IMMUNOLOGICAL SURVEY ON
THE INCIDENCE OF INFECTIOUS BRONCHITIS (IB) AND INFECTIOUS LARYNGOTRACHEITIS (ILT) IN POULTRY IN AND AROUND TRICHUR

BY

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THESIS

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of the requirement for the degree

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DECLARATION

I hereby declare that this thesis entitled "IMMUNOLOGICAL SURVEY ON THE INCIDENCE OF INFECTIOUS BRONCHITIS (IB) AND INFECTIOUS LARYNGOTRACHEITIS (ILT) IN POULTRY IN AND AROUND TRICHUR" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship, or other similar title of any other University or Society.

M.C. George

Mannuthy,
27-8-1979.

CERTIFICATE

Certified that this thesis, entitled "IMMUNOLOGICAL SURVEY ON THE INCIDENCE OF INFECTIOUS BRONCHITIS (IB) AND INFECTIOUS LARYNGOTRACHEITIS (ILT) IN POULTRY IN AND AROUND TRICHUR" is a record of research work done independently by Sri. M.C. George, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

29/8/

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CONTENTS

INTRODUCTION

INTRODUCTION

Kerala has agriculture based rural economy and most of the agriculturists in Kerala are either medium, small or marginal farmers. A significant percentage of income of small and marginal farmers comes from livestock and poultry. Kerala has a poultry population of 130.43 lakhs as per the 1977 census and on an average 1,050 millions of eggs are being produced every year. Most of these birds are reared under back yard condition. These birds are mainly kept for egg and meat. The two most important viral diseases which cause a drop in egg production are infectious bronchitis and infectious laryngotracheitis.

Avian infectious bronchitis (IB) is an acute, highly contagious respiratory disease of chicks characterised by tracheal rales, coughing and sneezing, produced by a virus belonging to the family Coronaviridae. In young chicks there may be nasal discharge and in laying flocks there is usually a drop in egg production. The first report of avian infectious bronchitis was made by Schalk and Hawn (1931) in North Dakota, U.S.A. The available literature shows that this disease is prevalent in India. The occurrence of infectious bronchitis was first established in India by Verma (1965) and subsequently by Adlakha (1966). Mahalingam et al. (1973) reported that IB

is not prevalent in Andhra Pradesh, Kerala, Karnataka and Pondichery. But Murthy et al. (1977) reported the incidence of IB in Andhra Pradesh and Karnataka. There is dearth of published literature regarding the incidence of IB in chickens in Kerala.

Infectious laryngotracheitis (ILT) is a disease affecting the respiratory tract of chickens characterised by signs of respiratory difficulty, gasping and expectoration of bloody exudate and is produced by a virus belonging to the family Herpetoviridae. This disease was first reported by May and Tittsler (1925) from United States. In India ILT was first reported by Singh et al. (1964) by isolating the virus from infected birds in Uttar Pradesh. Mahalingam et al. (1973) reported a very low percentage of incidence of ILT in Tamil Nadu, Karnataka, Pondichery and Andhra Pradesh and no incidence in Kerala. Murthy et al. (1977) reported a higher percentage of ILT in Andhra Pradesh and Karnataka.

Infectious bronchitis and infectious laryngotracheitis can be diagnosed on the basis of various tests like isolation of virus in chicken embryo, demonstration of lesions in chorioallantoic membrane as well as in the embryo and detection of neutralizing and precipitating antibodies in the serum samples of infected/convalescent

birds. Out of these methods serum neutralization and agar gel precipitation tests are usually employed for screening the flocks. The agar gel precipitation test has been preferred over serum neutralization test by Woernle and Brunner (1961), Ahmed and Monreal (1963) and Lezhava (1964). This test is simple, economical, requires less amount of reactants and result could be obtained within four to five days. More over large number of serum samples can be tested in a very short time.

Avian infectious bronchitis and avian infectious laryngotracheitis are the two main respiratory diseases of poultry which are responsible for very great economic loss to the poultry industry by way of decreased egg production, poor quality of eggs, loss of weight gain and decreased feed efficiency. Even though Mahalingam et al. (1973) reported that these two diseases are not prevalent in Kerala, there are often complaints from farmers that a sudden drop in egg production is noticed in their flocks. Since these two viral infections are associated with lowered egg production and no investigation has been done to identify the cause of lowered egg production due to these infections in Kerala, it was decided to undertake a systematic survey on the occurrence of these two viral agents among the poultry population in and around Trichur, by employing agar gel precipitation test.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Avian Infectious Bronchitis

Avian infectious bronchitis was first reported by Schalk and Hawn (1931) who observed the disease among young chicken in North Dakota, U.S.A. However, it was later observed that the disease was common in laying flocks as well. Subsequently several reports of the disease were made by Beaudette and Hudson (1933), Bushnell and Brandly (1933) and Beach and Schalm (1936) in United States. Woernle (1959) and Woernle and Brunner (1960) demonstrated infectious bronchitis antibodies by agar gel precipitation test. Woernle (1960) has not observed any difference between German strains, Beaudette strain, Connecticut and Massachusetts strain by agar gel precipitation test. Witter (1962) was able to demonstrate precipitating antibodies from seventh day of infection and it persisted for 20 to 94 days. Winterfield and Hitchner (1962) reported nephrosis associated with an outbreak of infectious bronchitis eventhough most of the lesions were confined to the bronchi. This syndrome was also reported from Australia by Cumming (1963) and appeared to be more severe than that occurred in United States.

