming)</td>
</tr>
<tr>
<td>(la)</td>
<td>iap</td>
<td>Plasmid</td>
<td>ADP-ribosylation of actin for la</td>
<td>Additional virulence factors</td>
</tr>
<tr>
<td>(lb)</td>
<td>ibp</td>
<td>Plasmid</td>
<td>Additional virulence factors</td>
<td></td>
</tr>
<tr>
<td>Enterotoxin (CPE)</td>
<td>cpe</td>
<td>Chromosome/plasmid</td>
<td>Activity</td>
<td>Cytotoxic, erythematous, lethal</td>
</tr>
<tr>
<td></td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>plbA</td>
<td>Chromosome</td>
<td>Fore-forming activity</td>
<td>Additional virulence factors</td>
</tr>
<tr>
<td>k</td>
<td>csaA</td>
<td>Chromosome</td>
<td>Hemolysin, specific to GM2</td>
<td>Additional virulence factors</td>
</tr>
<tr>
<td>k</td>
<td>ism</td>
<td>Plasmid</td>
<td>Additional virulence factors</td>
<td></td>
</tr>
<tr>
<td>v</td>
<td>nagH</td>
<td>Chromosome</td>
<td>Hemolysin, specific to cholesterol</td>
<td>Additional virulence factors</td>
</tr>
<tr>
<td>Neuraminidases</td>
<td>nhnH,J</td>
<td>Chromosome</td>
<td>Collagenase, gelatinase</td>
<td>Additional virulence factors</td>
</tr>
<tr>
<td></td>
<td>ureA,e</td>
<td>Plasmid</td>
<td>Protease</td>
<td>Additional virulence factors</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>Plasmid</td>
<td>Hyaluronidase</td>
<td>Additional virulence factors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dnase</td>
<td>Additional virulence factors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Neuraminidase</td>
<td>Additional virulence factors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Urease</td>
<td>Additional virulence factors</td>
</tr>
</tbody>
</table>

*The biological effects of t-toxin result from the action of the binding component (lb) and the enzymatic component (la), which is the intracellular active component. Cited from Trends in Microbiology Vol-7 No.3 March 1999

**Genotyping**

DNA-based techniques (PCR & hybridization) have been developed for *C. perfringens* typing and are a reliable alternative method in laboratory animals. In particular, genetic investigation is very useful for detection of cpe, which is rarely expressed under normal culture conditions. Strain definition by genotype is more indicative of potential virulence than is identification by conventional typing. However, the presence of a gene does not always correlate in the toxin synthesis although only the cpe gene in *C. perfringens* type E strains has been found to be silent. Genotyping is reliable of practical and allows a more accurate and complete determination of *C. perfringens* pathovars than does classical toxinotyping.

**Mode of action of C. perfringens enterotoxin**

With the exception of t-toxin which acts intracellularly; the *C. perfringens* toxins interact with the cell membrane leading to membrane disruption or pore formation. CPE is released after bacterial lysis. In the intestinal loop assay, CPE induces a significant secretion of fluid and ions into lumen, a reduction of glucose absorption and a slight desquamation of the epithelial cells of the intestinal mucosa. CPE binds by its 30 carboxy—terminal amino acids to a protein receptor, possibly a dimer of a 22-kDa protein found on the intestinal cell brush border and on the surface of verocells. CPE then associates with another membrane protein (70 kDa) to form a large complex (160kDa) leading to membrane insertion and pore formation. The rapid leakage of
small molecules (e.g. water, ions, nucleotides and amino acids) through the pores without the involvement of intermediate messengers; such as cyclic AMP or cyclic GMO; is responsible for the inhibition of protein synthesis.

**Necrotizing jejunitis (enteritis necroticans; pigbel)**

Caused by *C. perfringens* type C with severe lower abdominal pain and diarrhoea developed some hours after the patients had eaten rabbit, tinned meat or fish paste. Some cases showed blood and mucosal sloughs in the stools and a few deaths occurred due to peripheral circulatory failure or to intestinal obstruction caused by massive oedema of the intestinal mucosa. The area affected was principally the jejunitis. In India, Narayan and Rao (1970) and Pujari and Deodhare (1980) have reported the occurrence of this disease. It has also been suggested that *C. perfringens* type A may also occasionally cause the disease.

Necrotizing jejunitis in New Guineae was referred to the 'pigbel' syndrome (pigbel - abdominal discomfort after a large pork meal) because of its aetiological relationship to the widespread practice of pork feasting in the locality. The Pigbel has been defined as an acute, patchy, necrotising and inflammatory disease of the small bowel in High level Melanesians in New Guinea. Males are affected more frequently than females and the disease predominantly affects children. Clinically it is characterized by anorexia, severe upper abdominal pain, bloody diarrhoea and vomiting. It has been classified into four clinical types (1) An acute form (2) An acute surgical form (3) subacute surgical form (4) A mild form.

After *C. perfringens* being ingested it proliferates in the intestine and releases its beta toxin. Under normal circumstances the toxin is promptly destroyed by endogenous proteinases to which it is highly sensitive but if this does not take place severe toxic intestinal change may ensue.

Therapeutic antitoxin is of no value in the management of pigbel because toxic damage is inflicted some days before symptoms are displayed. Active immunization with *C. perfringens* type C toxoid, however, confers a high degree of durable protection.

**Infant Botulism**

Infant botulism is caused by the ingestion and germination of *C. botulinum* spores, followed by bacterial multiplication and toxigenesis in the intestine. The disease was first recognized by Pikett and his colleagues and its pathogenesis rapidly elucidated. Type A and proteolytic type B strains are responsible for almost all cases. The rare exceptions include cases due to a subtype B strain, a subtype Ba strain, a New Mexican strain that produced type F toxin but otherwise resembled *C. barati* and two Italian strains that produced type E but otherwise resembled *C. butyricum*. Most patients are between 2 and 26 weeks old. The disease, which usually begins with constipation, varies from a mild paralysis requiring treatment in hospital. The signs include ptosis and flaccidity of the fascial musculature. Mortality is low in patients admitted to hospital but it is evidenced that the disease may cause sudden infant deaths. Such deaths are associated with use of artificial foods rather than with breast-feeding or has been inadequately cooked. No cases have yet followed the consumption of fresh food, cooked or uncooked. In several instances those who consumed the affected food after cooking escaped, whereas those who consumed it before cooking.
suffered. The kind of food responsible for poisoning varies with the eating habits of the communities concerned. In Europe, it is sausages, ham, preserved meats, game plate and brawn. In America, home canned vegetables and fruits.

The environmental sources of spores are thought to include honey; about 10% of honey samples were found to contain type A or B spores and _C. botulinum_ is rarely present in the faeces of healthy human adults and symptomless carriage in infants occurs only occasionally. Both organisms and toxin sometimes are present in the faeces of infants for many weeks after clinical recovery from botulism.

**Botulism in Man**

Except in infants, human botulism is usually intoxication, not an infection. The causative organism _C. botulinum_, multiplies in the food before it is consumed and produces a powerful soluble toxin which on ingestion is absorbed mainly from the upper part of the intestine and gives rise to characteristic disease. The toxins produced by the 7 different types (A-G) of _C. botulinum_ differ in antigenic structure and in toxicity for a variety of experimental animals. All are neural toxin except for the second toxin (C2) of _C. botulinum_ type C. On bacteriological criteria _C. botulinum_ is classified into group I (type A and proteolytic strains of type B) Group II (type E and non-proteolytic strains of type B&F), III (type C&D strain) and IV (type G strain).

The recorded cases of human botulism are mainly due to A,B& E. The disease is a pure intoxication, food containing toxin inevitably contains _C. botulinum_ also. Most of the reported outbreaks of botulism have been caused by food that has been smoked, pickled or canned, allowed to stand for sometime and eaten without cooking, or has been inadequately cooked. No cases have yet followed the consumption of fresh food, cooked or uncooked. Of the type of botulism encountered the commonest is type A; it is well known for example in USA when it is caused mainly by home canned or preserved vegetables; type B is frequent when pork products are consumed. Type E botulism is of particular importance in Japan, Canada and Alaska. In Japan most of the outbreaks caused by type E have followed the consumption of _izushi_, a fermented food made of boiled rice, vegetable and new fish meat and allowed maturing for 2 months.

_C. botulinum_ A &B demand strictly anaerobic conditions while strain of type E show a somewhat more toleration behaviour towards oxygen.

Factors, which influence the development of _C. botulinum_ in foods, include (i) a fairly heavy initial contamination by the spores, (ii) insufficient heating, (iii) anaerobic conditions, (iv) too slow cooking, (v) use of this food without final cooking and (vi) home canning.

**Heat stability, growth conditions and other characteristics of Clostridial Food Poisoning**

Studies on heat stability of toxin of _C. botulinum_ has shown that temperature range for type A B varies from 10°C-50°C; Type E 40-45°C; type F 15-50°C and for _C. perfringens_ it ranges from 10-48°C. Hydrogen ion concentration growth limit for _C. botulinum_ is 4-5 and for _C. perfringens_ 5.0. The water activity for _C. botulinum_ is 0.93.

The incubation period for _C. perfringens_ C(F) toxin infection is 6hrs-6d. The duration of illness is several days with fever, vomiting nausea, diarrhoea. The case fatality rate is 40% and characteristic symptom includes enterocolitis.
The incubation period for \textit{C. botulinum} intoxication is 2-6 d, duration 8 months, may or may not cause vomiting, diarrhoea, fatality rate is 10-70\% with paralysis. The incubation period for \textit{C. perfringens} type A is 1-7hr, duration of disease is 1-2 days without fatality and with no characteristic symptom (Table 3 &4).

### Table 3: Food-borne Bacterial Intoxications in Man

**Heat Stability of Germs Resp. Toxins and Some Growth-Conditions**

<table>
<thead>
<tr>
<th>Germs</th>
<th>\textit{C. botulinum}</th>
<th>\textit{C. perfringens}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type A</td>
<td>Type E</td>
<td>Type F</td>
</tr>
<tr>
<td>Heat stability (100\°)</td>
<td>Germ</td>
<td>Toxin</td>
</tr>
<tr>
<td>10°-50°</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Temperature Range</td>
<td>optimum</td>
<td>35°</td>
</tr>
<tr>
<td>pH growth limit</td>
<td>4,5</td>
<td>5</td>
</tr>
<tr>
<td>Water activity growth limit</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Clostridial Food Intoxications

**(Incubation period, Duration and Clinical Symptoms in Man)**

<table>
<thead>
<tr>
<th>Toxins</th>
<th>\textit{C. perfringens} Type C(F)</th>
<th>\textit{C. botulinum} Type A, \textit{C. perfringens} Type A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation period</td>
<td>6h - 6days</td>
<td>2-6d</td>
</tr>
<tr>
<td>Duration</td>
<td>Several days</td>
<td>8 months</td>
</tr>
<tr>
<td>Fever</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>-</td>
<td>(+)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fatality rate</td>
<td>40%</td>
<td>10-70%</td>
</tr>
<tr>
<td>Characteristic symptoms</td>
<td>Enterocolitis</td>
<td>Paralysis</td>
</tr>
</tbody>
</table>

**Diagnosis**

Diagnosis of infant botulism is confirmed by examining the faeces for \textit{C. botulinum} organisms and for toxin. The latter is demonstrated by subjecting an extract to mouse inoculation and neutralization tests. An Enzyme Immuno assay has also been used successfully.

The symptoms of botulism in man are generally so characteristic that the disease may be diagnosed on clinical signs. The diagnosis should then be confirmed as rapidly as possible in the laboratory by employing.

1. Demonstration of toxin in the serum.
2. Demonstration of toxin in food and other material.
3. Demonstration of bacilli in food and other material.
**Prophylaxis:**

Human botulism is so rare and sporadic that prophylactic immunization is impracticable. Those who may be exposed to the hazards of intoxication as in biological warfare may be protected by immunization with toxoids.

