# Serological Survey of Infectious Bursal Disease in Chickens and Isolation of the Virus

BY

**B. JAYARAMAIAH**

Post-graduate College of Animal Sciences,
Indian Veterinary Research Institute,
IZATNAGAR, U.P.

**THESIS**

Submitted to the AGRA UNIVERSITY, AGRA,
In partial fulfilment of the requirements for the Degree of

**MASTER OF VETERINARY SCIENCE**

IN

**BACTERIOLOGY**

**INCLUDING IMMUNOLOGY AND VIROLOGY**

**1974.**

Dr. B.B. Mallick, D.Sc.,
Adviser,
Andhra Pradesh Agricultural University,
Tirupati campus,
TIRUPATI (A.P.)*

Date: 22-5-1974.

This is to certify that the entire research work presented in this thesis entitled "SEROLOGICAL SURVEY OF INFECTIOUS BURSAL DISEASE IN CHICKENS AND ISOLATION OF THE VIRUS" has been authentically carried out by Sri B. JAYARAMAIAH himself under my supervision and guidance.

B Mallick

(B.B. MALLICK)

* Formerly Project Coordinator (Respiratory Diseases of Poultry), Indian Veterinary Research Institute, IZATNAGAR, U.P.

## ACKNOWLEDGEMENTS

The author is indebted to Dr. B.B. Mallick, D.Sc., Adviser, Andhra Pradesh Agricultural University, Tirupati campus, Tirupati, Andhra Pradesh, formerly Project Coordinator, Respiratory Diseases of Poultry, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, whose guidance, constructive criticism and continuous support made this manuscript possible.

Sincere gratitude are expressed to Dr. C.M. Singh, M.S., Ph.D., Director and Dr. G.L. Sharma, M.Sc., Ph.D., Principal-cum-Joint Director, Indian Veterinary Research Institute, Izatnagar, U.P., for providing necessary facilities to carry out this research work and sanctioning Graduate Assistantship.

I am grateful to Dr. B. Sambamurti, M.S., Head, Department of Microbiology, College of Veterinary Science,Tirupati and Dr. P. Mahalingham, Research Officer, R.D.P. Laboratory, Madras Veterinary College, Tamil Nadu, for extending facilities for doing some portion of the work in their laboratories.

I sincerely thank Sarvasri V.A. Srinivasan and K.D. Pandey, Ph.D. Scholars and K.C. Verma, Assistant Bacteriologist (RDP) for their healthy criticism and suggestions.

I am equally thankful to all the staff of the R.D.P. Laboratory of Indian Veterinary Research Institute, Izatnagar.

The author is deeply grateful to his uncles Dr. M. Gopalakrishna, M.S., Honorary Surgeon and Professor, Government Medical College, Guntur and Dr. M.R. Chowdary, Ph.D., Director, Central Poultry Breeding Farm, Bangalore, for their encouragement throughout the carrier to persue his higher education.

Grateful thanks are also to Dr. M. Pattison, Central Veterinary Laboratory, Weybridge, England, U.K. and Dr. B.W. Calnek, Department of Avian Diseases, New York State Veterinary College, New York, U.S.A., for readily respond to our request to supply standard reference diagnostic antisera and antigen for this work.

Appreciation is expressed to Sarvasri N. Kondaiah, Y.U.B. Rao, Bramhanandam, M. Ramaiah and K. Janakiram Prasad, for their help.

## CONTENTS

# CHAPTER

# INTRODUCTION

# INTRODUCTION

The Indian Poultry Industry has made tremendous progress during the last decade due to the impetus given by the plan schemes. Massive introduction of foreign stock by government and private breeders and the heavy concentration of birds due to intensive system of rearing in large poultry enterprises, have accentuated the problem of infectious diseases among which viral infections are of primary importance.

Development and maintenance of flocks free from the viral maladies should be the national aim in order to avoid production losses due to morbidity and mortality from the infectious diseases.

Bursa of Fabricius is an organ which helps in defensive mechanism of chicken against diseases due to its lymphoid tissue. It is said to undergo slight to moderate changes in all diseases and also after vaccinations.

Infectious bursal disease (IBD) or Gumboro disease is an acute disease of chickens which has occurred since 1957 in U.S.A. (Cosgrove, 1962). The disease has since been recorded in many parts of the world (Luthgen, 1969). It has been revealed from the research that there are two distinct entities. One due to infectious bronchitis type of virus is responsible for the kidney lesions and termed as "Infectious

Avian Nephrosis". The other condition is due to a virus distinct from infectious bronchitis virus has particular affinity for bursa of Fabricius, causes "Infectious Bursal Disease".

Infectious bursal disease virus (IBDV) usually referred as Infectious Bursal Agent (IBA) that causes infectious bursal disease (IBD) in chickens with necrosis of lymphocytes has been recognised world over. In addition to the losses due to this disease itself, this malady perhaps devitalises the entire system of the infected chicken due to shake up of the immunological apparatus.

The existence of this disease in India was indicated on the basis of histopathology during the routine post-mortem examination and was described by histopathological examination and experimental infection of chickens with clinical materials without confirmation or isolation of the IBDV by Mohanty et al. (1971). No further attempt on this problem was made for the isolation and identification of IBDV if any and the serological survey of the disease.

Mortality in young chickens upto 3 weeks is not uncommon and in many occasions the disease is not properly identified due to low mortality and peculiarity of lesions in a vulnerable young age of the chicken. Infectious bursal disease has been

incriminated as a cause of considerable loss in brooder house chickens.

In India, until now the researches were directed towards the fatal diseases like New Castle Disease, chronic respiratory disease and Fowl Pox but with the growing poultry population coming up with the large organised poultry farms it has become necessary to prevent losses of other nature due to IBDV in younger age and reduction in the growth rate of broiler chickens.

With these points in view to find out the position of this disease in some of the major poultry rearing areas in this country by immunodiffusion technique useful for the diagnosis of IBDV especially for the early detection of an incidence of this infection, the investigation on the present problem was undertaken. The serological investigation will help in understanding the distribution of precipitating antibodies in field chickens in this country.

The isolation of the virus will help in confirming the prevalence, if any, investigated by serological survey and would help in understanding the biological characters of this viral pathogen and thereby help in understanding the magnitude of the problem.

# CHAPTER

# REVIEW OF LITERATURE

## REVIEW OF LITERATURE

### A. INFECTIOUS BURSAL DISEASE:

Cosgrove (loc.cit.) described this disease as an acute contagious disease of young chickens with increasing frequency which had occurred since the fall of 1957 in the Delmarva area of the U.S.A. The local name for this disease was given as "Gumboro disease" as the initial outbreaks occurred around an area known as Gumboro in Southern Delaware. The term "avian nephrosis" has been applied to this new disease because of the prominent lesion was tubular degeneration in the kidneys. Cosgrove (loc.cit) suggested that this infectious disease was caused by a virus as bacteriological examinations were negativ

Winterfield and Hitchner (1962) described a "nephritis-nephrosis syndrome" in chickens with similar renal damage from Delmarva area in the U.S.A. They have isolated two strains of infectious bronchitis virus namely "Holte" and "Gray". They have inoculated these two strains into susceptible chickens which produced clinical signs and lesions associated with those syndrome.

Cumming (1963) reported the disease in Australia where a clinical entity known as "Uremia" which had been recognised since 1948 (Hungerford, 1951).

Winterfield et al. (1962) recognised distinct differences between these two diseases and assigned the terms "infectious bronchitis variant viruses" to the "Holte" and "Gray" strains and "infectious bursal agent" to the infective agent associated with Gumboro disease.

Hitchner (1970 a) proposed the IBD to be a viral disease which caused acute pathognomonic changes in the bursa of Fabricius and discontinued the use of the terms "Nephrosis", "Nephritis", "Nephrosis-nephritis" and "Gumboro disease".

1. Distribution:

Since the first report by Cosgrove (loc.cit.), IBD was diagnosed in many areas of the U.S.A. (Winterfield and Hitchner, 1964), Mexico in 1962 (Giron, 1969), Puerto Rico (Bond et al., 1970) and Canada (Ide, 1970, cited by Faragher, 1972).

In Europe, the disease was recognised in Great Britain in 1962 (MAFF report 1961-62, cited by Faragher, 1972), Belgium in 1964 (Devos et al., 1966), Germany in 1965 (Landgr et al., 1967), Italy (Rinaldi et al., 1965), Switzerland (Riggenbach, 1967), Spain in 1968 (Badiola et al., 1969), Roumania (Adamesteanu and Adamesteanu, 1968), Greece (Dragnonas, 1969, cited by Faragher, 1972), Poland (Borzemska and

Golnik, 1969), France (Maire et al., 1969) and Yugoslavia (Herceg et al., 1971).

Bolotnikov (1966) stated that IBD had not been recorded in the U.S.S.R., although a similar pathological condition had been observed.

Infectious bursal disease has also been recorded in Israel in 1964 (Meroz, 1966), Malta (Cassar, 1968, cited by Faragher, 1972), South Africa (Coetzee, 1970, cited by Faragher, 1972), Japan (Shimizu et al., 1971) and Nigeria (Ojo et al., 1973).

In India, Mohanty et al. (loc.cit.) recorded gross and histopathological lesions similar to IBD.

Edgar and Waggoner (1965) reported that quails were susceptible to IBD.

Edgar and Yung Cho (1965) reported that wild sparrows have been known to die from this disease.

Giron (loc.cit.) inoculated with a Mexican strains of IBA to a group of 20 turkey poults, aged 10 weeks. All remained healthy without gross or microscopic changes associated with the infection in chickens when examined at intervals of 16 days. However, there is no firm evidence that IBD occurs in avian species other than the fowl.

## 2. Economic importance:

Cosgrove (loc.cit.) reported that the mortality rate was generally 1-15% averaging about 5% in 2-5 weeks old affected birds and it was typical that mortality was higher in 2-4 week old birds than in 4-5 week old birds. The disease usually appeared in a single pen of birds. After 2 or 3 weeks, it affected the adjoining pen, then entire house and eventually entire farm.

Hood (1963, cited by Faragher, 1972) reported the recurrence of the disease despite of most thorough cleansing and disinfection extending over a five week period.

Fraser (1964) reported losses due to IBD in England upto 15% within the flocks in case of a first outbreak of which 4-5% died on the day of peak mortality. The mortality returned to normal in about a week but the survivors did never quite pick up the condition again.

Parkhurst (1964 a) in a study of 115 outbreaks of IBD in broilers in the U.S.A. reported a mortality of 32.7% on one farm but found the mortality greater than 15% in two other outbreaks. The highest rate of mortality among outbreaks in Israel was 25%.

Parkhurst (1964 b) reported the characteristic pattern of mortality in the flock reaching a peak half way through the outbreak.

PATTERN OF MORTALITY

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| I. | Age of chicks (days) | 27 | 28 | 29 | 30 | 31 | 32 |
| | Mortality | 10 | 12 | 69 | 513 | 796 | 867 |
| | Mortality as % of total number at 27 days(7778) | 0.13 | 0.15 | 0.89 | 6.6 | 10.2 | 11.1 |
| II. | Age of chicks (days) | 33 | 34 | 35 | 36 | 37 | |
| | Mortality | 401 | 145 | 20 | 4 | 10 | |
| | Mortality as % of total number at 27 days(7778) | 5.16 | 1.86 | 0.26 | 0.051 | 0.13 | |

Parkhurst (1964 c) reported more than 30% losses. Meroz (loc.cit.) reported the decrease in the mortality rate in consecutive outbreaks.

Moultrop (1967) recorded the economic aftermath of an outbreak which was observed in terms of mortality rate which did exceed 10% and in the temporary reduction in growth rate of broiler chickens and more serious was in the farm where

flock after flock experiences a mortality of the order of 5% with accumulating financial loss.

Badiola et al. (loc.cit.) diagnosed IBD in 55 flocks in Spain. The average flock size was 6,600 birds and the mortality varied from 0.6 to 17% (average 5%).

DelBono et al. (1969) recorded 13 outbreaks of Gumboro disease in the North of Tuscany (Italy). The morbidity varied between 27 and 80% and the mortality between 1 and 24% respectively.

Bygrave and Faragher (1970) reported the overall mortality in outbreaks of IBD was usually within the range of 1-15% and the average was about 5% in flocks less than 6 weeks of age and it was lower in older chickens.

Ojo et al. (loc.cit.) reported the mortality rate of 12.5% with a morbidity rate of 60% in Nigeria. The disease was probably introduced through the importation of day old chickens.

3. Incidence:

Since the disease was recognised in 1957 numerous cases have been detected throughout the Delmarva area. On many farms it has recurred several times, one farm has had the

disease in ten successive flocks. The disease appears with equal frequency throughout the year (Cosgrove, loc.cit.).

Parkhurst (loc.cit.) and Hemsley (1965 b) stated that most farmers recognised the disease after the first outbreak and did not seek diagnosis when subsequent flocks were affected.

Meroz (loc.cit.) noted that outbreaks recurred in a third of instances atleast once, in 20% more than twice and in 9% more than three times and also found that the incidence was unaffected by the type of housing while vaccination against fowl pox or New Castle Disease was unrelated to the onset and duration associated with an outbreak of IBD.

Benton et al. (1967 a), DelBono et al.(loc.cit.) reported that light breeds of chickens were more susceptible than heavy breeds in terms of severity of the reaction to infection.

Winterfield (1969 a) reported that because of differences in the pathogenicity of different strains of IBA mild outbreaks of the disease were usually missed. Inapparent infection was reported to be widespread (Winterfield, 1969 b and Faragher, 1971).

4. Transmission:

Edgar and Yung Cho (loc.cit.) reported the spread of the disease from bird to bird by contact, by contaminated equipment,

litters, caretakers, feed, possibly air and wild sparrows which have been known to die of disease. It has been spread from farm to farm and area to area through contaminated feed. Man also act as an indirect vector in the transmission of the disease.

Yung Cho (1968) isolated the agent from litter, feed and water from diseased farms and from faeces of chickens 2-17 days after infection.

Benton et al. (loc.cit.) showed that IBA was highly contagious spreading readily by direct contact and the contaminated building remained infective for as long as 122 days after infected birds has been removed. Materials from infected environment were capable of initiating the infection in susceptible chickens.

Snedeker et al. (1967) collected lesser meal worms (Alphitobius diaperinus) and isolated IBA from a house eight weeks after an outbreak of IBD and saw characteristic lesions of the disease in chickens inoculated with a ground suspension of the meal worms.

Cheville (1967) demonstrated that insects may act as vectors in the natural transmission of the disease.

Brady (1970) commented that mites are exposed to the IBA and must have a much greater chance to pick it up than Alphitobius, partly because they are more numerous and all pervasive and partly because several species (e.g. caloglyphus) feed directly on poultry droppings.

5. Age susceptibility:

Cosgrove (loc.cit.) observed that IBD occurred in chickens within the age group of 2-15 weeks. Most severely affected were young birds upto five weeks old. Older birds may suffer from this disease, but the signs and mortality were less evident than in very young chickens.

Winterfield and Hitchner (loc.cit.) stated that at 3-5 weeks old chickens were more severely affected.

Hanson (1967) reported that the disease was most frequently occurred in chicks aged 3-5 weeks.

Parkhurst (loc.cit.), Meroz (loc.cit.), Badiola et al. (loc.cit.) confirmed the observation of Cosgrove that IBD occurred in chickens within the age group of 2-15 weeks.

Riggenbach (loc.cit.) stated the age of susceptibility was 6 weeks.

Badiola et al.(loc.cit.) diagnosed that birds aged 15-77 days were affected.

DelBono et al.(loc.cit.) reported the age of susceptibility was 45 days and Maire et al. (loc.cit.) found that average age of susceptibility was 29 days.

Ojo et al. (loc.cit.) observed the disease in 3-7 weeks old chickens in Nigeria.

6. The disease and its course:

Cosgrove (loc.cit.) reported that the onset of the disease is rapid. One of the earliest signs was found to be a whitish or watery diarrhoea with vent feathers soiled by urinary material followed by anorexia, depression, trembling, severe prostration and death. No respiratory signs were noted. In terminal stages trembling of the neck and body was very noticeable. The shanks showed dehydration but retain their normal yellow colour. The body temperature varied from normal to subnormal but no fever was present in any stage of the disease. The birds exhibited little interest in feed or water. The disease generally lasted 5-7 days in an affected batch of chickens (Parkhurst, loc.cit.; Meroz, loc.cit.).

Fraser (loc.cit.) reported ruffling of feathers and white mucoid diarrhoea with rapid onset and regression of mortality.

Parkhurst (loc.cit.) reported that affected birds rarely move, except towards a source of heat and then with an unsteady gait.

Barron et al.(1966) reported that in the early stages there was a resemblance of coccidiosis.

Riggenbach (loc.cit.) reported that in broiler chickens at 6 weeks of age there were sudden development of inappetence, depression, drowsiness, ruffled plumage with slight diarrhoea.

Giron (loc.cit.) noticed cyanosis of the comb and wattles and photophobia.

Maire et al. (loc.cit.) recorded convulsive trembling and greenish diarrhoea with white or haemorrhagic threads.

Bond et al. (loc.cit.) noticed loss of appetite restlessness, dehydration, slight tremor, birds tend to pick at the vents and passing large amount of urates in their droppings.

Mazurkiewicz et al. (1970) recorded the symptoms of weakness, disturbance in the locomotor system, tendency to sit on the tarsometatarsus, trembling and dehydration of legs appeared.

Bygrave and Faragher (loc.cit.) observed the course of the disease was over six days with diarrhoea, anorexia and trembling.

