AN ATTEMPT FOR ISOLATION AND CHARACTERIZATION OF LEPTOSPIRA

THESIS

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D E D I C A T E D

TO

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C E R T I F I C A T E

Certified that the research work embodied in the thesis entitled "An Attempt for Isolation and Characterisation of Leptospira" is an authentic original work conducted by the candidate Smt. M.R. Rajeswari, in the Division of Bacteriology & Mycology, Indian Veterinary Research Institute, under my supervision and guidance.

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C O N T E N T S

1. INTRODUCTION

Leptospirosis is universally spread among man and other mammals. The infection is caused by various sero-groups of leptospira. It assumes considerable importance from the public health point of view. The disease is shown to be prevalent among domestic animals such as cattle, sheep, goats, pigs, horses and dogs. It causes considerable economic loss to the livestock industry, and poses a great challenge to veterinarians in controlling the infection effectively.

The spread of the disease among livestock has been well documented in U.S.A., NewZealand, Australia and Japan. Some serotypes appear to be confined to certain endemic areas and show a host-specificity.

Abortion and non-viable full term calves are the principal clinical features of leptospiral infection. Abortion usually occurs in the last quarter of pregnancy and most often after two to five weeks incubation period. The most common clinical features of bovine leptospirosis are icterohaemoglobinuria, a typical mastitis characterised by blood-tinged milk with homogeneous clots (Mitchell, 1959). Icterohaemoglobinuria is more common feature in calves (Sutherland et al., 1949; Field and Sellers, 1950).

Leptospires are also known to be aetiological agents of abortion among sheep and goats. In U.S.A. Beamer et al. (1953) described ovine leptospirosis among young pregnant ewes causing abortions.

Swine are one of the most important animal species associated with endemic and epidemic occurrence of leptospirosis in domestic animals and man. An epidemic in Israel among young pigs about four months old was reported by Hooden (1956). The animals exhibited fever, symptoms of anorexia, listlessness, weakness and convulsions.

Lyubashenko and Novikova (1947b) were the first to report clinical cases of equine leptospirosis in U.S.S.R. The disease was characterised by fever for two or three days, followed by jaundice, patechiae on mucous membranes, haemolytic anaemia and haemoglobinuria in the terminal stages.

Canine leptospirosis is also universally distributed and the disease spreads from dog to dog by direct contact, or by contact with urine or contaminated fomites or by water. The disease is usually associated by icterohaemorrhagiae and canicola serotypes. Other serotypes are also occasionally found to be responsible for disease in dogs. The clinical

manifestations vary with the infecting serotype. Okell et al. (1925) proved that 'yellows' was caused by L. icterohaemorrhagiae. L. canicola on the other hand rarely causes jaundice. The clinical manifestations are usually azotaemic uraemia due to severe changes in the kidney (Klarenbeek, 1927).

Leptospirosis is also prevalent in India. The first report of human leptospirosis was reported by Chowdry (1933) and in canines by Ayyar (1932). Many workers reported serological evidence of infection later. There are very few reports of isolation of leptospires from clinical cases in support of serological evidence. But the infecting strains have often been isolated by other researchers abroad employing suitable culture media and other laboratory animals.

Since the information available is meagre, in our country, an investigation was undertaken to isolate and identify the leptospires from infected and suspected cases of livestock. Studies on serological evidence were also included.

As the disease also spread by coming in contact with contaminated water from chronic carriers or shedders, attempts were made to isolate the strains from surface-water samples

and kidney suspensions of rodents.

In view of the obvious difficulties of maintaining large number of leptospiral serotypes by regular subculturing, a trial to preserve the laboratory culture of L. pomona under liquid nitrogen was also undertaken.

2. REVIEW OF LITERATURE

2.1 HISTORICAL

Leptospirosis as a distinctive clinical entity was first recognised in Germany on the basis of description given by Weil (1886). Weil categorised leptospiral jaundice as a separate entity and differentiated it from the other recognised diseases. This form of jaundice, which was designated as "Weil's disease" was reported by Goldschmidt (1887). Within next few years this disease was reported from Britain (Young, 1889) and several other European countries (Jaeger, 1892).

Stimson (1907) observed finely coiled spiral organisms with hooked ends in the renal tubules of a man who died with jaundice during an outbreak of yellow fever in New Orleans, U.S.A. He described the morphological characteristics of the organism and cautiously named it Spirochaeta interrorans.

Wolbach and Binger (1914) isolated Spirochaeta biflexa by filtration of pond water in U.S.A. Attempts to culture this organism in a variety of media were unsuccessful.

The aetiology of the leptospiral disease was

established conclusively with the isolation of causal agent by Inada et al. (1916) in Japan, by Uhlenhuth and Fromme (1916) and Huebner and Reiter (1916) in Germany. All these workers placed the organism in the genus spirochaeta.

Noguchi (1917) studied the Spirochaeta icterohaemorrhagiae of Inada and American strains from wild rats in the U.S.A. and he found that all these resembled each other in morphology and in immunological tests. Since these organisms differed in appearance and movements from the other spirochaetes, Noguchi (1918a) introduced a new genus named Leptospira (Gr. Lepto = thin, spira = a coil) for organisms of this description, and the organism was named Leptospira icterohaemorrhagiae. During the next decade more strains of Leptospira were isolated in different parts of the world from various disease syndromes resembling Weil's disease. Many of these differed from L. icterohaemorrhagiae in their antigenic structure and some other properties and designated as new serotypes.

Among the domestic animals, the dog had been known to suffer from certain clinical conditions resembling those which are now known to be caused by leptospiral infection. The zoonotic importance of canine leptospirosis was shown by Krumbein and Frieling (1916) who reported two cases of

typical Weil's disease among officers living in the same room along with a dog suffering from jaundice.

2.2 MORPHOLOGY OF LEPTOSPIRA

Brucke and Haagen (1939) studied microscopically the external appearance of non-pathogenic water spirochaetes. Two years later Ruska (1941) published a photograph of a leptospira but without any detailed description. Babudieri (1949) noticed that all leptospirae have a rigid axial filament extending from one end to the other forming their skeleton protoplasmic spiral, wound tightly round the axistyle.

It was homogeneous and opeque to the electrons and was accompanied throughout its length by an undulating membrane. Leptospirae usually have hooked ends. Leptospira measures 6.20 /um or more by 0.1 /um in diameter (Bergey's Manual, 1974).

The leptospires are actively motile and exhibit three types of motility (1) rapid rotation around the long axis or spinning, (2) translation to and fro shunting movements occurs sometimes with a distinct pause or rest before the direction is reversed, (3) sinuous and boring or serpentine movements beautifully exemplified in the flakes of

agar in semisolid cultures (Turner, 1970).

Leptospiral organisms stain poorly with the usual bacterial stains but they may be best demonstrated by silver impregnation methods.

2.3 ANTIGENIC CHARACTER

All the three components of leptospira, axistyle, cytoplasmic cylinder and enveloping membrane, are highly antigenic.

The animal and human leptospirosis is caused by various serotypes. According to the recommendation of sub-committee on the taxonomy of leptospira (Turner, 1971) all the serotypes belong to a single species Leptospira interrogans of nonospecific genus. There are nearly 130 serotypes under 18 serogroups and are distinguishable from each other by their antigenic structure. Leptospires are classified on the basis of cross-agglutination and agglutinin absorption techniques employing antisera prepared in rabbits. All antigenic types of leptospires possess a common somatic antigen (lipopolysaccharide) but vary in their surface or agglutinating antigens. Difference in surface antigens are most readily demonstrated by agglutination of suspensions of either living or formalinized organisms. Complement fixation

and precipitin procedures are also sometimes employed. An antigen specific for genus leptospira is recognised through the haemolytic test based on erythrocyte sensitization by extracts of leptospira.

The 18 serogroups are icterohaemorrhagiae (comprising 13 serotypes), Javanica (6 serotypes), celledoni (2 serotypes), canicola (11 serotypes), Ballum (3 serotypes), Pyrogenes (9 serotypes), cynopteri (3 serotypes), Autumnalis (13 serotypes), Australis (10 serotypes), Pomona (6 serotypes), Grippotyphosa (2 serotypes), Hebdomadis (25 serotypes), Bataviae (7 serotypes), Tarassovi (11 serotypes), Semaranga (3 serotypes), and Panama, Shermani and Andamana comprising one serotype each (World Health Organization, 1967). During the past few years at least three more serotypes have been recognised. These include galtoni in canicola group (Tedesco et al., 1969), Tunis in tarassovi group (Bakos, 1969) and Ceylonica in Javanica group (Wityananda and Sulzer, 1969).

Recent studies by Hennebery and Cox (1968) and Cinco and Petelin (1970) show that the saphrophytic water leptospires which include semaranga serogroup, comprise an antigenically heterogenous group of organisms, which can

be further sub-divided into 16 serogroups and numerous serotypes.

2.4 MEDIA USED FOR ISOLATION OF LEPTOSPIRES

Leptospires grow readily in artificial media containing solutions of inorganic salts. Some of the media are supplemented with pooled rabbit serum (7-10 per cent).

Noguchi's (1912) medium contained mainly nutrient agar 0.5 to 1.0 part, Ringer's solution 3 parts; rabbit's serum 1 part, citrated rabbit plasma 0.5 part. The ingredients are sterilised separately and mixed.

Vervoort's (1923) medium consists of solution of 0.1 per cent peptone and 0.05 per cent sodium chloride in tap water, buffered by the addition of 5 to 10 per cent of phosphate solution and adjusted to pH 7.2. To this is added 10 per cent of inactivated rabbit serum or one drop per millilitre of whole rabbit blood. This was later modified by Wolff (1954).

Babudieri (1961) cited a modification (Dinger, 1932) of Noguchis medium. Tap water 100 ml, 6.0 ml of 3 per cent agar. These were sterilised in autoclave and cooled before 10 per cent inactivated rabbit serum was added.

The following media each of which is enriched with inactivated rabbit serum have proved to be reliable for the isolation and maintenance of leptospira strains :

1. Fletcher's medium (1923)
2. Korthof's medium (1932)
3. Stuarts medium (1946)
4. Tryptose phosphate agar medium (Cox and Larson, 1957)

Some rabbit sera (about 1-2 per cent) contain antileptospiral factors(Johnson and Muschel, 1966; Ryu, 1964) or leptospiral antibodies (Fuzi and Csoka, 1960; Nicolescu and Olinesco, 1968) which tend to inhibit the growth of leptospires. It is therefore advisable to use heat inactivated and Seitz filtered serum pooled from 20 or more preferably 100 rabbits and incorporated in culture media (Galton et al., 1962; Turner, 1970).

2.6 ISOLATION

Isolation of leptospires could be attended by direct or indirect culture from experimentally inoculated laboratory animals exposed to morbid materials from suspected cases (Cruickshank et al., 1975).

Direct cultures are usually attempted from freshly

drawn venous blood and cerebrospinal fluid from human cases during the first 1-10 days of illness while the patient is still febrile and before antibiotic treatment. Gochenour et al. (1953) inoculated minimal quantities of blood into medium tubes. Neil et al. (1956) inoculated serial dilutions of blood.