**Treatment:**

The toxin still in the stomach should be removed immediately by lavage with 2-5% bicarbonate solution. The stomach should be kept alkaline, as the toxins are liable at high pH and an alkaline milieu is probably unfavorable for the intragastric activation reported for type E toxin. In man, the early administration of antitoxin - polyvalent for types A, B & E or monovalent when the type of intoxication is known - lowers the case fatality rate.

**Prevention of Food intoxication**

1. Prevention of contamination
2. Destruction of germs in food
3. Creating conditions, which prevent multiplication of germs in foods.

**REFERENCES**

BACILLUS CEREUS FOOD POISONING


For long *Bacillus cereus* was taken as a laboratory contaminant and cause of 'sweet curdling' or 'bitty cream' of milk. Though there have been reports in the earlier literatures giving circumstantial evidences for its association with food poisoning cases, it was not until 1950 when Hague in Norway described an outbreak where *B. cereus* was implicated. Later in 1955, he was able to produce the symptoms in volunteers fed with *B. cereus*. Since then, the literature is replete with incidence of *B. cereus* food poisoning from all parts of the world establishing it as an important foodborne pathogen. It was the third most common cause of bacterial food poisoning between 1960-68 in Hungary.

Food poisoning:

*B. cereus* has been found to cause two distinct forms of human gastroenteritis besides being associated with a variety of extra-intestinal infections, viz., mastitis, systemic infections and gangrene in both man and animals. The foodborne syndromes are i) a late onset 'diarrhoeal syndrome characterised by abdominal pain, diarrhoea and rectal tenesmus usually resolving in 24 hrs. The symptoms start appearing 5-18 hrs after the ingestion of contaminated food. The diarrhoeal syndrome is mostly associated with foods like meat, soup, pudding, vegetable and mixed dishes besides many others; (ii) a rapid onset 'emetic syndrome' with predominating symptoms of nausea and vomition rarely accompanied with diarrhoea. The incubation period is usually 1-5 hrs. It is usually associated with starchy foods like fried rice, boiled rice and pastry. The symptoms subside within 24 hrs.

The infective dose has been reported to be (10^5 cells per grams of food. Diarrhoeal syndrome mimics the food poisoning caused by *Clostridium perfringens* type A, whereas symptoms of emetic syndrome closely resemble to that of *Staphylococcus aureus* food poisoning. A comparative clinical and epidemiological features of these has been presented in Table-1.

Properties and distribution

The aerobic endospore forming bacilli of the genus *Bacillus* was among the earliest bacteria to be described. The genus was proposed by Ferdenand Cohn in 1872 with *B. subtilis* as type species. A decade later in 1887, *B. cereus* was described as a species by Frankland and Frankland. *B. cereus* is a large aerobic or facultatively anaerobic Gram's positive, spore bearing bacilli. The temperature range for growth is 10-48°C with 28-35°C being optimum. Spores of certain strains of *B. cereus* are unusually resistant to heat and radiation. The salient feature characters have been listed in Table-2. The organism is usually regarded as saprophyte of ubiquitous distribution in the environment and can be isolated from soil, dust, cereals, spices, vegetables, dairy products besides many other foods. *B. cereus* has been found to be present in 90% of uncooked rice. No surprise, it has been regarded as a natural contaminant of many raw food ingredients.

1Scientist (Senior Scale), 2 Head of the Division, 4 M.V.Sc. scholar, Division of Veterinary Public Health, IVRI, Izatnagar-243 122 (U.P.) and 3 Scientist Division of Epidemiology, IVRI, Izatnagar-243122
Table 1: Clinical and epidemiological features of *B. cereus*, *S. aureus* and *Cl. perfringens* food poisoning

<table>
<thead>
<tr>
<th>Major source</th>
<th>Duration of illness (Hrs)</th>
<th>Diarrhoea, abdominal cramps</th>
<th>Nausea, Vomiting</th>
<th>I.P. (Hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>B. cereus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) Diarrhoeal</td>
<td>8-12</td>
<td>12-24</td>
<td>Predominant</td>
<td>Occasional</td>
</tr>
<tr>
<td>b) Emetic</td>
<td>1-5</td>
<td>6-24</td>
<td>Fairly common</td>
<td>Prevalent</td>
</tr>
<tr>
<td>2. <em>S. aureus</em></td>
<td>2-6</td>
<td>6-24</td>
<td>Fairly common</td>
<td>Prevalent</td>
</tr>
<tr>
<td>3. <em>Cl. perfringens</em></td>
<td>8-12</td>
<td>12-24</td>
<td>Prevalent</td>
<td>Rare</td>
</tr>
</tbody>
</table>

Detection of *B. cereus*

Like other foodborne pathogens, *B. cereus* can be detected by isolation and characterization or a number of other immunological techniques such as flow cytometry, infra-red spectroscopy or polymerase chain reaction (PCR). These methods could prove to be highly sensitive in detecting the pathogens of choice in a short time. Of these, isolation and identification of *B. cereus* can be done in all laboratories with a minimum of input and expertise. Of others PCR has been used to detect *B. cereus* in a variety of foods by a number of workers. No doubt ever since its introduction in the mid-1980's, PCR as a test has proved invaluable method to detect pathogens in food. PCR is a rapid test with high sensitivity as well as high specificity for quick detection and identification of specific pathogenic bacteria including *B. cereus* from different food materials. However, each of the PCR procedures reported in the literature describe a different procedure with varying sensitivity and specificity. This indicates a need for careful selection of primers besides modification in the protocol to meet the requirements. It is imperative to add that there is a need to develop a PCR protocol that can universally be used for detection of a variety of foodborne pathogens from a wide range of food as has been described by Wang et al. (1997).

In food microbiology, the DNA techniques have been used in association with bacteriological examination where the microorganism is usually enriched or even purified followed by its identification. No doubt, the best method would be to detect the pathogen directly from the food. However, to achieve this, the food microbiologists have to resolve many of the associated problems, only then the DNA based techniques may be adopted for routine use in the laboratory.

There are a number of bacteriological media for isolation and enumeration of *B. cereus* from different sources. These include mannitol phenol red egg yolk polymyxin agar (MYP), Kim and Goephert (KM) agar, polymyxin pyruvate egg yolk mannitol bromothymol blue (PEMB) agar besides some others. The most important character to identify *B. cereus* is egg yolk precipitation. On MYP agar, it produces rough colonies.
with a violet red background surrounded by white precipitate. On PEMB agar, B. cereus forms crenated or slightly rhizoid colonies with peacock blue colour surrounded by a zone of white precipitate. The isolates could be identified on the basis of biochemical tests. The laboratory processing for the isolation of B. cereus has been shown in Fig. 1. Serological testing to detect spore antigens could be used as a rapid confirmatory test.

A serotyping scheme based on flagellar antigens have been developed on the basis of which B. cereus is grouped into 23 serotypes. Of these, serotype I has been shown to be the major cause of emetic syndrome along with 8 and 12. Majority of the diarrhoeal outbreaks are caused by serotypes 1,2,6,8,10 and 19. The serotypes 1,8,12 & 19 have been shown to cause both diarrhoeal as well as emetic syndrome.

Shingawa et al. (1990) isolated 7 phages which were able to type 33 of 38 isolates from outbreak of vomiting syndrome. Ahmed et al. (1995) could type 97% of B. cereus isolates with 12 phages isolated from sewage. The phage typing for B. cereus has been shown to be good for epidemiological analysis of B. cereus food poisoning.

The molecular typing tools such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), pulse field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP) are rapid methods to identify and discriminate between B. cereus strains.

**B. cereus toxins and their detection**

B. cereus produces many toxins besides diarrhoeal and emetic toxins. There are a number of assays and commercial kits to detect diarrhoeal toxin but the emetic toxin is still a challenge for the food microbiologists.

The symptoms of B. cereus food poisoning are produced by two distinct toxins, viz., diarrhoeal and emetic. The toxin of B. cereus that produces diarrhoeal syndrome has been given a number of terms, viz., diarrhoeal toxins, diarrhoeagenic toxin, diarrhoeal agent, fluid accumulating factor, VP factor, dermonecrotic toxin and intestinonecrotic toxin. It is a protein of MW 50,000 produced during the experimental phase of growth in the presence of glucose (1%) over a wide range of pH with optimum being 7.0 to 7.5. Recently, it has been designated as enterotoxin complex or haemolysin BL having three components- B,L 1, and L2. Individually these have been found to be inactive but when combined together these factors are haemolytic, cytotoxic and dermonecrotic capable of producing vascular permeability ability. A 5(g dose may produce fluid accumulation in rabbit ileal loop. However, it also produces a variety of toxins which have been demonstrated to be different from the above two toxins. These have been identified as phospholipase C, hemolysin, mouse lethal toxin (MLT). The phospholipase produced by B. cereus has been reported to be different from that produced by C1. Perfringens. The MLT appears to be heat labile and sensitive to trypsin. The toxin is thought to stimulate adenylate cyclase cyclic AMP causing accumulation of fluid in the intestine. However, the mode of action is not precisely elucidated yet.

The bioassays (Table-3) used in detection and study of toxin produced by B. cereus includes mouse lethality, rabbit ligated ileal loop (RLIL) tests, vascular
permeability reaction (VPR) and cell culture assay for diarrhoeal toxin and monkey feeding tests for emetic toxin. The RLIL and VPR in rabbits and guinea pigs have been used extensively.

There are few serological tests, viz., reverse passive latex agglutination (RPLA) test and enzyme linked immunosorbent assay (ELISA) for detection of *B. cereus* enterotoxicity (Table-4). These two are commercially available in the form of kit. The results of the two kits are not comparable since the kits identify two different antigens. The monkey feeding test is the only test used to study the emetic toxin of *B. cereus*. Considerable research has been directed for identification, purification and characterization of *B. cereus* toxins by various workers. In spite of this, the nature of the toxin causing diarrhoeal syndromes has yet to be conclusively established.

*B. cereus* produces an emetic toxin distinct from diarrhoeal toxin. It is present in cell free segment (CFS) and produced in media rich in starch. The toxin produced only after sporulation is reported to be resistant to proteolytic enzymes such as pepsin and trypsin, heat and wide range of pH. Its MW is 10,000 and can cause vacuolation in Hep-2 cells. Recently, a putative emetic toxin-cereultide, has been identified capable of causing Hep-2 cell vacuolation. Non-availability of any suitable laboratory model and low MW of emetic toxin has hampered its detailed analysis. Rhesus monkey has been used to induce vomiting. Of late, cereultide has also been shown to cause emesis in Musk shrew (*Suncus murinus*) following oral as well as intraperitoneal application. Table 4 summarizes the salient properties of the two toxins of *B. cereus*.