Ojo et al. (loc.cit.) recorded that the disease was sudden in onset. The affected chicks were usually depressed

and off feed, yellowish white diarrhoea was common initially and diseased chicks were reluctant to move. The head was usually lowered with the beak buried in the litter. A little later an affected chick would fall on its side and succumb within a brief period. He reported the mortality pattern was low on the first day but increased rapidly from the 2nd to the 4th day and then declined rapidly from the 5th or 6th day onwards.

7. Macroscopic changes:

Cosgrove (loc.cit.) described the macroscopic changes which had died due to IBD. The carcasses were well developed and in good bodily condition. The skeletal muscles especially the breast and leg musculature showed severe dehydration. Some birds showed a small infarction on the edge of the left or right lobe of the liver. The most prominent lesion was renal damage. If the birds were necropsied early the kidneys were normal in colour but might show slightly pronounced tubules. Birds in advanced cases showed tubules and ureters filled with urates and kidneys were pale and whitish. Cosgrove (loc.cit.) observed that the bursa of Fabricius was enlarged about twice its normal size and when opened a white core was present. Inflammation of the mucosa of the bursa was also noticed. He observed low calcium content of serum which could account for the tetany manifestation.

Winterfield and Hitchner (loc.cit.) and Rinaldi et al. (loc.cit.) reported that bursa was edematous, yellowish in colour, with striations. Peribursal edema was reported by Wilson (1964) and Landgraf et al. (loc.cit.).

Barron et al. (loc.cit.) reported that kidneys exhibit some times in normal appearance and some times enlarged with prominent tubules and from pale grey to a dark brown colour.

Riggenbach (loc.cit.) commented on the absence of haemorrhages in skeletal muscles, bursa was edematous and enlarged weighing thrice its normal weight.

DelBono et al.(loc.cit) reported that haemorrhagic patches were more common on the muscles of the legs than on the breast or wings.

Lensing (1968) reported intestinal disorders. Luthgen (loc.cit.), Ojo et al.(loc.cit.) described that the crops were empty in the affected birds.

Maire et al. (loc.cit.) observed haemorrhagic lesions in the papillae of the proventriculus and in muscles, kidneys showed discolouration and dilation with urates, bursa was hypertrophied.

Bygrave and Faragher (loc.cit.) reported extensive haemorrhages in the skeletal muscles and the bursa of Fabricius was enlarged with peribursal edema.

Mazurkiewicz et al. (loc.cit.) reported the enlargement of liver, spleen, kidney, bursa of Fabricius, hyperaemia in the duodenum and inflammation of the intestines.

Provost et al. (1972) observed haemorrhages at the junction of the proventriculus and gizzard, on the sternum, thighs, hypertrophy of kidneys and bursa.

Ojo et al. (loc.cit.) reported that the carcasses were in fairly good condition though slightly dehydrated. Bursa was edematous, enlarged, highly inflamed, with occasional yellow cheasy exudate. Intestines were congested.

Mohanty et al. (loc.cit.) noticed enlargement with prominent striations and discolouration of bursa, a gelatinous material was often present inside the bursa. In advanced cases atrophy of bursa with accumulation of yellowish caseous material. They observed that birds with bursal lesions were having stunted growth and poor musculature.

8. Histopathological changes:

In many cases, the workers mentioned that the lesions were confined to the kidney tubules indicating degenerative changes, glomerular nephrosis, colliquative necrosis followed by hyperplasia of the follicle with vacuole formation, edema, degeneration and necrosis of lymphoid cells (Cosgrove, loc.cit.; Mandelli et al., 1966; Peters, 1967; Hasegawa et al., 1972).

Mohanty et al. (loc.cit.) observed necrotic and proliferative changes, heterophilic infiltration mixed with mononuclear cells in lymphoid follicles as well as interstinal tissue and mucosa of bursa. Bursal atrophy with depletion of the lymphocytes was seen in cases of prolonged duration. Whole bursa in most cases converted into a sac like structure filled with a caseous core.

9. Diagnosis and differential diagnosis:

Cosgrove (loc.cit.) stated that specific characteristic signs and lesions distinguished IBD as a specific entity.

Hanson (loc.cit.) established diagnosis of IBD from consideration of age, history of the flock, onset and course of the disease, clinical signs and post-mortem lesions of the affected chickens.

Wagner and Kosters (1968) diagnosed the isolates of IBD by agar gel precipitation test.

Luthgen (loc.cit.) confirmed the disease based on the results of inoculation of pathological material into susceptible fertile eggs.

Valdes et al. (1971) diagnosed the disease by immunofluorescence. Immunofluorescence showed the infectious agent in the proventriculus, gizzard, duodenum and kidney at 12 hours

and in spleen, bursa and kidney at 24 hours. It was found that the best organs for diagnosis by immunofluorescence were bursa of Fabricius and caecal tonsils.

Asdrubali and Gialletti (1971) was used direct fluorescent antibody technique which was simple and rapid procedure particularly when macroscopic lesions were not clear. This method allowed the distribution and localization of IBA after experimental infection. The viral antigen was first seen in macrophages of bursa of Fabricius. After 48 hours infection cells of the bursa showed a clear cytoplasmic fluorescence.

Nakano et al. (1972) confirmed the first outbreak in Brazil by typical symptoms, gross and histological lesions.

Cancellotti et al. (1972) used indirect immunofluorescence and neutralization test in cell culture.

Hirai et al. (1972) used immunodiffusion reaction to detect the IBDV. He prepared the antigen by inoculation of the agent into five day old susceptible chicks. After 4 days of inoculation the infected bursa was harvested and diluted 1:1 w/v and homogenized. The homogenate was frozen and thawed three times before used as antigen. The material was clarified by low speed centrifugation at 5,000 rpm and then

at high speed centrifugation 10,000 rpm for 30 minutes and 60 minutes respectively. The supernatent was used as antigen.

Barron et al.(loc.cit) while investigating IBD reported that coccidiosis resembled the disease in the early stages. They differentiated the disease by characteristic lesions and course of IBD.

Chinn and Coomber (1966) differentiated IBD from other conditions like, Fatty liver and kidney syndrome where the course of the disease was short, recovery was rare and the bursa of Fabricius was not involved.

10. Control:

There are several approaches to the control of this highly contagious disease. Cosgrove (loc.cit.) have tried numerous treatments without success. The therapeutic and prophylactic administration of antibiotics, sulphanamides, furazolidone, high levels of Vitamin A.or molasses in the drinking water (Parkhurst, loc.cit.) or intramuscular administration of calcium gluconate to correct the low calcium level had no beneficial effects.

Edgar and Yung Cho (loc.cit.) prepared a suspension of bursae from experimentally inoculated chickens. More than three million chickens were vaccinated with histories of IBD

where mortalities averaged 5% at 4-5 weeks of age. The chicks were vaccinated intra-ocularly or through drinking water at 3-7 days of age. Losses in vaccinated flocks from IBD averaged less than 0.7%.

Snedeker et al. (loc.cit.) propagated the strain of IBA in chicken embryo. The eighth embryo passage virus was evaluated as an immunizing agent. A filtered suspension was prepared from embryos which died during eighth passage and administered in the drinking water at 7-10 days of age, the agent produced a mild disease and an immunity which lasted atleast nine weeks. This virus would seem to have limited application as a vaccine. The virus was isolated from the lesser meal worm, suggesting that control of this insect might play a part in eliminating the disease from infected premises.

Benton et al. (1967 b) reported that disinfection with formaldehyde offers the best chance to reduce the viability of IBA but not certanity of success. Thorough cleansing, distant removal of litter, disinfestation and disinfection of buildings, surroundings and equipment depopulation and the isolation of premises have been carried out but without any success as judged by recurrence of IBD in subsequent batches of birds.

Dorn et al. (1968) prepared three vaccines by using homogenized bursa of Fabricius from natural cases of Gumboro

disease. Filtrate of bursa of Fabricius and a homogenate of chick embryos inoculated with bursal material were used for the immunization of chicks aged 2-12 days. This was administered in the drinking water. Losses were prevented in 67 vaccinated flocks involving over one million chickens on 27 farms where previous outbreaks of IBD had occurred. There was a slight clinical reaction between 2-21 days after vaccination, manifested by a drop in feed consumption and very slight mortality, in 4 flocks with intercurrent infections.

Yung Cho (loc.cit.) used a nonattenuated live IBA vaccine administered intra-ocularly or in the drinking water reduced losses from this disease significantly.

Bendheim (1969) reported that passive immunity to IBA was conferred on susceptible chickens by use of convalescence serum collected from broiler chickens which had recovered from IBD.

Winterfield (loc.cit.) showed that IBA isolate No. 2512 was capable of being propagated serially through 50 generations in susceptible chicken embryos. No clinical signs associated with IBD were observed in chickens which were vaccinated with materials from the 27th or 41st passage in laboratory trials. The vaccine was administered in the drinking water or by spray or by eye drop. Neutralizing antibodies against homologous and

heterologous IBA isolates were demonstrated in the serum of chickens which had recovered from IBD infection or vaccination. Chickens exposed to IBA at 3 days of age did not develop as significant neutralization titres as those exposed at 4 weeks or later. The neutralization test with the isolate No. 2512 proved valuable in assessing the immunity status of chickens. Groups of chickens vaccinated at 3-35 days of age were resistant to challenge by a virulent isolate. These results were confirmed in field trials by Winterfield and Fadly (1971).

Vitali (1971) reported that increased environmental temperature above 18°C tended to lower the mortality rate.

Bengelsdorff and Bernhardt (1971) done with virus strain-244 (isolated by Dorn) after numerous egg passages to baby mice until loss of virulence for chicks. Vaccine prepared from homogenisates of baby mice induced high concentration of virus neutralizing antibodies in layers and broiler chicks. These antibodies persisted for atleast 24 weeks and were transmitted in egg yolk. Vaccinal IBA excreted by the vaccinated chickens immunized susceptible chicks by contact.

Rinaldi et al. (1972) attenuated the strain isolated in Italy (1-65 PV) by 60 passages in fertile eggs. It was administered in drinking water to broiler chickens. The mortality, growth, feed transformation and carcasses condemned

at slaughter at 65 days of age was recorded for both vaccinated and control chickens and the results of vaccination was found to be satisfactory (mortality 0.95% for vaccinated and 4% for unvaccinated birds). Laboratory tests showed that attenuation was stable after 8 consecutive passages of the vaccine virus in chicks and that protection against challenge was present by the sixth day after vaccination.

B. AETIOLOGICAL AGENT:

A viral aetiology was suspected by various early workers (Cosgrove, loc.cit.; Rinaldi et al, loc.cit.; Ghenne, 1968; Luthgen, loc.cit.)

1. Isolation of virus:

Petek and Mandelli (1969) isolated the virus during an outbreak of the disease in a Venetian Poultry Farm and designated the virus as IBA/17.

Gelenczei and Lunger (1970) made the virus isolations from chickens affected by IBD. The IBA was isolated from bursa of Fabricius by chicken embryo inoculation. They obtained 5 isolates from different geographical areas.

Rinaldi et al. (1969) isolated a strain of IBA from an outbreak of Gumboro disease in Italy by means of inoculation into the amnio-allantoic cavity of embryonated chicken eggs.

Hitchner (1970 b) reported that chorioallantoic membrane (CAM) route of inoculation was preferred for initial isolation and materials for subpassage should be the CAM or embryo tissues but not allantoic fluid. He used kidney materials from natural cases for IBDV isolation.

Shimizu et al. (loc.cit.) isolated the virus from the affected bursa by inoculation of the material into 10 day old embryonated chicken eggs. They indicated that IBA was successfully isolated from chicken embryos.

Kosters et al. (1972) purified a virus from the allantoic fluid or CAM of IBA infected embryonated eggs for further characterization.

Winterfield et al.(1972) inoculated two different strains of IBDV in chicks. The virus was not isolated from tissues beyond ten days of post inoculation. At three days post inoculation, the virus was consistently isolated in the highest concentration from the bursa of Fabricius and spleen whereas the brain and blood were low in yield.

Benjamin et al. (1972) isolated IBDV (69-729) from birds showing clinical signs and lesions of IBD when inoculated into susceptible embryos it produced lesions characteristic to those produced by IBDV.

Hirai et al. ( 1973 ) succeeded in isolating seven strains which were identical with IBDV. During the past four years from 1968 to 1971 the authors received chickens affected with bursitis from seven farms involved in outbreaks of IBD in Gifu and Aichi prefectures.

2. Physicochemical properties:

a) Filtration:

Winterfield and Hitchner (loc.cit.) reported that IBA was filterable and size has been estimated by several studies.

Benton et al. (loc.cit.) passed the filtrate of embryo suspension through a series of Seitz filters and Millipore filters and the filtration studies indicated that the IBA was between 10 and 50 mμ in size.

Landgraf et al. (loc.cit.) passed the material through filters of 0.22 and 0.1 μ pore diameter and the virus did pass through these filters.

Yung Cho (loc.cit.) subjected three bursal suspensions to filtration through a series of Gelman triacetate filters of Average Pore Diameter (APD) 300, 200, 100 and 50 nm in size. All the filtrates were shown to be infective to chickens and concluded that the agent was less than 50 nm in size.

Rinaldi et al. (loc.cit.) stated that the agent passed through "Millipore" filters of APD 100 nm but was retained by filters of APD 50 nm.

b. Thermostability:

Landgraf et al. (loc.cit.) assessed the infectious bursal agent in fertile eggs. The embryos survived on exposure at 60°C for 30 minutes but not at 70°C and 80°C.

Benton et al. (loc.cit.) placed the bursal suspension in a waterbath at either 37°C or 56°C and reported that their strain of the agent withstood 37°C for 90 minutes and was still viable after exposure to 56°C for 5 hours. The infectivity was assayed in fertile eggs and chickens.

Yung Cho (loc.cit.) showed that the agent was infective for chickens after exposure at 60°C for 90 minutes at 25°C (room temperature) for 21 days and at -20°C for 3 years.

Faragher (loc.cit.) reported that the titre of infectivity for chickens of IBA after storage as a bursal suspension for upto 18 months at -58°C was undiminished.

c. Chemical stability:

Benton et al. (loc.cit.); Yung Cho (loc.cit.) and Rinaldi et al. (loc.cit.) reported that the IBA was resistant to treatment with ether, chloroform, merthiolate and trypsin.

Benton et al. (loc.cit.) and Yung Cho (loc.cit.) showed that it was stable at pH 2.0 for 60 minutes but unstable at pH 12.0 and in the presence of 1% formalin. Benton et al. (loc.cit.) reported that the agent was inhibited by Wescodyne (an iodine complex) but not by Staphene or Hyamine 2389 (quaternary ammonium compound), 1% phenol and thiomersal.

Faragher (loc.cit.) showed that IBA which was partially purified by fluorocarbon extraction and then by DEAE-cellulose chromatography, being eluted in a continuous gradient by 0.36 M-sodium chloride of 1.30 g/ml.

Yung Cho (loc. cit.) reported that the infectivity was not affected by exposure to Penicillin-Streptomycin for one hour.

3. Biological properties:

a. Propagation of IBA in embryos:

Wilson (loc.cit.); Mandelli et al. (loc.cit.); Snedeker et al. (loc.cit.); Landgraf et al. (loc.cit.) and Dorn et al. (loc.cit.) injected suspensions of tissues from affected chickens into embryonated eggs. Embryos died from the third day to fifth day afterwards after inoculation, with dwarfing, oedema, congestion and haemorrhages in the subcutis and in the region of the kidneys, swelling of the liver with

greenish discolouration and necrosis, enlargement of the spleen and pale foci in the heart muscle.

Wilson (loc.cit.) showed that serial passage of embryonic tissue resulted in failure to maintain the agent through three passages, as judged by mortality and lesions in embryos and by infectivity of the passaged material in chicken. Winterfield and Hitchner (loc.cit.); Benton (1964); Landgraf et al. (loc.cit.) also reported on this failure.

Benton (loc.cit.) pointed that eggs from certain flock failed to support the growth of the agent.

Snedeker et al. (loc.cit.) in the development of an egg adapted live vaccine found that embryonic mortality was nil, 75% and 95% after inoculation into the yolk sac, allantoic sac and CAM respectively, and that serial passage was successful with embryonic tissues but not with embryonic fluids. A titre of $10^{3.25}$ $ELD_{50}$ was obtained with an embryonic suspension after eight egg passages but similar preparations from earlier passages contained no appreciable virus.

Winterfield (loc.cit.) passaged IBA strain serially through 50 generations in chick embryos using allantoic sac route of inoculation. In early passages serial transfer was successful using embryonic tissue, and embryonic fluid contained

virus only after repeated passage. Embryonic mortality occurred between the third and sixth day after inoculation. The titres of both embryonic fluids and tissues were in the range of $10^{3.6}$ to $10^{5.0}$ per ml.

Petek and Mandelli (loc.cit.) reported small, white opaque pocks on the CAM of inoculated embryos.

Hitchner (loc.cit.) explained the difficulties encountered in previous attempts to propagate the IBA in embryonated eggs. He showed that for successful propagation eggs must be from a susceptible flock and the CAM route of inoculation was the most sensitive for detecting small amounts of the virus. The signs of infection were death of the embryo. Most mortality occurred between third and fifth day post inoculation. Affected embryos had edematous distension of abdomen, petechiae and congestion of skin and occasionally ecchymotic haemorrhages in the toe joints and cerebrum. Embryos that died approximately four or more days post inoculation had internal lesions consisting of mottled necrosis and ecchymotic haemorrhages of the liver, enlargement and paleness of the spleen, congestion and mottled necrosis of the kidneys, blanched heart musculature and frequently extreme congestion of lungs.