In India occurrence of IB was first reported by Verma (1965) and subsequently by Adlakha (1966). Their

studies were mainly based on serological tests. They used both serum neutralization and agar gel precipitation tests for screening the birds. Verma et al. (1968) reported the occurrence of IB infection in the Veterinary College Poultry Farm, Mathura. The diagnosis was based on the isolation of IB virus - like agents in the embryonated chicken eggs and by serum neutralization test. Characteristic embryo mortality and gross lesions in experimental studies proved that these agents were infectious bronchitis virus. Kumar and Mallick (1971) conducted a serological survey on the incidence of this disease based on serum neutralization and gel precipitation tests. Sera of birds from 28 poultry farms were examined and they have established the wide prevalence of this disease in India. The percentage of incidence of IB, noted by serum neutralization test was 87%, 78%, 53% and 94%, whereas the percentage of incidence noted by agar gel precipitation test was 50%, 50%, 11% and 27% respectively in Uttar Pradesh, Delhi, Punjab and Maharashtra. Dawson and Gough (1971) could not observe any appreciable difference in antigenicity between fifteen British strains of IB virus when compared with seven standard reference strain of IB virus by cross neutralization test in chicken embryos. Khanna et al. (1972) conducted

a study on the occurrence of avian respiratory mycoplasmosis, infectious bronchitis and infectious laryngotracheitis in Maharashtra and in some of the northern states of India based on a systematic serological survey. They have examined the sera of birds from 34 farms. Plate agglutination test was employed for avian respiratory mycoplasmosis and gel precipitation test was used for the detection of IB and ILT. On the basis of gel precipitation test the prevalence of IB was noticed in 20 farms.

Mathur et al. (1972) made an attempt to detect the occurrence of precipitating and neutralizing antibodies against IB and ILT in migratory birds, to understand the epizootology of these diseases. But they could not detect the same in migratory birds.

Mahalingam et al. (1973) conducted a detailed study on the occurrence of infectious bronchitis in South India. They reported that the incidence of IB was as low as 0.01% in Tamil Nadu. They also stated that this disease was not prevalent in Andhra Pradesh, Karnataka, Pondicherry and Kerala. Opitz and Kamara (1973) reported the incidence of avian infectious bronchitis in commercial poultry farms in Sierra Leone. The occurrence of neutralizing antibodies against IB virus was as high as 56%. In a similar study conducted in Orissa, Tripathy

and Kar (1973) observed only 8.2% IB infection in poultry. Lohr (1974) conducted a study to assess the prevalence of neutralizing and precipitating antibodies against IB in both laying and broiler flocks. He could detect positive reaction in 15 laying flocks out of the 20 flocks examined. Of the 15 positive flocks, seven flocks gave positive reaction both by serum neutralization and precipitation tests, while only five flocks gave positive neutralization reaction and the rest three flocks gave only positive precipitation reaction. Out of the 13 broiler flocks examined, nine flocks were positive for both the tests.

Singh and Malik (1974) conducted a study to find out the highest concentration of virus in the inoculated chicken embryos for conducting the agar gel precipitation test. They were able to detect the highest concentration of IB virus on the chorioallantoic membrane 72 hours after the inoculation which persisted upto the seventh day.

Capeno et al. (1976) confirmed the occurrence of infectious bronchitis virus in Uruguay by experimentally reproducing the disease by inoculating the two recently isolated strains of virus into the trachea of 1 to 25 days old chicks and studying the characteristic symptoms, lesions and histopathology. Murthy et al. (1977) reported

the incidence of IB in chicken in Andhra Pradesh and Karnataka based on serological survey. They collected sera from White Leghorn, Rhode Island Red and crossbred birds from 36 farms under private and public sector. They observed 11.53% infection in Andhra Pradesh and 17.77% infection in Karnataka state.

Lohr (1977) made a trial to find out the cause of sudden drop in egg production in laying flocks in New Zeland. He observed that IB was likely to be the most important single disease responsible for this condition.

Holmes and Darbyshire (1978) have studied the ability of nine strains of IB virus to induce chicken interferon. Out of the nine strains studied, six strains of IB virus were not susceptible to the inhibitory effects of chicken interferon.

Gough and Alexander (1978) compared the different serological tests like serum neutralization, haemagglutination inhibition, complement fixation and agar gel precipitation test for measuring the primary immune response to IB vaccine. The serum neutralization titre showed considerable variation for individual chicken and large number of birds were negative over a period of 14 weeks after vaccination. Positive haemagglutination inhibition titres were recorded for most birds at one week after vaccination and these

persisted for a period of 14 weeks. The results obtained with agar gel precipitation tests were transient, variable and did not compare well with results obtained by other tests. The highest titre among agar gel precipitation reactors was seen two to three weeks after vaccination. Most birds showed positive titres with complement fixation test some time after vaccination but titres were low and did not correlate with results obtained by other tests.

An immune electron microscopy agglutination technique was developed for serotyping avian infectious bronchitis virus using Connecticut and Massachusettes 41 serotypes by Odenwald et al. (1978). The positive cases were determined by finding the aggregation of virus and antibody on the grid.

Peters et al. (1979) conducted an experiment to find out the susceptibility of organ cultures of chicken kidney and oviduct to a vaccine strain of IB. The minimal infectious dose of the H 52 strain of infectious bronchitis virus for organ cultures of oviduct and kidney was compared in chicken of different ages. Organ cultures of oviduct were found to be highly susceptible to infection when compared to kidney organ culture regardless of the age of chicken and no difference in

susceptibility could be demonstrated between cultures of the magnum and uterus of mature oviduct.