**Table 2: Properties of *Bacillus cereus* diarrhoeal and emetic toxins**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Diarrhoeal toxin</th>
<th>Emetic toxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature</td>
<td>Protein</td>
<td>Peptide (?)</td>
</tr>
<tr>
<td>Molecular weight (daltons)</td>
<td>50,000 (G-100)</td>
<td>&lt;10,000 (Mol. sieve)</td>
</tr>
<tr>
<td></td>
<td>38-46,000 (SDS-PAGE)</td>
<td></td>
</tr>
<tr>
<td>Isoelectric point (pI)</td>
<td>4.9 - 5.3</td>
<td></td>
</tr>
<tr>
<td>Stability:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heat</td>
<td>destroyed at 55°C(20min)</td>
<td></td>
</tr>
<tr>
<td>Proteinases</td>
<td>pronase &amp; trypsin</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>sensitive</td>
<td></td>
</tr>
<tr>
<td></td>
<td>unstable at &lt;4 - &gt;11</td>
<td></td>
</tr>
<tr>
<td>Production:</td>
<td>late exponential</td>
<td></td>
</tr>
<tr>
<td>Growth phase</td>
<td>32°C - 37°C</td>
<td></td>
</tr>
<tr>
<td>Optimum temperature</td>
<td>complex nutrients</td>
<td></td>
</tr>
<tr>
<td>In lab. Media</td>
<td>occasionally pre-formed</td>
<td></td>
</tr>
<tr>
<td>In food</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biological activities:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monkey challenge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit ileal loop</td>
<td>diarrhoea 0.5 - 3.5h</td>
<td></td>
</tr>
<tr>
<td>Rabbit permeability</td>
<td>positive (150 µg)</td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td>increased (0.05 µg)</td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td>positive (12 µg iv)</td>
<td></td>
</tr>
<tr>
<td>Mouse lethality</td>
<td>cytotoxic (0.1 - 0.5 µg)</td>
<td></td>
</tr>
<tr>
<td>HFS, MRC-5, CHO cell lines</td>
<td>positive (multicomponent)</td>
<td></td>
</tr>
<tr>
<td>Antigenicity</td>
<td>various immunoassays</td>
<td></td>
</tr>
<tr>
<td>Detection</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>primate challenge only</td>
</tr>
</tbody>
</table>
Control of *B. cereus* food poisoning

The control methods include reducing the holding time of food between preparation and consumption, proper storage of food below 10°C which prevent multiplication of organisms, preparation of small quantities of rice as needed, etc. Cool cooked rice quickly retreating cooked rice thoroughly before serving also found to reduce the incidence and food borne outbreak by *B. cereus*.

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhoeagenic</td>
<td>- Monkey feeding</td>
</tr>
<tr>
<td></td>
<td>- Rabbit ileal loop</td>
</tr>
<tr>
<td></td>
<td>- Vascular permeability</td>
</tr>
<tr>
<td></td>
<td>- Guinea pig skin test</td>
</tr>
<tr>
<td>Emetic</td>
<td>- Rhesus monkey emesis</td>
</tr>
<tr>
<td>All</td>
<td>- Human foetal intestinal test</td>
</tr>
</tbody>
</table>

Table 3: Bioassays for *B. cereus* toxins

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Immunological test</th>
<th>Commercial kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhoeal</td>
<td>Reverse passive latex agglutination</td>
<td>BCET-RPLA (Oxoid)</td>
</tr>
<tr>
<td></td>
<td>ELISA</td>
<td>Tecra BDE-VIA (Tecra diagnostics)</td>
</tr>
<tr>
<td></td>
<td>Micro-slide double diffusion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aggregate haemagglutination</td>
<td></td>
</tr>
</tbody>
</table>

Table-4: Immunological methods in use for *B. cereus* toxins

Suggested readings:


FOODBORNE VIRUSES: IMPORTANCE, DIAGNOSIS & PREVENTION

S.V.S. Malik*, S.P. Chaudhari ** and S. Thakur***

Unlike bacteria, viruses do not multiply or produce toxins in foods; food items merely act as vehicles for their transfer. Epidemiological, clinical and laboratory investigations indicate that some of the viruses can potentially be transmitted via foods and feeds. All viruses known to be transmissible through foods and of concern to human health emanate from the human or animal intestine. Foodborne viruses can be classified into two main categories on the basis of their origin: [1] Those which reach food and water through environmental sources. The viruses originate in most cases from the intestinal tract of humans, and the contamination of food occurs during its production, processing, storage and transportation. As such, the contamination of food is of secondary nature. [2] Those which occur in food on account of carrier or diseased status of food animals themselves. This results in what is usually referred to as primary contamination of food. Most foodborne viruses are transmitted through man-to-man cycle and infection normally takes a faecal-oral route. Among various enteric viruses, the major causes of gastroenteritis in man are Norwalk, hepatitis A, hepatitis E and rota viruses.

There has been speculation that more than 50% of the outbreaks of unknown etiology are due to viruses. It is estimated that more than 100 different viruses are excreted in the faeces of human being. Approximately, 5% of confirmed foodborne outbreaks in the United States during 1983-1987 are attributed to viruses. However, the extent of virus-caused disease may be grossly underestimated. Throughout the world there have been several epidemics of foodborne diseases (FBD) about which there is lack of sufficient information for public health institutions to take appropriate measures primarily because of the scarcity of rapid and sensitive techniques to detect foodborne viruses. To further complicate the situation, the infectious doses of some viruses are low. Several of the tests to detect viruses rely upon tissue culture line; however, some food borne viruses of considerable public health importance, such as hepatitis A virus, rotavirus, calcivirus and Norwalk agent, are difficult to grow in the laboratory. A recent study based on data from 61 sources revealed considerable under reporting and lack of data on FBD throughout various countries, with viruses being the second most important cause of FBD in USA. Two agents, Norwalk virus and hepatitis A, were the fifth and sixth most frequent causes, respectively. Despite the scarcity of the problem, rotavirus, poliovirus, hepatitis E virus, astrovirus, echovirus 4, Snow Mountain virus, Hawaii agent and small gastroenteric viruses have also been implicated as other important causes of FBD.

There are many documented incidences in which international trade in products derived from the animal resulted in the introduction of foreign animal diseases into the domestic animal population. The introduction of foreign animal diseases via animal products can be very costly. The predicted cost of a wide-scale outbreak of FMD in the USA in the first year alone would be in excess of $4 billion in direct losses with approximately 10 times greater indirect losses. These outbreaks emphasize the needs to regulate export of animal products from nations in which economically devastating animal disease is endemic. In developed nations, the most important viral diseases from economical point of

*Scientist (Senior Scale), **Ph.D. Scholar, ***M.V.Sc. Scholar, Division of Veterinary Public Health, IVRI, Izatnagar-243 122
view are African swine fever (ASF), Swine vesicular disease (SVD), Hog cholera (HC) and foot and mouth disease (FMD).

Epidemiology:

Foods derived from animals are frequently processed by heating, lowering pH, salting, dehydration and, irradiation etc., to preserve and/or change the organoleptic quality of the food before serving. Processed foods are not necessarily free of viral agents. Because of the practice of feeding garbage to livestock, virus survival in such products prepared from infected animal is a concern. The lack of stability of virus in food may be the factor that has prevented transmission of some viruses via foods. However, with few exceptions, principally involving shellfish contaminated with hepatitis-A or the Norwalk agent, which are especially heat resistant, no documented outbreak depended on the ability of viruses to withstand even limited heating in food.

Foods may be contaminated either at source (primary contamination) or at the time and place of preparation (secondary contamination). The most common source of food contamination is either the faeces- contaminated fingers of an infected food handler or water polluted with faeces, though the possibility of insects serving as mechanical vectors for faecal viruses can not be excluded entirely, although vectors and aerosols have not been firmly implicated as source of contamination. Any kind of food is at risk of hands-on contamination. An instance of contamination via a fomite may occur when cafeteria trays with which food is in contact becomes contaminated by an infected worker.

Primary contamination:

The most clearly implicated food in the transmission of viruses is the bivalve mollusks (oysters, clams, cockles, and mussels); most illness associated with consumption of this type of shellfish is viral. Some outbreaks in various parts of the world have involved several hundred people or more. Bivalve mollusks are filter feeders that inhabit shallow inshore coastal waters, which may be polluted with sewage. During their natural feeding process, mollusks extract particulate matter, including bacteria and viruses, from the very large volumes of water that pass over their gills. These mollusks are often not cooked properly but may be subjected to a brief heat treatment only and, oysters are frequently eaten raw.

Another potential source of primary viral contamination is the application of polluted water and sewage sludge to fruit and vegetable crops. Viruses may be transferred by direct surface contact during irrigation and fertilization, and it has been suggested from experimental studies that mammalian viruses could be taken up through the roots from ground waters.

Secondary contamination:

It arises from infected food handlers, and is largely associated with cold food items that require much food handling- sandwiches and salads; surfaces in food preparation areas may become contaminated. The viruses involved are highly infectious in low doses. There is circumstantial evidence that some gastroenteritis outbreaks may be caused by symptom-free excretors of viruses. The possibility of insects serving as mechanical vectors for fecal viruses cannot be excluded entirely.
The point of secondary contamination is most often during the final preparation and serving of food. It is seldom in food processing, storage, or distribution, unless one considers the preparation and spreading of icings on baked goods to be processed. Shellfish are different in that they may accumulate viruses and are usually contaminated before harvesting, but it is true of practically no other food.

**Hepatitis viruses:**

**Hepatitis A virus (HAV):** This RNA virus causes infectious hepatitis in man and higher primates. Although in the developed world waterborne outbreaks are rare and foodborne outbreaks are uncommonly reported, waterborne and foodborne spread of HAV has long been recognized in the endemic areas, mostly identified in the developing countries. It is difficult to associate illness with a specific food item because of the long incubation period of HAV (2 to 6 weeks); for the same reason, foodborne transmission is probably under recognized. Although, many food items have been implicated, shellfish accounts for a high proportion of outbreak. Soft fruits, such as raspberries and strawberries, either raw or processed into ice cream and other foods have also been implicated.

Children below 5 years of age are more susceptible to the infection. The virus is excreted in faeces of infected subjects and causes liver dysfunction. Malaise, fever, headache, vomiting, diarrhoea, upper right quadrant tenderness and dark coloured urine manifest the disease. Jaundice is common in the later phase, especially among persons above 18 years. Many of the infected subjects may harbor the virus without showing clinical signs. However, in the occupationally exposed groups, especially those involved in sewage disposal, the seroprevalence of the infection is 2.2 times higher than the normal population.

**Hepatitis E virus (HEV):** A more recently identified enteric form of hepatitis (designated hepatitis E) has been associated with large waterborne outbreaks in some developing countries. In India, hepatitis E (HEV) infection was first identified during the 1956 outbreak in Delhi, involving over 29000 cases. Another large epidemic involving 79,000 people occurred in Kanpur during 1990-91. In the disease-endemic countries, HEV is implicated in causing more than 50% of the sporadic cases of acute viral hepatitis. Hepatitis E virus is most prevalent in Africa, Asia and Central America, whereas in developed nations infection has primarily been recognized in travelers returning from endemic areas.

The virus mainly spreads through the contaminated water used for drinking or washing/processing foods. However, there is a potential for foodborne transmission of this virus particularly by shellfish. HEV infection is a self-limiting disease that does not progress to chronicity. Complete recovery is normally achieved within 2 months, with a 0.1-1% mortality rate from fulminant hepatitis. An increase in disease severity is observed in pregnant women, with a mortality rate of up to 25% during the third trimester. Persons, especially males, in the age group of 18-45 years have been reported to suffer most with the disease.

**Non-A, Non-B (NANB) viruses:** Hepatitis due to then NANB group of viruses is not as clearly identified as that due to hepatitis A (HAV), hepatitis B (HAB), and the delta agent (HDV). Enteric NANB is reported to be a 25-27 nm RNA virus with an outer shell and an inner core. There has been very little advance on the characterization of NANB viruses.
Hepatitis due to faecal-oral NANB infection has been commonly reported in epidemic forms from India, USSR, Japan, Indonesia, Thailand, Burma, Nepal, Bangladesh and Algeria. The problem has been extensively studied in India. The mode of transmission of enteric NANB is similar to that of hepatitis A. Adults suffer more than children in epidemics. Pregnant women contract a severe form of the disease and have a higher mortality.

**Gastroenteritis viruses:**

Viruses of several morphological groups have been associated with gastroenteritis. However, more than 90% of foodborne outbreaks in which a virus has been identified have been attributed to one of the small round structured viruses (SRSVs) of the Norwalk group, whereas, calciviruses, astroviruses, parvoviruses and rotaviruses have only rarely been implicated. When sewage pollution is involved, as in shellfish contamination, more than one virus type may be detected. Instances of gastroenteritis 24 h after eating shellfish, followed by hepatitis A 3 to 4 weeks later, have been recorded.