A growth curve study with IBDV strain 2512 showed peak virus titers in the CAM, embryo and allantoamnionic fluids occurred by 72 hours post inoculation(Hitchner, loc.cit.).

Leyk (1971) observed the following changes in the embryonated eggs inoculated with IBDV viz., subcutaneous edema, haemorrhages, dwarfing and liver necrosis.

b. Propagation of IBA in cell-culture:

Mora (1966) and Landgraf et al. (loc.cit.) reported distinct cytopathic effects (CPE) following infection with IBA of cell cultures of chicken tissue macrophages and chick embryo fibroblasts.

Petek and Mandelli (loc.cit.) studied the CPE of IBDV strain 17 in chick embryo kidney cell culture. Irregular eosinophilic inclusion bodies in paranuclear position in the cytoplasm of many cells and formation of large multinucleated cells were observed before and during the progressive vacuolization and destruction of the monolayers.

Shimizu et al. (1970) attempted without success to propagate a strain of IBA in chicken kidney cell culture, but demonstrated an interfering factor in chicken tissue infected with IBA.

Lukert and Davis (1970) reported the growth and characterization of IBA in cells derived from the bursa of Fabricius and kidney of chickens. Infection of bursal cultures was detected by cytopathology and immunofluorescence. The CPE was

not readily observable in kidney cell cultures but infection could be detected by immunofluorescence.

Gelenczi and Lunger (loc.cit.) isolated 5 strains from the bursa of Fabricius of chickens affected with IBD by propagation in avian cell cultures. They concluded that a mixed infection of IBA and a reovirus was present in the bursa and stated that the two viruses were separated by certain tissue culture techniques and identified.

Kosters and Paulsen (1971) described the propagation of IBA in chicken embryo kidney cell cultures. Multiplication of IBA was not inhibited by 5-Iodo-2'deoxyuridine or cytosine arabinofuranoside. The CPE was seen after 3-5 days of incubation.

Rinaldi et al. (loc.cit.) propagated the IBDV on chicken embryo fibroblasts having antibodies to IBD. Counts of plaque formation units were observed in chicken embryo fibroblast cultures.

Rinaldi et al. (loc.cit.) observed a growth curve showed a highest count of plaque forming units after 30 minutes incubation at 37°C. Titration in chicken embryo fibroblasts proved more sensitive than in eggs or unweaned mice.

Mandelli et al. (1972) showed the cultural, cytological and ultramicroscopical characteristics of a strain IBDV (1/PV). The CPE appeared in the first passage only in pig kidney cell culture but in all 15 passages in chicken kidney. Progressive lysis of the monolayer began at about 48 hours. No CPE were observed in mouse, calf or guinea pig kidney cell cultures. In cell lines CPE appeared in one passage only in rabbit cornea and in two passages only in rabbit kidney. No CPE was observed in pig, hamster and calf kidney, He La or amnion cell lines. No inclusion bodies or syncytia were seen under normal microscope. With acridine orange staining, the fluorescence could not be localized (although it can be for reovirus). Under ultramicroscope viral particles were seen in the cytoplasm of necrotic fibroblasts and in intercellular spaces. Particles were either loose or in crystalline form typical of reovirus.

c. Experimental infection of chickens:

Helmboldt and Garner (1964) found that the inoculation of IBA into susceptible chickens at 21 days was followed by necrosis of the lymphoid elements. This began as early as 2-4 days after inoculation and reached its peak in 3-4 days. Microscopic lesions were noted in the bursa of Fabricius, spleen, thymus, caecal tonsils. They speculated that the relative resistance to infection of one day old chicks was due to some type of parental immunity.

Mandelli et al. (1966) also found that one day old chicks to be resistant to experimental infection, while chickens aged 20-70 days were susceptible.

Cheville (loc.cit.) investigated the cytopathological changes in the bursa of Fabricius, spleen and thymus of chickens following experimental infection with IBD at 28 days of age by light, fluorescent and electron microscopy. Clinical signs of IBD was apparent three days later with all the birds recovering. Viraemia, severe panleucopenia, elevation of the body temperature and splenomegaly coincided with increase in size and weight of the bursa of Fabricius and subsequently atropy of the bursa was rapid. Aggregations of viral particles were seen in the lysosomal debris within the macrophages, heterophils and endothelial cells but not in lymphocytes. He also reported the specific immunofluorescent findings on the pathogenesis of experimentally infected chicks with IBD.

Mandelli et al. (1967) reported viral particles similar to those seen by Cheville (loc.cit.) on electron microscopic examination of bursae from both experimental and naturally infected chicks.

Badiola et al. (loc.cit.) studied the microscopic changes in the bursa of Fabricius of chickens for 30 days after experimental infection and reported no regeneration of lymphoid follicles.

Malhotra (1969) described small foci of regenerated lymphoid tissue in some follicles of the bursa by the 14th day after inoculation. Clinical signs appeared at least 24 hours earlier in chicks infected by intravenous and by cloacal routes as compared with ocular and oral routes.

Mandelli et al. (1969) studied the clinical and pathological effects in groups of chickens inoculated with two strains of IBA at one day of age. No clinical signs were apparent after infection except perceptible slowing of the growth rate.

Mohanty et al. (loc.cit.) inoculated the bursal suspension of the naturally infected birds after filtration into susceptible chicks by intraperitoneally and simultaneously given a drop of inoculum on the eyes and nostrils. The clinical signs were evident on second day after three serial passages. Dullness, depression and death occurred on the third or fourth day post inoculation and the gross lesions were confined to bursa with prominent striations and discolouration.

d. Experimental infection in laboratory animals:

Landgraf et al. (loc.cit.) inoculated five mice by the intravenous route with a filtered suspension of bursa from naturally infected chickens. The mice remained unaffected and no lesions were seen five days later.

Rinaldi et al. (loc.cit.) found that only white mice were sensitive to infection with IBDV. Mice aged 1-11 days and inoculated into the peritoneal cavity and older mice aged 12-14 days and inoculated intracerebrally showed nervous signs and heavy mortality after 5-13 days. The post-mortem findings were of encephalomyocarditis.

4. Antigenicity:

Wagner and Kosters (loc.cit.) reported that IBA did not haemagglutinate erythrocytes from horse, ox, sheep, goat, dog, fowl, rabbit, mouse, rat or man.

Faragher (loc.cit.) found that a suspension of IBA containing $2.51 \times 10^4$ fowl infective doses 0.05 ml did not agglutinate erythrocytes from the sheep, guinea pig, rat, man or fowl at room temperature, 37°C or 4°C over the pH range of 6.0 - 7.6.

Landgraf et al. (loc. cit.); Badiola et al. (loc.cit.) and Rinaldi et al. (loc.cit.) reported briefly the development of specific neutralizing antibodies in the serum of naturally and experimentally infected chickens.

Winterfield (loc.cit.) stated that if the bursa was removed surgically chickens did not develop neutralizing antibodies.

Winterfield (loc.cit.) showed that chickens which had recovered from natural infection or which were vaccinated with an attenuated strain of IBDV developed neutralizing antibodies in their serum against homologous and heterologous strains of the virus. Neutralizing antibodies were demonstrated in the serum samples from U.S.A., Great Britain, Italy, Brazil, Venezuela and Chile. Serum samples from Rhodesia, Mexico, Japan, Spain, Hawaii and Ireland were negative.

Dorn et al. (loc.cit.) investigated the susceptibility of broiler breeds in Germany by embryo susceptibility test. Out of 40 flocks, 29 were not susceptible.

Luthgen (1970) concluded that commercial flocks in Germany largely of foreign origin have an acquired immunity, only few local flocks of poultry did contact the disease.

Wagner and Kosters (loc.cit.) reported a positive reaction in the agar gel diffusion precipitation test between convalescent serum from naturally infected chickens and a homogenate of CAM from infected embryos. The reaction was specific and no strain differences were apparent between 12 isolates of IBA.

Faragher (loc.cit.) determined the optimal conditions for immunodiffusion with sera from IBD survivors and found to be similar to other avian virus systems.

Kosters (1971 b) detected serum precipitins from 1-5 weeks and antigens in the CAM of incubated eggs from 2-12 weeks after inoculation of fowls aged 17 months with IBDV.

Allan et al. (1972) demonstrated that chickens which had been inoculated with IBA at one day of age, the reduction in the serological response to New Castle disease vaccine was confirmed and the susceptibility of chickens to challenge despite prior New Castle disease vaccination.

5. Classification:

Yung Cho and Edgar (1969) characterized IBDV. The characteristics of the IBDV in their study presented most like the picorna virus group except that IBDV was more heat stable than the picorna viruses. However, they reported that more work must be done before IBDV can be definitely classified as belonging to the picorna group.

Petek and Mandelli (loc.cit.) reported that the CPE produced by the infection in chicken embryo kidney cell culture was characteristic of an avian reovirus and also resistance shown by the virus to various chemical and physical agents and its appearance under electron microscopy.

Gelenezei and Lunger (loc.cit.) isolated the reovirus from bursa of Fabricius of chickens suffering from IBD. The

isolates showed typical characteristics of IBA inoculated into chicken embryos and chickens. All the isolates changed their characteristics following serial passages in certain avian cell culture. The size, morphology of the tissue culture adapted isolates as demonstrated by the electron microscopy and their resistance to various physical and chemical treatments was to that of avian reovirus. The isolates propagated in chicken embryo and duck embryo fibroblast cell cultures and demonstrated all characteristics that are typical to IBA.

Rinaldi et al.(loc.cit.) isolated the IBDV from an outbreak in Italy and reported that the properties of the virus were similar to those of reovirus.

Kosters et al. (loc.cit.) purified an RNA virus from the allantoic fluid or CAM of IBDV infected embryonated eggs which had physicochemical and morphological characteristics of an avian reovirus.

Lunger et al. (1972) discussed the probable taxonomic position of the IBDV. He studied the sequential morphological events associated with the avian IBD by thin section Electron microscopy of tissue samples taken at 24 hours intervals over a ten day period. It is clear from the study of Snedeker et al. (loc.cit.) that IBDV can be transmitted to chickens via

inoculation of lesser meal worm suspensions. This observation coupled with the remarkable morphological similarity of IBA replication to maturation events associated with Nodamura virus an arthropod borne Picorna virus, suggests that IBA may also be related to this virus category except for the relatively large size of IBA (60 mμ). The evidence presented in the above study as well as physicochemical characterization demonstrates that IBDV meets many of the criteria of Picorna viruses as cited by Murphy et al. viz., a) cubic symmentry, b) maturation within a cytoplasmic matrix in association with a viroplasmic matrix and cytopathology, c) a marked tendency to form crystalline virus arrays in infected cells, d) ether and chloroform resistance, e) presence of an RNA core. There are evidences based on both morphological and immunological considerations for assuming that IBDV is not an avian reovirus.

C. SEROLOGICAL SURVEY:

Wagner and Kosters (loc.cit.) prepared specific antigen from chicken embryo allantoic membrane infected with IBDV to identify the isolates by agar gel precipitation test.

Schneider and Hass (1969) investigated serological diagnosis for finding precipitating antibodies after oral infection with IBDV. Precipitating antibodies were detected between 8 to 24 days of post inoculation. In flock survey,

the authors found that a relatively high proportion of birds had antibodies which might have arisen from multiple reinfection.

Kosters and Geissler (1971) studied the distribution of IBD by agar gel precipitation test using bursal homogenates from infected chicks as antigen. Of 1087 birds received alive for diagnosis, 174 (15%) gave positive results. Of 1284 blood samples from 33 farms, 198 (16%) from 11 farms were positive.

Cancellotti et al. (loc.cit.) done the serological survey and detected the antibodies in yolk samples by indirect immunofluorescence and neutralization in tissue culture in primary chicken fibroblasts. Serum samples from 59 out of 60 flocks from Northern Italy were positive for IBD antibodies.

Faragher (loc.cit.) detected the precipitins associated with IBD in 1000 out of 2552 serum samples from 97 out of 186 flocks in Great Britain. The proportion of broiler flocks giving positive reaction was significantly higher than the proportion of layers or parent flocks.

Hirai et al.(loc.cit.) showed the distribution of precipitating antibodies in chicken sera by gel diffusion test in Japan. They concluded that a peak titer of precipitating antibodies was observed among the 61 to 100 day old chickens.

# CHAPTER

# MATERIALS AND METHODS

# MATERIALS AND METHODS

## MATERIALS

### 1. Embryonated eggs:

Embryos of 10-12 days age were used for inoculation of materials which were collected from the post-mortem room of the Institute. These eggs were incubated in the egg incubator until used. The details of the embryos used for virus isolation are given in Table 1.

TABLE 1.

DETAILS OF THE EMBRYO INOCULATION USED FOR VIRUS ISOLATION

| Passage No. | Total No. of specimens inoculated. | No.of embryos used per specimen. | Total No. of embryos used. |
|---|---|---|---|
| 1 | 68 | 5 | 340 |
| 2 | 68 | 5 | 340 |
| 3 | 68 | 4 | 272 |
| 4 | 3 | 5 | 15 |
| 5 | 2 | 5 | 10 |
| 6 | 2 | 5 | 10 |
| 7 | 2 | 5 | 10 |

2. Chickens:

Chickens suspected to infectious bursal disease were used for collection of materials after sacrificing.

3. Serum samples:

Serum samples were collected from the poultry farms of Uttar Pradesh, Karnataka, Tamil Nadu and Andhra Pradesh as detailed in Table 2.

TABLE 2.

DETAILS OF SERUM SAMPLES COLLECTED FROM DIFFERENT STATES

| S. No. | Name of the State | Name of the Farm | Age of the birds in months. | No.of sera samples collected. |
|---|---|---|---|---|
| 1. | Uttar Pradesh | Experimental Poultry Farm, I.V.R.I., Izatnagar. | 2 | 23 |
| | | -do- | 15 | 27 |
| 2. | Karnataka | Central Poultry Breeding Farm, Hessarghatta, Bangalore. | 8-11 | 40 |
| 3. | Andhra Pradesh | College of Veterinary Science, Tirupati. | 2 | 110 |
| | | Private Poultry Farm, Chittoor. | 12 | 15 |
| | | Private Poultry Farm, Nellore. | 3 | 15 |
| 4. | Tamil Nadu | Institute of Vet.Preventive Medicine,Ranipet. | 12 | 80 |
| | | Poultry Research Station, Madras. | 2½ | 12 |
| | | Tiruchchirapalli | 10 | 40 |

4. Diagnostic standard reference antisera (freeze dried):

a) Diagnostic antisera against infectious bursal disease virus "52/70" were received through the courtesy of Dr. M. Pattison, Poultry Department, Central Veterinary Laboratory Weybridge, England, U.K. was used in this study for confirming the isolates.

b) Diagnostic standard antiserum (freeze dried) against infectious bursal disease virus and antigen (inactivated) were also received from Dr. B.W. Calneck, Department of Avian Diseases, New York State Veterinary College, New York, U.S.A. for this study.

5. Materials collected for virus isolation:

During routine post-mortem examination at the following places materials were collected as detailed in Table 3 for the isolation of IBDV. Bursa of Fabricius and kidneys were collected from 2-8 weeks old birds showing lesions indicative of IBD. The materials were collected in 50% glycerine buffer saline and kept at 4°C for 5-20 days until processed. The materials collected from ailing birds had shown in Table 4.

TABLE 3.

MATERIALS COLLECTED FROM POST-MORTEM BIRDS

| No.of specimens collected | Source | Specimen numbers | Age of chicks in weeks | Materials collected. | Post-mortem findings. |
|---|---|---|---|---|---|
| 60 | Experimental Poultry Farm, I.V.R.I., Izatnagar. | B1-B60 | 1-8 | Bursa of Fabricius and kidney | Atropy, congestion and enlargement of bursa, cheasy exudate in the bursa. Nephrosis, urates in the ureters, congestion of keel muscle and legs, liver and heart showed petichae. |
| 2 | Poultry Research Station, Madras. | B61-B62 | 5 | Bursa of Fabricius | Enlargement of bursa, nephrosis. |

TABLE 4.

MATERIALS FROM AILING BIRDS

| No.of specimens collected. | Source | Specimen numbers | Age in weeks | Materials collected | Symptoms and lesions. |
|---|---|---|---|---|---|
| 4 | Experimental Poultry Farm, I.V.R.I., Izatnagar. | B63-B68 | 4 | Bursa of Fabricius and kidney | Dullness, unsteady gait. Atropy of bursa and nephrosis. |

6. Chemicals:

Chemicals of "Analar" grade were used for the preparation of the following solutions.

a) Phosphate Buffer Saline (PBS):

| | | |
|---|---|---|
| Sodium chloride | .. | 0.800 gm. |
| Dipotassium hydrogen phosphate | .. | 0.121 gm. |
| Potassium dihydrogen phosphate | .. | 0.034 gm. |
| Distilled water | .. | 100 ml. |

This was sterilized by autoclaving at 121°C for 15 minutes. This was used for washing CAM and for the dilution of the virus.

b) 50% Glycerine Buffer Saline:

Same as phosphate buffer saline and made it upto a volume of 200 ml by adding 100 ml of glycerine.

c) Hank's Balanced Salt Solution:

| | | |
|---|---|---|
| Sodium chloride | .. | 8.000 gm. |
| Potassium chloride | .. | 0.400 gm. |
| Calcium chloride ($2H_2O$) | .. | 0.185 gm. |
| Magnesium sulphate ($7H_2O$) | | 0.100 gm. |
| Magnesium chloride ($6H_2O$) | | 0.100 gm. |
| Disodium hydrogen phosphate | | 0.048 gm. |

| | | |
|---|---|---|
| Potassium dihydrogen phosphate | | 0.060 gm. |
| Glucose | .. | 1.000 gm. |
| Distilled water (Triple) | | 970 ml. |
| Phenol red 0.4% | .. | 5 ml. |

d) Hank's growth media:

It consisted of Hank's balanced salt solution 875 ml and the following chemicals and antibiotics:

| | | |
|---|---|---|
| Lactalbumin hydrolysate | | 5.000 gm. |
| Yeast extract | .. | 1.000 gm. |
| Inactivated calf serum | .. | 10% |
| Penicillin | .. | 100 units per ml. |
| Streptomycin | .. | 100 mg per ml. |

The pH was adjusted to 7.2 to 7.4 (Cunningham, 1966).