Infectious Laryngotracheitis

Infectious laryngotracheitis was first reported by May and Tittsler (1925) who described an outbreak in North America. Beaudette (1930) first reported that this disease was caused by a filterable agent and this was then called infectious bronchitis. Since the bronchi were not involved in this disease and lesions were confined to larynx and trachea, Beach (1930) and Graham et al. (1930) used the term infectious laryngotracheitis for this disease. Hudson and Beaudette (1932) reported that the chicken and pheasants were the only recognised hosts for ILT. Seddon and Hart (1935) studied the occurrence of ILT in fowls in New South Wales and identified the disease on the basis of typical growth of the virus on chorio-allantoic membrane of chicken embryo. Beaudette et al. (1948) have grown the virus in embryonated turkey and chicken eggs. The lesions noticed on chorioallantoic membrane were grayish plaques of about three to four mm in diameter. Satriano et al. (1957) isolated a mild strain of ILT virus from the lacrimal fluid of 19 days old Cornish cross chicks which did not show any

respiratory signs. Cover and Benton (1958) isolated virus similar to or identical with ILT virus from chicken showing symptoms of chronic respiratory disease.

Woernle and Brunner (1961) studied the importance of agar gel precipitation test in the diagnosis of ILT, using antigen prepared from chorioallantoic membrane of infected chick embryos and serum from infected fowls. Specificity of reaction was confirmed by comparative tests with avian infectious bronchitis, fowl pox and Newcastle disease. Jordan and Chubb (1962) determined the influence of the concentration of sodium chloride on agar gel precipitation test. They found that 8% salt concentration was necessary for the detection of threshold amount of either antigen or antibody. Using the agar gel precipitation test the identity of ILT precipitating antibodies was demonstrated in 40 samples of field sera from Great Britian, one from Australia and two from New Zealand. They compared egg inoculation and gel precipitation test for the diagnosis of ILT and its differentiation from fowl pox. They could not isolate the virus from the trachea of birds died of this disease, but the sera collected from these birds gave a positive agar

gel precipitation test. Ahmed and Monreal (1963) used agar gel precipitation test for the detection of latent form of ILT in fowls in Germany. Out of the 139 sera collected from seven farms, 41% was positive. The disease was subclinical in nature with complications of other respiratory disease. Jordan (1964) reported that ILT virus was more readily grown when inoculated by the "dropped" chorioallantoic membrane method than by the shell membrane method. Lezhava (1964) employed agar gel precipitation test for the diagnosis of ILT in fowls. He has inoculated several strains of ILT into chicken embryos and their presence was demonstrated by agar gel precipitation test using the supernatant from a centrifuged homogenate of infected chorioallantoic membrane as antigen and immune serum prepared in fowls or rabbits as antibody. The wells in agar were eight to ten mm apart. Similar results were also obtained by agar gel precipitation test using intra tracheal exudate of dead and slaughtered birds from infected farms, as antigen. Precipitating antibody was detected in serum of birds which had recovered from the disease or from the birds which were vaccinated against ILT.

Singh et al. (1964) reported the occurrence of ILT

for the first time in India by isolating the virus in embryonated chicken eggs, from the lung of infected birds in Uttar Pradesh. Panda and Singh (1967) have characterised the ILT virus of poultry by cultivation, propagation and serum neutralization test in developing chick embryos. Prasad and Malik (1968a) have isolated two strains of ILT virus, one from the Veterinary College, Poultry Farm, Mathura and other from Poultry Farm, Babugarh. The identification of the virus was made by various tests including, virus neutralization with known ILT hyper immune serum. They could neither isolate Newcastle disease virus or fowl pox virus nor detect antibody against Newcastle disease or fowl pox in the sera of these birds. Prasad and Malik (1968b) randomly collected 150 serum samples from Mathura and Babugarh and tested them for the presence of ILT virus antibodies. Only two samples gave positive reaction. Verma and Malik (1968) conducted a serological survey by employing agar gel precipitation test for the detection of ILT in various parts of India. They have observed 66.1% and 65.7% infection at the Veterinary College Poultry Farm, Mathura and Regional Poultry Farm, Mathura respectively. They have also noticed a low percentage of infection in Hyderbad but

no incidence in Ranipet. Zaheer et al. (1969) detected the presence of ILT in chicken in Andhra Pradesh from an outbreak by studying the symptoms, morbidity, mortality, histopathological picture and serological evidences. Khanna et al. (1972) conducted a study to find out the occurrence of avian respiratory mycoplasmosis, infectious bronchitis and infectious laryngotracheitis in Maharashtra State and some of the northern states in India. By employing the agar gel precipitation test they could detect the prevalence of ILT in 23 farms out of the 34 farms they have screened. Mahalingam et al. (1973) conducted a serological survey to detect the occurrence of avian respiratory mycoplasmosis, infectious bronchitis, infectious laryngotracheitis and chicken embryo lethal orphan virus in southern states of India. Of the 1,784 pooled serum samples examined, 544 were positive for avian respiratory mycoplasmosis, 17 for infectious bronchitis, 44 for infectious laryngotracheitis and 348 for chicken embryo lethal orphan virus. The incidence was higher in birds above six months of age. They have also reported that ILT was not prevalent in Kerala, but noticed the incidence of 0.001% ILT in Tamil Nadu, 1.46% in Karnataka, 3.57% in Pondichery and 0.02% in Andhra

Pradesh. Lohr and Saywell (1976) detected the prevalence of ILT antibodies in poultry in New Zealand. By using serum neutralization test in chicken embryonic kidney cell culture, they have observed ILT antibodies in sera of 14 flocks out of 54 flocks (26%) examined. A neutralizing index of 1.5 or more was considered positive. All the ten broiler flocks examined were negative. Sharma and Mehrotra (1976) conducted a study to find out the incidence of ILT infection in poultry in Rajasthan by employing gel precipitation and serum neutralization tests. Out of the 290 serum samples tested by agar gel precipitation test 63 samples were positive (21.7%). The serum samples negative by agar gel precipitation test when tested by serum neutralization test, gave a serum neutralization index of 0.7 which was considered negative. Murthy et al. (1977) employing the agar gel precipitation test reported an incidence of 38.63% of ILT infection in Andhra Pradesh and 15.55% in Karnataka.