Menges et al. (1958, 1960) obtained good results with cultures made with descending dilutions of urine collected from dogs by bladder tap with strict aseptic precautions. Roth et al. (1961) and Robertson et al. (1964) claimed success in isolation of leptospires from naturally voided urine by dilution method.

Galton (1959), McGowan and Karstad (1965), Sulzer et al. (1968) attempted cultures of leptospires from pieces of kidneys from fatal cases as well as from abattoir material in connection with surveys of leptospiral infection in animals.

Kenzy et al. (1958), Yanagawa et al. (1963), Stalheim (1966) indicated that kidney tissue suspensions contained substances which in dilutions of 1:50 to 1:500 inhibited growth of leptospires but at higher dilutions (1:5000) there was no effect.

Podgwaite et al. (1955) from cattle, Beamer et al.

(1953) from sheep, Bryan et al. (1953), Pavlovic and Matic (1964), Manirique and Roberts (1968) from pigs isolated leptospires successfully from the aborted foetuses.

Fennestad and Borg-Petersen (1958, 1958a) stated that the possibilities of isolation of leptospires from the aborted bovine foetal materials depend to a great extent on the time lapsed between foetal death and abortion. The isolation attempts usually failed due to progressive autolytic changes if the foetus remained in uterus for 24 hours or longer after its death.

Various workers used different laboratory animals as a supplementary procedure in isolation work. Hesden (1954) used gerbilles (Meriones), Roberts and Turner (1968) used chinchilla, Byrne et al. (1965), Fisher et al. (1958) utilized 1 to 2 day old chicks; Burki (1960) used hamsters; Faine (1962) inoculated Swiss mice less than 10 g and Turner (1970) used deermice (Peromyscus).

Stavitsky (1945) found sulphadiazine as useful agent to inhibit the contaminating organisms in the clinical material. Galton et al. (1962) obtained encouraging results with vancmycin and bacitracin. Johnson and Rogers (1964) found 5 fluorouracil very active in inhibiting the growth

of many bacteria and at the same time not affecting the growth of leptospires in concentration of 200 to 400 µgm per millilitre of medium. Turner (1970) widely used this substance for primary isolation.

2.6 DEMONSTRATION OF LEPTOSPIRES

Wolff (1953) described Schuffner's technique of staining with Giemsa whereby leptospires were stained violet. Hoyer (1956) reported a method of negative staining with Congo red. Ryu (1963) claimed good results with basic fuchsin after treatment of smears with sodium bicarbonate. Blender et al. (1964) developed a single technique of silver staining. The tissue impression smears, urine sediment and culture smears were first treated with an alkaline formalised solution of tannic acid and ferric chloride for 5 to 10 minutes and then after washing, stained with silver nitrate solution. The leptospires appeared as black spirals on a golden brown background.

Sturdza et al. (1966) described a procedure of dry dark field microscopy for examination of tissue impression smears. Dried impression smears of kidney cortex were covered with 10 per cent acetic acid for 5 to 10 minutes and then washed with water. Thick smears were also treated

with 0.25 per cent trypsin for 1 to 3 minutes. When examined with dry dark field illumination, leptospires were detected in kidneys of 12 out of 72 slaughtered pigs compared with only one when untreated cell suspensions were examined.

Lillie (1954) demonstrated leptospires by Lavaditi's method in specially prepared silver impregnated tissue sections. Warthin and Starry (1920) described a procedure whereby paraffin sections could be stained with silver solution. Bridges and Luna (1957) further improved this method. The technique followed by Fielding (1941) gave good results.

White and Ristic (1959), White *et al.* (1961) employed fluorescent antibody technique (FA) for detecting leptospires in naturally infected bovines and dogs. Maestrone (1963) adopted same technique and demonstrated leptospires in various tissues of aborted bovine equine and swine foetuses. Smith and Reynolds (1966) and Smith *et al.* (1967) concluded that while cultural and histopathological examinations were superior to FA technique in demonstrating leptospires in fresh materials, FA technique was superior to other methods in detecting leptospires in contaminated and autolysed materials. Cook (1970) found FA technique as good as silver staining methods for demonstration of leptospires in tissues.

Henry et al. (1971) used specific immunofluorescence staining for detection of leptospires in soil and water under laboratory and field conditions.

2.7 SEROLOGICAL TESTS

Martin and Pettit (1918) reported agglutination of leptospires by antibodies in the serum.

The microscopic agglutination test using living organisms as the antigen (MAL test or the agglutination lysis test of Schuffner and Mochtar, 1927) was considered to be the standard reference procedure for sero diagnosis of leptospirosis and for evaluating the sensitivity of other tests.

Stoenor (1954) and Howarth (1956) described capillary tube and plate agglutination tests. Galton et al. (1958) devised an improved macroscopic slide test where antigen pools comprising three antigens per pool were used for preliminary screening followed by tests with individual antigens of the reacting pools. Turner and Reed (1965) regarded this test less favourable with animal sera. Stoenor (1953, 1954) reported a good correlation between the results obtained with the plate agglutination and microscopic agglutination tests.

Four to 14 day old fluid cultures having uniform suspension of approximately 200 million organisms per millilitre were used as antigen so that the final serum antigen mixture should contain approximately 100 million organisms per millilitre (World Health Organization, 1967). Turner (1968) recommended that highest serum dilution in the serum antigen mixture showing 50 per cent agglutination i.e. agglutinated clumps with less than 50 per cent free organisms represent the end point reaction or the agglutinin titre. The World Health Organization (loc. cit.) suggested inclusion of representative serotypes of 14 serogroups in the test.

Sturdza and Elian (1961), Elian and Nicoara (1964) reported that complement fixation (CF) test with group specific antigen prepared from serotype patoc of Semaranga serogroup gave good results for screening human sera from cases suspected of leptospiral infection. Yark (1952), Moore and Rice (1956) found CF test was useful for screening animal sera. They detected pomona infection using homologous antigen. Sturdza et al. (1960), Robertson and Boulanger (1963), Nicolescu and Lelutiu (1967) reported unsatisfactory results by using single antigen for detecting heterologous infection. Robertson and Boulanger (loc. cit.), Nicolescu (1967) suggested that mixed or pooled antigen prepared from

several serotypes prevalent in the given area can be used to detect infections caused by heterologous serotypes.

Chang and McComb (1954), McComb et al. (1957) observed broad serological reactivity with sensitised erythrocyte agglutination (HA and SEA) test. Cox (1955) observed same results with sensitised erythrocyte lysis (HL & SEL) test.

Torten et al. (1966) using a group specific patoc antigen which was treated with suspected human serum samples in dilutions ranging from 1:50 to 1:450 and then stained with 1:100 dilution of rabbit antihuman fluorescein conjugated serum, observed nearly 100 per cent correlation between indirect fluorescent antibody (IFA) test and the microscopic agglutination test. Hirschberg et al. (1968) also reported similar results with the IFA technique using antigen of individual serotypes of leptospira. Burger and Fuchs (1968) stated that IFA technique was more sensitive than agglutination technique on the basis of examination of 556 bovine serum samples of which 39 contained agglutinins of one or more leptospiral serotypes.

Combiescu et al. (1958, 1960), Addamiano and Babudieri (1963) and Correa et al. (1970) correlated positive agglutination reactions between patoc and the antigens belonging to

other leptospiral serogroups. Addamiano and Babudieri (loc. cit.) observed that high percentage of serum samples from animals (cattle, horse, pig, sheep and dog) which were positive for agglutinins to pathogenic leptospires gave negative results with patoc and sao paulo antigens.

2.8 INTERPRETATION OF AGGLUTININ TITRES

Broom and McIntyre (1948); Babudieri and Gaspardis (1959, 1965); Turner (1968) regarded the low antibody titres observed frequently in the animal sera represent residual titres consequent to the previous infection.

Bohl and Ferguson (1952), Borg-Petersen and Fennestad (1962), Wolff et al. (1962), Turner (1968), Lepherd (1969), Shotts and Hayes (1970) and others accepted agglutinin titres of 1:100 and higher observed with living antigens as significant of present and past infection. Johnson (1939), Field and Sellers (1950), Michna (1969) considered agglutinin titres of 1:10 to 1:30 with 100 per cent agglutination lysis as significant. Turner (1968) considered 1:10 titre significant with 75 to 100 per cent agglutination with formalised antigen while others considered 1:300 (Keast et al., 1964) or 1:400 or higher titres (Alexander and Evans, 1962) as reliable evidence of infection. Fuhner (1951)

reported cross reactions with other serotypes in the serogroup and multiple heterologous reactions with serotypes belonging to other serogroups. Broom (1953) confirmed these findings. According to Alexander et al. (1963) multiple heterologous reactions may be due to successive infections with different serotypes. Topciu et al. (1967) believed that polyvalent reactions represent cross reactions resulting from a single infection.

2.9 INCIDENCE OF LEPTOSPIROSIS AMONG FOREIGN ANIMALS

The first report of leptospirosis in cattle was published in U.S.S.R. by Michin and Azinov (1935) who isolated *L. grippotyphosa* from calves with acute infectious haemoglobinuria. Semskow (1941) observed the disease in swampy areas of southern Russia especially during the months of May and August. Within next few years the disease was reported in many other countries such as Australia (Johnson, 1942), Argentina (Savino and Rennella, 1944), United States of America (Jungherr, 1944). Nikolajev (1946) also identified the disease in Russia. Boulanger and Smith (1957) recorded serological studies with cattle sera in Canada.

Bernkopf (1946) and Bernkopf et al. (1948) isolated *L. hovia* from cattle during an outbreak in Israel and later

detected a high percentage of significant reaction in bovine serum samples from several other middle Eastern countries. Sutherland et al. (1949) elucidated the aetiology of red water disease of calves in Australia and isolated L. pomona. Bovine leptospirosis due to L. pomona infection has been recorded from Italy by Babudieri (1949b). Field and Sellers (1950) reported leptospirosis in England. Te Punga and Bishop (1953) in NewZealand; Hooden (1955a) in Israel; Borge-Petersen and Fennested (1956b) in Denmark recorded leptospirosis. Morse and Melhutt (1956) recorded experimental leptospirosis with L. pomona in pregnant heifers. Turner et al. (1958) isolated serotypes canicola from urine of a sick two-day old calf whose dam had homologous antibodies. Karasch (1964) isolated the serotypes canicola, ictero haemorrhagiae and grippotyphosa from slaughtered cows.

2.9.1 Cattle

The first isolation of L. hardjo from cattle was made by Roth and Galton (1960) in Louisiana, followed by Robertson et al. (1964) in Canada. Sullivan and Stallman (1969) and Sullivan and Callan (1970) reported isolation of L. hardjo from cattle in Australia.

Kujumgiev (1963) in Bulgaria reported leptospirosis

in buffaloes. Ryu (1969) recorded positive findings to 10 leptospiral serotypes in sera of buffaloes of Taiwan, Malaysia and Thailand. Michna and Campbell (1969) isolated Leptospira sejroe from the kidneys of aborting cattle.