) Hank's maintenance media:

It consisted of above ingredients but the calf serum as added at the rate of 5% in case of chicken embryo kidney nd 2% in case of chicken embryo fibroblast cell cultures.

) Trypsin solution:

| | | |
|---|---|---|
| Sodium chloride | .. | 4.000 gm. |
| Potassium chloride | .. | 0.200 gm. |
| Glucose | .. | 0.500 gm. |
| Distilled water | .. | 500 ml. |
| Trypsin | .. | 1.250 gm. |
| Phenol red 0.4% | .. | 2.5 ml. |

Trypsin solution was filtered through Seitz filter. This was used for cell dispersion of tissues through enzymatic digestion.

g) Sodium bicarbonate solution:

| | | |
|---|---|---|
| Sodium bicarbonate | .. | 1.4 gm. |
| Distilled water | .. | 100 ml. |

This was sterilized by autoclaving at 121°C for 10 minutes. This was used to adjust the pH of the Hank's Balanced salt solution and Hank's growth and maintenance media.

7. Glassware:

"Corning" brand of glassware was used for tissue culture work.

8. Filters:

Seitz filters and Millipore filters of 0.22 $\mu$ was used for filtration of the isolates.

9. Equipment and other instruments:

Bacteriological incubators, Refrigerators, Deep freeze, Magnetic stirrers and minor instruments like stainless steel scissors, forceps etc. available at the Respiratory Diseases of Poultry Laboratory of I.V.R.I. were used.

## METHODS

### 1. Collection of materials from naturally infected birds:

Bursa of Fabricius and kidney were collected aseptically during the routine post-mortem examination and birds suspected for IBD from young growing birds of about 2-8 weeks of age. The materials were collected from the birds with the help of scissors and forceps into the penicillin vials containing 50% Glycerine buffer saline as preservative. Sixty eight specimens had been collected both from the post-mortem and the ailing birds. The materials were kept at 4°C until processed.

### 2. Processing of materials for embryo inoculation:

The bursa and kidney were washed with PBS before maceration. The maceration was done in the pestle and mortar using sterile sand, and 10% suspension was made by adding PBS containing 1,000 units of Penicillin and 10 mg. of Streptomycin per ml. The suspensions were kept at 37°C for 30 minutes and inoculated into blood agar slant for bacterial sterility. The suspensions were clarified by low speed centrifugation at 1,000 rpm for 30 minutes. The supernatants were collected as the stock of the isolates and stored frozen at -20°C until use. Freezing and thawing of suspensions were done thrice before inoculation into embryonated eggs.

3. Embryo inoculation for virus isolation:

Embryos of 11 to 12 days of age were used for CAM route of inoculation which is most sensitive for initial isolation of IBDV (Hitchner, loc.cit.) than yolk sac and allantoic cavity route. Clean and preferably white shelled eggs were used to facilitate easy candling. The embryos were candled and the edge of the air space was marked. A triangle area was also made on the side of the egg which was away from underlying blood vessels. The shell surface was sterilized with 70% alcohol. The triangle area was drilled with dental drill. Over the centre of air space a hole was made. At the site of inoculation a slit of 5 m.m. long and 3 m.m. wide was made and the shell was cut away. The shell membrane was thus exposed without perforation. The shell membrane of the inoculation site was pierced with sterile 23 gauge needle and then gentle suction was applied to the perforation of the air sac with a rubber bulb. This caused the CAM to drop away from the closely adherent shell membrane so that an artificial air space was made between the two. The eggs were examined over a candler to confirm that the air sac space had been displaced and the membrane had dropped. The suspension of 0.05 ml was deposited in the membrane with 1 ml tuberculin syringe fitted with 23 gauge needle and was distributed evenly by gently tilting the embryos. Then the holes in the air sac and at the site of

inoculation were sealed with transparent adhesive tape. Inoculated eggs were placed in horizontal position and incubated at 37°C for 5 to 6 days. The candling of embryos were done twice daily. Mortality during the first 24 hours was taken as non-specific. Mortality occurred between the second to sixth post inoculation days were harvested. Only the CAM was collected for further passages or as a source of virus material. Lesions in both CAM and embryos including its organs were recorded. All the 68 specimens were given three passages, and the specimen B7 and B38 were passaged upto seventh passage level.

4. Harvesting of chorioallantoic membrane:

The egg shell of the embryo was cleaned with 70% alcohol and with sterile blunt forceps the air space was broken and the CAM was ruptured. The contents of the embryo including the embryo was poured into one petridish. The CAM attached to the shell membrane was slowly separated with the help of forceps and CAM was transferred into a clean sterile petridish. The CAM was rinsed in PBS and the CAM was washed twice or thrice and drained the excess fluid and the membranes were collected in the sterile test tube and were stored at -20°C in the deep freeze until used but not beyond 10 to 15 days.

## 5. Preparation of materials for cell cultures:

### i) Glassware other than pipettes:

All the new glassware except pipettes were soaked in 33 percent of hydrochloric acid overnight. Next day they were thoroughly washed in tap water to remove the acid. Used glassware were washed in water without overnight soaking in acid. Infected and contaminated culture glassware were first autoclaved and after cooling they were brushed and rinsed several times in running tap water to dislodge any loosely adherent debris.

Initially prepared glassware were immersed in dilute detergent solution (1.4% det solution) and this was brought to boil and simmer for 30 minutes. After cooling, those were brushed until all adherent particles were removed. Then they were drained in an inverted position and washed with tap water. The washed ones were rinsed sequentially in metal distilled and deionised water. The above procedure were done by rinsing in two basins, one for metal distilled water and another for deionised water. Then they were well drained and covered with good quality aluminium foil folded for about 3 cms below the mouth. All of them were sterilized in hot air oven at 160°C for one hour.

### ii) Pipettes:

Pipettes were thoroughly washed in hot water using an

automatic washer of reflex type. At least 20 reflex cycles were carried out keeping the mouth piece of the pipette downwards. Then they were soaked in metal distilled water and deionised water as mentioned for other glassware . The mouth piece was plugged with nonabsorbent cotton wool and wraped in paper and finally sterilized in hot air oven at 160°C for one hour.

iii) Magnetic Stirrer:

Magnetic stirrers were immersed in 33 percent hydrochloric acid. They were then washed thoroughly in tap water and transferred to metal distilled and deionised water. The stirrer was sterilized by autoclaving at 121°C for 10 minutes.

iv) Instruments:

Instruments like scissors, forceps, scalpel were subjected to boiling in 'Det' solution for one hour before rinsing them in boiling water several times. They were then rinsed in metal distilled water and deionised water before they were finally dried and sterilized in hot air oven at 160°C for one hour.

v) Seitz filter:

Metal portions of the Seitz filter were treated as per the other instruments. After assembly prior to sterilization,

deionised water was passed through filter pad to remove loose asbestos fibres and toxic materials. Assembled Seitz filter was finally sterilized by autoclaving at 121°C for fifteen minutes.

vi) Muslin cloth:

Muslin cloth was rinsed in tap water and boiled in water. Then these were transferred to metal distilled and deionised water. The mouth of the filtered flask was covered with 3 layers of muslin cloth and was covered with aluminium foil and sterilized.

vii) Rubber tubings and corks:

All the new rubber tubings and corks were washed in tap water and boiled in distilled water containing 0.1% sodium carbonate for 30 minutes and rinsed in running water. Then these were boiled in 0.5% N/1 HCl for 30 minutes and rinsed well in tap water and then transferred to metal distilled and deionised water sequentially. Used rubber articles were washed in 'Det' solution (1.4%) before treating them in the manner described for the new ones. These were packed in the test tubes and covered with aluminium foil and autoclaved at 121°C for 15 minutes.

All sterilized apparatus were stored in closed cabinets to protect from dust until used.

viii) Preparation of culture room:

The culture room was kept clean and it was sprayed with 70% alcohol before the day of use. Ultra violet lamp was put on the day previous to work for one hour and on the day immediately before use for 30 minutes.

6. Preparation of chicken embryo kidney cell culture:

Embryonating chicken eggs of 18-20 days old were examined for living condition. 70% alcohol was applied over the shell with cotton tipped applicator. The egg shell was broken open along the margin of air sac with sterile forceps. With the same forceps (flamed) shell membrane was removed and then with a pair of angle forceps the inner membrane was slit open and the embryos were removed into sterile petridish containing Hank's balanced salt solution (BSS) with antibiotics. Picked up the skin over the xiphoid cartilage with forceps and make a transverse cut of the skin with scissors. Pulled the skin anteriorly from the pectoral muscles. Holding the posterior portion of the body up right at the caudal end, cut the viscera loose with small curved scissors and discarded. Using the curved forceps the kidneys were removed and placed them in petridishes containing of about 20 ml of BSS at room temperature (25°C). The kidneys were washed twice with BSS and transferred into a beaker and minced with a sterile curved scissors. Then washed

with BSS. The minced tissues were transferred into a conical flask and with a magnetic stirrer, trypsin a concentration of 0.25% about 20 ml previously warmed to 37°C was added aseptically for enzymatic digestion and dispersion. The tissues covered with trypsin solution were stirred with the magnetic stirrer in such a way to avoid frothing. The trypsinisation was carried for about 30 minutes. The recovery of cells through extractions were collected and strained through 3 layers of muslin cloth to remove clumps and connective tissue and collected into conical graduated centrifuge tube. Then the dispersed cells were sedimented by low speed centrifugation 1000 rpm for 10 minutes and decanted the trypsin. Then the cells were resuspended in Hank's BSS and centrifuged at 1000 rpm for 10 minutes. Then the cells were resuspended in Hank's growth medium. The centrifuged cells were pooled and finally resuspended in growth medium so that approximate cell concentration was $1 \times 10^6$ cells per ml.

Assessment of viable cells:

The number of viable cells was determined by direct count in haemocytometer using trypan blue as the vital stain. To one ml of cell suspension 0.5 ml of trypan blue was added. The viable cells did not take the stain. The cell count of the suspension was made with the haemocytometer. Finally the

dilution was made with growth medium so that the cell concentration was 0.3 to 0.5 million of cells per ml.

7. Preparation of chicken embryo fibroblast cell culture:

Ten day old embryonated chicken eggs were taken and examined for living condition. The shell was swabbed with 70% alcohol. The egg shell was broken open along the margin of air sac with sterile forceps, then the shell membrane was removed with angle forceps. The inner membrane was slit open and the embryo was removed into a sterile petridish containing Hank's BSS with antibiotics. The head, appendages and visceral organs were removed and was washed twice with BSS. They were transferred into sterile beaker and minced to fragments of about 1-2 cm. with the help of curved scissors. The fragments were washed with BSS and then transferred to a conical flask (250 ml) with a magnetic stirrer. The further procedure was same as that of chicken embryo kidney cell culture.

8. Processing of material for tissue culture infection:

The CAM of the isolates B7, B38, B39 and S at the fourth passage level were triturated with the help of a pestle and mortar. This was diluted with BSS 1:10 w/v containing antibiotics and kept at 37°C for 30 minutes and then stored in the freezing chamber of the refrigerator.

After one freezing and thawing, the suspension was centrifuged at 2,500 rpm for 10 minutes and the supernatant was used to infect the chicken embryo kidney and chicken embryo fibroblast cell cultures. The completely formed monolayers were washed once with Hank's BSS to remove the debris before infection of the culture tubes.

9. Characterization of isolates by filtration:

The fresh CAM of embryos infected with specimen B 7 and B38 were triturated at the fifth passage level and diluted with PBS 1:10 w/v. The suspension was centrifuged with low speed centrifugation and then with high speed centrifugation. Then the supernatant was filtered through a millipore filter of 0.22 μ APD. The filtrate was inoculated into 5 embryos with each isolate and observed for about 5 days. The unfiltered suspension also inoculated into 2 embryos as control.

10. Preparation of antigen for immunodiffusion reaction:

Twelve day old embryonated chicken eggs were inoculated via CAM with our indigenous isolates B38/3 and B7/4. Embryos died after 24 hours of post inoculation was taken as non-specific. From the second day onwards the embryos those died and those which were alive upto five days after inoculation, were chilled and the CAMs were harvested. The CAM was stored in the freezing chamber of a refrigerator for one day. After

that, pooled CAM was triturated thoroughly in pestle and mortar along with 1 gm of sterilized sand and diluted to 1:5 w/v with phosphate buffer saline solution. The suspension was treated with Penicillin and Streptomycin and allowed to stand for 30 minutes at 37°C and was centrifuged at 2500 rpm for 10 minutes. The supernatant was transferred with a sterile pipette into a sterilized 'Corning' test tube and kept in the freezing chamber of a refrigerator. Freezing and thawing of the antigen was done 3 times before using as antigen. The antigen was tested for sterility and only sterile antigen was used for immunodiffusion reaction.

11. Preparation of control antigen:

Control antigen was also prepared as described above but with CAM from 17 days old embryonated eggs.

12. Procedure for immunodiffusion reaction:

The procedure followed here is essentially that of Witter (1962) with slight modification, which was developed by Kumar and Mallick (1972) as given below:

Diffusion medium:

| | | |
|---|---|---|
| Sodium chloride | .. | 8 gm |
| Agar (Difco) | .. | 1 gm |
| Distilled water | .. | 100 ml |
| Merthiolate | .. | 1:10,000 |

The medium was buffered at pH 7.2 and melted in a steamer and filtered through a pad of an absorbant cotton and again steamed for 15 minutes.

Preparation of glass slides:

Thirteen millilitres of melted agar was poured into a clear sterile glass slide of 8 x 6 cm and 4 ml on the glass slide of 8 x 3 cm and kept at room temperature to solidify then stored in the refrigerator at 4°C for 24 hours to set the agar.

Five wells of 4 mm diameter, one central and four peripheral were punched in the medium by means of cork borer. The distance between the central well and the peripheral was 4 mm. The punched agar slab was removed with the help of a 20 gauge needle and the bottom of the wells were sealed with a drop of melted agar.

13. Serological test (immunodiffusion reaction):

The isolates B7 and B38 were tested against standard reference antisera of both European and American strains of IBDV received through the courtesy of Drs. M. Pattison (U.K.) and B.W. Calnek (USA). The antiserum was filled in the central well and antigens were filled in the peripheral wells along with two wells of control antigen (normal CAM). The

slides were kept in the petridishes having water soaked layer of absorbent cotton. The petridishes containing slides were covered immediately to avoid drying. The slides with the petridishes were incubated at 37°C and some sets in the refrigerator at 4°C.

14. Reading of the agar gel slides:

The slides were observed after every 12 hours at oblique transmitted light. The observation was continued for at least 10-12 days. Identification of a line of precipitation was possible through its coalescence with the reference of precipitation.

15. Collection of sera samples from the field (Survey samples):

Blood was collected from the wing vein of birds with a sterile 5 ml syringe to which 20 gauge needle was attached. About 3 ml of blood was collected into a sterile test tube. All the sterile precautions were taken for collection. The collected blood was kept in a slanting position for easy collection of serum. After clotting of blood the clot was broken with a pipette and the tubes were kept in the refrigerator overnight to get a maximum possible amount of serum from it. They were centrifuged to separate the serum from the clot at 2500 rpm for 15 minutes.

The serum was collected in 3 ml corning test tubes and one drop of merthiolate in concentration of 1:100 were added to it and then the test tubes were sealed and kept in a refrigerator and those were tested after 4 to 20 weeks.

16. Preparation of antigen for testing sera samples:

The virus which had been isolated and was filtered through Millipore filter of APD 0.22 $\mu$ and inoculated into 12 day old chicken embryos. After 5 days post inoculation the embryos were opened and the CAM of those embryos which had shown characteristic lesions in embryos were harvested and kept at -20°C of the freezing chamber. After 24 hours CAMs were triturated with the help of pestle and mortar and the dilution of 1:5 weight/volume with normal saline solution was made. The suspension was kept at -20°C in the deep freeze and was given 3 freezing and thawingsbefore using as antigen for testing field sera samples. The antigen was also tested against standard reference antisera received from U.K. and U.S.A.

17. Screening of field sera samples employing immunodiffusion reaction:

The sera samples collected from various farms were tested against IBDV antigen by immunodiffusion reaction. The preparation of diffusion medium and the preparation of glass

slides was made as described previously. The antigen was filled in the central well and the field sera samples were filled in the peripheral wells along with one well as control. The slides were kept in the petridishes having a water soaked layer of absorbent cotton and the petridishes along with the slides were incubated at 37°C for 8 days. The observations were made for every 12 hours upto 8 days before discarding the test slides.