Meulemans and Halen (1978) compared three methods of diagnosis of ILT. They could detect only 60% of cases by direct immunofluorescence method from cases detected by virus isolation in chick kidney

cells or in embryonating eggs. So they advocated coupling of direct immunofluorescence on tracheal sections with virus isolation and immunofluorescence in chick kidney cells as the good practical method for detecting ILT infection in sick birds. Ide (1978) has studied the sensitivity and specificity of the fluorescent antibody technique for detection of ILT virus. He has compared the efficacy of fluorescent antibody test with isolation of virus by inoculating clinical materials into chorioallantoic membrane of chicken embryo. He has observed a high degree of correlation between these tests.

MATERIALS AND METHODS

MATERIALS AND METHODS

Infectious Bronchitis

Seed Virus.

The infectious bronchitis virus (Mathura strain) was obtained from Indian Veterinary Research Institute, Izatnagar.

Preparation of Infectious Bronchitis Antigen.

Freeze dried ampuoles containing the seed virus was diluted in one ml of sterile distilled water and 0.2 ml each of the diluted viral suspension was inoculated into the allantoic cavity of 25 numbers of ten days old embryonated chicken eggs, collected from IB free flocks. Five, ten days old embryonated eggs were kept as control by inoculating 0.2 ml each of sterile normal saline into the allantoic cavity. The embryos were examined daily for any change. After the fourth day of inoculation, the embryos were killed by keeping them in the refrigerator at 4°C for one hour. The virus inoculated and the control embryos were examined for the presence of lesions. The allantoic fluid and chorioallentoic membrane from the eggs inoculated with the virus, were aseptically harvested. After freezing and thawing, the chorioallantoic membranes were finely triturated in sterile mortar and pestle

using sterile aberassive sand. To the triturated material, the harvested allantoic fluid was added at the rate of one ml per chorioallantoic membrane. After thorough mixing, the suspension was centrifuged for 20 minutes at 2,500 rpm in a refrigerated centrifuge maintained at 4°C. The supernatant so obtained after centrifugation was used as antigen for gel precipitation test. This was stored at -20°C in two ml vials and was used within one month. The remaining allantoic fluid was used for preparation of hyper immune sera.

Preparation of Infectious Bronchitis Hyper Immune Serum.

Six White Leghorn male chicks of six to eight weeks old obtained from the University Poultry Farm, Mannuthy were used for the production of IB hyper immune serum. The blood from these birds was collected and serum was seperated. This serum was tested against the common poultry infections and was found negative. Four birds were given 0.25 ml each of the allantoic fluid harvested earlier, by intratracheal route and 0.25 ml each through subcutaneous route on the first day. Two birds were kept as control. Three more injections in increasing doses of 0.75 ml, 1.0 ml, and 1.5 ml each were given in equally divided

doses by subcutaneous and intramuscular routes on every alternate day . Two weeks after the last injection, blood from each of these birds was collected and serum separated and stored at -20°C in the deep freeze in separate vials. The blood from the control birds was also collected on the same day, serum separated and stored at -20°C in the deep freeze.

Infectious Laryngotracheitis

Seed Virus.

The infectious laryngotracheitis virus (vaccine strain) was obtained from Indian Veterinary Research Institute, Izatnagar.

Preparation of Infectious Laryngotracheitis Antigen.

Freeze dried ampuoles containing the seed virus, was diluted in one ml of sterile distilled water and 0.2 ml each of the diluted viral suspension was inoculated into the chorioallantoic membrane of 25 numbers of ten days old embryonated chicken eggs, collected from ILT free flocks. Five, ten days old embryonated eggs were kept as control after inoculating 0.2 ml each of sterile normal saline into the chorioallantoic membrane. The eggs were candled every day. Fifth day after inoculation, the embryos were killed by keeping them at 4°C for one hour. The allantoic fluid

and chorioallantoic membrane from the eggs inoculated with the virus were aseptically harvested. The chorioallantoic membrane of eggs inoculated with the virus was compared with that of control. Using the above material, the antigen for gel precipitation test was prepared as in case of IB antigen. The antigen prepared was stored at -20°C in two ml vials and used within one month.

Preparation of Infectious Laryngotracheitis Hyper Immune Serum.

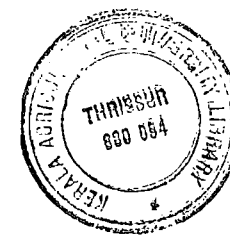
Infectious laryngotracheitis hyper immune serum was prepared in the same way as that of IB except that the suspension of ILT virus infected chorioallantoic membrane was injected instead of allantoic fluid.

Collection of Serum samples from field.

A total of 2,110 samples of serum were collected from different breeds of poultry viz. White Leghorn and Rhode Island Red, belonging to various poultry units located in Trichur and from Desi birds kept by farmers in and around Trichur. Serum samples were also collected from birds slaughtered at different hotels in Trichur (Table 1). These serum samples were collected over a period of six months.

Preparation of Agar.

Composition.



Noble agar	1.00 g
Sodium chloride	8.50 g
Merthiolate	0.01 g
Distilled water	100.00 ml

The ingredients were mixed and the contents allowed to boil. The medium was stored at 4°C in the refrigerator until used.

Preparation of Slides.