2.9.2 Sheep

Wirth (1937) was the first to report serological evidence of leptospiral infection in sheep. Nefed'ev (1949) reported grippotyphosa infection in various parts of Russia. Schlossberger (1951) found significant titres of ictero-haemorrhagiae antibodies in 15 sheep in West Germany. Hartley (1952) reported two outbreaks of leptospirosis with pomona in sheep and lambs. He could find out leptospires on histopathological examination of liver and kidney tissues. Seddon (1953) reported leptospirosis in sheep in Australia. Benner et al. (1953) reported ovine leptospirosis with the history of abortion. Ovine leptospirosis was also reported by Hochmann (1955, 1957) in East Germany; Karakasevic (1957) in Macedonia; Hakioglu (1956) in Turkey; Rafyi and Maghami (1959) in Iran; Cacchione et al. (1961) in Argentina.

2.9.3 Goats

Wirth (1937) reported caprine leptospirosis caused by icterohaemorrhagiae. Semskov (1941) reported outbreaks

of grippotyphosa infection simultaneously in bovines and goats in U.S.A. Hoeden (1953c) reported outbreaks of leptospirosis in goats caused by grippotyphosa in Israel. Humbert (1955) recorded caprine leptospirosis associated with L. pomona infection in U.S.A. Hartley and Hakioglu (1970) described an outbreak of icterohaemoglobinuria in goats which was attributed to grippotyphosa with possible association with plant poisoning.

2.9.4 Pigs

Alexander et al. (1964) showed that swine were one of the most important animal species associated with endemic and epidemic occurrence of leptospirosis in domestic animals and man. Lorey (1932) putforth serological evidence of L. icterohaemorrhagiae infection in pigs. Klarenbeek and Winsser (1937) isolated serotype icterohaemorrhagiae from a jaundiced piglet. Johnson (1939) reported isolation of pomona from pigs in Australia. Pomona infection in swine was recognised by Mochtar (1940) in Indonesia, Gsell and Rimpau (1944b) in Switzerland; Savino and Renella (1944) in Argentina.

Another serotype, canicola which is frequently associated with swine leptospirosis was first isolated in

pigs by Williams et al. (1963) in U.S.A. Seiler et al. (1956) recorded high rate of canicola infection in pigs by serological investigations. Hoeden (1956) reported several outbreaks of swine leptospirosis caused by canicola. Kemmones et al. (1962) reported canicola infection as a cause of abortion and reproductive failures in pigs. McErlean (1964) and Michna (1965) showed that L. canicola caused swine abortion. Manirique and Roberts (1968) isolated a strain of canicola from aborted foetuses of swine.

Collier (1948) in Indonesia, reported serological evidence of leptospiral infection in pigs with serotypes autumnalis and pyrogenes. Fennestad (1956) documented serological evidence with grippotyphosa, bataviae, sejroe and poi in Denmark. Kmety et al. (1966) reported ballum infection in Czechoslovakia. Liebermann and Muller (1961) demonstrated antibody titres for bataviae, grippotyphosa and icterohaemorrhagiae in sows with abortion history. Hanson et al. (1971) isolated grippotyphosa from a sow.

2.9.5 Horse

Kathe (1943) reported the presence of grippotyphosa agglutinins in apparently healthy horses in Germany. Lyubashenko and Novikova (1947b) reported clinical cases of

equine leptospirosis in Russia. Rimpau (1947) pointed out the relationship between the recurrent iridocyclitis (periodic ophthalmia) and leptospiral infection in equines on the basis of the presence of leptospiral antibodies in the serum of affected animals. Yager et al. (1950), Bohl and Ferguson (1952) in U.S.A., Gsell (1952) in Czechoslovakia, Rossi and Kolochine-Erber (1954) in France, Kemenes et al. (1960) in Hungary agreed with Rimpau's findings by their observations.

2.9.6 Dogs

Canine leptospirosis, which is usually associated with ictero-haemorrhagiae and canicola infections, is world wide in distribution and reported to occur in at least 68 countries (Animal Health Yearbook, 1968). Gkell et al. (1925), Klarenbeek (1938), Hills (1948) recorded leptospirosis in dogs caused by icterohaemorrhagiae infection (Yellow's) characterized by acute course and accompanied by jaundice. Bloom (1953) noted higher incidence in the males than in females. Alston and Broom (1958) showed that the incidence of canine leptospirosis was quite high in most of the European countries by examining serum samples for leptospiral antibodies. Hoeden (1953) showed the serological evidence of

canine leptospirosis caused by several other serotypes which included grippotyphosa, pomona, bataviae, autumnalis, australis and a number of serotypes of Hebdomadis serogroup. Alexander et al. (1957) showed that dogs in U.S.A. harboured at least 10 leptospiral serotypes. Topsiu et al. (1973) isolated serotype bataviae from a dog in North Vietnam. Carlos et al. (1971a) isolated serotypes autumnalis, icterohaemorrhagiae, pyrogenes and grippotyphosa in Philippines.

2.9.7 Rodents

Anderson and Wagles (1931) recovered leptospiral strains from rats. Galton et al. (1962) considered small rodents especially rats as the primary carriers of leptospires.

2.10 INCIDENCE OF LEPTOSPIROSIS AMONG ANIMALS IN INDIA

Chowdry (1903) was the first worker to record the occurrence of leptospirosis in India in Andaman islands in human cases. This was later recognised as Weil's disease. Later on Wooley (1911, 1913) and de Castro (1922) also investigated jaundice cases in Andamans. But Barker (1926) had the credit of presenting microscopic evidence of the presence of leptospires in the morbid materials of affected cases. Taylor and Goyle (1931) isolated a number of strains of Andamana and grippotyphosa serotypes in Andamans.

DasGupta (1939, 1940) also reported isolates of grippotyphosa and andamana strains. Tripathy (1977) recorded serological prevalence of leptospirosis in cattle, sheep and goats.

2.10.1 Cattle

Adinarayan et al. (1960) reported the occurrence of leptospirosis among cattle population of a farm in Uttar Pradesh. Venkataraman and Jagannathan (1961) reported an outbreak of leptospiral infection in bovine in Madras State. Pandey and Sekariah (1961) tested sera samples of 1500 healthy cattle and buffaloes slaughtered in Bombay, Calcutta and Madras. They reported that 4.4 per cent of these animals were positive for L. pomona and L. grippotyphosa. Later several States have since then documented serological screening. The status of the disease in the Indian sub-continent has been reviewed by Bhatnagar et al. (1967); Rao and Surendran (1970); Palit and Sharma (1971a) and Rajasekhar and Manjiah (1971).

Khera (1972) in studying epidimiological aspect of the disease, recorded serological evidence of leptospirosis in buffaloes of various States of India. James and Adinarayan (1973) reported the isolation of leptospires from liver of a foetus whose dam had antibody titre against L. wolffi serotype. Murthy and Khera (1973, 1974) investigated prevalence of

leptospirosis in India by screening 1908 sera samples and reported positive findings. Singh and Uppal (1973) projected the prevalence and distribution of leptospirosis in relation to livestock health and productivity in India.

2.10.2 Sheep

In India serological evidence of leptospiral infections in sheep have been recorded by many workers. Ball and Sheik (1958) examined random serum samples in Bombay region and found significant agglutinin titres of various leptospiral serogroups. Mukherjee et al. (1962) found high titres of leptospiral antibodies in the serum samples of the sheep from a flock in U.P. where several abortions had occurred in the flock. Sawhney and Saxena (1967) recorded agglutinin titres of leptospiral antibodies with sheep serum. Khera (1972) has established serological evidence of leptospiral disease in sheep in various parts of the country. He further evidenced the predominant prevalence of L. hebdomadis infection in sheep.

2.10.3 Goats

In India, Ball and Sheik (1958) attempted a serological survey of the capridae and encountered a number of reactors. Pande and Sekariah (1960) claimed to have recovered cultures

of *L. pomona* and *L. sejroe* (Hebdomadis group) from goats. Mukherjee *et al.* (1962), Pargaonker and Ramakrishna (1963), Pargaonker (1964) resorted to serological screening to elucidate the incidence. Iyer and Nanda (1965) recognized the white spot lesions in the kidney of goats and suspected it to be due to leptospirosis. Sawhney and Saxena (1967), Bhatnagar et al. (1967) and Sawhney (1968) recorded serological incidence of leptospirosis. Kharole and Rao (1968) demonstrated leptospira in sections of 11 kidneys and living organisms in two fresh specimens under dark field microscopy. Khanna and Iyer (1971) have reviewed and discussed the pathology of white spot lesions. Serological results to substantiate the incidence of caprine leptospirosis were recorded by Palit and Sharma (1971a); Rajasekhar and Ranjith (1971). Khera (1972) recorded evidence for the predominance of *L. pomona* serotypes in goats. Nigam *et al.* (1974) reported serological evidence of *L. pyrogenes* infection in 2 bucks and one aborted goat out of 82 animals screened.

2.10.4 *Pigs*

In India Bhagwat (1964) established the incidence of leptospirosis among pigs. Sawhney and Saxena (1967) detected agglutinins against *L. pomona* in a fatal case of a pregnant sow. Balaprakasam and Seshadiri (1968) recorded an interesting

case of peripheral leptospiraemia in piglets. However, tissue sections did not reveal the organism. Bhatnagar et al. (1967), Rajasekhar and Nanjiah (1971) recorded serological evidence of leptospirosis in pigs.

2.10.5 Horse

In India, excepting the serological findings reported by Ball and Sheik (1958), Sawhney and Saxena (1967), Rajasekhar and Nanjiah (1971) and Rajasekhar et al. (1977), very little information is available on clinical and other aspects of equine leptospirosis.

2.10.6 Dogs

Ayyar (1932) was first to report the occurrence of leptospiral disease in canines, and serologically confirmed the disease due to *L. icterohaemorrhagiae*. DasGupta and Sen (1945) isolated the culture of *L. canicola* from a dog and Joseph and Kalra (1966) recovered a strain of *L. icterohaemorrhagiae* from a case of jaundice in dog. Bhatnagar et al. (1967), Ball and Sheik (1958) and Rajasekhar and Nanjiah (1971) reported significant titres of agglutinins of various leptospiral serotypes in dog serum samples.

2.10.7 Bolenta

DasGupta (1940) from Calcutta and Lahiri (1941) from Bombay isolated unidentified serotypes of the icterohaemorrhagiae group from murine species.

2.11 LONG TERM PRESERVATION

Kirchner and Graham (1959) advocated the use of solid media for preserving leptospires for longer periods. They stabbed the cultures into solid media in tubes. Rosseler and van Rell (1966) reported that leptospires can be preserved successfully by the use of liquid nitrogen. Alexander et al. (1972) reported successful preservation of leptospiral cultures under liquid nitrogen. Otsuka and Manaka (1961) reported exceptional and promising results of preserving leptospiral strains by freeze drying. Annear (1956) reported successful drying of concentrated leptospiral cultures on plugs of starch peptone and subsequent recovery of leptospiral growth in liquid media.

3. MATERIALS AND METHODS

3.1 MATERIALS COLLECTED FOR ISOLATION OF LEPTOSPIRA

Tissue samples, urine and blood were collected with strict aseptic precautions for isolation of leptospira. Uterine discharges from aborted animals, pieces of kidneys, liver and lungs from aborted foetuses were also collected for the above purpose. Urine samples were procured from animals with the history of abortion and reproductive disorders. Kidney pieces from slaughtered animals, wild rats and laboratory mice were examined.