# CHAPTER

# EXPERIMENTAL RESULTS

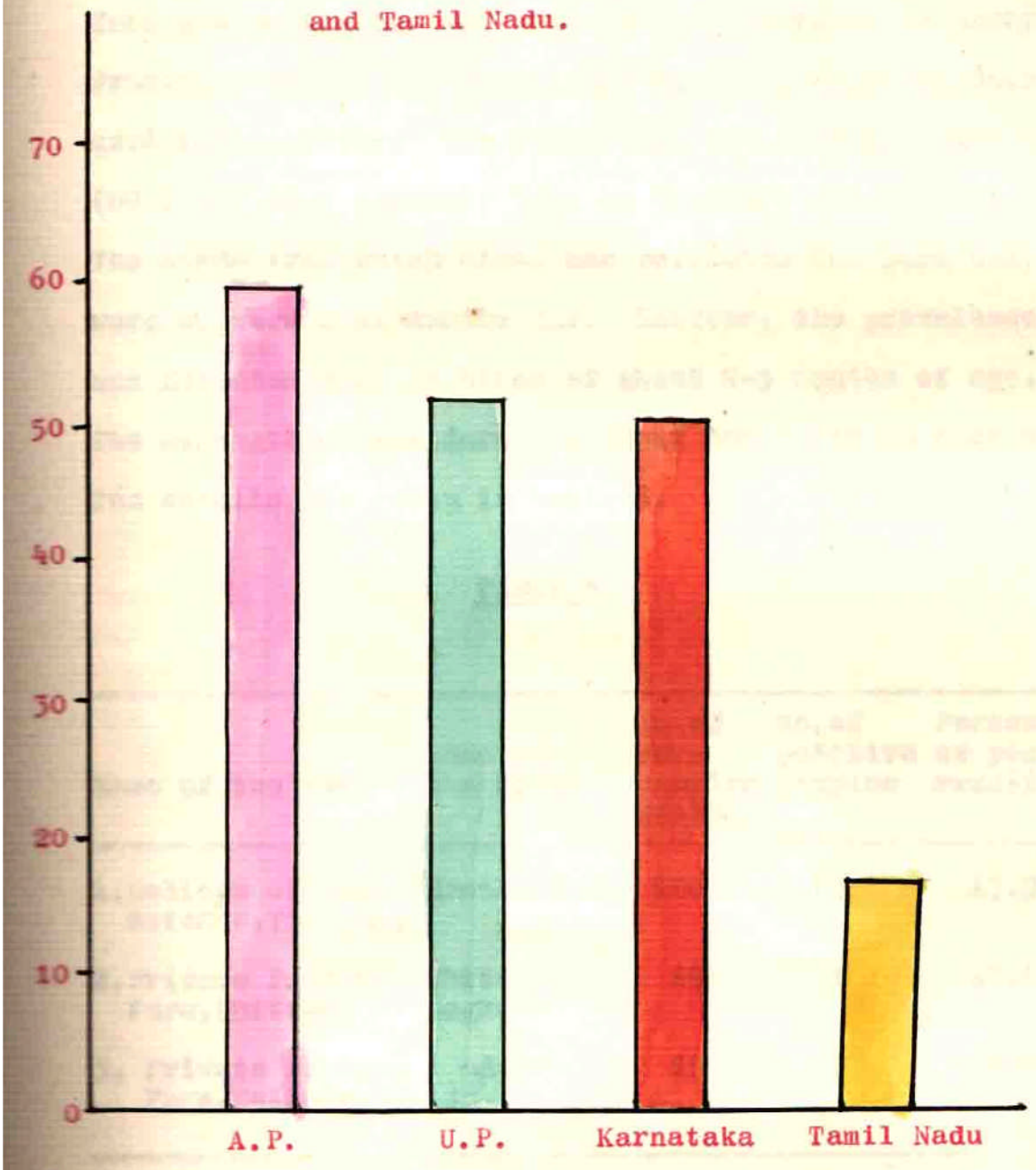
## RESULTS

### I. SEROLOGICAL SURVEY:

#### 1. Distribution of precipitating antibodies against IBDV in chickens of some organised farms in four States:

A total of 362 individual sera samples were examined from 8 organised poultry farms of Andhra Pradesh, Tamil Nadu, Uttar Pradesh and Karnataka. The sera samples were from some broiler, white leghorn and Austrolop breeds of chickens. The age of these chickens were 2 to 12 months. A maximum 60 percent of prevalence of precipitating antibodies were observed from a private farm in Andhra Pradesh followed by Uttar Pradesh (52.1 percent), Karnataka (50 percent) and Tamil Nadu (16.6 percent). Details of prevalence is shown in Fig. 1.

Fig. 1: The comparative percentage of prevalence of precipitating antibodies in chickens in Andhra Pradesh, Uttar Pradesh, Karnataka, and Tamil Nadu.



A. Distribution of precipitating antibodies against IBDV in chickens in Andhra Pradesh.

The prevalence of precipitating antibodies in chickens from one public and two private poultry farms in Andhra Pradesh revealed the prevalence of 17.3, 20.0 and 60.0 per cent respectively. The prevalence was more in White Leghorn (60.0 and 20.0 percent) than in broilers (17.3 percent). The birds from which blood was collected for sera samples were between 2-12 months age. However, the prevalence was detected more in birds of about 2-3 months of age. The strength of the farm was about 500 birds in each case. The details are given in table 5.

TABLE 5

| Name of the Farm | Name of the breed | No.of sera samples tested | No.of positive samples | Percentage of positive reaction |
|---|---|---|---|---|
| 1.College of Vet. Science,Tirupati | Broilers | 110 | 19 | 17.3 |
| 2.Private Poultry Farm,Chittoor | White Leghorn | 15 | 3 | 20.0 |
| 3. Private Poultry Farm,Nellore | -do- | 15 | 9 | 60.0 |

B. **Distribution of precipitating antibodies against IBDV in chickens in the State of Tamil Nadu:**

The prevalence of precipitating antibodies in chickens from three public farms in Tamil Nadu showed the prevalence of 16.6, 10.0 and 5.0 percent. The birds from which blood was collected for sera samples were between 2½ months to 12 months. However, the prevalence was detected more in birds of 2½ months of age. The farm size was about 5000 birds in each case. The farms were wide spread at a distance of 70 to 100 miles in the State. The details are given in table 6.

TABLE 6

| Name of the Farm | Name of the breed | No.of sera samples tested | No.of positive samples | Percentage of positive reaction |
|---|---|---|---|---|
| 1.Poultry Research Station,Madras | White Leghorn | 12 | 2 | 16.6 |
| 2.Institute of Vet. Preventive Medicine, Ranipet | -do- | 80 | 8 | 10.0 |
| 3.Tiruchchirapalli | -do- | 40 | 2 | 5.0 |

C. Distribution of precipitating antibodies against IBDV in chickens in Uttar Pradesh:

The prevalence of precipitating antibodies in chickens from experimental poultry farm in Uttar Pradesh showed the prevalence of 52.1 percent in broilers and 14.0 percent in White Leghorns. The birds from which blood was collected for sera samples were between 2-12 months age. However, the prevalence was detected more in chickens about 2 months of age. The farm size was about 25000 birds. Number of strains of birds received at week old age were brought to this farm from various parts of the world like Israel, U.S.A., Australia and Japan and reared. The details are given in table 7.

TABLE 7

| Name of Farm | Name of the breed | No.of sera samples tested | No.of positive samples | Percentage of positive reaction |
|---|---|---|---|---|
| 1.Experimental Poultry Farm, I.V.R.I., Izatnagar | Broiler | 23 | 12 | 52.1 |
| 2. -do- | White Leghorn | 27 | 4 | 14.0 |

D. Distribution of precipitating antibodies against IBDV in chickens of Karnataka:

The prevalence of precipitating antibodies in chickens from Government Farm from Bangalore region of Karnataka showed the prevalence of 10.0, 30.0, 50.0 and 30.0. The prevalence is much more in White Leghorn (M strain) received from abroad (50 percent) than in Austrolop breed of 10 per cent which was in the farm since long. The birds from which the blood was collected for sera samples were between 8-11 months age. The farm size was about 8000 birds with individual flock of about 500-800. Details are given in table 8.

TABLE 8

| Name of the Farm | Name of the breed | No.of sera samples tested | No.of positive samples | Percentage of positive reaction |
|---|---|---|---|---|
| 1.Central Poultry Breeding Farm, Hessarghatta, Bangalore | Austrolop | 10 | 1 | 10.0 |
| 2. -do- | Broilers | 10 | 3 | 30.0 |
| 3. -do- | 'M' strain White Leghorn | 10 | 5 | 50.0 |
| 4. -do- | 'T' strain White Leghorn | 10 | 3 | 30.0 |

## II. ISOLATION AND IDENTIFICATION OF IBD VIRUS:

### A. Isolation in developing chicken embryos:

Twelve day old chicken embryos were used to passage 68 specimens for three times. The details of embryo lesions at first passage level observed with 25 specimens are given in table 9, whereas the same with 22 specimens at second passage level and with only 12 specimens at third passage level are given in table 10 and table 11 respectively.

At the second passage level, out of these twenty two specimens, eleven viz., B7, B14, B15, B16, B17, B25, B38, B40, B44, B60 and S had shown lesions at both first and second passage.

At the third passage level out of those 12 specimens showed lesions, seven (B7, B25, B38, B39, B40, B60, B64) had shown lesions at both second and third passage. Only 5 specimens B7, B25, B38, B40, B60 were constantly showing lesions in all the three passages.

TABLE 9

**Isolation of virus: Materials passaged in chicken embryos**

**Details of embryo lesions in FIRST PASSAGE LEVEL**

| S.No. | Lab.No. of the specimen | Age of bird in weeks | Characteristic lesions observed in embryos | No.of specimens passaged | No.of specimens from which virus was isolated | Percentage of isolates. |
|---|---|---|---|---|---|---|
| 1. | 2. | 3. | 4. | 5. | 6. | 7. |
| 1 | B4 | 3½ | Dwarfing, congestion of kidney, liver and legs, petichal haemorrhages on the liver. | | | |
| 2 | B7 | 5 | Little edema of CAM, liver and kidney congested. | | | |
| 3 | B14 | 5 | Little edema and necrosis of CAM, liver and kidney congested, heart also very much congested. | 68 (B1 to B68) | 25 | 36.8 |
| 4 | B15 | 4 | Edema and congestion of CAM, mottled necrosis of liver, kidney and heart congested. | | | |
| 5 | B16 | 4 | Extensive edema of CAM, liver and kidney congested. | | | |
| 6 | B17 | 4 | Edema of CAM, liver shows necrosis and petichal haemorrhages, mottled necrosis, kidney congested. | | | |

| 1. | 2. | 3. | 4. | 5. | 6. | 7. |
|---|---|---|---|---|---|---|
| 7 | B21 | 5 | Liver enlarged, icteric, mottled necrosis. | | | |
| 8 | B23 | 4½ | Opaque white pock lesions on the CAM with edema, liver showed necrosis, kidney congested. | | | |
| 9 | B24 | 5½ | CAM showed necrosis, edema and pock lesions, liver showed greenish discolouration. | | | |
| 10 | B25 | 4½ | Liver pale in colour, necrosis and congestion. | | | |
| 11 | B26 | 4½ | Necrosis and congestion of liver. | | | |
| 12 | B28 | 8 | CAM haemorrhagic, liver and kidney congested. | | | |
| 13 | B29 | 5 | Edema of CAM, liver and kidney congested. | | | |
| 14 | B38 | 1 | Little edema of CAM, congestion of liver, kidney tubules prominent. | | | |
| 15 | B40 | 2½ | CAM haemorrhagic, dwarfing, liver enlarged and congested, kidney congested. | | | |
| 16 | B41 | 4 | CAM edematous, liver very much congested. | | | |
| 17 | B42 | 6 | CAM haemorrhagic, liver congested, kidney showed enlargement. | | | |

| 1. | 2. | 3. | 4. | 5. | 6. | 7. |
|---|---|---|---|---|---|---|
| 18 | B44 | 2 | One lobe of the liver necrosed and the other congested. Kidney showed congestion. | | | |
| 19 | B55 | 8 | CAM haemorrhagic, liver showed greenish discolouration, kidney congested. | | | |
| 20 | B56 | 8 | CAM edematous, liver congested and enlarged, kidney congested. | | | |
| 21 | B57 | 4 | One lobe of liver congested and the other pale, kidney very much congested. | | | |
| 22 | B60 | 2½ | CAM showed necrosis and edema, small white opaque pocks on the CAM, liver and kidney congested, spleen enlarged. | | | |
| 23 | B63 | 5 | CAM showed edema, liver and kidney congested. | | | |
| 24 | B65 | 4 | One lobe of the liver enlarged and the other atropied, white foci on the liver. | | | |
| 25 | S | - | Dwarfing, liver and kidney congested, subcutaneous haemorrhages, slight enlargement of right lobe of liver shown Fig. 3. | | | |

The remaining 43 specimens did not show any detectable lesions in embryos.

## TABLE 10

**Isolation of virus: Materials passaged in chicken embryos**

**Details of embryo lesions in SECOND PASSAGE LEVEL**

| S.No. | Lab.No. of the specimen | Age of bird in weeks | Characteristic lesions observed in embryos | No.of specimens passaged | No.of specimens from which virus was isolated | Percentage of isolates. |
|---|---|---|---|---|---|---|
| 1. | 2. | 3. | 4. | 5. | 6. | 7. |
| 1 | B1 | 5 | CAM edematous, congestion of liver and kidney. | | | |
| 2 | B2 | 2 | Edema and opaque pocks on the CAM, dwarfing and congestion of liver. | | | |
| 3 | B5 | 4½ | CAM haemorrhagic, pock lesions on the CAM, congestion of liver. | | | |
| 4 | B7 | 5 | CAM edematous, liver enlarged and icteric, kidney congested. | 68 (B1 to B68) | 22 | 32.3 |
| 5 | B9 | 3½ | CAM haemorrhagic, one lobe of liver enlarged and the other atropied, congestion of liver. | | | |
| 6 | B10 | 5 | Slight edema of CAM, congestion and mottled necrosis of liver, muscles congested. | | | |
| 7 | B14 | 5 | Extensive edema of CAM, congestion and greenish discolouration of liver. | | | |

| 1. | 2. | 3. | 4. | 5. | 6. | 7. |
|---|---|---|---|---|---|---|
| 8 | B15 | 4 | Congestion of liver, one lobe of the liver enlarged and greenish discolouration. | | | |
| 9 | B16 | 4 | CAM edematous, dwarfing, one lobe of liver congested and other lobe is green. | | | |
| 10 | B17 | 4 | Liver congested and also showed greenish discolouration. | | | |
| 11 | B20 | 4½ | Edema of CAM, one lobe of the liver congested. | | | |
| 12 | B25 | 4½ | Little edema of CAM, liver showed greenish discolouration. | | | |
| 13 | B38 | 1 | CAM edematous, enlarged liver, greenish discolouration of one lobe of the liver and the other atropied. Shown Fig. 4. | | | |
| 14 | B39 | 1 | Liver enlarged and yellowish discolouration of right lobe, kidney and liver showed congestion. Shown Fig. 5. | | | |
| 15 | B40 | 2½ | Enlargement, congestion and greenish discolouration of liver, kidney also congested. Shown Fig.6. | | | |
| 16 | B44 | 2 | Extensive edema and pock lesions on the CAM, necrosis, congestion and enlargement of liver, kidney congested. | | | |

| 1. | 2. | 3. | 4. | 5. | 6. | 7. |
|---|---|---|---|---|---|---|
| 17 | B45 | 2 | Congestion and enlargement of liver, kidney congested. | | | |
| 18 | B46 | 3 | Liver showed necrosis, kidney congested. | | | |
| 19 | B47 | 4 | CAM edematous, necrosis and congestion of liver. | | | |
| 20 | B60 | 2½ | CAM edematous, dwarfing, liver enlarged, congestion of kidney and liver. | | | |
| 21 | B64 | 4 | Congestion of liver, necrotic borders on the liver, kidney showed prominence of tubules. | | | |
| 22 | S | | Dwarfing, subcutaneous haemorrhages, congestion of liver. | | | |

The remaining 46 specimens did not show any detectable lesions in embryos.

TABLE II

**Isolation of virus: Materials passaged in chicken embryos**

**Details of embryo lesions in THIRD PASSAGE LEVEL**

| S.No. | Lab.No. of the specimen | Age of bird in weeks | Characteristic lesions observed in embryos | No.of specimens passaged | No.of specimens from which virus was isolated | Percentage of isolates. |
|---|---|---|---|---|---|---|
| 1. | 2. | 3. | 4. | 5. | 6. | 7. |
| 1 | B7 | 5 | Extensive edema and necrosis of CAM, liver and kidney congested, one lobe of the liver enlarged. | | | |
| 2 | B25 | 4½ | Edema of CAM, liver showed greenish discolouration. | | | |
| 3 | B38 | 1 | CAM edematous, enlargement and greenish discolouration of the liver, atropy of the left lobe of the liver. | 68 (B1 to B68) | 12 | 17.6 |
| 4 | B39 | 1 | Dwarfing and subcutaneous haemorrhages of the embryo, liver showed greenish discolouration. | | | |
| 5 | B40 | 2½ | Liver and kidney very much congested and enlarged. | | | |
| 6 | B48 | 4 | CAM edematous, liver very much congested. | | | |

| 1. | 2. | 3. | 4. | 5. | 6. | 7. |
|---|---|---|---|---|---|---|
| 7 | B51 | 4 | Kidney congested and liver icteric. | | | |
| 8 | B57 | 4 | Pock lesions on the CAM, liver and kidney congested. | | | |
| 9 | B60 | 2½ | Liver enlarged, greenish discolouration, congestion of kidney. | | | |
| 10 | B62 | 6 | Kidney and liver congested, one lobe of the liver enlarged. | | | |
| 11 | B64 | 4 | CAM haemorrhagic, greenish discolouration of liver. | | | |
| 12 | B65 | 3 | CAM edematous, congestion of liver and kidney. | | | |

The remaining 56 specimens did not show any detectable lesions in embryos.