Viral gastroenteritis has a variable incubation period (usually 5 to 50 h) which may be dose dependent, and symptoms often include both vomiting and diarrhoea. Secondary cases (person-to-person transmission) are a characteristic feature. Reports of foodborne outbreaks of gastroenteritis in developed countries have increased in recent years possibly as a result of increase surveillance and awareness. However, because the illness is mild, most cases of viral gastroenteritis cases remain unreported; hence the true scale of the problem is unknown. Although symptoms may be mild and short lived, large foodborne outbreaks may have considerable economic impact in terms of working days loss. They may disrupt or even lead to the closure of the hospital and school.

**Norwalk virus:** This virus is a well-known cause of acute foodborne gastroenteritis (endemic diarrhoea). In the United States, this virus may be leading cause of nonbacterial gastroenteritis in adults. The virus was discovered in Norwalk (Ohio) in 1968 among students and teachers of a school. It has two serotypes. The infection primarily affects young ones and, is usually self-limiting. Transmission occurs through consumption of faecally contaminated foods involving a man-to-man cycle, especially in densely populated areas. The cases occur round the year with a prevalence ranging from 30-50% in the exposed groups. The incubation period of the disease is 18-48h, the illness lasts for 1-2 days and seroconversion occurs in 79% cases.

**Rotaviruses:** Rotaviruses are the most common cause of severe diarrhoea worldwide, with most of the cases occurring during winter season. Though occasionally implicated in foodborne illnesses, the viruses mainly transmitted through faecal-oral route, especially in the infected nurseries. In developing countries, rotaviruses may cause 6,00,000 to 8,70,000 deaths each year, accounting for an estimated 20-25% of all deaths due to diarrhoea and 6% of all deaths among children < 5 year of age. In India, rotaviruses account for 5-7% of acute diarrhoea in children attending hospitals. The rotaviral infection, which mostly occurs as concurrent infection with other enteropathogens, leads to diarrhoea within 2-4 days of the consumption of contaminated food/water lasting for 2-5 days. Mortality in affected persons can be as high as 20%.
Rotaviruses derived the name from Latin word- "rota" meaning wheel because of their resemblance with it. These have been classified into 7 groups (A-G) and 4 serotypes. Group A, B, and C rotaviruses have been found in both humans and animals while group D, E, F and G have been found to infect not only human beings but also birds like chicken, turkeys, and animals like calves, piglets, foals, dogs, cats, deer, rabbits, and mice. Prevalence studies show that 29.4% of the infected animals excrete the virus in faeces. Human origin virus shares a common antigen with Nebraska calf diarrhoea virus (NCDV), simian agent (SA)-11 and offal agent of sheep and goat. Most of the rotaviruses have been found to cross inter-species barrier under experimental conditions. Natural transmission of the virus has been documented between bovine and porcine species; and also between man and calf.

**Parvoviruses:** These viruses are important in cat and dog, however, their clinical significance in man is yet to be established.

**Astroviruses:** These viruses following of an incubation period cause diarrhoea, fever, headache and vomiting, especially in children. However, the pathogenic potential of these viruses is yet to be ascertained.

**Calciviruses:** Human calciviruses cause diarrhoea outbreaks associated with consumption of contaminated food and water. Sero-epidemiological studies in developing countries suggest that these viruses can cause acute gastroenteritis in children. Recently, it has been decided by the International committee for Taxonomy of Viruses (ICTV) to divide the family of Calciviridae into four genera namely, Vesivirus, Lagovirus, Norwalk-like virus (NLV) and Sapporo-like virus (SLV). The latter two viruses occur in man and cause gastroenteritis. Calciviruses got their name from the Latin word -calix, which means chalice or cup- referring to the cup-shaped depressions that can be seen on some, but not all, of these viruses; Norwalk-like viruses (NLVs) do not have this distinct capsid surface pattern. Porcine enteric calcivirus, which is associated with diarrhoea in pigs, is genetically related to Sapporo-like human calcivirus and, to date it is the only cultivable enteric calcivirus. Calciviruses as a whole vary remarkably with regard to the disease symptoms and the course of infection.

**Enteroviruses:** Enteroviruses include a large number of viruses including poliovirus (3 serotypes), Echovirus (34 serotypes), Coxsackie virus (24 serotypes is characterized as types A and B), New enterovirus types 69-71 (4 serotypes) and Hepatitis A (enterovirus 72) virus (one serotype). Polio/poliomyelitis virus which causes meningitis, paralysis and fever was the first to be associated with foodborne illness. The disease, which can persist for 4 weeks or more, is widespread in many developing countries. Currently, WHO is co-ordinating with endemic countries to ensure its global eradication programme. The Echoviruses causes meningitis, respiratory disease, diarrhoea, rash and fever. The disease is mostly mild in nature and, Echoviruses 4 and 5 are particularly important in case of human infections. The Coxsackie virus, which originated from Coxsackie city in New York, is associated with meningitis, respiratory illness and fever. Type B can cause myocarditis and congestive heart failure. The new enterovirus types 69-71 are associated with meningitis, encephalitis, respiratory disease, rash, acute haemorrhagic conjunctivitis and fever.

**Tick-borne encephalitis viruses:** These viruses, an appropriate example of foodborne viral illness, are the causal agents of Russian summer spring encephalitis (RSSE) and Central European encephalitis (CEE) in Russia and Europe, respectively. The
virus is excreted in the milk of animals, especially goats, which become infected following a tick-bite. However, the details of its epidemiology are largely lacking.

**Adenoviruses:** These viruses which have more than 30 serotypes are divided into 6 subgenera (A-F). These have been isolated from the faeces and urine of man and many species of animals. In human beings, respiratory illness and conjunctivitis manifest the infection that is usually associated with F group virus, especially among the children.

**Cytomegalovirus:** The virus, which causes respiratory illness and hepatitis, has a limited role in causing diarrhoea. The virus is also found in urine.

**Foot and mouth disease virus:** The virus causes vesicular lesions on the tongue and hooves of ruminants and swine. The virus has 7 serotypes (A, O, C, Asia-1, SAT-1, SAT-2, SAT-3). Meat and milk obtained from infected animals is the source of infection for healthy animals, animals in disease-free zones/countries and man. The agent is transmitted through direct contact and aerosol. The virus has also been detected in the urine of infected animals. The lesions in children are found on buccal mucosa and skin.

**Diagnosis:**

Detection methods for viruses in foods are primarily of value for verification that a food was a source of virus in an outbreak rather than to monitor foods for presence of viruses because of the expense, relative lack of sensitivity and time consumption of such methods. Increasingly quick, efficient, sensitive, inexpensive methods are being developed for detecting enteroviruses, and perhaps rotaviruses in shellfish and other foods. Such methods will be of use if it can be shown that these viruses are good indicator of the presence of hepatitis-A virus and the Norwalk-like viruses in the food tested but they will not detect either of the last named agents in food and neither will apply in any other currently available methods.

Detection of gastroenteritis viruses largely depends on examination of faecal specimens by electron microscopy. The number of SRSVs rapidly falls below detectable level and so specimen should ideally be collected within 48 hours of onset of symptoms. Freezing and thawing may some time destroy the morphological integrity of variance but not the infectivity of the sample. Specimens should therefore be stored without freezing before examination. It is not practicable to examine food remnant because the number of virus particles will be too low for currently available detection method. The general view is that at least 106 virus particles per mm of specimen are required for detection by electron microscopy. Viruses with very distinct morphological features—e.g., rotavirus—will be more readily detectable than SRSVs whose features are less clearly defined, and it may be necessary for greater number of these viruses to be present for definite identification.

Since HAV is excreted mainly before infection is apparent, faecal specimens are inappropriate. Laboratory diagnosis of infection depends on the detection of specific anti-HAV IgM in serum. HAV can be grown in cell culture and this provides the opportunity to investigate some foodborne outbreaks more thoroughly. However, primary virus isolation is a lengthy procedure and isolation from food source has never been achieved. Of late, HAV has been isolated from a drinking water supply that had been responsible for an outbreak in the USA, but this involve culture of highly
concentrated water samples for up to 21 weeks. Specific gene probes to detect HAV in fecal samples have been developed but these have not been successfully applied to food and environment specimens. Further work is needed to enhance their sensitivity.

Attempts by several investigators to develop gene probes for detection of SRSVs are hampered by the failure to grow the virus in vitro and the difficulty in extracting virus directly from clinical specimens. Despite their difficulties, new developments in molecular biology technique offer the most promising approach for detection of virus in food and water and in associated illnesses.

Tests using gene probes and/or polymerase chain reaction (PCR) are being developed. When gene probes are used to detect PCR-amplified DNA, sensitivity can be less than 10 virus particles, but in many cases, sensitivity is limited by the procedure used to prepare samples. Although a RNA-containing virus cannot be detected directly by PCR, its genome can be copied into a strand of DNA (DNA) by using a reverse transcriptase, which serves as template strand, and the segment is then amplified as in the PCR, this is referred to as reverse transcription polymerase chain reaction (RT-PCR) method.

**Norwalk Virus:** Norwalk virus is difficult to detect because it cannot be grown in cell culture. A RT-PCR has been used to detect Norwalk virus in stools and in shellfish. RT-PCR sensitivity was judged to be 100 fold higher than the use of gene probes alone, which could detect between 10^4 and 10^5 hepatitis A virus particles. Confirming PCR product with hybridization to a virus specific probe can boost the sensitivity of a RT-PCR based test. A rotavirus-specific probe and a monoclonal enzyme immunoassay to detect virus in stool have already been developed.

The sensitivity of PCR-based assays for Norwalk virus detection has reportedly increased by using an RT-PCR-OP (oligoprobe) method. The detection of small round structured viruses (SRSVs) that resemble Norwalk viruses has been reported by using RT-PCR and subsequent detection of the PCR product with Southern blotting and hybridization with a probe targeted to an international region of the amplified segment. Shellfish associated with four outbreaks of human gastroenteritis have been found positive for SRSVs. Some SRSVs are difficult to detect because of nucleotide sequence diversity within the group. Of late, primer sets for Norwalk viruses and Snow Mountain agent have been devised and then used to classify the viruses by employing four sets of internal nonisotopically labeled gene probes.

**Rotavirus:** An AP-labeled oligonucleotide probe has been reported for use in a dot blot format to detect as little as 1 ng of rotavirus RNA. By using individual genes as probes, subgrouping of rotaviruses isolated from humans is now possible.

Serotype-specific cDNA molecules by using RT-PCR have been produced to generate gene probes to detect rotaviruses of porcine origin. RT-PCR targeted to the major outer capsid glycoprotein gene (vp7) has been developed for the detection of rotavirus in fecal samples from humans with a sensitivity of about 2 ng (equivalent to 10^8 viral genomes). A PCR typing method has also been reported in which each serotype resulted in a characteristic size fragment.

**Hepatitis A virus (HAV):** There appears to be nucleotide sequence diversity among strains of HAV isolated from human and non-human sources, which suggests the construction of universal gene probes to detect these viruses. Nevertheless, a probe
for a dot blot assay to monitor waterborne HAV has been developed. A radioactive
cDNA probe has been developed to detect HAV in stool samples and in estuarine
samples. In the latter cases, humic acids caused only slight decreases in sensitivity
and proteinase K treatment and phenol extraction could lessen interference by organic
components.

As most methods for obtaining viral nucleic acids from clinical samples
are time-consuming, a novel method has been reported in which virus particles
in fecal samples were selectively captured by employing anti-HAV monoclonal
antibodies, viral RNA was released by heating and amplified by RT-PCR. Southern
blot analysis of its PCR products using gene probes yielded a detection rate of
90% with sensitivity of 3-30 virus particles. A comparison of solid-phase
radioimmunoassay (RIA) and cDNA-RNA hybridization resulted in 50 and 65%
positive samples, respectively. More recently, antigen capture and RT-PCR has
been reported to test seeded oyster samples. Using probes to detect the PCR
products, a sensitivity of about four virus particles was obtained.