During the investigation three hundred and twenty four tissue specimens, 43 urine, two blood and eight surface water samples were used for isolation work. Table 1 shows details of materials collected from different species of animals for investigation. Eight surface water samples were also included in the work.

3.2 CULTURAL PROCEDURES

3.2.1 Culture Media

During the course of study following culture media were used for the maintenance of various leptospira strains and for bacteriological examination of specimens.

Table 1

Statement showing total number of tissue specimens collected from different species of animals and number of water samples tested

Sl. No.	Type of material collected	SPECIES OF ANIMALS								Total
		Cattle	Sheep	Goat	Pig	Dog	Wild rats	Swiss mice	Water samples	
1.	Urine	43								43
2.	Uterine discharge	35								35
3.	Vaginal swab		3		2					5
4.	Liver, kidney and lungs from aborted foetuses	2			2					4
5.	Kidney cortex		26	5	3		10	140		183
6.	Blood					2				2
7.	Surface water samples									8
									Grand total	280

(i) Korthof's Medium (Alston and Broom, 1958)

Peptone	...	0.8 g
Sodium chloride (NaCl)	...	1.4 g
Sodium bicarbonate ($NaHCO_3$)	...	0.02 g
Potassium chloride (KCl)	...	0.04 g
Calcium chloride (CaCl)	...	0.04 g
Potassium dihydrogen phosphate (KH_2PO_4)	...	0.24 g
Disodium hydrogen phosphate ($Na_2HPO_4 \cdot 2H_2O$)	...	0.88 g
Distilled water	...	1 litre

The above medium was steamed at 100°C for 20 minutes and filtered through double thickness Whatmann No. 1 paper, bottled in 100 ml amounts and autoclaved at 115°C for 15 minutes. Heat inactivated Seitz filtered, pooled rabbit serum was added to the base with aseptic precautions to give a final concentration of 8 to 10 per cent.

(ii) Stuart's Medium (Galton et al., 1962)

L-asparagine	...	0.132 g
Ammonium chloride (NH_4Cl)	...	0.368 g
Magnesium chloride ($MgCl_2.6\ H_2O$)	.	0.406 g
Sodium chloride (NaCl)	...	1.808 g
Disodium hydrogen phosphate ($Na_2HPO_4.2\ H_2O$)	...	0.666 g

Potassium dihydrogen phosphate (KH_2PO_4)	...	0.037 g
Phenol red	...	0.01 g
Distilled water	...	995 ml
Glycerine	...	5 ml

The above reagents were mixed thoroughly and sterilized by autoclaving at 15 lbs for 15 minutes. It was cooled to less than 50°C and 8-10 per cent inactivated rabbit serum was added.

(iii) Fletcher's Medium (Galton et al., 1962)

Peptone	...	0.3 g
Beef extract	...	0.2 g
Sodium chloride (NaCl)	...	0.5 g
Agar	...	1.5 g
Water buffered to pH 7.4	...	920 ml

The above base was sterilized by autoclaving at 15 lbs for 15 minutes. When the mixture was cooled to 50°C, 8 to 10 per cent inactivated rabbit serum was added.

(iv) Cox Tryptose Agar (Cox and Larson, 1957)

Bacto tryptose	...	20 g
Bacto dextrose	...	2 g
Sodium chloride (NaCl)	...	5 g

Disodium hydrogen phosphate ($Na_2HPO_4.2\ H_2O$)	...	2.5 g
Agar	...	10 g
Distilled water	...	1000 ml

Inactivated rabbit serum was added upto 10 per cent concentration.

(v) Bovine Albumin Polysorbate 80 Medium (Ellinghausen and McCullough, 1965b)

Stock Solutions

(1) Phosphate buffer (x 25 concentrated) :

Disodium hydrogen phosphate (Na_2HPO_4)	...	16.6 g
Potassium dihydrogen phosphate (KH_2PO_4)	...	2.172 g
Distilled water	...	1000 ml

(2) Salt concentrated x 20 :

Sodium chloride ($NaCl$)	...	38.6 g
Ammonium chloride (NH_4Cl)	...	5.35 g
Magnesium chloride ($MgCl.6\ H_2O$)	.	3.81 g
Distilled water	...	1000 ml

(3) Cupper sulphate ($Cu\ SO_4.5\ H_2O$)	...	30 mg per 100 ml distilled water

(4) Zinc sulphate ($Zn\ SO_4 \cdot 7\ H_2O$) ... 80 mg per 200 ml distilled water

(5) Iron sulphate ($FeSO_4 \cdot 7\ H_2O$) ... 500 mg per 200 ml distilled water

(6) Vitamin B_{12} ... (a) Conc. 10 mg per 100 ml distilled water

(b) Working solution 10 ml concentrate in 90 ml distilled water

(7) Thiamine hydrochloride .. 200 mg per 100 ml distilled water

(8) Tween 80 ... Dissolved 10 ml of Tween 80 in 90 ml distilled water at 60°C by adding it drop by drop

To 700 ml distilled water, added 40 ml buffer (x 25 conc. stock), 50 ml salts (x 20 conc. stock), 1.0 ml of cupper sulphate solution, 10 ml of zinc solution and 20 ml of iron solution. At this point haze developed. The mixture was shaken for 5 minutes. 200 g L. <u>cynting</u> was added and shaken for 3 minutes. The mixture was filtered through double thickness Whatmann No. 1 paper. 20 ml of vitamin B_{12} working solution and 0.1 ml thiamine stock solution was added to the filtrate. 120 ml Tween 80 (1%) solution was added and the total volume was brought to 1000 ml. Freshly prepared 5 per cent bovine albumin solution (5 g bovine albumin fraction V in 100 ml single strength phosphate buffer i.e. 40 ml x 25 conc. stock solution in 960 ml D.W.) whose pH was adjusted

to 7.4 by adding 0.4 N NaoH was added to the basal mixture and sterilized by Seitz filter.

5-fluorouracil was incorporated in the aforesaid culture media, in a final concentration of 200 micrograms per ml (according to Johnson and Rogers, 1964) and was used for purification of contaminated culture and for attempting cultures from various specimens.

3.2.2 Tissue Specimens

Aseptically collected tissue pieces were transferred into a sterile petri dish and Korthof's, Fletcher's, Stuart's or bovine albumin medium approximately equal to four times the volume of weight of tissue pieces was added. The tissue pieces were minced thoroughly with a pair of sterile scissors and the suspension was allowed to stand for 8 to 10 minutes. Then a loopful of supernatant was taken on a slide, a cover slip was applied to cover it and examined for the presence of leptospires by dark field microscopy (with patch stop) at x 100 magnification.

At least 4 bottles each containing 9-10 ml of medium were inoculated with tissue suspension. Two media bottles were inoculated with a small piece of tissue approximately equal to the size of millet seed and two or three drops of

supernatant of the suspension so as to make approximately 10 fold dilution of inoculum. Further ten fold serial dilutions were made upto 10^{-4} and in some cases upto 10^{-5} in bottles containing medium. Inoculated medium bottles were incubated at 29-30°C for 10 days and examined daily.

The tissue suspension, which on microscopic examination showed leptospiral like organisms, were inoculated into laboratory animals. Young guinea pigs weighing 125-150 gm were injected intraperitoneally with 1 ml of supernatant from each tissue suspension. Daily temperature was recorded twice - morning and evening. The animals were kept under observation for 15-21 days. If the animals died during observation period post-mortem was conducted and cultures attempted from their kidneys. Serum samples were collected from such of those animals which survived upto the end of observation period for detecting leptospiral antibodies.

3.2.3 Urine Samples

Urine collected from known aborted cases was centrifuged at 1500 r.p.m. for 15 minutes and the supernatant was inoculated into Korthof's and bovine albumin media with or without 5 fluorouracil in serial dilutions upto 10^{-4} and sometimes upto 10^{-5}.

3.2.4 Blood Samples

Two to three bottles containing 9-10 ml of medium were inoculated each with 1-2 drops of venus blood from dogs suffering from high fever and jaundice.

3.2.5 Kidneys of Free Living Rats and Swiss Mice

Kidneys from trapped rats and Swiss mice were collected for cultural work. Whole kidneys were used for this purpose. Laboratory Swiss mice were selected at random from mice colony of the Biological Products Division. Korthof's and bovine albumin fraction V media were used for making kidney suspensions and for making dilutions. Millipore filters of .45 μ diameter were used to filter kidney suspensions in order to minimise contaminants.

3.2.6 Surface Water Samples

Water samples were collected from stagnant water pools and ponds in a sterile 20 cc McCartney bottles. Before collecting water samples from stagnant pools, the ground surface below the water level was disturbed with a stick in order to disperse the particulate matter into the upper layer of water. After the heavier particles settled down in about 10-12 minutes, the cap of the bottle was removed and with one hand the bottle plunged into the water in inverted

position (bottom above) and filled in gradually. The cap was placed immediately after collection. The technique of Cox (1966) was followed for isolation of leptospires. After the samples were kept at room temperature for 3 days then the samples were filtered through millipore filters of average pore size 0.45 microns. The filtrates were kept at room temperature in a cupboard for three weeks and at the end of 3 weeks period a loopful of samples were placed in the centre of two plates of synthetic medium tryptose phosphate serum agar. Two bottles of each bovine albumin and Korthof's medium were inoculated with a sample. With some samples ten fold dilutions upto 10^{-4} were made and inoculated two bottles of each of bovine albumin and Korthof's medium with all dilutions starting from higher dilution and coming down to lower dilution. The plates were sealed with adhesive tape to prevent evaporation and incubated at 28-30°C for 10 days and examined daily after 4th day for leptospiral colonies. Fluid medium bottles were incubated at 28-30°C for 10 days and then kept at room temperature. The bottles were examined daily for leptospiral organisms and were put into observation upto 4 months, examining samples at weekly intervals.

3.3 SERUM SAMPLES

Serum samples from bovine, ovine caprine and porcine were collected by personal visit to Andhra Pradesh Government

livestock farms Warangal, Kakinada and Chintapalli. Serum was also collected from Gannavaram piggery and from places like Vijayawada and Hyderabad. Some samples that were received from field on personal request were also included in the test. Most of the samples were from animals with known abortion history or other reproductive disorders. Some samples were also from unspecified illness and unknown clinical history and some were collected at random from slaughter house.

In all 283 sera samples from different species of animals were tested as discerned in Table 2.

Table 2

Total number of sera tested from different species of animals

Sl. No.	Species of animals	No. of sera tested
1.	Cattle	183
2.	Sheep	29
3.	Goats	24
4.	Pigs	18
5.	Dogs	5
6.	Horses	5
7.	Rats	4
8.	Swiss mice	10
9.	Guinea pigs	3
10.	Rabbits	2
	Total	283

Generally the samples were examined within a few days of their collection on receipt in the laboratory.

Serum collected from laboratory animals used in the test and from rats and Swiss mice used for isolation work was also subjected for microscopic agglutination test.

3.3.1 Leptospiral Antigen

Fourteen antigens belonging to 11 serogroups were employed in the serological tests for detecting leptospiral antibodies. All the fourteen antigens of leptospira were supplied by the Leptospirosis Laboratory. Table 3 shows the details of various serotypes representing serogroups used in this study. The antigen strains were maintained in bovine albumin medium prepared according to Ellinghausen and McCullough (1965b) by sub-culturing at one week to one month interval.