Clean micro slides of size 75 mm x 25 mm x 1 mm were used for conducting agar gel precipitation test. First they were coated with 1% agar in distilled water and dried. Two and a half millilitre of agar prepared above, was poured into each slides and allowed to set by keeping it at room temperature and at 4°C for ten minutes each.

Procedure.

A template with a central and six peripheral wells at 5 mm equidistance was made. The wells were 4 mm in diameter. By placing the template underneath the glass slides already prepared, wells were cut using a glass tube of 4 mm in diameter. With the help of a suction pump the agar material inside the well was removed.

Testing of Hyper Immune Serum.

Two wells of 4 mm in diameter each were cut on the agar gel slide at a distance of 5 mm apart. The antigens and hyper immune sera prepared earlier were taken from the deep freeze and thawed. The antigen was filled in one well of the slide and the corresponding anti serum in the other well. They were incubated at 37°C in a humid chamber. The gel slides were examined at 18, 24, 36, 48 and 74 hours interval and the reactions assessed. Infectious bronchitis and infectious laryngotracheitis were tested in different slides. Similarly the sera collected from control birds were also tested using IB and ILT antigens.

Testing of Field Serum Sample.

The serum samples collected from the field were pooled. One pool contained sera of ten birds. Altogether there were 211 pooled serum samples made from 2,110 individual serum samples. The procedure for conducting the gel precipitation test was similar to that of hyper immune serum except that the agar gel slides with seven wells were used. Four pooled samples were tested in a single micro slide using one positive and one negative control. Infectious bronchitis and infectious laryngotracheitis were tested separately using the same pooled serum samples.

Staining of Positive Slides. (Kwapinski, 1972)

Composition of stain.

Brom cresol blue	1 g
Sodium acetate - acetic acid buffer, 0.2 M, pH 3.6	1,000 ml

Composition of Decolourizer No. 1.

Methyl alcohol	45 ml
Glacial acetic acid	10 ml
Distilled water	50 ml

Composition of Decolourizer No. 2.

Ethyl alcohol (absolute)	40 ml
Glacial acetic acid	10 ml
Distilled water	50 ml

The positive slides were soaked in two changes of normal saline for 24 hours and then in distilled water for another 24 hours. The agar was covered with a moist Whatman's filter paper No. I and dried at 37°C. When the agar completely dried the filter paper was stripped off. The sticked pieces of filter paper were removed by wetting the agar slightly with a decolourizer and rubbing gently. The dried agar slides were immersed in brom cresol blue stain for 15 minutes and were washed in decolourizer No. 1 twice (each wash for 20 minutes) and then in decolourizer No. 2 for 20 minutes. Then the slides were dried at 37°C for one hour and were mounted.

RESULTS

RESULTS

Infectious Bronchitis

Chicken Embryo Inoculation.

The Mathura strain of infectious bronchitis virus inoculated into ten days old embryonated eggs did not cause mortality of embryos even after the fourth day of inoculation. However, when sacrificed on the fifth day the infected embryos revealed distinct dwarfing and curling when compared to the uninfected embryos. No pock lesions were seen on the chorioallantoic membrane.

Testing of Hyper Immune Serum.

The hyper immune serum prepared against infectious bronchitis virus when tested with IB antigen in agar gel slides, produced a distinct single line of precipitation within 36 hours of incubation (Fig. 1). This line was close and curved towards the antigen well.

Testing of Field Serum Samples.

Two hundred and eleven pooled serum samples were tested against IB antigen for detecting the presence of precipitating antibodies against IB virus. But none of the samples gave a positive precipitin line.

Infectious Laryngotracheitis

Chicken Embryo Inoculation.

The vaccine strain of infectious laryngotracheitis virus inoculated into ten days old embryonated eggs did not

cause any mortality of embryos even after the fifth day of inoculation. When compared to the controls the chorioallantoic membrane of infected embryos showed minute pocks scattered all over the membrane.

Testing of Hyper Immune Serum.

The hyper immune serum prepared against the infectious laryngotracheitis virus when tested with ILT antigen in agar gel slides produced a single line of precipitation of a diffuse nature within 36 hours of incubation (Fig. 2). This line was close and curved towards the antigen well.

Testing of Field Serum Samples.

Two hundred and eleven pooled serum samples were tested against ILT antigen for detecting the presence of precipitating antibodies against ILT virus. But none of the samples produced a positive precipitin line.

DISCUSSION

DISCUSSION

Infectious Bronchitis

Chick Embryo Inoculation.

Mathura strain of infectious bronchitis virus inoculated into ten days old embryonated eggs did not cause mortality of embryos even after fourth day of inoculation. But in contrast to the uninfected embryos to infected embryos when sacrificed on the fifth day showed curling and dwarfing, which have been considered as pathagnomonic in IB. No pock lesions were seen on the chorioallantoic membrane. Asplin (1948), Fabricant (1949) and Van Roekel (1955) noticed the curling and dwarfing of embryos when inoculated with IB virus. Verma et al. (1968) observed similar lesions by inoculating suspensions of infected lung and trachea into the chicken embryos. Hofstad (1972) also recorded similar lesions in chick embryos inoculated with IB virus. The result of the present study was similar to the findings described by these earlier workers.

Testing of Hyper Immune Serum.