## Characterization of IBDV:

### 1. Filtrability of the isolate:

The isolate B7 was filtered through the Millipore ilter of APD 0.22 µ and the filtrate was checked for bacterial terility and inoculated into 12 days old chicken embryos. he filtered material did also show growth characters and ssions similar to those before filtration. Details are shown n table 12.

TABLE 12

Characterization of isolate

Embryo infectivity after filtration of the isolate at the 7th passage level.

| so-ate o. | No.of embryos inocu-lated. | Lesions observed before filtration | Filtra-tion th-rough Millipore filter of APD 0.22µ | No.of embryos inocu-lated after filtra-tion | Lesions observed after filtra-tion |
|---|---|---|---|---|---|
| 37 | 5 | Extensive edema and necrosis of CAM, dwarfing, liver showed congestion, enlargement, kidney congested. | Yes | 5 | Extensive edema and necrosis of CAM,dwarf-ing, liver showed conge-stion,enlarge-ment, kidney congested. |
| Control | 2 | CAM, liver and kidney normal. | No | 2 | CAM, liver and kidney normal. |

2. **Identification of isolates by immunodiffusion reaction with standard reference sera from U.K. and U.S.A.:**

The isolate B7 which was filtered through Millipore filter of APD 0.22 μ was subjected with immunodiffusion reaction with standard IBDV reference antisera obtained through the courtesy of Dr. M. Pattison, Central Veterinary Laboratory, Weybridge, England and Dr. B.W. Calnek, Department of Avian Diseases, New York State Veterinary College, New York. The isolate showed positive reaction within 18 hours at 37°C with both the antisera shown in Fig. 2.

Another isolate B38 had also shown precipitation line against both antisera from U.K. and U.S.A. The precipitation line appeared only after 48 hours at 37°C or 8-12 days after when subjected at 4°C. The result of precipitation reaction was reproduceable. The details of immunodiffusion reaction with standard reference diagnostic sera are given in table 13.

TABLE 13

Identification of isolates by immunodiffusion reaction with standard reference diagnostic sera from U.K. and U.S.A.

| No.of isolates. | Tested against reference antisera | | Incubation temperature | Line appeared after hrs/days | Tested after filtration through 0.22 μ APD Millipore filter. |
|---|---|---|---|---|---|
| | U.K. | U.S.A. | | | |
| 7 | + | + | 37°C | 18 hours | Yes |
| 38 | + | + | 4°C | 8-12 days | No |
| 38 | + | + | 37°C | 48 hours | No |

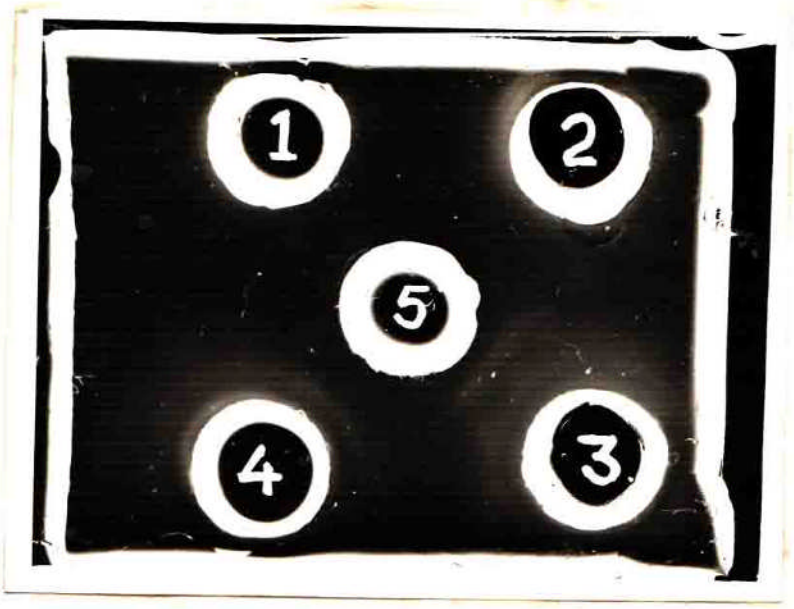
3. Growth characters of some of the isolates in chicken embryos upto seventh passage level:

The isolates B7 and B38 were passaged in embryos upto seventh passage level. The characteristic lesions observed with both the isolates were edema and necrosis of CAM, enlargement and congestion of liver and kidneys. The peculiarity of the lesions were that the right lobe of the liver in almost all embryos was enlarged few times more than the left lobe. These lesions were observed consistently in all the passage level i.e. from fourth to seventh and the lesions were progressive towards higher passage level. The details of the embryo lesions and mortality are given in table 14.

## TABLE 14

Isolates of IBD virus showing lesions upto seventh passage.

| Specimen No. | 4th passage | 5th passage | 6th passage | 7th passage |
|---|---|---|---|---|
| B7 | CAM showed extensive edema, necrosis, shown Fig. 8. White opaque pocks on the CAM. Liver congested, enlarged, mottled necrosis, kidney tubules are prominent and enlarged and congested, shown Fig. 7. | Extensive edema, necrosis of CAM, liver showed necrosis, white foci, pale in colour, kidney showed congestion. | Extensive edema and necrosis of CAM, dwarfing, liver showed necrosis enlargement, kidney also congested and enlarged. | Extensive edema of CAM, necrosis of CAM, liver enlarged and congested, necrotic borders in the liver. |
| | Mortality: 20% | Mortality: 20% | Mortality: 15% | Mortality: 25% |
| B38 | Little edema of CAM, congestion, enlargement and greenish discolouration of liver. | CAM edematous, liver icteric, white foci on the liver, one lobe of the liver enlarged and greenish discolouration. | Little edema of CAM, liver very much enlarged, kidney showed congestion. | Little edema of CAM, necrosis, enlargement and greenish discolouration of liver, kidney showed congestion with prominence of tubules. |
| | Mortality: 30% | Mortality: 20% | Mortality: 20% | Mortality: 15% |




1. Control antigen (normal CAM suspensio
2. Control antigen (normal CAM suspensio
3. B7/5 CAM suspension
4. B38/5 CAM suspension
5. Diagnostic antisera against IBDV.

Fig. 2: Immunodiffusion reaction with the isolates tested against diagnostic antisera of IBDV received through the courtesy of Dr. Pattison, Central Veterinary Laboratory, Weybridge, England, U.K.


Fig. 3: Embryo inoculated with isolate No. BS/1 at the 1st passage level through CAM route at 12th day showing congestion and slight enlargement of liver specially the right lobe and subcutaneous haemorrhages, dwarfing of the embryo when opened after 5 days post inoculation.

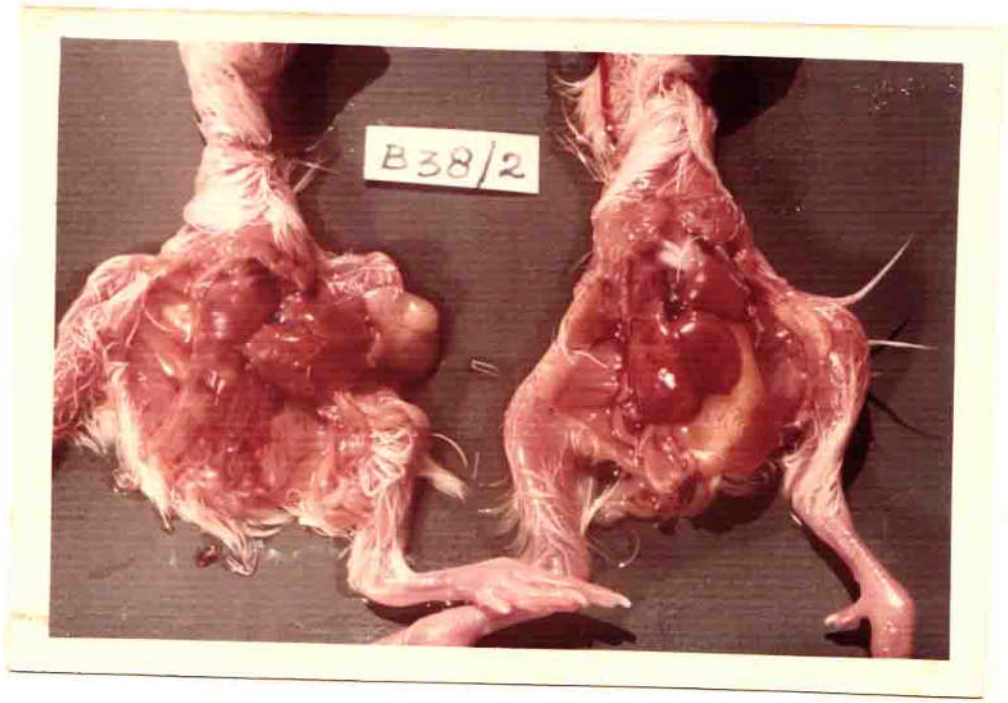



Fig. 4: Embryos inoculated with isolate No.B38/2 at 2nd passage level through CAM route at 12th day showing enlargement of liver specially the right lobe with general congestion and patches of peticheal haemorrhages and also congestion and haemorrhages in kidneys when opened after 5 days of post inoculation.

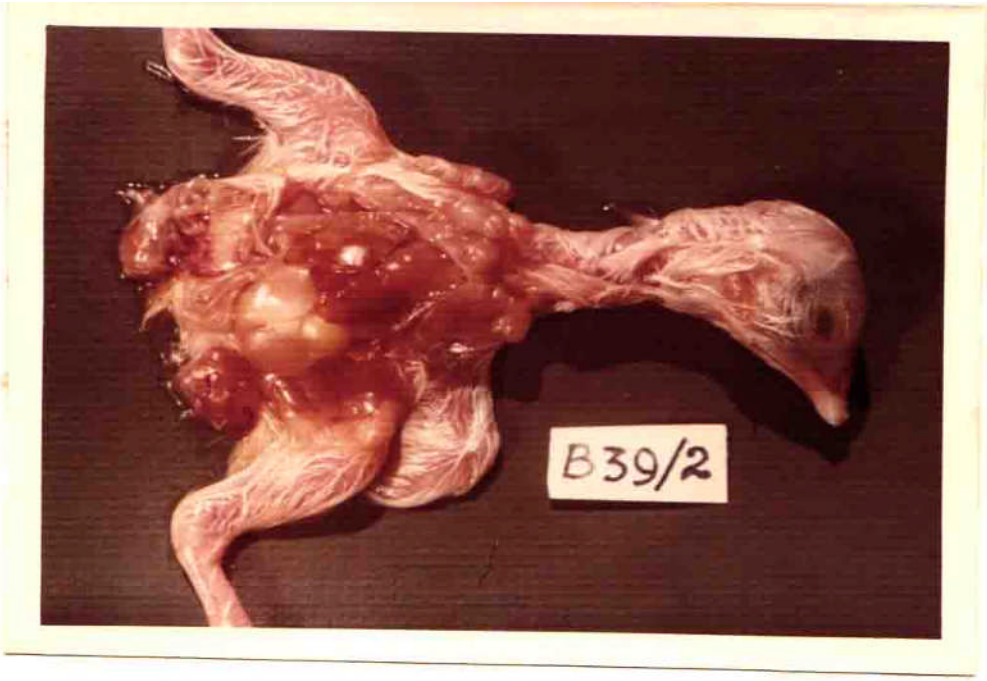



Fig. 5: Embryo inoculated with isolate No.B39/2 at 2nd passage level through CAM route at 12th day showing enlargement of right lobe with yellowish discolouration and left lobe congestion in left lobe when opened after 5 days of post inoculation.


Fig. 6: Embryos inoculated with isolate No.B40/2 at 2nd passage level through CAM route at 12th day showing very much enlargement and severe congestion of liver, greenish discolouration of liver when opened after 5 days of post inoculation.

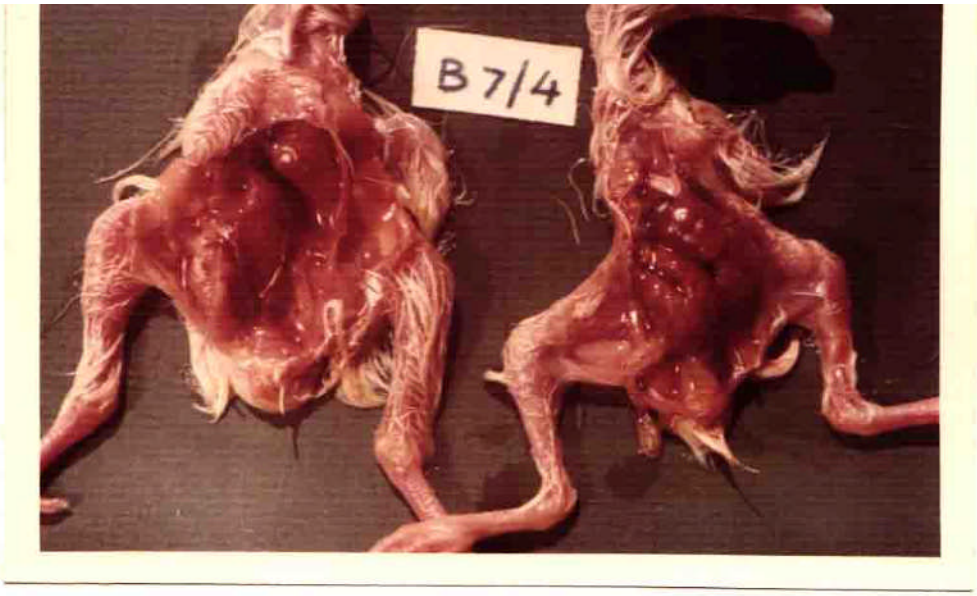



Fig. 7: Embryos inoculated with isolate of B7/4 at the 4th passage level through CAM route at 12th day showing congestion, enlargement with prominence of tubules of kidney, enlargement and congestion of liver when opened after 5 days post inoculation.

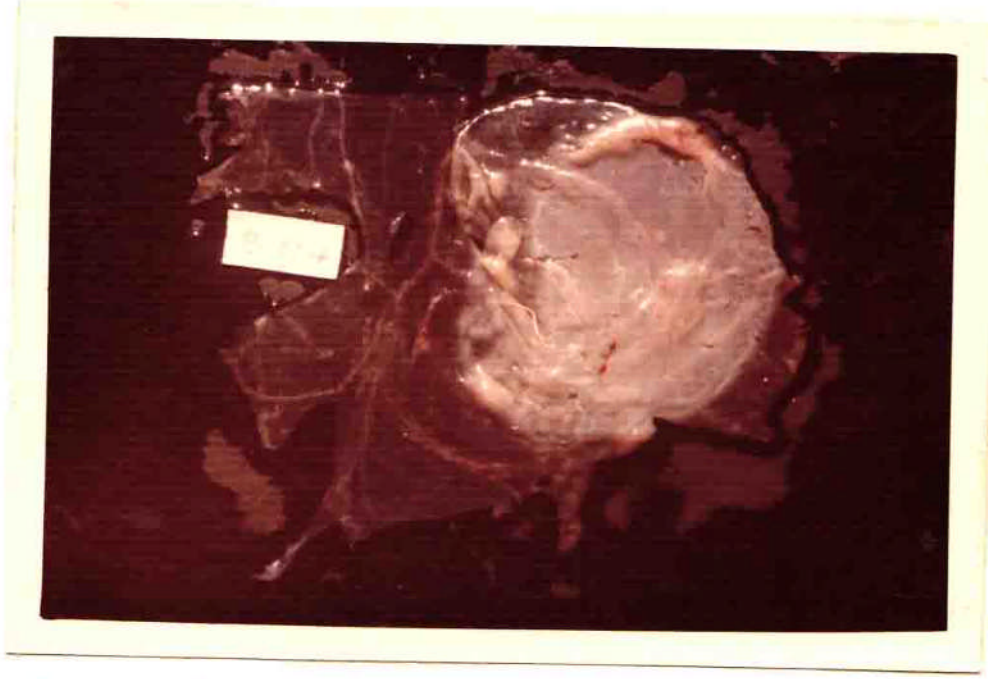
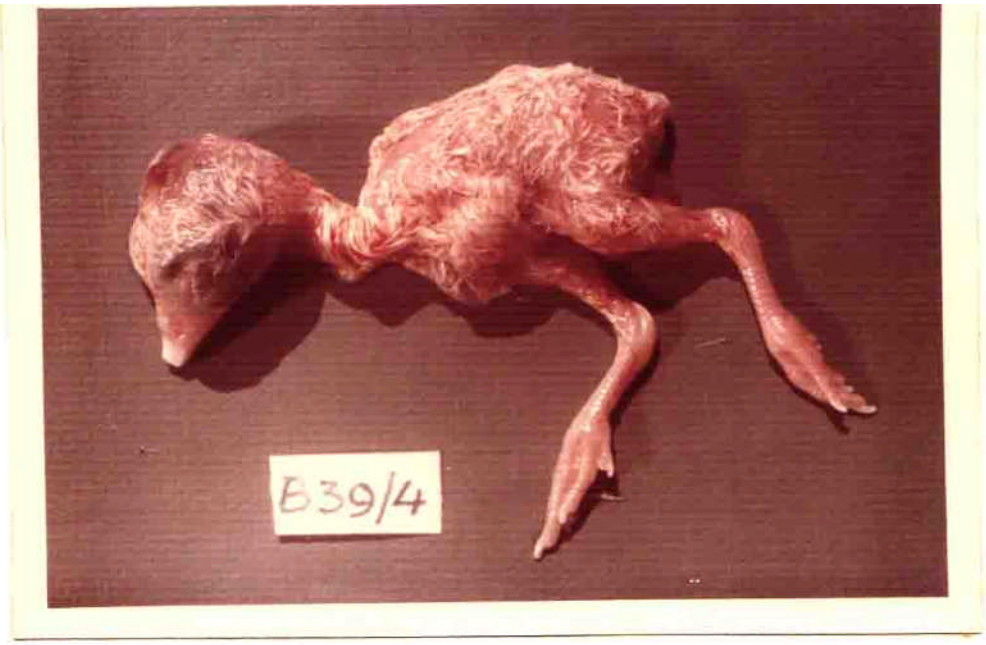


Fig. 8: CAM showing pronounced edema and necrosis with small white opaque pock like areas, while inoculated with isolate No.B7/4 at 4th passage level, when opened after 5 days post inoculation.




ig. 9: Embryos inoculated with specimen No. B39/4 at the 4th passage level through CAM route at 12th day showing subcutaneous haemorrhages stunting when opened after 5 days post inoculation.