A good growth of 4 to 12 day old cultures of leptospires containing a uniform suspension of 150 to 200 million cells per millilitre was used as antigens.

3.3.2 Microscopic Agglutination Test

The serum samples were centrifuged at 3000 r.p.m. and the supernatant was subjected to serological tests. All the

Table 3

Leptospiral antigens used in the investigation

Sl. No.	Serotype (strain)	Sl. No.	Serogroup
1.	RGA (Strain)	1.	Icterohaemorrhagiae
2.	canicola	2.	Canicola
3.	pyrogenes	3.	Pyrogenes
4.	butumbo	4.	Cynopteri
5.	autumnalis	5.	Autumnalis
6.	pomona	6.	Pomona
7.	borincana	7.	Hebdomadis
8.	wolffi		
9.	bataviae	8.	Bataviae
10.	Van tienen (strain)		
11.	tarassovi	9.	Tarassovi
12.	patoc	10.	Semaranga
13.	sao paulo		
14.	andamana	11.	Andamana

serum samples were examined singly by microscopic agglutination test with a battery of living suspension of leptospiral antigens.

Serum samples were examined by preliminary screening by using dilutions of 1:30, 1:100 and 1:300 and those found positive for agglutinins of one or more antigens were further examined in 4 fold dilutions of 1:100, 1:400 and 1:1600 and 1:6400. Since the two fold dilution steps were too close and 4 fold dilution steps too wide, some samples were examined by Dutch dilution or inter-locking two fold dilution scheme (Schuffner and Bohlander, 1939; Wolff, 1925, 1954) (Fig. 1).

The test was carried out in a perfectly washed clean perspex haemagglutination trays each having 80 cups of approximately 1 ml capacity (Fig. 2). Sterilized pasteur pipette calibrated to deliver 30 drops per ml of normal saline solution was used in the test. Serum dilutions of 1:15, 1:60 and 1:150 were made in test tubes and three drops of 1:150, 1:60 and 1:15 of each serum dilutions were delivered in rows 3, 4 and 5 respectively from 1 to 14 cups. First two rows were used for 50 and 100 per cent antigen controls. Similarly the dilutions of other serum samples were delivered in cups in the next set of three rows using separate sterile pipette. The antigens 1 to 14 were then added columnwise, equal drops of antigen was added to serum dilutions thus raising the final concentration to 1:300, 1:100 and 1:30. The mixture of serum dilutions and antigens were mixed by gentle agitation of trays in horizontal plane and incubated

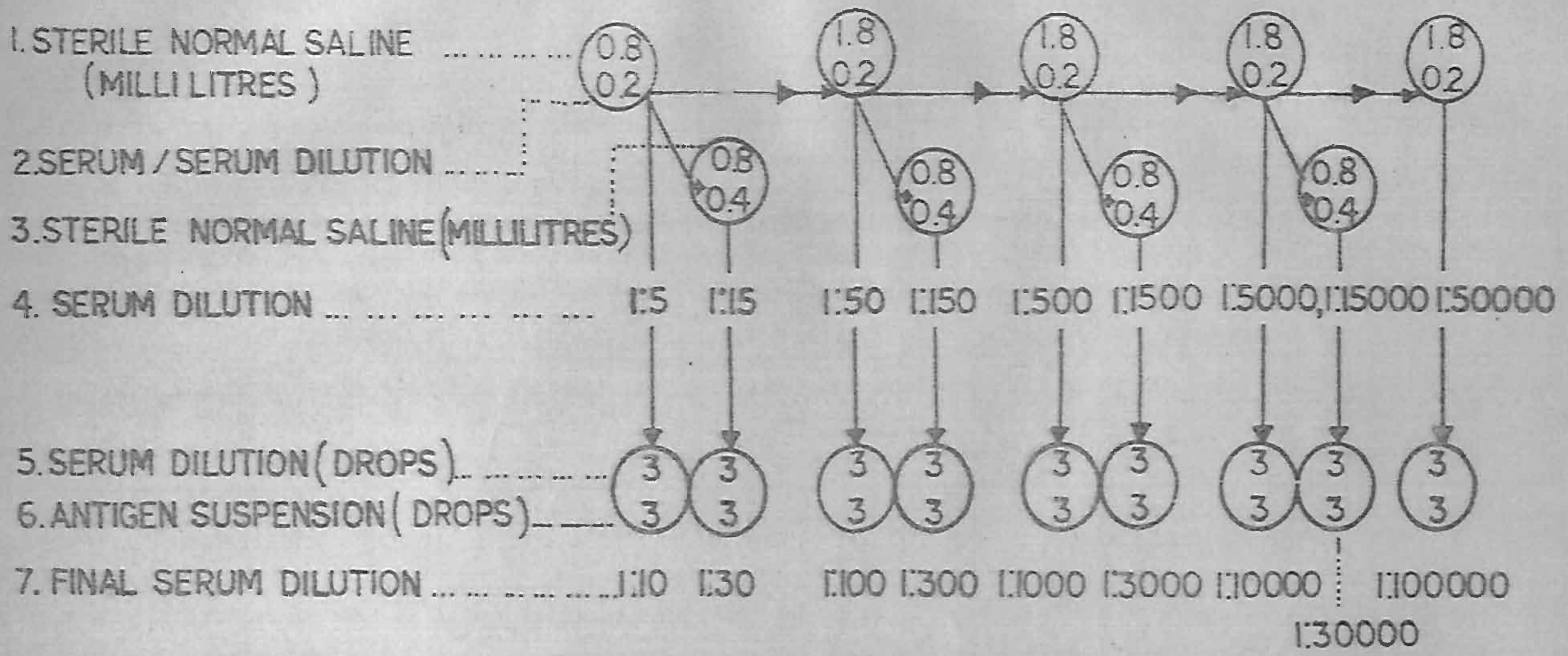


FIG.1. 'DUTCH DILUTION' OR "INTERLOCKING TEN-FOLD DILUTION" SCHEME.

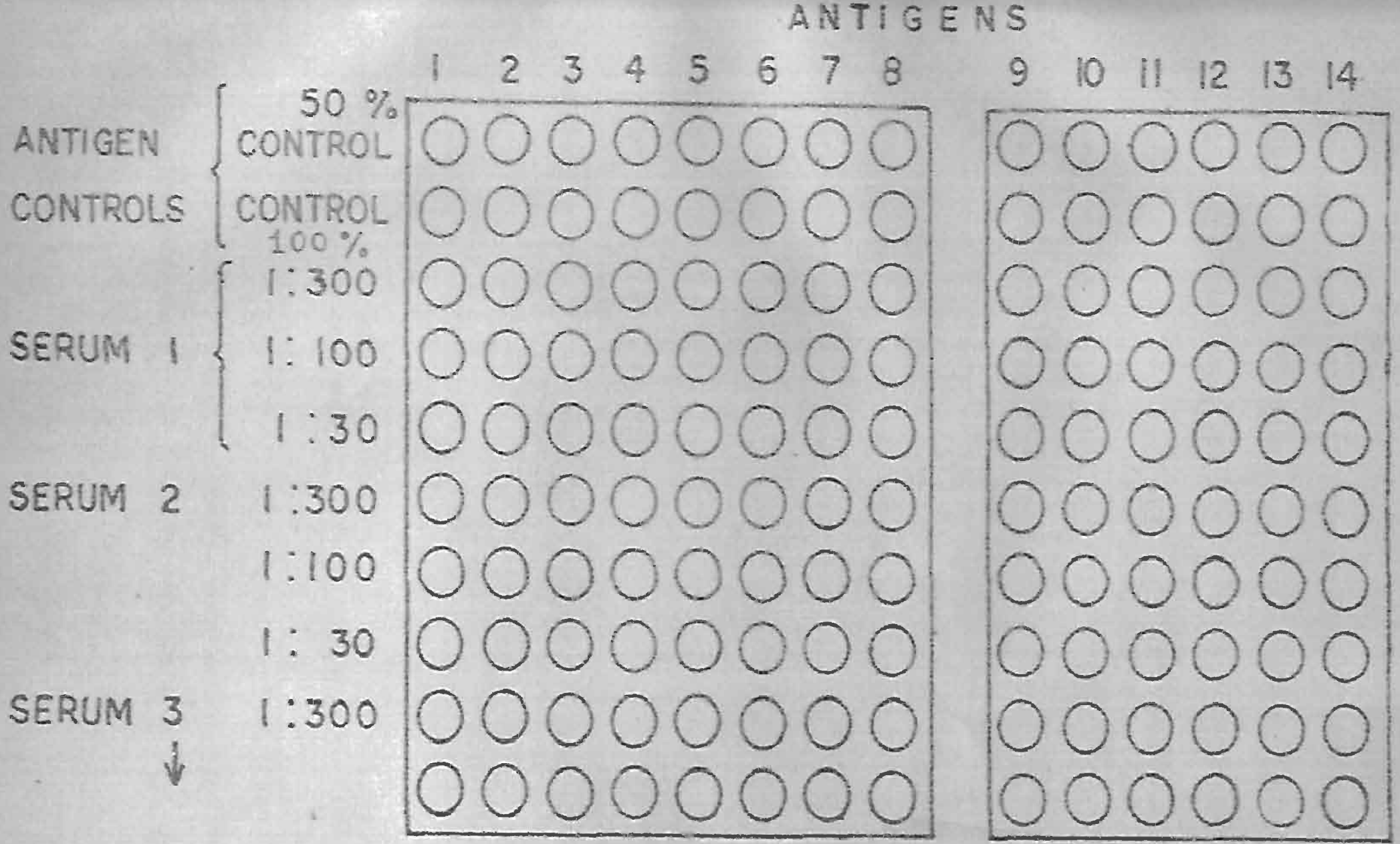


FIG.2_SCREENING PROCEDURE BY MICROSCOPIC AGGLUTINATION TEST WITH LIVE ANTGENS(MAL TEST)

at 30°C for 3 hours. In antigen control row (1) three drops of normal saline solution and three drops of each antigen were added. In next antigen control row (2) six drops of each antigen was added. At the end of the incubation period the trays were removed from the incubator and the antigen serum dilutions were mixed by gentle pipetting. The test was read by examining a drop from each dilution by dark ground microscopy (with patch stop) at x 100 magnification for agglutination and the proportion of free leptospires. The test results were recorded as follows :

Complete agglutination or only one or two organisms in an occasional field with the formation of large clusters ...	4+
Approximately 75 per cent agglutination with about 25 per cent free leptospires ...	3+
About 50 per cent leptospires clumped or agglutinated and about 50 per cent free leptospires ...	2+
Approximately 25 per cent leptospires clumped and about 75 per cent free leptospires ...	1+
No appreciable reduction in concentrated leptospires and absence of leptospiral clumps ...	Negative

The highest serum dilution which gave 2+ (50 per cent agglutination) or higher reaction was scored as positive.