The hyper immune sera prepared against IB virus when tested with IB antigen on agar gel slides produced a distinct single line of precipitation, close and curved towards the antigen well, within 36 hours of

incubation at 37°C, in humid chamber. This curving and closeness of precipitin line towards the antigen well could probably due to higher concentration of antibodies in the sera and higher molecular weight of antigen (Cunningham, 1978). Verma (1965), Adlakha (1966), Tripathy and Kar (1973) and Lohr (1974) have noticed only a single line of precipitation when the positive serum was tested with IB antigen. But Woernle (1959) and Kumar and Mallick (1971) have noticed two precipitin lines in certain positive samples tested with IB antigen. The IB antigen they used contained more than one strain of IB virus and the two precipitin lines which they have observed may be due to the difference in the antigenicity of strains used. Since in this study only one strain (Mathura strain) was used as antigen and for producing hyper immune sera, only one line of precipitation was noticed and this is in agreement with the findings of most of the workers.

Testing of Field Serum Samples.

A total of 2,110 serum samples were collected, pooled into 211 groups and tested by agar gel precipitation test. None of the samples gave a positive reaction. In India IB was first reported by Verma (1965)

and subsequently by Adlakha (1966). Their studies were mainly based on serological tests. They employed both serum neutralization and agar gel precipitation tests for screening the birds against IB. In these tests they noticed that both the tests gave almost the same results. But Kumar and Mallick (1971) employing serum neutralization test noticed a higher percentage of incidence of IB infection than with agar gel precipitation test. They observed that this was due to the early disappearance of precipitating antibodies.

In the present study serum samples were collected from different localities in Trichur. These serum samples could be considered as representative samples because, the collection was made not only from birds maintained in a confined system, but also from birds reared in individual houses and from birds slaughtered in hotels. The sera collected from houses and hotels were of Desi birds. The management practices adopted by farmers were such that the birds were always open to infection. The sera collected from birds slaughtered in hotels were also a good material for conducting serological screening against IB, because these birds were brought from different villages by the vendors.

The farmers dispose of their birds only when they become unproductive. Since the serum samples were collected from different sources and over a period of six months, precipitating antibodies against IB virus, if present, could have been detected from the serum samples by agar gel precipitation test. Serum samples were tested by agar gel precipitation test only against Mathura strain of IB virus, because no antigenic difference was observed between different strains of IB virus (Woernle, 1960). Since all the samples tested gave a uniformly negative result to agar gel precipitation test, it was inferred that IB infection was not prevalent in Trichur and its suburbs.

Mahalingam et al. (1973) observed that IB was not prevalent in Andhra Pradesh, Karnataka, Pondichery and Kerala. Murthy et al. (1977) in their study found that IB was prevalent in Andhra Pradesh and Karnataka. No survey was conducted to find out the prevalence of IB infection in Kerala after 1973. Nair (1979) could not encounter lesions suggestive of IB infection in any of the birds autopsied during the period 1960 to 1979 in the Department of Pathology, College of Veterinary and Animal Sciences, Mannuthy. The results of present

study agree with that of Mahalingam et al. (1973) and Nair (1979).

Infectious Laryngotracheitis

Chick Embryo Inoculation.

Vaccine strain of ILT virus inoculated into ten days old embryonated eggs did not cause mortality of embryos even after the fifth day of inoculation. On examination of these embryos on the sixth day, the chorioallantoic membranes have shown minute pocks distributed all over the membrane. Burnet (1934) noticed two types of pocks on the chorioallantoic membrane of ILT virus infected chick embryos, viz. large pocks with opaque periphery and necrotic centre produced by virulent virus and small pocks without necrosis produced by less virulent ILT virus. Singh et al. (1964) noticed pin point to pin head sized white pocks on the chorioallantoic membrane of embryos inoculated with suspension of lung and trachea of infected birds. They observed that the pocks increased in size upon serial passage. This was attributed to the increased virulence of the virus on serial passage. When the virulence was enhanced, the size of the pocks was also found to increase correspondingly. But Panda and Singh (1967) have noticed only one type of pocks of one to two mm diameter on the chorioallantoic membrane of embryos

inoculated with virulent ILT virus. Since a mild virulent vaccine strain was used in the present study for chick embryo inoculation, only small pocks were produced on the chorioallantoic membrane. This finding agrees with the observations of Burnet (1934) and Singh et al. (1964).

Testing of Hyper Immune Serum.

The hyper immune sera prepared against ILT virus when tested with ILT antigen on agar gel slides, produced a single diffused line of precipitation, close and curved towards the antigen well, within 36 hours of incubation at 37°C in a humid chamber. The curving and closeness of the precipitation line towards the antigen well could probably due to higher concentration of antibodies in the sera and higher molecular weight of the antigen (Cunningham, 1973). Most of the workers have noticed a single line of precipitation when the ILT positive serum was tested by agar gel precipitation method. But Jordan and Chubb (1962) have noticed two lines of precipitation in a few positive cases of ILT. Prasad and Malik (1968b) have also noticed two lines of precipitation in a case of ILT. But Verma and Malik (1968) could observe two lines of precipitation only in 14.7 of samples tested.

However, they have observed two lines of precipitation with hyper immune sera collected ten days after the last injection. So they assumed that the antibody fraction responsible for the second line of precipitation might have only a short span of life resulting in the early disappearance from the blood. In the present study, the hyper immune serum was collected fifteen days after the last injection. The antibody fraction responsible for the second line might have disappeared from the blood when the serum was collected on the 15th day. So a single line of precipitation was obtained. This agrees with the findings of the above workers.

Testing of Field Serum Samples.

A total of 2,110 serum samples were collected. They were pooled into 211 groups and tested by agar gel precipitation test. None of the samples gave a positive reaction. Woernle and Brunner (1961) employed agar gel precipitation test for the first time for demonstrating ILT precipitating antibodies in the serum of infected birds. Later Jordan and Chubb (1962), Ahmed and Monreal (1963) and others employed this test to find out the incidence of ILT in the field. In India agar gel precipitation test was used by Prasad and Malik (1968b) for testing a limited number of serum samples for diagnosing ILT

infection in a Poultry Farm attached to the Veterinary College, Mathura. They got only two positive cases out of the 150 serum samples tested. Later Verma and Malik (1968) have reported 66.1% incidence of ILT infection from the same farm. They also tested 73 serum samples from Ranipet, Madras but none of the samples gave positive reaction.