In an attempt to detect HAV in seeded oysters using a sample preparation
method developed for Norwalk virus; HAV could not be detected by RT-PCR when
added to whole oysters but could be detected after being spiked into extracts of
oysters. Subsequently, it was determined that HAV was removed from the
supernatant during the flocculation step. This finding illustrates the difficulty in
extrapolating methods, from one microbe and samples type to others.

A single stranded RNA probe specific for HAV has been found to show a
sensitivity of 5-1000 infectious units and, used to detect the virus in water samples
during an outbreak. In a study using riboprobes, it has been found that 67% of
shellfish examined contained HAV RNA and, there was no correlation between
viral contamination and fecal coliform counts. On comparison of IEM, RIA and
hybridization with a digoxigenin-labeled cDNA probe for the detection of human
HAV in raw and treated sewage, RIA appeared the least sensitive and no
relationship was established between the presence of HAV and enterovirus
concentration.

Enteroviruses: These RNA-containing viruses are a diverse group of pathogens
and common causes of childhood infections, the most notable of which is polio.
It is difficult to detect these viruses with gene probes, as titers of virus are usually
low in clinical samples. Because the complete nucleotide sequences of several
enteroviruses have been determined, RT-PCR methods have been applied.

A 154 bp-conserved region at the 5' end of the enteroviral genome has
been targeted. The sensitivity of gene probe assay is reported to be in the range
of 10^6 molecules (about 10^4 particles capable of growth in tissue culture); more
importantly, the time required to identify these viruses has been shortened from
4-8 days to less than 1. By using positively charged nylon membranes to capture
enteroviruses from water samples, isolation of RNA with oligonucleotides bound
to magnetic beads has been achieved. The detection limit is reported as 20 TCID
and 7 of 40 (17%) of water samples tested were found positive.

Recently, a multiplex has been developed for the simultaneous detection
of the human enteroviruses, hepatitis A virus (HAV) and Norwalk virus. Multiplex
PCR offers advantages over cell culture methodology and monoplex PCR because it allows for rapid and cost-effective detection of several human enteric viruses in a single reaction tube.

Gene probe based tests have been applied to detect and characterize most of the important pathogenic foodborne viruses. However, most of the reported work is based on clinical application and environmental testing. Still a number of problems remain, the most challenging of which might be sample preparation. There are limitations in the application of PCR and RT-PCR that restrict their usefulness for measuring the virological safety of food. The most serious limitation is that molecular techniques fail to discriminate between viable and inactivated viruses even though inactivated viruses pose no threat to the consumer and may be present at levels substantially higher than the virulent forms. Other disadvantages include a lack of assay sensitivity and specificity, high assay costs, and the level of technical expertise not available in most food-testing laboratories. Innovative techniques, therefore, need to be developed to foster further improvement in rapid, convenient and sensitive assays for foodborne viruses.

<table>
<thead>
<tr>
<th>VIRUSES</th>
<th>GROWN IN CULTURE</th>
<th>METHOD OF DETECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parvo</td>
<td>Yes</td>
<td>EM, HA</td>
</tr>
<tr>
<td>Calci (Norwalk, NLVs)</td>
<td>No, except porcine calcivirus</td>
<td>EM, RT-PCR</td>
</tr>
<tr>
<td>Entero</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polio</td>
<td>Yes</td>
<td>Variety of methods, RT-PCR</td>
</tr>
<tr>
<td>Coxsackie A &amp; B</td>
<td>Variety of cultures needed, lab. Animals</td>
<td></td>
</tr>
<tr>
<td>Echo</td>
<td>Yes</td>
<td>Variety of methods, RT-PCR</td>
</tr>
<tr>
<td>Hepatitis A (Enterovirus 72)</td>
<td>Yes, Slow, Cell associated, direct isolation, quantitation questionable</td>
<td>EM, Immunological, RT-PCR</td>
</tr>
<tr>
<td>Rota</td>
<td>Yes, direct isolation</td>
<td>EM, Immunological, RT-PCR</td>
</tr>
<tr>
<td>Reo</td>
<td>Yes</td>
<td>Variety of methods</td>
</tr>
<tr>
<td>Papo</td>
<td>Yes, difficult to culture</td>
<td>EM, FAT, ELISA, RT-PCR</td>
</tr>
<tr>
<td>Astro</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Hepatitis E</td>
<td>Yes, difficult to culture</td>
<td></td>
</tr>
</tbody>
</table>
Hepatitis E infection. Rapid notification of local health officials, collection of clinical specimens, and institution of infection control measures are necessary if viral gastroenteritis transmission is to be limited in institutional settings. The Codex committee on food hygiene has recommended the adoption of Hazard Analysis and Critical Control Point (HACCP) as the basis for food safety control. Ozone applications in the food industry are mostly related to decontamination of product surface and water treatment.

It has been suggested that 69°C is a critical temperature in the thermal processing of animal products because ASF, SVD, HC and FMD viruses are inactivated at this temperature. However, meat derived from animals infected with FMD is not rendered free of the virus by the usual commercial procedures of ripening, boning, salting and storage. Aging of meat stored in a chilling room at a temperature not below 20°C during 24 h is one of the requirements of the European Economic Community (EEC) for meat exporting countries affected with FMD. The objective of this regulation is to ensure that a pH below 6.0 has been reached before deboning, to guarantee inactivation of FMD virus that might be present. There is a possibility of using electric stimulation as an alternative method in the aging of meat to destroy FMD virus. There is a great potential and promise for development and application of food processing methods that can destroy the pathogenic viruses in foods.

Further readings suggested:


The genus *Campylobacter* was proposed in 1963 to include what were until then called "Microaerophilic Vibrios". Several species of Campylobacters are now encountered in animals, but only *C. jejuni* and *C. coli* are common causes of enteritis in man. However, a third species namely *C. lari* has also occasionally been found to be involved. All of these species grow at 42°C and thus have been grouped as thermophilic *Campylobacter*.

*Campylobacter* spp. are widely distributed in nature. Most of the species are adapted to the intestinal tract of warm blooded animals and birds. *C. jejuni* and *C. lari* are particularly adapted to birds, which probably form the largest reservoirs of these species. Pigs are the main hosts of *C. coli*. Such variation in occurrence of *Campylobacter* spp. may be due to differences in diet habits of these animals.

In some of the developed countries, the frequency of isolation of this organism from diarrhoeal cases has exceeded than that of conventional enteropathogens like *Salmonella* and *Shigella*. In contrast to reports from developed countries where asymptomatic infection is rare, most studies in developing countries have reported large number of inapparent infections particularly among infants and children. Thus, illness to infection ratio is much lower in developing countries than that observed in developed countries.

**Mode of infection to man:**

Ingestion of contaminated food products, unpasteurized milk and water is the principal mode of infection in man. *Campylobacteriosis* also occurs as an occupational disease involving the workers engaged in processing of food and handling of food animals and their products. Farmers, veterinarians, game keepers, kennel maids and those engaged in animal husbandry may acquire infection from infected animals. Direct man to man and congenital mode of transmission have also been suggested.

**Clinical features:**

*Campylobacteriosis* in man is usually characterized by moderate to severe diarrhoea. Following an average incubation period of 3 to 5 days, there is a febrile period accompanied by malaise, headache, dizziness, myalgia and abdominal pain. This is followed by watery diarrhoea. Malodorous stools can sometimes contain blood and mucus. *C. jejuni* has also been recorded from cases of urinary tract infection, cholecystitis, meningitis, reiter's syndrome, reactive arthritis and Gullain Barre syndrome.

*Campylobacter* is mainly localized in jejunum and ileum. It may also infect colon and even rectum. The pathogenicity can be attributed to three possible mechanisms viz. enterotoxigenicity, enteroinvasiveness and translocation.

*Campylobacter* can cause enteritis in several species of domestic animals like dogs, cattle, sheep, pigs etc. Cases of bovine mastitis and abortion or still birth in ewes and cattle have also been observed.

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**CAMPYLOBACTERIOSIS AND VIBRIOSIS**

Ashok Kumar* and N.D. Verma**
Laboratory animal models:

Campylobacter spp. does not induce the reactions in classical animal models like Sereny test, sucking mouse assay. In search of suitable in vivo methods to test the pathogenicity of *Campylobacter*, various models like gnotobiotic beagle puppies, chickens, new born calves, lambs, monkeys etc have been tried without much success. The rabbit ligated ileal loop assay gave some promise but results are inconsistent in this biological model. Rat ileal ligated loop assay is considered to be a better choice amongst the models attempted till now.

Methods of detection:

(a) Isolation:

*Campylobacter* is a fastidious organism. The samples collected for isolation of *Campylobacter* should be kept at 4°C or on ice. Cary-Blair transport medium may be used for transportation of samples to laboratory.

For the isolation of *Campylobacter* organisms, the samples can be directly inoculated onto selective media for *Campylobacter* spp. However, where the number of organisms is less, enrichment of the samples is required. Thioglycollate broth, Brucella broth, Preston broth and Campylobacter enrichment broth are some media used for enrichment of the samples.

The selective media used for isolation of thermophilic *Campylobacter* usually consist of a basal medium, blood, growth and selective supplements. Ferrous sulphate, sodium metabisulphite and sodium pyruvate (FBP) are commonly used growth supplements. Different combinations of various antimicrobial agents like vancomycin, bacitracin, novobiocin, rifampicin, polymyxin B, colistin, trimethoprim, cephalothin, cyclohexamide and amphotericin B have been successfully tried. Various media viz. Skirrow's selective medium, Butzler selective medium, Blaserwang's medium and Preston medium are commercially available. Sometimes blood free media like Preston blood free medium are also used.

Incubation is done at 42-43°C under microaerophilic conditions (5% O₂, 10% CO₂, 85% N₂) which may be obtained in an anaerobic jar by evacuation/replacement method or using *Campylobacter* gas generating envelop with catalyst or anaerobic gas generating envelop without catalyst. *Campylobacter* is a slow growing organism. Therefore, an incubation period of about 48 hours is required.

Colonies of *Campylobacter* are flat, light, opaque and nearly always tend to spread along the growth line. Thermophilic *Campylobacter* are oxidase positive, Gram negative and S-shaped or spiral in morphology. Characteristic cork screw like mortality can be seen under dark field microscopy. Their inability to grow at 25°C distinguishes them from *C. fetus*.

*C. jejuni* can be differentiated from *C. coli* by its ability to hydrolyse the hippurate. Both *C. jejuni* and *C. coli* are susceptible to nalidixic acid where as *C. lari* is resistant. The species may also be distinguished by DNA/DNA hybridization.
Serological Methods:

Various serological methods such as agglutination, complement fixation, radioimmunoassay and enzyme linked immunosorbent assay have been developed to detect antibodies in human patients. Rapid changes in IgA and IgM have been reported to be diagnostic in early phase of disease. The antigen like acid glycine extract has been employed in some of the studies.

Other Methods:

Attempts have also been made to identify *Campylobacter* species by analysing the protein band patterns of outer membrane proteins, soluble proteins or whole cell proteins. A major 45 KDa outer membrane protein has shown the promises in this direction. It can elicit antibodies in rabbits, which react strongly with *C. jejuni / coli* and weakly with *C. lari*.