3.4 PRESERVATION OF LEPTOSPIRES BY LIQUID NITROGEN

L. pomona serotype was received from Leptospirosis Laboratory and cultivated in bovine albumin fraction V medium. Eight day old culture was used for preservation in liquid nitrogen. It is non-pathogenic strain. The microscopic count was carried out over a period of eight months.

Glycerol was added to the culture as a protective additive to give a final concentration of 10 per cent W/V and were slowly taken to the liquid phase (-196°C) of the liquid nitrogen.

Prior to freezing, the microscopic count of the culture was determined by Petroff-Hauser counting chamber. Microscopic count was made on frozen samples taken out by day after freezing and at weekly and monthly intervals. Immediately after taking out samples from liquid nitrogen the culture samples were rapidly thawed by immersion in a 37°C water bath. The culture in vials were pooled and motility was tested and count of leptospires was determined by microscopic count by using Petroff-Hauser counting chamber. The culture was diluted serially at ten-fold increments upto 10^{-6} and sub-culturing was done from each dilution in three to five bovine albumin medium with 0.5 ml of diluted culture

and incubated at 28°C and observed for growth upto 10-15 days. The cultures were examined for the presence of leptospires at weekly intervals upto 6 weeks.

Table 4

Table showing details of materials collected from various sources for isolation and the results thereof

Sl. No.	Species of animals	History and locality	Type of material collected	No. of samples collected	Culture media used	Experimental animals used	Results	Remarks
			SUSPECTED SAMPLES					
1.	Cattle	Abortion and reproductive disorders - Andhra Pradesh (A.P.)	Urine	43	Korthof's & Fletcher's	-	Negative	
			Uterine discharges	20*	"	Rabbits	"	*Two samples showed organisms resembling leptospires which proved negative
		Abortion - IVRI Key Village Centre	Uterine discharges	16	Korthof's & Stuart's	-	"	
		Aborted foetuses - (A.P.)	Pieces of kidney, liver and lungs	2	Korthof's & Fletcher's	Guinea pigs	"	
2.	Sheep	Abortion - (A.P.)	Vaginal discharges	3	Korthof's	-	"	
3.	Pigs	Abortion - (IVRI)	Vaginal swab	2	Korthof's & Stuart's	-	"	
		Aborted foetuses - (IVRI)	Pieces of liver, kidney and lungs	2	"	-	"	
4.	Dogs	Fever and jaundice - (A.P.)	Blood	2	Korthof's	-	"	
			Total	89				

Table 4 (contd)

Sl. No.	Species of animals	History and locality	Type of material collected	No. of samples collected	Culture media used	Experimental animals used	Results	Remarks
			HEALTHY TISSUES					
1.	Sheep	Slaughtered - (A.P. and IVRI)	Kidney cortex	25	Korthof's & Fletcher's	-	Negative	
2.	Goats	Slaughtered-(A.P. and IVRI)	Kidney cortex	5	Korthof's	-	"	
3.	Pigs	Slaughtered - (A.P.)	Kidney cortex	3	Korthof's & Fletcher's	-	"	
4.	Wild rats	Trapped - (IVRI)	Kidneys	10	Korthof's Tryptose agar	-	"	
5.	Swiss mice	Healthy - Mice Colony, Division of Biological Products, IVRI	Kidneys	40*	Korthof's	Guinea pigs	"	*Two suspensions revealed leptospira like organisms which proved negative on culturing
		From other Sections	Kidneys	100	"	-	"	
			Total	183				
			Grand Total	272				

Table 4 (contd)

Sl. No.	Species of animals	History and locality	Type of material collected	No. of samples collected	Culture media used	Experimental animals used	Results	Remarks
			WATER SAMPLES					
1.	-	Collection of washings of Dairy sheds (IVRI)	Surface water	2	Korthof's & Tryptose agar	-	Negative	
		Collection of washings of piggery sheds (IVRI)	Surface water	2	"	-	"	
		Pond (IVRI)	Surface water	2	"	-	"	
		Pond (Izatnagar)	Surface water	2	"	-	"	
			Total	8				

slaughtered sheep (25), goats (5) and pigs (3) were the source material for isolation. The results of these attempts are presented in the above table. In spite of the best efforts it was not possible to isolate them in culture.

A perusal of the results in the same table would however show that leptospires like organisms could be seen under the microscope in the kidney suspensions of two out of forty Swiss mice, received from Nice colony of the Division of Biological Products. Not a single kidney of mouse received from other sources could reveal leptospires like organisms microscopically, in spite of testing as many as 100 mice.

4.1.3 Water Samples

Water samples were included in the study since it is well established fact that this source forms an important vehicle for transmission of communicable disease including leptospirosis. As many as eight samples representing a cross section of possible sources of spread were selected. The technique of sampling has been mentioned in the previous section. The samples from these sources were negative to microscopic examination either initially or after incubation of cultures.

4.2 MICROSCOPIC AGGLUTINATION TEST WITH LIVE ANTIGEN

Serology was included as a part of the study with a view to elicit information about the status of the disease in a population. Two hundred and eighty three sera samples (Table 2) (cattle 183; sheep 29; goats 24; pigs 18; dogs 5; horses 5 and laboratory animals 19) were screened for the presence of leptospiral antibodies. The sera samples were titrated against a battery of leptospiral antigens of 14 in number representing eleven serogroups. The results of cattle sera tested were shown in Table 5.

It was observed that out of 183 sera samples from cattle 65 were positive for one or the other antigens and some to more than one antigen. The study was confined to Andhra Pradesh Livestock farms and hospital samples which also included nearby key village centres, and crossbreeding units. Out of a total of 91 sera samples from clinical cases of abortion from the Veterinary hospitals 32 were positive. The predominant serogroups were Hebdomadis (12) followed by Pyrogenes (7) and Pomona (5), Tarassovi (3) and other antigens.

On the other hand out of 92 sera collected from farm animals, 33 were positive for one or more of the antigens. Sera positive to more than one or two serogroups were also

Table 6

Table showing number of cattle sera tested against 14 antigens representing eleven serogroups and results thereof

Locality of collection of sera	No. of sera tested	Serotypes (or Strains)														Sera positive to			Total sera positive
		RGA (strain)	canicola	pyrogenes	butembo	autumnalis	pomona	borincana	wolffi	bataviae	van Tienen (strain)	tarassovi	patoc	saopaulo	andamana	Single serogroup	Two serogroups	Three serogroups	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14				
		Serogroups																	
		Icterohaemorrhagiae	Canicola	Pyrogenes	Cynopteri	Autumnalis	Pomona	Hebdomadis		Bataviae		Tarassovi	Semaranga		Andamana				
		1	2	3	4	5	6	7		8		9	10		11				
Sera from Veterinary Hospitals																			
Hyderabad	46	1	-	4	1	1	3	-	4	-	-	1	-	-	-	15	-	-	15
Vijayawada	45	-	-	3	1	1	2	-	8	-	-	2	-	-	-	17	-	-	17
Total	91	1	-	7	2	2	5	-	12	-	-	3	-	-	-	32	-	-	32
Sera from Government Livestock Farms																			
Warangal	34	-	-	1	-	-	1	-	-	-	-	-	-	-	-	2	12	-	14
Kakinada	23	-	-	-	-	-	3	-	7	-	-	-	-	-	-	10	-	-	10
Chintapalli	35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	9
Total	92	-	-	1	-	-	4	-	7	-	-	-	-	-	-	12	12	9	33
Grand Total	183	1	-	8	2	2	9	-	19	-	-	3	-	-	-	44	12	9	65

encountered. Hebdomadis appeared to be the predominent serogroup followed by pomona. Out of 65 positive cattle sera, forty four sera had agglutinins to one or other single serogroup with a significant titre of 1:100 (Table 6). Twelve sera samples showed agglutinins to two serogroups with high titre (1:300) to one serogroup as compared to those of other cross reacting serogroup (1:100) (Table 7). Nine sera samples had agglutinins to three serogroups showing high titre (1:300) to one serogroup as compared to those of other two cross reacting serogroups (1:100) (Table 8).

As has been mentioned above that 65 sera samples were positive out of 183 from cattle of hospital and farm animals. The intensity of the infection that was measured through microscopic agglutination test has been presented in Tables 6, 7 and 8. The results of the 44 samples of sera positive to single serogroup were given in Table 6.

The twelve samples that were positive to leptospiral serogroups have been shown in Table 7. Eight samples were positive to pomona and hebdomadis, 2 were positive to autumnalis and hebdomadis, one was positive to pomona and tarassovi and the remaining one to hebdomadis and tarassovi.

A perusal of the results in Table 8 pertaining to nine positive samples of sera from farm animals shows that

Table 6

Table showing titre of 44 sera positive to single serogroups

Species of animal	No. of sera tested	No. of sera positive	Name of serogroup having agglutinins in the serum	No. of sera	Titre* recorded
Cattle	282	44	Ictero-haemorrhagiae	1	100
			Pyrogenes	8	100
			Cynopteri	2	100
			Autumnalis	2	100
			Pomona	9	100
			Hebdomadis	19	100
			Tarassovi	3	100
		Total		44	

*Titres expressed as reciprocal to serum dilutions.

Table 7

Table showing titres of 12 sera positive to two serogroups

Species of animal	Locality	Names of two serogroups having agglutinins in the serum	No. of sera	Agglutinin titre* to serogroup Autumnalis	Pomona	Hebdomadis	Tarassovi
Cattle	Warangal	Pomona-Hebdomadis	8	100		300	
				100		300	
				100		300	
				100		300	
				100		300	
				300		100	
				300		100	
				300		100	
		Autumnalis-Hebdomadis	2	100		300	
				100		300	
		Hebdomadis-Tarassovi	1			300	100
		Pomona-Tarassovi	1		300		100
		Total sera	12				

*Titres expressed as reciprocal to serum dilutions

Table 8

Table showing titres of 9 sera positive to three serogroups

Species of animal	Locality	Names of three serogroups having agglutinins in the serum	No. of sera	Agglutinin titre* to serogroup Autumnalis	Pomona	Hebdomadis	Tarassovi
Cattle	Chintapalli	Autumnalis-Pomona-Hebdomadis	4	100	100	300	
				100	100	300	
				100	300	100	
				100	300	100	
		Pomona-Hebdomadis-Tarassovi	3		100	300	100
					100	300	100
					300	100	100
		Autumnalis-Hebdomadis-Tarassovi	1	100		300	100
		Autumnalis-Pomona-Tarassovi	1	100	300		100
		Total	9				

*Titres expressed as reciprocal to serum dilutions

four were positive for Autumnalis-Pomona and Hebdomadis agglutinins, while three samples were positive for Pomona-Hebdomadis and Tarassovi. One sample was positive to Autumnalis-Hebdomadis and Tarassovi and the remaining sample was positive to Autumnalis-Pomona and Tarassovi.

The results of other domestic and laboratory animal sera tested are furnished in Table 9. Out of 29 sheep sera samples only five were positive to leptospiral antibodies at 1:100 titre and the only serogroup encountered was *L. pomona*. All these were sheep from Hyderabad.

Four out of 24 goat sera from the same place were also positive for pomona with significant titre of 1:100.

L. pomona antibodies of 1:100 titre were also detected in three sera samples out of 18 from Gannavaram piggery.

The serum samples from dogs and horses as well as from laboratory animals did not reveal antibodies to any of the serotypes.