In the present study the sera employed for screening birds against ILT was the same as that used for screening birds against IB infection. These samples have such a diversity in breed, age, location and time of collection which make them suitable for screening field infection by agar gel precipitation test. Mahalingam et al. (1973) have noticed a low percentage of infection of ILT in Karnataka and Andhra Pradesh but no evidence of infection in Kerala. But Murthy et al. (1977) noticed a higher percentage of ILT infection in Karnataka and Andhra Pradesh. No survey was conducted to find out the prevalence of ILT infection in Kerala since the study of Mahalingam et al. (1973).

Nair (1979) could not encounter lesions suggestive of ILT infection in any of the birds autopsied during the period 1960 to 1979 in the Department of Pathology,

College of Veterinary and Animal Sciences, Mannuthy. The results of the present study regarding the occurrence of ILT infection in and around Trichur, concur with the observations of Mahalingam et al. (1973) and Nair (1979).

Agar gel precipitation and serum neutralization tests were the usual methods employed for mass screening of birds against ID and ILT infections (Verma and Malik, 1968). Inspite of the fact that the precipitating antibodies appear and disappear rapidly in the birds after infection making the agar gel precipitation test comperatively less sensitive than serum neutralization test, it was still considered to be the test of choice. It was simple, economical and requires only less amount of reactants. A large number of serum samples can be tested within a short period of time with this test.

According to Verma et al. (1968) in an area showing high incidence of infection, it is immaterial if a few cases are not detected by the agar gel precipitation test. On the other hand in an area showing low percentage of infection as detected by agar gel precipitation test, it is advisable to conduct serum neutralization with "agar gel precipitation test negative samples", in order to

find out the exact incidence. In the present study all the samples tested gave negative result with agar gel precipitation test. So it was considered unnecessary to conduct serum neutralization test with the serum samples, as all the serum samples may not be from birds of same immune status.

In the present study serum samples were collected from diverse sources, spreading over a long period and precipitating antibodies, if present in the poultry population, would have been detected at least in a few samples by agar gel precipitation test. Since all the samples gave uniformly negative results, it was considered beyond doubt that IB and ILT infections do not exist at least in and around Trichur.

SUMMARY

SUMMARY

A total of 2,110 serum samples were collected from the birds of different ages, comprising of White Leghorn, Rhode Island Red and Desi birds from different farms, households and hotels located in Trichur district. These serum samples were tested against infectious bronchitis and infectious laryngotracheitis by agar gel precipitation method. The serum samples were grouped into 211 pools. Precipitating antibodies to infectious bronchitis and infectious laryngotracheitis were tested separately on different agar gel slides.

The antigens used for testing these field samples were prepared using the chorioallantoic membrane and allantoic fluid of infected embryos. The potency of these antigens was tested by conducting agar gel precipitation test with corresponding antisera prepared in White Leghorn male chicks of six to eight weeks of age.

When the field serum samples were tested with this antigen, none of the samples gave a precipitin line either to infectious bronchitis or to infectious laryngotracheitis. Since all the samples gave uniformly negative result to agar gel precipitation test it was assumed that these two viral diseases are not prevalent in and around Trichur.

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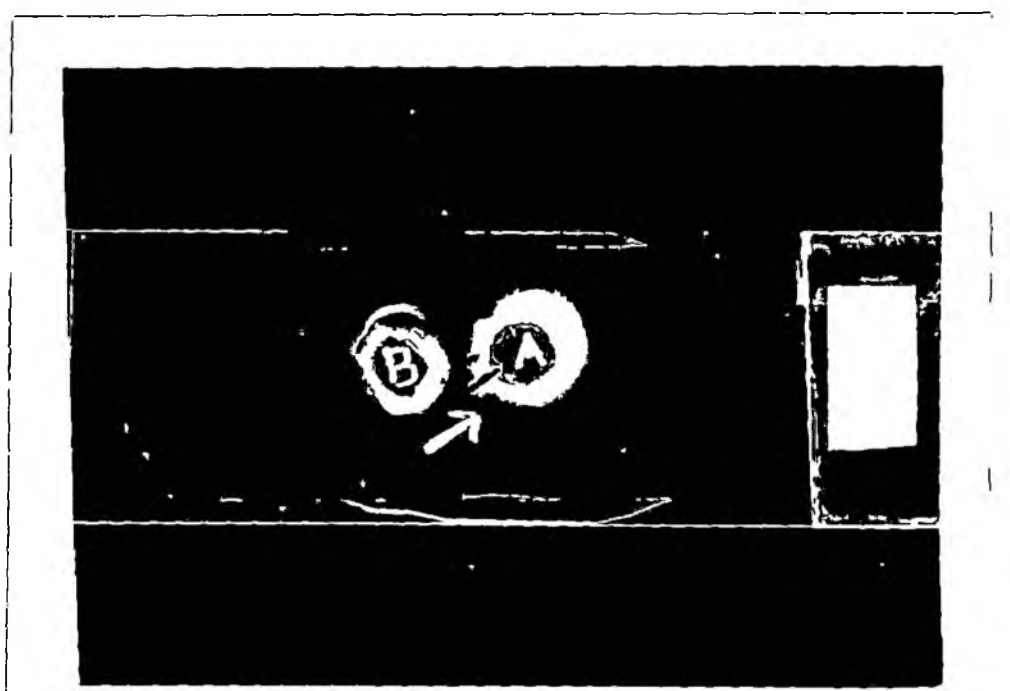
TABLES