PCR based assays that target specific DNA sequences like flageller genes i.e. fla A and fla B from conserved regions have been developed. These can detect thermophilic *Campylobacter* in faecal samples, both before and after selective enrichment (Rasmussen et al., 1996). Lamoureux et al. (1997) developed an immunomagnetic hybridization system using monoclonal antibodies against specific outer membrane proteins.

typing system:

*Campylobacters* are relatively inactive in the traditional physiological tests used in microbiology and so there is a need to develop effective typing schemes which can facilitate their discrimination at subspecies level. A variety of systems have been described. The techniques used are auxotyping, resistotyping, bacteriophage typing, plasmid analysis, chromosomal restriction pattern, DNA fingerprinting, electrophoresis of proteins, biotyping and serotyping. The last two methods are most commonly applied.

Among the several biotyping schemes proposed by different workers, Lior's extended biotyping scheme has widely been accepted. The scheme is based on three tests viz. hippurate hydrolysis, rapid H₂S production and DNA hydrolysis test.

Various serotyping schemes utilizing slide agglutination technique, tube agglutination test, passive haemagglutination technique, fluorescent antibody technique and coagglutination system have been developed. It was finalized by the International Committee on serotyping of *Campylobacter* during the third International Workshop on *Campylobacter* Infections in Ottawa (1985) that only two systems, first based on soluble heat stable antigens using passive haemagglutination test and second based on heat labile antigens using slide agglutination test should be used.

Prevention and Control:

An ideal method to control the Campylobacteriosis which indeed, is difficult to implement, is the development of *Campylobacter* free farms. Thermophilic *Campylobacter* can rarely multiply outside the animal body. Better hygienic practices during animal management may alone reduce the hazards of infection through contaminated food and environment. The emphasis should be given to eliminate or at least reduce the cross contamination between the birds within flock or between the different flocks.
Enforcement of strict hygienic rules during slaughter of animals and post slaughter processing of meat, irradiation of packaged foods, pasteurization of milk and chlorination of water are the important means to combat food borne diseases like Campylobacteriosis. The efforts to develop whole cell killed, live attenuated or subunit vaccines are in the process (Scott, 1997; Kopecko, 1997).

Special educational programmes directed against groups at risk should be developed specially for those engaged in food processing.

Vibriosis

The genus Vibrio has been classified in the family Vibrionaceae. Morphologically, the bacteria of this genus are straight or curved shaped. They are Gram negative, motile, non-sporing, oxidase positive and capable of both fermentative and respiratory metabolism. The several species of genus Vibrio are pathogenic to man. At least 8 species viz. V. cholerae, V. parahaemolyticus, V. vulnificus, V. fluvialis, V. furnisi, V. hollisae, V. metschnikovii and V. mimicus have been implicated in the food-borne diseases. The first 3 species are responsible for most of the foodborne infections related with Vibrio spp.

Vibrios are usually transmitted through contaminated food and water. Cholera is mainly transmitted through water. Fruits and vegetables may also be contaminated with polluted water. V. parahaemolyticus is more common in seafoods and sea water.

The incubation period may vary from a few hours to days. The symptoms may usually appear within 24 hours. Diarrhoea is the main symptom. However its nature and severity may vary depending upon the species involved. In case of cholera classical ‘rice water’ stool results in enormous loss of fluid, electrolyte disbalance, dehydration and circulatory collapse. V. vulnificus is comparatively more invasive and causes primary septicaemia.

Isolation of Vibrio spp.

Isolation of Vibrio spp. from clinical cases can be achieved by the use of selective media such as thiosulphale citrate bile salts sucrose (TCBS) agar. However, primary enrichment is required to isolate these organisms from carriers or food and water samples. Enrichment is done in alkaline peptone water (pH 8.2) with 3% sodium chloride or sodium taehrocholate broth. In case of V. parahaemolyticus, glucose salt Teepol (GST) broth or alkaline peptone water with 3% sodium chloride can be used for enrichment.

The genus is identified on the basis of the following tests:

1. Gram’s reaction - ve
2. Oxidase +ve
3. Sodium ion requirement +ve
4. Gas from glucose - ve
5. Inhibition by vibriostate 0/129 (150 µg) +ve
6. Inhibition by methylene blue +ve
7. Acid from inositol - ve

After confirmation of genus, V. cholerae can be differentiated from other species by its ability to produce acid in sucrose and mannose but not in arabinose.
Biotyping of *V. cholerae* divides these organisms in two biovars i.e. classic and El Tor. Each biovar is subdivided into 3 serological types namely Inaba, Ogawa and Hikojima. By serotyping, Vibrio can be grouped as *V. cholerae* 01 and *V. cholerae* non 01. The later group is also referred as non cholera Vibrio (NCV) or non agglutinating vibrio (NAG). Recently, a new serotype (0139) has been recognized as causative agent for cholera, hence now non-01 and non O 139 are considered as NAG. The other schemes like phage typing and vibriocin typing are also in use.

The Kanagawa haemolysin reaction employing Wagatsuma agar is a widely used test to determine the pathogenic potential of *V. parahaemolyticus* isolates. The serotyping of *V. parahaemolyticus* is based on thermostable O (somatic) and thermolabile K (capsular) antigens.

**Rapid detection of Vibrio**

Fluorescent antibody technique is used for rapid detection of *V. cholerae* in water. *V. parahaemolyticus* can be detected by fluorogenic assay for trypsin like activity. Enzyme linked immunosorbent assay and reverse passive latex agglutination assays are used to test the haemolysin of *V. parahaemolyticus*. DNA probes targeting some of the specific genes have been developed.

**Prevention and control:**

Cholera is a notifiable disease. The National Cholera Control Programme has now been merged with diarrhoeal diseases control programme. The preventive measures include:

1. Immunization of population at risk with cholera vaccine.
2. Proper disposal of sewage.
3. Purification and chlorination of water.
4. Proper cooking of foods and prevention of secondary contamination of cooked foods.

**REFERENCES**

HAZARD ANALYSIS AND CRITICAL CONTROL PROGRAMME

V.N. Bachhil*

Introduction

Hazard analysis and critical control point (HACCP) and microbiological specifications under 2 and 3 class attribute schemes are two excellent means of ensuring food safety and enhancing storage stability of the perishable food products. The application of HACCP offers most effective methods to use the resources for monitoring the CCP. Hazard means unacceptable contamination, survival or growth of the hazardous organism. In fact, this is a preventive system of control, particularly the microbiological hazards. It encompasses analysis of ingredients, products and processes to determine components and areas to be controlled for safe food production. Microbiological specifications for foods may avoid or minimize the risk to human health. Safety of the product can be determined by assessing pathogenic and 'indicator bacteria' and keeping quality, state of freshness and handling history by "total counts" as mentioned earlier.

2-3 attribute schemes may be worked out on the basis of severity of hazard e.g. n=5, c=2, m=10, M=50 for E.coli and n=5, C=0 for Salmonella in fresh and frozen meats to reduce or eliminate public health risk. Many models of HACCP and various microbiological specifications have been proposed by various agencies.

1. Sources of infection:

I) Infected animals: If food animal infected with zoonotic agent is slaughtered, it often poses a threat to the handlers as well as consumers. Fecal contamination of carcass during slaughter: It is common way for transmission of enteric infection. During slaughter of animals, the intestinal contents may cross contaminate the carcass if proper hygienic and sanitary measures are not taken.

II) Infected human: Infected man engaged in handling/processing of meat and meat products at any point of food chain may be a source of contamination to these commodities.

III) Environment: Water, soil, air and equipment may serve as vehicles for various infectious agents.

IV) Ingredients incorporated in meat products: Meat is a very nutritious medium for the growth of infectious agents. It gets in the chain through other ingredients incorporated to manufacture various meat products.

2. Processing procedures:

Processing of meat is very important as fault in handling or processing of meat may spoil the microbiological quality of meat. Most of the organisms are killed when the meat is subjected to proper heat. However, toxins produced by some bacteria like *S. aureus* and spores of *C. botulinum*, *C. perfringens* and *B. cereus* are resistant to heating temperatures that are usually applied to the meat and meat products. Various preserving methods like high or low temp. low pH, low...
water activity, irradiation and curing salts used to prolong the storage life of food have varying effect on different zoonotic agents.

3. **Food habits:**

The food habits vary in different socio-economic groups within a country and in different countries. Therefore, making the respective population vulnerable to different zoonotic agent(s).

4. Population movement and increased trade in meat industry: These factors further aid to transmission of zoonoses in new areas.

**Hazard Analysis and Critical Control Point (HACCP):**

HACCP is a system of food quality control to render foods safe. Emphasis is laid on controlling food-borne hazards particularly the food-borne infections and intoxication during production itself. Hazard refers to unacceptable contamination, survival and/or growth of hazardous microorganisms. In case of slaughter house hygiene, it should also be extended to any pathological condition of carcasses. Hazard analyses (HA) includes factors affecting possible microbial multiplication in food production. This can be used to prescribe microbiological specifications for safety and monitoring ultimate food quality. HA relates with sensitivity or hazardous microbiological quality of ingredients handling factors as they affect product safety and stability. Microbiological status and the sanitary conditions of processing and storage are the two important aspect of HACCP.

Application of HACCP provides the most effective use of control sources by emphasizing monitoring of various critical control point rather than analyzing the end product only.

**Critical Control Points (CCP)**

These may be locations, processes, practices, procedures or ingredients that need careful analysis for its role in eliminating or minimizing the food-borne hazards. There are in fact, the factors or determiners whose failure or loss can render the food hazardous or risky to consumers. CCP may be microbial quality or quantity; time-temp. relationship, personal hygiene, sanitation and equipment cleanliness etc. Good manufacturing practices requiring regular monitoring can be referred as CCP eg. intrinsic quality of raw material (animal), production, processing, packaging, heating, storage and distribution etc. Trouble spots in production and processing chain should be worked out and microbiological counts or presence/absence test may be carried out repeatedly to find control points. These can be tabulated mainly under two heads:

1. **Raw material control:**
   a). Microbiological
   b). Pathological (animal)
   c). Physical
   d). Chemical
2. **Process Control**

   a) Time temperature relationship  
   b) Prevention of cross contamination  
   c) Inefficient cooling  
   d) Post process contamination  
   e) Holding of foods at higher or ambient temp.  
   f) Process delays due to machinery/electricity failure

**CCP in slaughter house:**

As far as slaughter house hygiene is concerned, under routine abattoir conditions, there are 5 major sources of contamination viz. Air, water, handling processing and the animals itself.

*Stolle (1988)* reported that lateral surfaces of carcasses are more prone to contamination and there exists significant interaction between contamination rate and the site of carcasses. It is improper handling of the carcasses that is more important than other factors which should be considered as CCP. It is essential to prevent cross contamination of carcasses during dressing operation.

HACCP should be worked out for each abattoir or for each system followed in abattoirs. It may be noted that it is not possible to eliminate all the risk factors at various stages of carcass processing. However, the risk can be certainly reduced by recognizing and controlling the critical control points.

**HACCP and the Microbial Groups:**

HACCP should be worked out of specific pathogens, groups of pathogens, indicator organisms and the spoilage organisms. Most relevant pathogens in relation to slaughter house and the carcass processing are: Salmonella, E.coli, Shigella, *V.parahaemolyticus*, *Y.enterococlitica*, *Aeromonas*, *Listeria monocytogenes*, *S. aureus*, *S.cereus* and *Clostridium perfringens* etc.

Among important spoilage groups are proteolytic, psychrotrophs, Lipolytics and Yeast and Moulds.

Indicator organisms are another important microbial groups used to assess the sanitary conditions of handling, processing and storage etc. These include coliforms, faecal coliforms, enterobacteriaceae and total counts.

**Priority Products:**

The hazard and the CCP should be first recognized for abattoir processes, chilled and frozen raw meats and poultry, frozen frog legs, precooked frozen shrimps and prawns as these are the foods are of great concern in international trade.