4.3 PRESERVATION OF LEPTOSPIRES BY LIQUID NITROGEN

Liquid nitrogen was found to be a very good method for preserving fragile leptospires. The results of limited study have been presented in Table 10. It would be seen that the initial count which was 38.5×10^8 organisms had

Table 9

The results of sera of other domestic and laboratory animals

Species of animals	Locality	No. of sera tested	No. found positive	Agglutinins present to serogroup*
Sheep	Hyderabad	20	5	Pomona
	IVRI	9	-	
Goats	Hyderabad	16	4	Pomona
	IVRI	8	-	
Pigs	Gannavaram	18	3	Pomona
Dogs	Hyderabad	5	-	
Horses	IVRI	5	-	
Rats	IVRI	4	-	
Swiss mice	IVRI	10	-	
Experimental Animals used in Investigation				
Guinea pigs	IVRI	3	-	
Rabbits		2	-	
	Total	100	12	

*The sera were screened against 14 serogroups as shown in Table 5.

Table 10

Results of preservation of leptospira under liquid nitrogen

Time of preservation	Motility	Microscopic count
4 hours	+	Not done
24 "	+	38.6×10^6
7 days	+	34.2×10^6
15 "	+	34.2×10^6
1 month	+	34.3×10^6
2 months	+	33.2×10^6
3 "	+	34.3×10^6
4 "	+	33.8×10^6
5 "	+	33.4×10^6
6 "	+	32.8×10^6
7 "	+	31.6×10^6
8 "	+	31.7×10^6

come down to 31.7×10^6 after 8 months of storage. The same table would also show that the sudden drop after its exposure to liquid nitrogen appears to be maintained during the observation period. Addition of glycerine to the culture appeared to be favourable.

6. DISCUSSION

It is well known that communicable diseases affecting man and animal are of considerable importance for any effective health control programme. Without an effective method for their control, it would be difficult to imagine man and animal in healthy conditions. Man's dependence on foods of animal origin has also led to the desirability of controlling animal diseases. In this context, leptospirosis assumes a very important position when considered in terms of public health point of view. It is a zoonotic disease naturally transmitted between vertebrate animals and man.

Leptospirosis causes abortion, flaccid mastitis, haemoglobinuria and incapacitates the animals. The fruitful results achieved by vigorous efforts made during the last two decades to control animal brucellosis by means of vaccination, testing and segregation etc. brought the importance of leptospirosis into light as a widely prevalent disease.

In India leptospirosis among animals was reported 45 years ago by Ayyar (1932) in Andaman island. Since then several States have documented the serological evidence of leptospirosis (Adinarayana et al., 1960; Pande and Sakariah, 1961; Sawhney and Saxena, 1967; Rajasekhar and Manjiah, 1971; Khera, 1972; Rajasekhar et al., 1977 and by many other workers).

A strong serological reaction is an evidence of presence of leptospiral infection. But isolation of etiological agent from a seropositive case is a concrete proof of infection. The urine of seropositive animals and the blood from acute cases are the materials of choice for the isolation of leptospires.

Serological surveys of animals help to determine the principle reservoirs of maintaining hosts of leptospires. The detection of urinary excretions is of fundamental importance in this respect. Serological surveys should always be supplemented by the culture of kidney cortex material and urine or by the inoculation of such materials into susceptible laboratory animals or by both methods and then the demonstration of leptospires.

ISOLATION ATTEMPTS FROM SUSPECTED AND HEALTHY TISSUE

As far as isolation of leptospires is concerned there is a common belief that parasitic leptospires are difficult to isolate which is not wholly true. With the availability of suitable media and laboratory animals the infecting strain can often be isolated from suitable materials.

Turner (1965) states that following factors are responsible for isolation failures :

1. Contamination with other organisms.
2. Failure to inoculate source material when it contains leptospires in less number.
3. Death of a few organisms which may result from the presence of antibodies or inhibitory substances associated with the lipid fraction of emulsified kidney tissue or residual traces of detergent in imperfectly cleaned glassware.
4. Use of media which do not promote the growth of leptospires. This may be due to the presence of inhibitory factors or the absence of necessary growth factors.
5. Inadequate incubation of cultures. These should be observed upto three months.
6. Failure to use suitable laboratory animals.

Isolation of leptospires has successfully been done in foreign countries. Turner *et al.* (1958) isolated seretype canicola from urine of a sick two day old calf whose dam had homologous antibodies. Roth and Galton (1960) isolated *L. hardjo* from cattle. Karash (1964) isolated the serotypes canicola, icterochaemorrhagiae and grippotyphosa from slaughtered cow.

In India there are very few reports about the isolation of leptospires from clinical cases. Taylor and Doyle (1931)

and DasGupta (1939, 1940) reported isolation of grippotyphosa and andaman strains in Andaman islands. DasGupta and Sen (1945) isolated L. canicola from a dog. Pando and Sekariah (1960) claimed to have recovered cultures of L. pomona and Sejroe from goats. Joseph and Kalra (1966) recovered a strain of L. icterohaemorrhagiae from a dog suffering from jaundice.

Only limited number of morbid materials were available for attempting isolation of leptospires at the time of undertaking present investigation. There were several difficulties in collecting suitable material for isolation work under the existing field conditions. In addition, the broad spectrum antibiotics therapy complicated the problem. Treatment with penicillin, tetracyclines, erythromycin or streptomycin kills the leptospires and tend to reduce the chances of successful isolation of infecting strains (Turner, 1965).

The leptospirosis is more prevalent in southern States than the northern States of the country. The scientific reports of I.V.R.I. for the years 1973, 1974 and 1976 reveal the same. Adinarayan (1972) claimed to have isolated leptospires in Kerala. Murthy and Khera (1973, 1974) reported serological evidence of infection in the livestock farms Kakinada, Banavasi, Vishakapatnam and Warangal of Andhra Pradesh.

The results in Table 4 would indicate the primary isolation of organisms resembling leptospires from two cases of abortion in cattle from Andhra Pradesh. But on further passage, the primary isolate could not be passed on the medium as well as in experimental animal. The possible reasons for this have been elucidated above.

In view of the above findings it can be said that isolation of this organisms may be easier to attempt in endemic areas of Andhra Pradesh and Kerala.

Two more isolations in mice tissues received from the Division of Biological Products of I.V.R.I. were also recorded. The behaviour of this culture was similar to those isolated from cattle. Leptospiral isolation from rodents were recorded by earlier workers (Anderson and Wagle, 1931; Galton et al., 1962).

ISOLATION ATTEMPTS FROM SURFACE WATER

Surface water contamination with infected urine of domestic animals and wild life was considered to be most important factor in the epidemiology of leptospirosis (Gillespie and Ryno, 1963; Desch and McCulloch, 1966). Saphrophytic leptospires also occur in surface water. Hennebery and Cox (1968) and Cinco and Petelin (1970) studied water leptospires and distinguished them into numerous

serogroups, each containing many serotypes. Carroll and Leclair (1969) isolated strains of serotype patoc from animals suffering from febrile illness, jaundice and haemoglobinuria suggesting the possibility of this group being associated with diseased conditions in domestic animals. The serotype patoc belongs to serogroup Semaranga of water leptospires.

It was not possible to confirm or negate the findings of these authors due to negative results obtained from water samples. As such this source namely water from ponds and other sources could not be incriminated in the overall epedimiology.

PREVALENCE OF LEPTOSPIROSIS

Ever since Ball and Sheikh (1958) presented serological evidence of leptospiral infection among sheep, horse and dogs in Maharashtra State, numerous reports about the occurrence of leptospirosis among the domestic livestock had come to light. Adinarayan et al. (1960) reported occurrence of leptospirosis among cattle population of a farm in Uttar Pradesh. Their serological studies indicated the possible involvement of one or more of the serotypes of Hebdomadis group. Venkataraman and Jagannathan (1961) reported an outbreak of leptospiral infection in bovine in Madras State. They presented histological and serological evidence of the disease along with clinical

observations. Rao and Surendran (1970) observed antibodies in aborted cattle against six serotypes namely L. pomona, L. australis, L. autumnalis, L. hebdomadis, L. medanensis and L. sankozhing, the last three belonging to Hebdomadis group. Khera (1972) investigated incidence of leptospirosis on the countrywide basis and reported its prevalence in many States of India and recorded most common infection with serogroups Hebdomadis and Pomona among animals of Andhra Pradesh. Murthy and Khera (1973, 1974) examined sera samples from Andhra Pradesh and reported prevalence of leptospirosis due to L. wolffi, L. pyrogenes, L. ballum and L. tarassovi.

SIGNIFICANT TITRES

In the present investigation microscopic agglutination test was the procedure employed for diagnosis of leptospirosis. Though agglutination of leptospires by the specific serum occurs in the presence of present or past infection (Broom and McIntyre, 1948; Babudieri and Gaspardis, 1965), there is lack of uniformity on the interpretation of serological results. Some workers have considered 1:10 to 1:30 with 100 per cent agglutination lysis as specific (Johnson, 1939; Field and Sellers, 1950) or 1:10 titre with 75 to 100 per cent agglutination with formalised antigen (Turner, 1968). Palit and Sharma (1971a) regarded titre of 1:300 and above as positive.

But most workers accept titres of 1:100 or higher as indicative of leptospiral infection (Wolff et al., 1962; Turner, 1968 and Khera, 1972).

In the present investigation 1:100 and higher titres were regarded as significant because (i) the serum samples were collected from unknown clinical history. The time of collection varied from few weeks to several months after their recovery from disease, (ii) the serum samples during transit period were exposed to temperatures suitable for bacterial contamination. So the observed titres were expected to be lower than otherwise titres.

Even though the number of serum samples examined were quite less (Table 5), sera that were positive showed significant titres (Tables 6, 7 & 8). The present investigation has revealed leptospiral infection among animals in Andhra Pradesh. The samples collected were from widely separated livestock farms and hospitals of the State.

The serological results in Table 6 indicated that infecting serotypes belonged to seven serogroups and among these Hebdomadis group infection was predominant and followed by Pomona and other serogroups. These findings are in agreement with those of Khera (1972) and Murthy and Khera (1974). Singh and Uppal (1967) examined 208 sera samples from Andhra Pradesh

and recorded antibodies against four serogroups namely Hebdomadis, Semaranga, Autumnalis and Bataviae, whereas the findings in the present investigation differ by encountering agglutinins for serogroups Icterohaemorrhagiae, Pyrogenes, Cynoptori, Autumnalis, Pomona, Hebdomadis and Tarassovi.

In the present study out of 283 sera tested, 56 showed agglutinins to antigens of one or the other single serogroup (Tables 5 & 9) and twenty one sera exhibited varying titres with different combination of antigens with more than one serogroup (Tables 6 & 7). The sera which had agglutinins of 2 to 3 serogroups reacted more strongly with antigens of one serogroup and less strongly with antigens of one or more other serogroups. The combination of multiple reactions did not have any definite pattern. However, in few instances multiple agglutinins were encountered in serum samples of a given farm to antigens of only those serogroups which appeared to be prevalent there. The occurrence of various combinations of multiple agglutinins was reported by many workers in animal sera (Chung, 1968; Carlos et al., 1971a and Khera, 1972). The present finding also reflects similar views.