Table I. Details of serum samples collected from the field

Sl. no.	Name of poultry farms/hotels	Breeds of Fowls						Total No. of sera collected	Total No. of pools tested
		White Leghorn		Rhode Island Red		Desi birds			
		No. of sera collected	No. of pools tested	No. of sera collected	No. of pools tested	No. of sera collected	No. of pools tested		
1.	Kerala Agricultural University Poultry Farm, Mannuthy.	450	45	50	5	—	—	500	50
2.	Bethany Convent Poultry Farm, Kottappady.	60	6	—	-	—	—	60	6
3.	Chacko's Poultry Farm, Annakara.	40	4	—	-	—	—	40	4
4.	Ambassador Hotel, Trichur.	—	-	—	-	140	14	140	14
5.	Hotel Liberty, Trichur.	—	-	—	-	120	12	120	12
6.	Kinsotel, Trichur.	—	-	—	-	150	15	150	15
7.	From individual houses.	—	-	—	-	1,100	110	1,100	110
	Total	550	55	50	5	1,510	151	2,110	211

PLATES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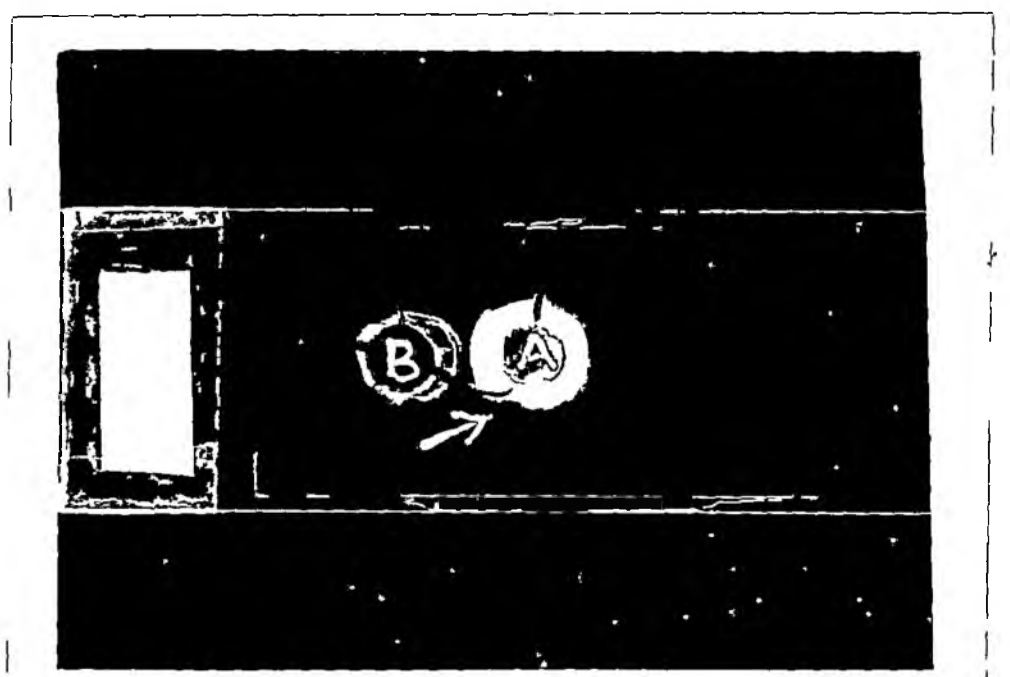
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IMMUNOLOGICAL SURVEY ON
THE INCIDENCE OF INFECTIOUS BRONCHITIS (IB) AND INFECTIOUS LARYNGOTRACHEITIS (ILT) IN POULTRY IN AND AROUND TRICHUR

BY

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ABSTRACT OF A THESIS

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of the requirement for the degree

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AB TRACT

Infectious bronchitis and infectious laryngotracheitis are the two viral diseases of poultry responsible for economic loss to the poultry industry by way of decreased egg production, poor quality of eggs, decreased feed efficiency and loss of weight gain. These diseases have been reported from the neighbouring states of Kerala. In the present study a serological survey was carried out to understand the prevalence of these two diseases in the poultry population in and around Trichur.

A total of 2,110 serum samples have been collected from the field, comprising of White Leghorn, Rhode Island Red and Desi birds belonging to different age groups. Serum samples were collected from organised farms, from birds kept by farmers and from the birds slaughtered in different hotels at Trichur.

These serum samples were tested against the infectious bronchitis and infectious laryngotracheitis, by employing agar gel precipitation test. The chorioallantoic membrane and allantoic fluid of infected embryos were used for the preparation of antigens for agar gel precipitation test. The potency of antigens was tested by conducting the agar gel precipitation test with corresponding hyper immune sera prepared in

White Leghorn male chicks of six to eight weeks of age. A line of precipitation was obtained in both cases which was close and curved towards the antigen well, because of the high concentration of antibody in the sera and due to the high molecular weight of the antigen. In the case of infectious bronchitis the line of precipitation was distinct where as in case of infectious laryngotracheitis it was diffused.

The antigen, those efficacy was tested using hyper immune sera, was used to test samples of sera collected from the field. The samples were pooled to 211 groups and tested for the presence of infectious bronchitis and infectious laryngotracheitis precipitating antibodies separately by agar gel precipitation test. None of the samples gave precipitin line either to infectious bronchitis or to infectious laryngotracheitis. So it was assumed that both of these viral diseases are not prevalent in Trichur and its suburbs.