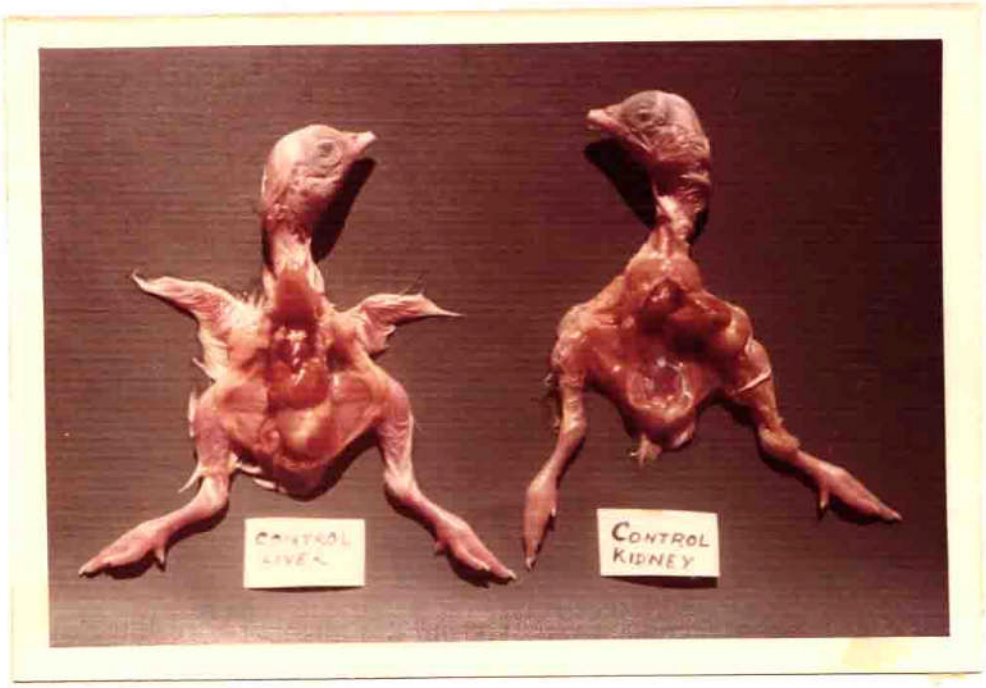



Fig.10: Control. Healthy embryos 17 days old are showing normal liver and kidney.

## 4. Cultivation of some of the isolates in chicken embryo kidney and chicken embryo fibroblast cell culture:

The isolates B7, B38, B39 and S were passaged in cell cultures of chicken embryos, kidney and chicken embryo fibroblasts. Three passages were given in both the cell cultures and the details are given in table 15 and table 16. Except slight toxic effect which were observed in both the systems of chickens embryo fibroblasts and chicken embryo kidney cell cultures, no other apparent change was observed, while three subsequent passages were made.

## TABLE 15

**Passage of some of the isolates in cell cultures**

**In chicken embryo kidney cell culture**

| Isolate No. | Passage No. | No.of tubes infected | Passage No. | No. of tubes infected | Passage No. | No. of tubes infected | Remarks |
|---|---|---|---|---|---|---|---|
| B7 | 1 | 5 | 2 | 5 | 3 | 5 | No cytopathic effect |
| B38 | 1 | 5 | 2 | 5 | 3 | 5 | -do- |
| B39 | 1 | 5 | 2 | 5 | 3 | 5 | -do- |
| S | 1 | 5 | 2 | 5 | 3 | 5 | -do- |

## TABLE 16

**Passage of some of the isolates in chicken embryo fibroblast cell culture**

| Isolate No. | Passage No. | No. of tubes infected | Passage No. | No. of tubes infected | Passage No. | No. of tubes infected | Remarks |
|---|---|---|---|---|---|---|---|
| B7 | 1 | 4 | 2 | 4 | 3 | 5 | No cytopathic effect |
| B38 | 1 | 4 | 2 | 4 | 3 | 5 | -do- |
| B39 | 1 | 4 | 2 | 4 | 3 | 5 | -do- |
| S | 1 | 4 | 2 | 4 | 3 | 5 | -do- |

# CHAPTER

# DISCUSSION

## DISCUSSION

The investigation on the problem of "Serological survey of infectious bursal disease in chickens and isolation of virus" revealed prevalence of precipitating antibodies against IBDV were 60.0, 20.0 and 17.3 percent in Andhra Pradesh; 16.6, 10.0 and 5.0 percent in Tamil Nadu; 52.1 and 14.0 percent in Uttar Pradesh and 50.0, 30.0, 30.0 and 10.0 percent in Karnataka amongst different breeds on various farms. The evidence obtained from this survey indicated that the distribution of precipitating antibodies against IBDV were quite prevalent in the sera samples collected from these 4 States. Benjamin et al. (loc.cit.) demonstrated the prevalence of 90.5 percent antibodies against IBDV in 19 to 21 flocks tested, Dorn et al.(loc.cit.) and Winterfield (loc.cit.) indicated that majority of the flocks tested possessed antibodies against IBDV. The present sera samples were taken only from a limited number of chickens and the sera of the suitable age group of birds (2-3 months) could not be obtained. Perhaps the low percentage of reactors might be due to these factors. This bottleneck made it impossible to assess the exact prevalence of this disease.

The limited time and scope for collection of sera samples put an obvious limitation to this work. However, since IBD is a highly infectious disease (Cosgrove, loc.cit.;

o et al., loc.cit.), finding out positive cases with sera mples collected from different parts of the country is ghly significant and indicated that the disease is widely evalent. With the limited time and facilities at hand, ra for the serological survey were collected only from farms of Andhra Pradesh, Tamil Nadu, Uttar Pradesh and rnataka and the results showed that the prevalence of the ease is a great concern in all the farms in these States. precipitation test was used usefully to detect the distri- ion of precipitating antibodies in chicken sera against V by Hirai et al. (loc.cit.) in Japan and the test was nd as a useful tool in our hand too.

During the routine postmortem examination the dead birds xperimental poultry farm (I.V.R.I., Izatnagar, Uttar lesh) revealed the typical lesions of IBD viz., enlargement ursa of Fabricius, lot of cheasy exudate in the bursa, ppy of bursa, haemorrhages on the muscles, nephrosis, es in the ureters, as mentioned by Cosgrove (loc.cit.); erfield and Hitchner (loc.cit.) and Rinaldi et al.(loc.cit.). this farm a collection of 66 specimens of bursa of icius and kidney were made and passaged in chicken embryos 5 times. The result of embryo inoculation revealed that od number of embryos were showing typical lesions of IBDV ne first passage level. Two of such materials were also

llected from Poultry Research Station, Madras. Out of ese two specimens only one showed good embryo lesions dicative of the presence of IBDV. Both the farms received number of week old chicks from various countries like rael, Australia, U.S.A. and Japan where IBD was repeatedly ported (Meroz, loc.cit., Shimizu et al., loc.cit., Winter-eld and Hitchner, loc.cit.).

Regarding the isolation of IBDV, it was found that a rge number of materials taken from typical cases of IBD elded a large percent (36.8%) of isolates. The isolates ich were suspected at the first passage level based on bryo lesions had shown lesions in 44 percent embryos at d passage level and did show 31.8 percent lesions in chicken bryos at 3rd passage level. The CAM route has been used for l the passages as described by Hitchner (loc.cit.) and CAM s the materials of choice. However, when this highly in-ctious disease was found to be widely prevalent and the bryos used were from the infected flocks, it was logical at those (embryos) would not support the growth of the virus grow in good titers at early passages. As in other viral eases, parental antibodies from immune dams through the yolk might have interfered with the virus growth (Gango-lhyaya and Mallick, 1970).

The virus isolate B7 was filtered through Millipore
ter of APD 0.22 μ and then grown in chicken embryos.
isolate B7 showed constant lesions (viz., edema and
rosis of CAM, liver showed congestion and enlargement,
ney also showed congestion) in all the 3 passages. This
late, grown after filtration, showed positive immuno-
fusion reaction with standard IBDV reference diagnostic
isera obtained through the courtesy of Dr. M. Pattison,U.K.
Dr. B.W. Calnek, U.S.A., confirmed the presence of IBDV.
isolate B38, which had shown similar lesions in embryos
those of B7, did also give positive immunodiffusion reaction
h antisera. All the isolates are judged by their lesions
mortality in embryos were obtained by pathological materials
wing similar lesions. All the isolates were from only one
m (Experimental Poultry Farm, I.V.R.I.) except one isolate
from Poultry Farm, Madras. Therefore, the confirmation
le for only two isolates (B7, B38). The rest of the speci-
ns showing similar embryo lesions were considered to be
itive for IBDV isolates. All the isolates could not be
nfirmed due to acute shortage of most valuable standard
ference sera which was obtained only in a very small quantity.

The confirmed isolates B7 and B38 were passaged between
h to 7th passages to study growth characteristics and
serving embryo lesions. The embryo lesions were produced

tantly from 4th to 7th passage level with each isolates.
was done as there has been paucity of informations regard-
the growth characteristics of IBDV in embryonating chicken
. Both these isolates were growing very well with constant
ons. The isolate B7 showed edema, necrosis and white
ue pock like lesions on the CAM, congestion and enlargement
iver, kidneys were also congested, with prominence of
les and embryo mortality of 20 to 15 percent. The isolate
showed comparatively less edema of CAM, liver and kidney
estion, one lobe of the liver was enlarged with greenish
olouration. However, the mortality pattern observed by
hner(loc.cit.) was not found to occur with these two iso-
s, that might be due to the fact that the embryonating
were obtained from the same flock where from the patho-
cal material for isolation of virus was also collected.
as, therefore, logical that the embryos would have got good
tity of maternal antibodies in the yolk and proper biolo-
l characters would not have been revealed by growing the
ates in such embryos.

To avoid the interference due to antibodies in embryonat-
eggs the isolates B7, B38, B39 and S were passaged in both
cken embryo kidney' and 'chicken embryo fibroblast' cell
ures upto 3rd passage level. None of the isolates had
n in either of the two cell cultures. Shimizu et al.(loc.cit)

mpted without success to propagate a strain of IBA in
ken kidney cell cultures. Rinaldi et al. (loc.cit.),
(loc.cit.) and Kosters and Paulsen (loc. cit.) described
propagation of IBA in chicken embryo kidney and chicken
ryo fibroblast cell cultures. Perhaps it would have been
irable to do more passages to get the virus adopted in
se two cell culture systems. Due to shortage of time more
sages could not be done.

Prevalence of precipitating antibodies can be detected
y comparatively for a short time in the birds after viral
ection and the sera samples should be obtained at suitable
which is the proper post infection time for this disease
natural occurrence. Suitable number of samples from a
rticular flock size should also be taken for such a survey
mar and Mallick, loc.cit.).

Regarding the representative isolation of virus, mate-
als should be taken from various farms situated at diffe-
nt parts of the country. The sera samples had been taken
ly from 8 farms in 4 States. The specimens for virus iso-
tion were collected only from two sources. Therefore,
sirable sampling of sera for serological survey and mate-
als for virus isolation could not be obtained due to obvious
mitation of time and other material facilities.

However, the detection of a high percent (60%, 52.1%, and 16.6%) of positive cases on immunodiffusion reaction loying an antigen prepared from the indigenous isolate 37 and confirmed by using an inactivated antigen prepared n standard U.S.A. strain (which was supplied by Dr. B.W. nek) has shown that the disease is prevalent in this ntry. This was further confirmed by getting a high percent .8, 32.3, and 17.6 in first, second and third passage level pectively) of IBDV isolates from the post-mortem material wing lesions of IBD. As mentioned earlier that two of se isolates were confirmed by using standard reference gnostic antisera from both U.K. and U.S.A.

Therefore, it has been proved that IBD is emerging in s country and it will depend on the future research worker undertake country wide systematic survey for this important ease and also study the virus in further details.

# CHAPTER

# SUMMARY

## SUMMARY

The Indian Poultry Industry for the production of animal protein in the form of eggs and meat needs tremendous improvement. In recent years the Government of India has launched the National Schemes to improve the poultry production, as this has got most quick prospect for enhancing the national economy. Poultry farming has been brought to the magnitude of an industry from the backyard poultry keeping with few birds. Intensive method of rearing has become essential due to obvious reasons. Management and balance nutrition are very necessary but suitable health care has become the key for success in poultry production.

Amongst many infectious diseases of viral origin Infectious Bursal Disease (IBD) has been reported increasingly from all parts of the world and emerging to be a problem in many parts of the world. An attempt was made to conduct serological survey and isolate the virus from natural cases from chickens on various organised farms in different States.

In our serological survey, precipitating antibodies against Infectious Bursal Disease Virus (IBDV) was detected in 71 individual sera samples out of 362 sera samples tested

by immunodiffusion reaction employing an antigen prepared from one of our isolates B7. Sera samples from Andhra Pradesh showed a high percentage (60%) of positive reactions. The antigen was prepared from the chorioallantoic membrane of infected chicken embryo. The positive cases varied from 5 to 60 percent among age groups of 2-12 months of the sera samples tested. The positive percentage was found to be higher at 3 months and 2 months of age in White Leghorn and broilers respectively. The positive percentage of sera samples from other States like Uttar Pradesh, Karnataka and Tamil Nadu were 52.1, 50.0 and 16.6 respectively. Of the 362 individual sera samples tested, 71 individual sera samples from the 8 farms have been found to possess antibodies against Infectious Bursal Disease. These eight farms are situated wide apart in four different States.

The antigen used for testing the field sera samples was prepared from the isolate B7 and was confirmed by the standard reference diagnostic antisera received from Dr. M. Pattison, U.K. and from Dr. B.W. Calnek, U.S.A. The precipitating antibodies detected with the indigenous antigen was also confirmed by testing with inactivated antigen received through the courtesy of Dr. B.W. Calnek. Another isolate (B38) was also confirmed by testing with the reference sera from both the U.K. and U.S.A. sources.

The materials collected for virus isolation was from the routine post-mortem cases of chickens showing lesions of enlargement of bursa of Fabricius, cheasy exudate in the bursa, nephrosis, urates in the ureters and haemorrhages in the muscles. Sixty eight specimens were collected both from post-mortem room and ailing chickens. All the sixty eight specimens were given three passages by taking chorioallantoic membrane for the subsequent passage into 12 day old chicken embryos, produced lesions in embryos similar to those of infectious bursal disease described by other workers like White Opaque Pocks and edema on the chorioallantoic membrane, enlargement and congestion and necrosis of the liver, congested and enlarged kidneys. Two isolates viz., B7 and B38 were passaged upto 7th passage level to confirm the constancy of lesions in embryos. The lesions produced by these two isolates were found to be persistant and very characteristic in 4th, 5th, 6th and 7th passage levels. The failure of mortality in the chicken embryos suggested that maternal antibodies from the dams might have been transferred in the egg yolk and inhibited the death of the embryos by interfering with the optimum growth of the virus.

The isolate B7 was filtered through Millipore filter of Average Pore Diameter 0.22 $\mu$ and showed similar growth characters and lesions in embryos as that of before filtration.

The observations made with embryo lesions, immuno-
fusion reaction and filtration studies suggested that the
ate B7 was an infectious bursal disease virus.

Attempts to cultivate four isolates of infectious bursal
ase virus in chicken embryo kidney and chicken embryo
oblast cell cultures had failed.

Therefore the prevalence of precipitating antibodies was
rved in 71 sera samples out of 362 sera samples from eight
s in four States of Andhra Pradesh, Tamil Nadu, Uttar
esh, Karnataka and isolation of number of isolates of
ctious bursal disease virus revealed that infectious bursal
ase is prevalent in the various parts of this country.
future work on the systematic countrywide survey will give
idea of exact prevalence of the disease and help in under-
ding the magnitude of this problem.

# BIBLIOGRAPHY

## BIBLIOGRAPHY

Adamesteanu, I. and Adamesteanu, C. (1968). Gumboro disease in chickens. Revtazooteh. Med. Vet., 18: 48-53. (Cited by Faragher, J.T., 1972, Vet.Bull.42:361-369).

Allan, W.H., Faragher, J.T. and Cullen, G.A. (1972). Immunosupression by the infectious bursal agent in chickens immunised against New Castle disease. Vet. Rec. 90: 511-512.

Asdrubali, G. and Gialletti, L. (1971). Diagnostic and pathogenesis of Gumboro disease by means of immuno-fluorescence. Nuova. Vet. 47: 223-229. (Biol.Abst. 54: 20523).

Badiola, C., Oliver, F. and Parla, J. (1969). Infectious bursal disease (GD). 20. (Vet. Bull. 39, abst.3926).

Barron, N.S. (1966). The kidney/liver syndrome in chickens. Vet. Rec. 78: 624-626.