HACCP consists of 1-analysis of hazard, 2-establishing the CCP and 3-monitoring the CCP for proper control measures (WHO, 1982). HA has to be carried out keeping in view the type of raw material (in the present case animals or carcasses),
fate of pathogens whether it will survive and multiply during distribution and storage and the effect of intrinsic and extrinsic factors like water activity, pH, temperature, humidity etc.

**Monitoring of CCP**

It may be divided into 3 major categories:

a) Physical - Water activity, pH, time temperature relationship.

b) Chemical - Concentration of disinfectants and preservatives.

c) Microbiological - This pertains to microbiological analysis covering pathogenic, spoilage and indicator organisms as stated earlier (Bachhil, 1987).

Meat production under routine abattoir procedures does not destroy food-borne pathogens and spoilage flora. Keeping this in view two types of CCPs can be considered.

1) CCPs that are effective in eliminating the hazard or assuring the quality.

2) Those that do not eliminate but only reduce the risk or hazard i.e. these are not absolute CCP. In abattoirs, animals, environment, handling, processing, chilling are the major aspects that need attention.

Major factors responsible for contamination/or infection are animals at farm and slaughter houses or contact with other animals, animal feeds, pastures and water.

Lairage should be regularly cleaned and disinfected to allow minimum holding time. CCPs in sheep, goat and buffalo abattoirs, could be skinning, evisceration, carcass cutting and chilling. For pigs, CCPs could be scalding, dehairing process, evisceration and chilling.

Skin contact and improper or careless evisceration can transfer enteric infection to carcasses and handlers. Knives should be disinfected at about 80°C before cutting. Scald temperature should be maintained at 62-65°C. Systematic presentation of effective and non-absolute CCPs are diagrammatically/shown in Fig.1 (Simosen et al., 1987).

CCPs for other products (Simosen et al., 1987), are as follows:

- Fresh sausages: Salmonella, free animals temperature of processing 15°C water, ice quality.

  Preservative concentration eg. 450 ppm sodium metabisulphite

- fermented sausages: Fermentation 18-44°C.

- Pork pies Raw material (raw meat) Processing temperature (8-10°C) Pastry Ingredients (fat, floor, water, etc.)

Considerations for devising sampling plan:

There are three most important factors that need consideration before devising a sampling plan. These are:-
1. Distribution of microbes: The knowledge of the distribution of microbes in or on the meat is very important for devising a sampling scheme. True mean microbial counts should be calculated on the basis of investigations at various stages of production to determine critical points. This depends upon the type and the extent of processing, which the product has received. Processing changes the microbial distribution in meat and meat products and it is advisable that variance of microbial distribution in food is kept to minimum. This, in addition of other methods, can be achieved by increasing the sample size, i.e., weight and number of samples within the practical limits. Microbial population can be defined by the types of microbe present, their levels and the way they are distributed.

2. Hazard: The choice of sampling plan to assess the hazard from microbial species depend upon (i) severity of the disease and (ii) the distribution of pathogenic organisms or their toxins in meats. The type of health hazard considered by ICMSF (1974) are:

   i). Low hazard: Includes non pathogenic organisms e.g. coliform etc.

   ii) Severe hazards: This includes the hazards caused by S.typhi, S.paratyphi, S.choleraesuis, Sh. dysenterae, V. cholerae, C. botulinum, B. melitensis and C. perfringens.

   iii). Moderate hazards:

   a) Potentially extensive spread: This group includes salmonellosis (S.typhimurium and others) Sh. sonnei, Sh. flexneri, V. parahaemolyticus and enteropathogenic E.coli.

   b) Limited spread: This group includes pathogens like S. aureus, B. cereus, B. abortus and C. perfringens.

   Clinical severity is the most important factor for deciding the sampling scheme. The more the severity the more stringent the plan. The most relevant pathogens occurring in meat products are:

   *Salmonella*: Red meat and poultry
   *Staphylococcus*: Processed and fermented meats
   *C.perfringens*: Cooked meats
   *B. cereus*: Processed semi cooked meats, meat pies
   *E.coli*: Red meats and poultry

1. Processing techniques: Standards should be formulated keeping in view the effect of a particular process on the survival and growth of microbes in meat and meat products. The process may include thermal processing, ionizing radiation, freezing and chemical preservatives etc.
1. Water
3. Other animals/birds

Animals
(Sheep, goat, pig, buffalo etc.)

Transportation (PS)

(PS) Abattoir

(PS) Lairage

Stunning

Killing

Pigs
- Scalding (PE)
- Dehairing (MS)
- Singeing (PE)
- Evisceration (MS, PE)
- Chilling (E)

Sheep, goat, buffalo
- Evisceration
- Chilling (E)
- Cutting (E)
- Deboning

Poultry
- Bleeding
- Scalding (PE)
- Picking (MS)
- Singeing
- Washing (PE)
- Evisceration (PE, MS)
- Inspection (PS)

Packaging ← Chilling ← Washing<br>(E, PS) (PE)

E= Effective CCP (absolute); PE= Partially effective; CCP (not absolute) MS: Major source of contamination.
PS= Possible sources of contamination (Source = Simosen et al., 1987)
Some recommended proposed microbiological specifications for meat & meat products.

<table>
<thead>
<tr>
<th>Product</th>
<th>Test</th>
<th>Plan</th>
<th>n</th>
<th>c</th>
<th>m</th>
<th>M</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen</td>
<td>SPC</td>
<td>3</td>
<td>10</td>
<td>5</td>
<td>5x10^5</td>
<td>10^8</td>
<td>Bachhil (1986)</td>
</tr>
<tr>
<td></td>
<td>E.coli</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>10</td>
<td>50</td>
<td>-do-</td>
</tr>
<tr>
<td></td>
<td>Salmonella</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-do-</td>
</tr>
<tr>
<td>Frozen raw</td>
<td>SPC</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>10^6</td>
<td>10^7</td>
<td>ICMSF (1974)</td>
</tr>
<tr>
<td>Comminuted meat</td>
<td>Salmonella</td>
<td>2</td>
<td>5</td>
<td>1(0)</td>
<td>-</td>
<td>-</td>
<td>-do-</td>
</tr>
<tr>
<td>Non-frozen ground</td>
<td>SPC</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>10^7</td>
<td>5x10^7</td>
<td>Pivnick et al. (1976)</td>
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<tr>
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<td>E.coli</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>10^2</td>
<td>5x10^2</td>
<td>-do-</td>
</tr>
<tr>
<td></td>
<td>S. aureus</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>10^2</td>
<td>10^3</td>
<td>-do-</td>
</tr>
<tr>
<td></td>
<td>Salmonella</td>
<td>3</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-do-</td>
</tr>
<tr>
<td>Precooked (semi-cooked meat)</td>
<td>E.coli</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>10</td>
<td>Bachhil (1986)</td>
</tr>
<tr>
<td></td>
<td>S. aureus</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>10</td>
<td>100</td>
<td>-do-</td>
</tr>
<tr>
<td></td>
<td>Salmonella</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-do-</td>
</tr>
<tr>
<td></td>
<td>B. cereus</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>5x10^7</td>
<td>10^9</td>
<td>-do-</td>
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<tr>
<td></td>
<td>C. perfringens</td>
<td>3</td>
<td>5</td>
<td>3</td>
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<td>10</td>
<td>-do-</td>
</tr>
<tr>
<td>Cooked canned meats</td>
<td>S. aureus</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>Bachhil (1986)</td>
</tr>
<tr>
<td></td>
<td>E.coli</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-do-</td>
</tr>
<tr>
<td></td>
<td>B. cereus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. perfringens</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dried and frozen whole egg.</td>
<td>APC</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>5x10^4</td>
<td>10^8</td>
<td>FAO (1975)</td>
</tr>
<tr>
<td></td>
<td>Coliforms</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>10</td>
<td>10^3</td>
<td>-do-</td>
</tr>
<tr>
<td></td>
<td>Salmonella</td>
<td>2</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-do-</td>
</tr>
<tr>
<td>Dried and frozen</td>
<td>APC</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>5x10^4</td>
<td>10^8</td>
<td>-do-</td>
</tr>
<tr>
<td></td>
<td>Coliforms</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>10</td>
<td>10^3</td>
<td>-do-</td>
</tr>
<tr>
<td>Chilled and frozen meats</td>
<td>SPC</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>10^8</td>
<td>10^7</td>
<td>Jayaraman (1983)*</td>
</tr>
<tr>
<td></td>
<td>E.coli</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>10</td>
<td>100</td>
<td>-do-</td>
</tr>
</tbody>
</table>

*Formulated on the basis of information from
### Standards of quality for edible fat

<table>
<thead>
<tr>
<th>Type of fat</th>
<th>Saponification Value</th>
<th>Range of Iodine</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef fat</td>
<td>193-46</td>
<td>35-46</td>
<td>Rule a-10 Appendix B 01</td>
</tr>
<tr>
<td>Mutton fat</td>
<td>192-195</td>
<td>35-46</td>
<td>Rule a-10 Appendix B 02</td>
</tr>
<tr>
<td>Goat fat</td>
<td>193-36-45</td>
<td>36-45</td>
<td>Rule a-10 Appendix B 03</td>
</tr>
<tr>
<td>Lard</td>
<td>192-198</td>
<td>52-65</td>
<td>Rule a-10 Appendix B 04</td>
</tr>
</tbody>
</table>

### Use of class II preservatives—restrictions part X-rule 55

<table>
<thead>
<tr>
<th>Article of Food</th>
<th>Preservative</th>
<th>Not to exceed PPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sausage and sausage meat containing raw meat and condiments</td>
<td>Sulphur dioxide</td>
<td>450</td>
</tr>
<tr>
<td>Cooked pickled meat including hams and bacon</td>
<td>Sod. Or Pot. nitrite (Calculated in Sod. nitrite)</td>
<td>200</td>
</tr>
</tbody>
</table>

### Poisonous metals—Part XI Rule-57

<table>
<thead>
<tr>
<th>Metal</th>
<th>Food</th>
<th>PPM by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>Canned meat, meat, meat extracts</td>
<td>5.0</td>
</tr>
<tr>
<td>Copper</td>
<td>Foods not specified</td>
<td>0.1</td>
</tr>
<tr>
<td>Arsenic</td>
<td>Foods not specified</td>
<td>1.1</td>
</tr>
<tr>
<td>Tin</td>
<td>Foods not specified</td>
<td>250.00</td>
</tr>
<tr>
<td>Zinc</td>
<td>Foods not specified</td>
<td>50.00</td>
</tr>
</tbody>
</table>
Restriction on use of antioxidants in edible oils and fats

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Not exceeding in concentration</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.H.T.</td>
<td>0.02%</td>
<td>Part XII-Rule-59</td>
</tr>
<tr>
<td>B.H.A.</td>
<td>0.02%</td>
<td>Part XII-Rule-59</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.01%</td>
<td>Part XII-Rule-59</td>
</tr>
</tbody>
</table>

FLAVOURING AGENTS, PART XIII

<table>
<thead>
<tr>
<th>FLAVOURING AGENT</th>
<th>FOOD PRODUCT</th>
<th>MAX.LIMIT</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono Sodium Glutamate</td>
<td>Meat products</td>
<td>500 ppm*</td>
<td>Rule 64-AA</td>
</tr>
</tbody>
</table>

Not added to food meant for infants below 12 months.