Topicu et al. (1967) elucidated that multiple agglutinins in a serum was the result of single infection but was not due to successive infections with different serotypes. But antibody

absorption studies of Pike et al. (1961) and Turner (1968) disclosed that no single antigen agglutinated all the antibodies of the serum reacting against many serotypes.

Samples collected from Government Livestock Farm, Warangal showed the presence of agglutinins of L. wolffi (Hebdomadis group), L. autumnalis, L. Tarassovi and L. pomona whereas the samples from Chintalpalli farm showed agglutinins to L. pyrogenes, L. wolffi (Hebdomadis group), L. pomona, L. autumnalis and L. tarassovi (Tables 5 & 6). These findings were similar to the reports of Murthy and Khera (1973, 1974). The samples from Kakinada farm showed agglutinins against Pomona and Hebdomadis serogroup.

In the present investigation 46 sera samples from Veterinary Hospitals, Hyderabad were tested. Leptospiral agglutinins against seven serogroups, namely Icterohaemorrhagiae (1), Pyrogenes (4), Cynopteri (1), Autumnalis (1), Pomona (3), Hebdomadis (4) and Tarassovi (1), were encountered. Pargaonkar and Ramakrishna (1963) and Pargaonkar (1964) reported serological evidence of leptospiral infection with Pomona serogroup in sheep and goats of Hyderabad. Whereas 45 sera samples tested from Veterinary Hospitals, Vijayawada revealed presence of agglutinins against six serogroups only. The serogroups recorded were Pyrogenes (3), Cynopteri (1), Autumnalis (1), Pomona (2), Hebdomadis (3) and Tarassovi (2).

The prevalence of serogroups recorded in Andhra Pradesh in the present investigation are depicted in Fig. 3.

Leptospiral antibodies have been reported in the aborted as well as in the healthy goats and sheep by many workers namely Pargoankar and Ramakrishna (1963); Pargoankar (1964); Mukherjee et al. (1968); Rajasekhar and Nanjiah (1971); Khera (1972) and Nigam et al. (1974).

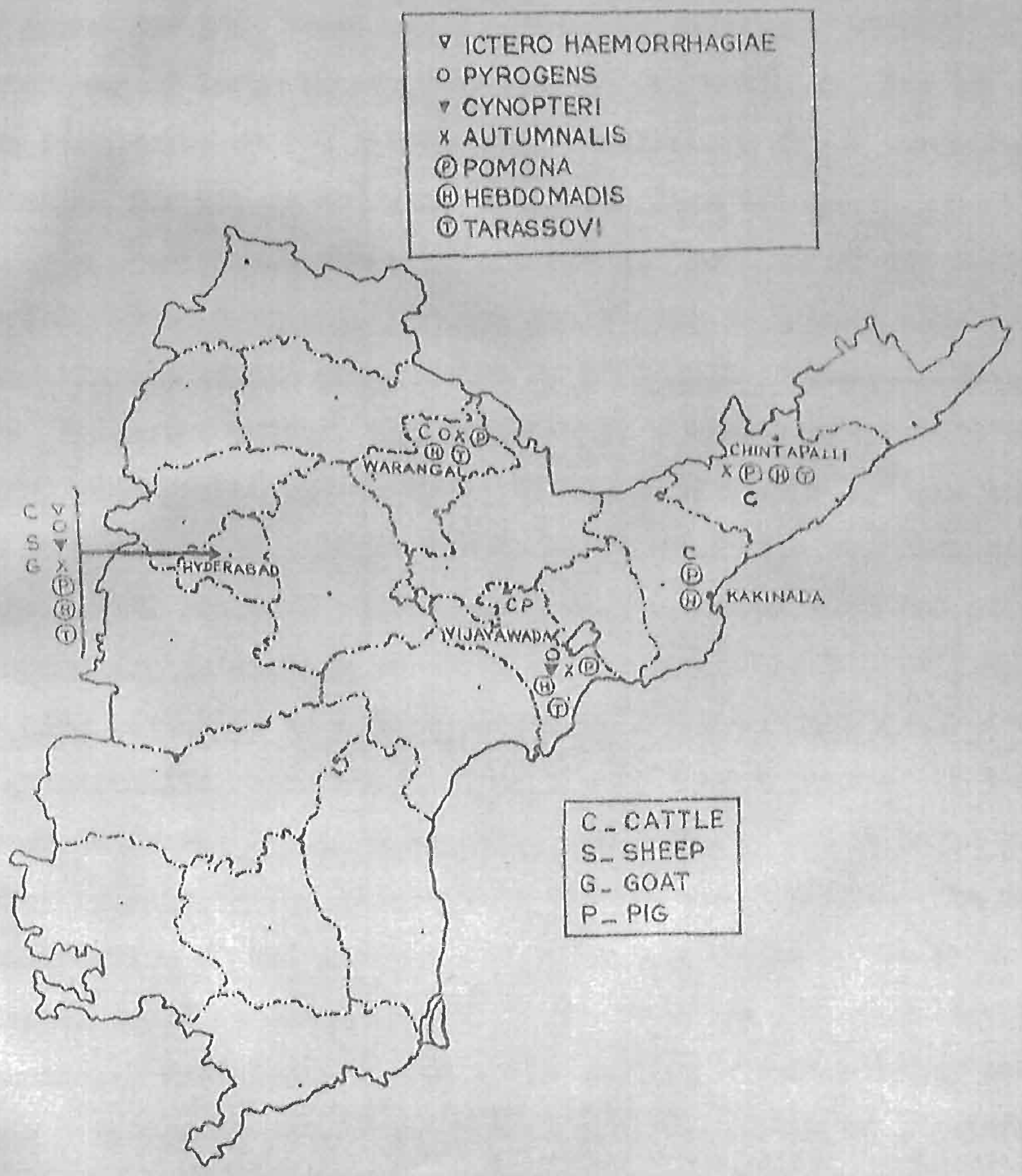
Pargoankar and Ramakrishna (1963) examined 186 samples of sera (102 from sheep and 84 from goats) obtained from a slaughter house in Hyderabad (A.P.) and reported 12.1 per cent samples showing leptospiral antibodies against L. pomona. In the present investigation only L. pomona antibodies were detected in the sera of goats and sheep. These findings are in agreement with those of Pargoankar and Ramakrishna (1963) and Khera (1972).

In India Bhagwat (1964) established the incidence of leptospirosis among pigs. Sawhney and Saxena (1967) detected agglutinins against L. pomona in a fatal case of a pregnant sow. Balaprakasam and Seshadri (1968) recorded an interesting case of peripheral leptospiraemia in piglets. Bhatnagar et al. (1967) and Rajasekhar and Nanjiah (1971) recorded serological evidence of leptospirosis in pigs. In the present investigation only L. pomona antibodies were recorded in the sera of pigs.

No leptospiral antibodies could be demonstrated with dogs, horse and laboratory animal sera.

FIG. 3_OCCURANCE OF REACTORS TO SEROGROUPS OF LEPTOSPIRA

IN ANDHRA PRADESH



PRESERVATION OF LEPTOSPIRES

Leptospires are maintained in laboratory by regular transfer in suitable media. In recent years the need to develop a satisfactory means for long term preservation has increased because (i) maintenance of large number of cultures are increased due to continuous disclosure of new types, (ii) requirement for a large battery of strains for use as antigens in serological tests.

In the present study an attempt was made to preserve avirulent laboratory strain L. pomona, cultivated in bovine albumin medium, in the liquid phase (-196°C) of liquid nitrogen refrigeration. Preserved culture was tested upto 8 months of storage which yielded good growth and microscopic count of 31.7×10^6 and was used as a source of antigen in the serological tests. Alexander et al. (1972) reported successful preservation of virulent strain of Leptospira interrogans serotype canicola in Stuart's medium. They used glycerine as a cryoprotective and presented qualitative and quantitative observations over a period of 5 years. In the present study the strain used being non-pathogenic its testing in laboratory animals for infectivity was not carried out. The period of observation of the preserved culture was limited. No definite inference could be drawn due to limited studies although liquid nitrogen was definitely a good preservative. Further study of longer duration of preservation is required to come to a conclusion about the routine use of liquid nitrogen as a preservative.

6. SUMMARY

Eighty nine suspected materials from aborted animals, aborted foetuses, blood from fever and icterus cases and urine from animals with history of abortion were collected from Government Livestock farms and other places of Andhra Pradesh for isolation of leptospira. These tissues were examined bacteriologically and isolation was tried directly by inoculating the material into suitable medium.

In view of the importance of leptospiral infection resulting by coming in contact with surface water contaminated with infected urine of domestic animals and carriers of free living small rodents, a number of kidneys of Swiss mice (140), rats (10) caught by trapping and water samples (2) were also included in the study and were processed for leptospiral isolation.

One hundred and eighty three healthy tissues (kidney suspensions of slaughtered sheep (25), goats (5) and pigs (3)) were also the source material for isolation.

In all a total of 272 samples of suspected and healthy tissues were used for isolation work.

Serum samples were collected for testing from animals

with the history of abortion, other reproductive disorders, cases of icterus and slaughtered animals. The serum samples were examined by microscopic agglutination test in order to evaluate the prevalence of serogroups among the livestock of Andhra Pradesh. A battery of live antigens representing 11 serogroups, namely, Icterohaemorrhagiae, Canicola, Pyrogenes, Cynopteri, Autumnalis, Pomona, Hebdomadis, Bataviae, Tarassovi, Semaranga and Andamana, were used in the microscopic agglutination test.

Two hundred and eighty three samples were collected from cattle, sheep, goats, pigs, dogs, horses and laboratory animals of suspected as well as non-clinical cases of leptospirosis and were screened for the presence of leptospiral agglutinins.

Out of the above mentioned sera samples, one hundred and eighty three were collected from cattle belonging to Government Dairy farms (Kakinada, Chintapalli and Warangal) and from Veterinary hospitals, key village and crossbreeding units of Vijayawada and Hyderabad. The remaining sera samples were collected from other domestic and laboratory animals.

Sixty five out of 183 sera samples examined from cattle were found positive for leptospiral agglutinins with a significant titre of 1:100 and above.

Out of sixty five positive sera, 44 sera had agglutinins to one or other single serogroup with a significant titre of 1:100, whereas twenty sera samples had agglutinins to more than one serogroups showing high titre to one serogroup (1:300) as compared to those of other cross-reacting serogroups (1:100). Infection with hebdomadis serogroup appeared to be more prevalent followed by Pomona and other serogroups. Only L. pomona agglutinins were detected in the sera of sheep, goats and pigs.

Out of 29 sera samples of sheep examined five had agglutinins to Pomona serogroup.

Four out of 24 goat sera were positive for Pomona.

L. pomona antibodies were also detected in three sera samples out of 18 pig sera.

Sera samples from dogs and horses as well as laboratory animals did not reveal agglutinins for leptospiral antigens.

Attempts were made to preserve avirulent laboratory strains of L. pomona, grown in bovine albumin fraction V medium in liquid nitrogen (at -196°C). The preserved cultures were tested upto 8 months which yielded good growth and microscopic count of 31.7×10^6 and was used as a source of antigen in the microscopic agglutination test.

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