Brady, J. (1970). Litter mites and their effect on poultry. Wld's Poult. Sci. J. 26: 658-668.

Bendheim, U. (1969). Prevention of Gumboro disease in chickens by the use of convalescent serum. Refuah. Vet.26: 25-27 (Vet. Bull. 39. abst. 4348).

Bengelsdorff, H.J. and Bernhardt, D. (1971). Serological examinations after vaccination of chicks with mouse-adapted infectious bursitis virus. Proc. 19th World Vet. Congr. Mexico. (Vet. Bull. 42. abst. 2537).

Benjamin, L., Antillon, A. and Fernandez, P. (1972). Identification of the infectious bursal disease virus in Mexico. Avian Dis. 16. 241-248.

Benton, W.J. (1964) Nephrosis nephritis syndrome (Gumboro disease). Proc. 8th Poultry Path. Conf. New Jersey. 10-11.

Benton, W.J., Cover, M.S. and Rosenberger, J.K. (1967 a). Studies on the transmission of infectious bursal agent (IBA) of chickens. Avian Dis. 11: 430-438.

Benton, W.J., Cover, M.S., Rosenberger, J.K. and Lake, R.S. (1967 b). Physicochemical properties of the infectious bursal agent (IBA). Avian Dis. 11:438-445.

Bolotnikov, I.A. (1966). Immunological characteristics of the viruses of Gumboro disease and avian infectious bronchitis. Veterinariya. 43: 8. 25-28 (Vet.Bull. 37, abst. 1704).

Bond, D.S., Edgar, S.A., Calderin, G.G. and Ramos, B.N.(1970). Infectious bursal disease in Puerto Rico broilers. Avian Dis. 14: 404-405.

Borzemska, W. and Golnik, W. (1969). A case of Gumboro disease (infectious bursitis diagnosed in Poland. Medycyna wet. 25: 644-645. (Cited by Faragher, J.T., 1972. Vet. Bull. 42: 361-369).

Bygrave, A. and Faragher, J.T. (1970). Mortality associated with Gumboro disease. Vet. Rec. 86: 758-759.

Cancellotti, F., Aprile, P.N. and Petek, M. (1972). Serological survey of Gumboro disease. Detection of antibodies in yolk samples by indirect immunofluorescence and neutralization in tissue culture. Nuova. Veterinaria. 48: 206-209 (Vet. Bull. 43. abst.2094).

Cheville, N.F. (1967). Studies on the pathogenesis of Gumboro disease in the bursa of Fabricius, spleen and thymus of chicken. Am.J. Path. 51: 527-551.

Chinn, W.T. and Coomber, D.H. (1966). Fatty liver and kidney syndrome. Vet. Rec. 78: 210-221.

Cosgrove, A.S. (1962). An apparently new disease of chickens. Avian nephrosis. Avian Dis. 6: 385-389.

Cumming, R.B. (1963). Infectious avian nephrosis (uraemia) in Australia. Aust. Vet. J. 39: 145-147.

Cunningham, C.H. (1966). A laboratory guide in Virology. Sixth ed. Burgess Publishing Company, Minnesota.

DelBono, G., Agrimi, P. and Braca, G. (1969). Gumboro disease. Annali. Fac. Med. Vet. Pisa. 21: 13-45 (Vet. Bull. 40, abst. 1318).

Devos, A., Viaene, N., Spanoghe, L. and Vanimpe, J. (1966). Comparative study of postmortem findings at the Gent Poultry Clinic during 1965. (Cited by Faragher, J.T. (1972).(Vet. Bull 42: 361-369).

Dorn, P., Kronthaler, P. and Schindler (1968). The course and control of Gumboro disease. Berl. Munch. tierarzt. Wschr. 81: 272-275. (Vet. Bull. 39, abst. 1300).

Edgar, S.A. and Waggoner, R. (1965). Pathogens of Coturnix coturnix japonica. Quail Quarterly 2, 11-14.(Cited by Faragher, J.T. (1972) (Vet. Bull. 42: 361-369).

Edgar, S.A. and Yung Cho (1965). Avian nephrosis (Gumboro disease) and its control by immunization. Poult.Sci. 44: 1366.

Faragher, J.T. (1971).Studies on Gumboro disease of the fowl. Ph.D. thesis (Cited by Faragher, J.T. 1972, Vet.Bull. 42: 361-369).

Faragher, J.T. (1972). Infectious bursal disease in chickens. Vet. Bull. 42: 361-369.

Fraser, D.M. (1964). Gumboro disease. A review. Vet. Rec.76: 430-431.

Gangopadhyaya, K. and Mallick, B.B. (1970). Studies on New Castle (Ranikhet) Disease. I. Serological reaction with 'F' strain vaccination in chickens hatched from eggs of immunized hens. Indian Vet.Jour.47: 931-938.

Gelenczei, E.F. and Lunger, P.D. (1970). Isolation of a reovirus from bursa of Fabricius of chickens affected by infectious bursal disease. J. Am. Vet. med. Ass. 156. 1270-1271.

Ghnne, P. (1968). Gumboro disease. Ann. Med. Vet. 112: 515-522. (Biol. Abst. 51: 62606).

Giron, P.C. (1969). Algunos aspectos de la nefrosis aviaria y de la enfermedad producida por el agents infeccioso de la bolsa de Fabricio en Mexico. Tec.Pecu.No.1. 98-104. (Cited by Faragher, J.T. 1972, Vet.Bull.42: 361-369).

Hanson (1967). Postmortem lesions diagnostic of certain poultry diseases. Vet. Rec. 80:109-119 & 122.

Hasegawa, I., Tomizawa, M. and Horiuchi, T. (1972). Pathological changes in the field chickens with Gumboro disease. Bull. Nat. Inst. Anim. Hlth. No.65. 13-16 (Natn. Inst. Anim. Hlth. 2.No.4.234).

Helmboldt, C.F. and Garner, E. (1964). Experimentally induced Gumboro disease (IBA). Avian Dis.8:561-575.

Hemsley, L.E. (1965 b). A survey of disease outbreaks in broiler chicken flocks and their economic importance. Vet. Rec. 77: 473-476.

Herceg, M., Mazija, H. and Kralj, M. (1971). Investigation of Gumboro disease in fowls. Vet. Arh. 41: 67-71.

Hirai, K., Shimakura, S. and Hirose, M. (1972). Immunodiffusion reaction of avian infectious bursal virus. Avian Dis.16: 961-964.

Hirai, K., Shimakura, S., Chang, C., Adachi, Y., Kawamoto,E., Taguchi, N., Suzuki, Y., Itakura, C., Funahashi,F., Tusushio, Y. and Hirose, M. (1973). Isolation of infectious bursal disease virus and distribution of precipitating antibodies in chicken sera. Jap. J. Vet. Sci. 35: 105-113.

Hitchner, S.B. (1970 a). The differentiation of infectious bursal disease (Gumboro) and avian nephrosis. 14th World Poultry Congr., abstr. Sci. Commun. Sect.ii.447.

Hitchner, S.B. (1970 b). Infectivity of infectious bursal disease virus for embryonating eggs. Poult. Sci.49: 511-516.

Hungerford, T.G. (1951). Diseases of poultry. 2nd ed. 243-246.

Kosters, J. and Geissler, H. (1971). Serological studies on the distribution of avian infectious bursitis. Tierartzliche. 26: 573-575 (Vet.Bull.42:abst.2543).

Kosters, J. (1971 b). Infectivity of the bursal agent for adult hens. Proc. 19th World. Vet. Congr. Mexico.790.

Kosters, J. and Paulsen, J. (1971). Multiplication of the causal agent of infectious bursitis of fowls, in chick embryo kidney cell culture. Zentralblatt fur Veterinarmedizin. 18B. 366-372.

Kosters, J., Becht, H. and Rudolph, R. (1972). Properties of the infectious bursal agent in chicken. Med. Microbiol. immunol. 157: 291-298.

Kumar, P.N. and Mallick, B.B. (1972). Studies on infectious bronchitis in chickens. Ind. J. Anim. Hlth. 11: 17-23.

Landgraf, H., Vielitz, E. and Kirsch, R. (1967). Occurrence of an infectious disease affecting the bursa of Fabricius (Gumboro disease). Dt. tieraztl. Wschr. 74: 6-10 (Vet. Bull. 37, abst. 2293).

Lensing, H.H. (1968). Gumboro disease. Tijdschr. Diergeneesk. 93. 1452-1460. (Vet.Bull.39, abst. 1299).

Leyk, W. (1971). Macroscopic and microscopic changes in chick embryos after experimental infection with the agent of infectious bursitis in pullets. Vet.Bull.42, abst. 3355.

Lukert, P.D. and Davis, R.B. (1970). Growth and characterization of infectious bursal agent in cell cultures. J. Am.Vet. med.Ass. 156: 1271.

Lunger, P.D. and Maddux, T.C. (1972). Fine structure studies of the avian infectious bursal agent. 1. In vivo viral morphogenesis. Avian Dis. 16: 874-893.

Luthgen, W. (1969). Gumboro disease. Vet. Med. Rev.No.1.3-17.

Luthgen, W. (1970). Vergleichende Untersuchungen zur Verbreitung der Gumboro-disease in Wirtschafts-und Rassegefluge-bestanden mit Hilfe des Bruteitestes. Dt.tierarztl. Wschr. 77: 61.63.(Cited by Faragher, J.T. (1972), Vet. Bull. 42: 361-369).

Malhotra, F.C. (1969). Pathology and Pathogenesis of infectious bursal disease. Diss. abst. 30B: 1436-1437.

Maire, C., Renault, L., Alamagny, A., Dreuille, M. and Albouy, R. (1969). Existence of Gumboro disease in France. Recl.Med. Vet. 145: 75-84 (Vet. Bull. 39: abst. 3927).

Mandelli, G., Rinaldi, A. and Cervio, G. (1966). Elementi di diagnosi differenziale tra le malattia di Gumboro e la nefrite nefrosi del pollo. Atti del 5° Convengo di Patologia Aviare, Varese, June 1966, 89-98. (Cited by Faragher, J.T. (1972), Vet. Bull,42:361-369).

Mandelli, G., Rinaldi, A., Cerioli, A. and Cervio,G.(1967). Aspetti ultrastrutturali delli borsa di Farizio nella malattia di Gumboro del pollo. Atti Soc.ital. Sci. Vet. 21: 615-619 (Cited by Faragher, J.T. 1972, Vet.Bull. 42: 361-369).

Mandelli, G., Rinaldi, A., Cessi,D., Cervio, G., Pascucci,S., and Valeri, A. (1969). Infezione sperimentale del pulcino di un gierno col virus della malattia di Gumboro. Atti Soc. ital. Sci. vet. 23:1070-1074. (cited by Faragher, J.T.(1972) Vet. Bull. 42:361-369).

Mandelli, G., Lodetii, E., Rinaldi, A. and Cervio, G.(1972). Cultural, cytological and ultramicroscopical characteristics of a strain of Gumboro disease virus (IBA I/PV). Folia Veterinaria. 2:399-425. (Vet.Bull.43).

Meroz, M. (1966). An epidemiological survey of Gumboro disease. Refuah. Vet. 23: 199-201. (Vet.Bull. 37, abst.5303).

Mazurkieziez, M. and Wachnik, Z.(1970). Infectious bursitis in chickens. Med. Vet. 26:165-168. (Biol.Abst.52, 102481).

Mohanty, G.C., Pandey, A.P. and Rajya, B.S. (1971). Infectious bursal disease in chickens. Curr. Sci. 40:181-184.

Mora,E.C. (1966). Virus infections of cultured macrophages and lymphacytes. Poult. Sci. 45: 1106-1107.

Moultrop, I.M. (1967). Infectious bursal disease (Gumboro). Proc. 70th Ann. Meet. U.S. Livestock Sanit.Ass. 438-439. (Cited by Faragher, J.T. 1972, Vet. Bull. 42: 361-369).

Nakano, M., Portugal, M.A.S.C., Saliba, A.M., Nobre,D. and Narimatsu, M.N. (1972). Outbreak of Gumboro disease in Brazil, Postmortem diagnosis. Biologica. 38: 60-61. (Vet.Bull. 42, Abst. 5821).

Ojo, M.O., Oduye, O.O., Noibi, L.M. and Idowu,A.L. (1973). Gumboro like disease in Nigeria. Trop.Anim.Hlth. Prod. 5: 52-56.

Parkhurst, R.T. (1964 a). On the farm studies of Gumboro disease in broilers. Avian Dis. 8: 584-596.

Parkhurst, R.T. (1964 b). Pattern of mortality in avian nephrosis. Poult. Sci. 43: 788-790.

Parkhurst, R.T. (1964 c). Avian nephrosis (Gumboro disease) in U.S.A. broilers: Treatment trails. Wld's Poult. Sci.J. 20: 208-211.

Petek, M. and Mandelli, G. (1969). Biological properties of a reovirus isolated in an outbreak of Gumboro disease. Atti. Soc. ital. Sci. Vet. 22: 875-879. (Vet. Bull. 39: abst.4246).

Peters, G. (1967). Histology of Gumboro disease. Berl. Munch.tieraztl. Wschr. 80: 394-396. (Vet.Bull.38. abst. 2851).

Provost, A., Borredon, C. and Bocquet, P. (1972). Two avian diseases new to chad: Infectious laryngotracheitis and Gumboro disease. Vet. Bull. 43: abst.1590.

Riggenbach, C. (1967). Appearance of Gumboro disease in Switzerland, Schweizer, Arch. Tierheik. 109: 398-400. (Vet.Bull.38. abst. 1576).

Rinaldi, A., Cervio, G. and Mondelli, G. (1965). Comparsa di una nuova forma morbosa dei polli negli allevamenti avicoli della Lombardia verosimilmente identificabile con la considdetta 'Malattia di Gumboro'. Sel.Vet. 6: 207-209. (Cited by Faragher, J.T. 1972, Vet.Bull. 42: 361-369).

Rinaldi, A., Mandelli, G., Cervio, G., Valeri, A., Cessi, D. Pascucci, S. (1969). Aetiology of Gumboro disease. I. Isolation and characterization of virus. IVth Congr. World Vet. Poultry Ass. 303-307. (Vet. Bull. 41. abst. 3376).

Rinaldi, A., Cessi, D., Cervio, G. and Lodetti, E. (1972). Attenuation of Gumboro disease virus and vaccination trials under laboratory and field conditions. Nuova Veterinaria. 48: 216-223. (Vet.Bull.43. abst.2534).

Schneider, J. and Hass, K. (1969). Investigation for serological diagnosis of infectious bursitis disease of chicken. Berl. Munch.tiear Wchr. 82: 270-272. (Biol.Abst. 51: 12045).

Shimizu, F., Shimizu, M. and Tsubahara, H. (1970). Interfering factor in chicken tissues infected with infectious bursal agent against Western equine encephalomyelitis virus. Natn.Inst. Anim.Hlth. 10: 92-93.

Shinizu, F., Hasegawa, I. and Tomizawa, M. (1971). Isolation of infectious bursal agent from field chickens in Japan. Nat. Inst. Anim. Hlth. 11: 4. 223.

Snedeker, C., Wills, F.K. and Moultrop, I.M. (1967). Some studies on the infectious bursal agent. Avian Dis. 11: 519-528.

Wagner, K. and Koster, J. (1968). Serological examinations into infectious bursitis in young pullets. Berl. Munch. Tierazti. Wschr. 81: 464-466. (Biol.Abst. 51:10302).

Wilson, P.H. (1964). Studies on the aetiology of an infectious bursal condition of chickens. M.S. thesis. (Cited by Faragher, 1972, Vet. Bull. 42: 361-369).

Witter, R.L. (1962). The diagnosis of infectious bronchitis of chickens by the agar gel precipitation test. Avian Dis. 6: 478-492.

Winterfield, R.W. and Hitchner, S.B. (1962). Aetiology of an infectious nephritis-nephrosis syndrome of chickens. Am.J.Vet.Res. 23: 1273-1279.

Winterfield, R.W., Hitchner, S.B., Appleton, G.S. and Cosgrove, A.S. (1962). Avian nephrosis, nephritis and Gumboro disease. L and M News and Views.3:No.5 (Cited by Faragher, J.T.(1972) (Vet.Bull.42: 361-369).

Winterfield, R.W. and Hitchner, S.B. (1964). Gumboro disease. Poult. Dis. 206-207.

Winterfield, R.W. (1969 a). Current observation on infectious bursal disease. Proc.18th Western Poulty Dis. Conf. 52-53.

Winterfield, R.W. (1969 b). Immunity response to the infectious bursal agent. Avian Dis. 13: 548-551.

Winterfield, R.W. and Faldy, A. (1971). Infectious bursal disease immunity. Proc. 19th World Vet. Congr., Medico. 502-504. (Vet. Bull. 42: Abst. 1865).

Winterfield, R.W., Fadly, A.M. and Bickford, A. (1972). Infectivity and distribution of infectious bursal virus in chickens persistence of the virus and lesion. Avian Dis. 16: 622-632.

Valdes, L.L.E., Lucio, M.B. and Antillon, R.A. (1971). Study of Gumboro disease clinically post-mortem and by immunofluorescence. Veterinaria. 2: No. 3-5-10 (Vet. Bull. 42: Abst. 5820).

Vitali, E. (1971). Gumboro disease. Practical note.clinica. Vet. 93: 468-472. (Vet. Bull. 41, abst. 4860).

Yung Cho (1968). A study of infectious bursal disease and its control by immunization. Diss. Abst.28B. 286-287.

Yung Cho, Edgar, S.A. (1969). Characterization of infectious bursal agent. Poult. Sci. 48: 2102-2109.