Restriction on the use of insecticides rule-65

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Food Product</th>
<th>Not to exceed tolerance LT. ppm (mg/kg)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin, Dieldrin</td>
<td>Meat, egg</td>
<td>0.2</td>
<td>On whole product basis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>On shell free basis</td>
</tr>
<tr>
<td>D.D.T.</td>
<td>Meat, poultry, fish, Egg</td>
<td>7.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Feithrothion</td>
<td>Meat</td>
<td>0.03</td>
<td>On whole wt. basis</td>
</tr>
<tr>
<td>Lindane</td>
<td>Meat and poultry</td>
<td>2.0</td>
<td></td>
</tr>
</tbody>
</table>

Meat Food Products Order, 1993

Schedule-4.11: No meat food product shall contain any poisonous elements (specified) in excess of the quality specified below

<table>
<thead>
<tr>
<th>Poisonous Element</th>
<th>Not to exceed P.P.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>2.5</td>
</tr>
<tr>
<td>Copper</td>
<td>2.0</td>
</tr>
<tr>
<td>Arsenic</td>
<td>2.0</td>
</tr>
<tr>
<td>Tin</td>
<td>250</td>
</tr>
<tr>
<td>Zinc</td>
<td>50</td>
</tr>
</tbody>
</table>
REFERENCES


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CONTROL OF NON-BIOLOGICAL CONTAMINANTS IN FOOD OF ANIMAL ORIGIN

S. K. Dwivedi*

Non-biological contaminants (with reference to livestock) comprise physical and chemical agents that contaminate livestock products during production, slaughter, processing or packing. Attempt has been made to compile information on chemical contaminants of interest to animal health, production and marketing in general and veterinary public health in particular. Emphasis is given to common residues occurring in the milk, meat and egg, viz., veterinary drugs (antimicrobials), industrial effluents, pesticides and heavy metals.

In the developed world, the chances of human exposure attributed to the hazardous contamination of milk, meat and egg by these agents is rare on account of strict public health regulations. Nevertheless, they pose serious impediment to human health in developing and underdeveloped world. Additionally, international trade regulations require their monitoring to satisfy the barrier of minimum permissible limit.

During the recent years, large-scale contamination of human food with both biological and chemical contaminants has raised concern in the mind of people on food safety. In India, we are at tremendous health risk due to indiscriminate use of antimicrobials, farm chemicals, growth hormones and contamination of food and water from industrial effluents comprising heavy metals and toxic chemicals. Danger of pesticide in the environment and food and abuse of hormones (emerging role of diethylstilbestrol [DES] with cancer in daughters of DES treated women) are the issues even in the developed world. The question of DES being used in livestock to augment production, are emerging issues to be addressed on priority in our country, too, in addition to the recent controversy of using oxytocin in animals for letting down of milk. The issue has not yet been settled though more points are against its use. Administration of hormones for livestock fattening and the use of bovine somatotropin (BST) in dairy cattle are of prime concern in the mind of general public with respect to food safety front.

Food of animal origin can be contaminated by thousands of man made chemicals and those which are of particular concern to human health and livestock trading are antimicrobials, growth promoting hormones and adjuncts, polyhalogenated hydrocarbon pesticides, industrial chemicals and heavy metals. However, primary concern is still the food from animals contaminated by bacterial toxins, (botulism, staphylococcal enterotoxin), mycotoxins, (aflatoxin and ochratoxin) and algal toxin (saxitoxin and shellfish). A detailed study on the subject, I am sure, is taken care by a majority of the speakers in this course. However additional literature may, if necessary, be referred (Anon, 1994; Waltner and Ewen, 1994a).

If we peep into the area of food production, processing and preservation, the question of use of additive ranks number one on the priority. Additives commonly used include: nitrite (if its toxic metabolite nitrosamines are allowed to form prior to ingestion). Similarly iodine, bromide and chlorine are used for sanitation and are contaminants following their careless use. Other non-biological contaminants in food of animal origin include minute pieces of wood, plastic and even metals released from food processing equipment.
What has been greatly neglected in the past is analysis of risk and its management following low-grade exposure of environmental contaminants. It is heartening to note that in recent years excellent publications have emerged on this aspect (Anon, 1994; Hathaway, 1993; Waltner and McEvwen, 1994b) and it is now possible to deal with these by use of safety factors (a safety factor of 100 has been used to account for human-animal differences and also within the susceptibility of human population). Threshold doses have been used to denote acceptable daily intakes (ADIs) of chemical residues in food over life time. Routes of exposure and estimated daily consumption of contaminants will help to decide maximum residual limits (MRLs) in various food (milk, meat). In veterinary practice ADIs and MRLs could be utilized to decide the withholding period of the animals treated with drugs to ensure that residues do not appear in animal products. The WHO and FAO have significantly contributed to the hazards identification and their risk assessment (establishment of ADI and MRL guidelines) with special reference to chemical contaminants. An important aspect of assessment of chemical exposure is estimation of their residual level in the food and also within the consumers (e.g., measurement of organochlorines in blood and tissues). The results are expressed in terms of MRLs or in some case tolerance or legal levels. These levels indirectly reflect ADIs.

**Residues of industrial effluents and other environmental pollutants:**

Heavy metals and agricultural chemicals which contaminate animal and its environment like seed and wood preservatives, may gain access to milk, meat or egg. Wood preservative (pentachlorophenol) may contaminate animal houses and pens made of treated wood. Similarly grain preservatives (hexachlorobenzene) may gain access into animal body if such grains are used as animal feed, may be by accident.

Persistence of industrial chemical like DDT, at one time widely used has now been banned in many part of the world on account of its dangerous level recorded in human and animal tissues entering into the body through food chain. Cadmium, lead and to some extent mercury contaminate milk and meat when they are ingested through animal pasture contaminated by industrial contaminants. Lead exposure has been a problem since decades but recent studies has indicated alarming effects of its low-level exposure in children causing toxicities. Slaughterhouse condemnation of liver and kidney with high level of cadmium necessitate further studies in this direction. It is reported that lead level is rarely elevated above MRL in milk and meat but their continuous consumption do have cumulative effects. Iodine level in veterinary drugs, it is reported, has been causing concern in some countries on account of dangerously high content recorded in milk of treated animals. This has resulted into withdrawal of license of iodine based medicine from feed and feed producing animals in some countries.

**Pesticide residues:**

In most of the countries, the pesticides used in agriculture are usually herbicides, the residues of which have been recorded in fruits and vegetables, and are therefore, less concern to food of animal origin. It is the insecticides and fungicides which concerns most in animal and poultry products. Amongst these the organochlorine pesticides e.g. DDT, Heptachlor, Hexachloroethane, Lindane which are lipid soluble, are prone to bioaccumulation. In India the levels detected in the milk and meat are well above permissible limits.
Recent studies suggest that environmental contaminants including organochlorines and other pesticides may be associated with endocrine disturbances in animals and possibly in man. Further, environmental contamination with oestrogen or other hormone like activity may interact with each other to exert toxic effect.

**Exogenous hormonal residues:**

Hormones have been used for augmentation of milk and meat production in dairy animals in various parts of the world. The issues of concern are whether their residues are responsible for the health problems in man? What will be the economic and social implications of banning their use? At present, hormones are used legally in many countries for increasing meat and milk production. From the food safety point of view, these substances could safely be divided into a) those that are present in the animal body and eventually in man and b) those that are synthetic compounds and are administered in animals, e.g., steroidal and non-steriodal xenobiotics. Amongst the naturally occurring compounds are the testosterones, progesterones, oestrogen and somatotropins. The bovine somatotropin (BST) is a genetically engineered hormone for commercialization (recombinant bovine somatotropin [rBST]).

Xenobiotic compounds do not occur in animal tissue naturally and their safe levels have been established. Their withdrawal times to prevent entry in human food in harmful level have also been delineated. Survey conducted in developed countries revealed that they are rarely present in illegal levels in food from animals. However, the data in India is woefully lacking.

When the question of assessing the risk of naturally occurring hormone in human food is taken-up, it is important to understand that if no difference occurs in the concentration of exogenous naturally occurring hormone in the edible tissue between treated and untreated animals, there should be no reason of concern for public safety.

**Antimicrobial residues:**

Studies conducted in North America on the antibiotic residues in human food of animal origin indicated that most of the antibiotics used in animal practice are present in safe limits and that they are relatively non-toxic. However, some of the antibiotic have posed serious concern to human health when present in high concentrations. Chloramphenicols is one such drug which has been associated with aplastic anaemia and bone marrow depression and subsequently leukemia. Bioassay indicates that nitrofurazones and some of the antiparasitic drugs (dimetridazole) are also incriminated to be carcinogenic. Penicillin, an important narrow spectrum antibiotic, known to cause allergy in human in 1: 100 000 cases, is not of much concern in Europe and America, but its actual incidence in India is again lacking. Similarly, the residues of sulphonamides and tetracyclines are on record to produce allergic reactions in recommended dosage, there is little evidence to prove that their residues are present in food from animal origin. Again these reports are based on the work conducted in USA and Canada and status report in India or for that matter any of the developing countries is not available. Still, I have reasons to believe that indiscriminate use of antibiotic in animals might result into serious human health hazard on account of drug resistance by the microbial flora of humans consuming products from animals treated with antibiotics in low doses. Therefore, prudent use of antibacterials in veterinary practice in immediately required.
Control measure to contain non-biological contaminants

The methods used for control of non-biological contaminants includes:

1. Regulations on availability and use of chemicals in the country and its enforcement.

2. Strict residue monitoring and surveillance.

3. Govt./Industry or NGO's sponsored Quality Assurance and Hazard Analysis and Critical Control Programs. (HACCP)

The monitoring of milk, meat and egg for antibiotic residues is routinely done in many countries. In India, we have now started realizing its impact on export potential of livestock and poultry and their products. Nevertheless, much has to be done in all the three areas delineated above. The situation in developed countries is highly encouraging and reports indicate that the violative level of antibiotic in the food from animal origin is decreasing. Violative levels of antibiotics are consequence to their non-prudential use in treatment of sub-clinical or clinical infectious diseases. A host of antibiotic and sulfonamide are used in animal health management which include penicillin, tetracycline, aminoglycosides and many others. Administration of these antibiotics prior to slaughter in certain class of animals, viz., culled dairy cattle and buffalo, pose more chances of contaminations. The pharmacokinetics and physical properties of some of the antibiotics (aminoglycosides), indicate presence of its residues for a longer period in kidney to be detected after slaughter.

"Extra-label" treatment (veterinarian induced error) is another contributory factor to the residues in the milk and meat. In this practice, the antibiotics are administered in way which differs (in dose, route, frequency, animal species) from the directions prescribed by the manufacturer. Since the withdrawal time of a drug is determined on the basis of the recommended dose schedule, any violation to this will result into drug resistance or drug residues in edible tissues. The other type of error committed by humans (more common in India) is failure of withdrawal of milk for public consumption following treatment of mastitis. The blame cannot be given to farmers alone as it is well known, particularly for India, that people do not have knowledge of withdrawal period of milk and meat following treatment with antibiotics. In USA, on the contrary, dairy quality assurance program has resulted into safe and responsible use of antibiotics in animals. I am, therefore, of the view that education of veterinarians on the prudent use of antibiotics and also to the farmers on the harmful effects of antibiotic residues in the food is very important. The situation in India on the drug residues in food from animal origin is poorly studied and a status paper on this aspect is the demand of the time. Also, continued monitoring of chemical residues by the food industries is necessary. Managemental practices involving less use of antibiotics and shift to non-antibiotic and non-hormonal methods of animal production may also be discussed vis-a-vis food safety. In this context, it would not be out of place to mention that the indigenous methods of livestock health management will reduce the dependence on antibiotics and other chemicals used for the livestock pest management.
REFERENCES


APPENDIX

List of Practicals:

1. Molecular biology techniques for diagnosis of zoonotic and foodborne diseases.
2. Detection of S. aureus and its enterotoxin
3. Detection of Aeromonas and Plesiomonas
4. Detection of E. coli
5. Recent advances in diagnosis of salmonellosis
6. Diagnosis of brucellosis
7. Diagnosis of hydatidosis
8. Detection of L. monocytogenes
9. Diagnosis of tuberculosis
10. Diagnosis of Rabies
11. Diagnosis of Mycotic zoonosis
12. Detection of Campylobacter and Vibrio

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