SUSCEPTIBILITY OF DUCKS TO NEWCASTLE DISEASE VIRUS (NDV) AND THEIR ROLE IN THE TRANSMISSION OF THE DISEASE TO CHICKEN

BY

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "SUSCEPTIBILITY OF DUCKS TO NEWCASTLE DISEASE VIRUS (NDV) AND THEIR ROLE IN THE TRANSMISSION OF THE DISEASE TO CHICKEN" 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.

Mannuthy,
31-7-1981.

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CERTIFICATE

Certified that this thesis, entitled "SUSCEPTIBILITY OF DUCKS TO NEWCASTLE DISEASE VIRUS (NDV) AND THEIR ROLE IN THE TRANSMISSION OF THE DISEASE TO CHICKEN" is a record of research work done independently by Smt.D.Sudharma, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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CONTENTS

INTRODUCTION

INTRODUCTION

Asia is considered to be the homeland of wild ducks or the mallard from which the modern domestic ducks have been evolved in Europe and America through scientific breeding, feeding and management practices. Ducks occupy second place to chicken for the production of table eggs in India. Ducks are traditionally reared in coastal areas, where they can swim and feed on aquatic fauna. The extensive inland waterlogged area form an excellent natural habitat for waterfowls.

Newcastle disease (ND) is a virus infection of birds which can cause mortality even up to 100 per cent in susceptible chicken. It was first reported in the Dutch East Indies (Kraneveld, 1926) and described in the following year by Doyle (1927) when a series of outbreaks occurred near Newcastle-on-Tyne from which the disease got its name. Later the disease was reported from almost all the countries. At present ND occurs as a series of clinical entities that range from inapparent to fulminating fatal illness which is determined primarily by the strain of the virus.

The mode of spread of ND is influenced by transport of live birds, movement of personnel and infected materials between poultry premises, wind, free-living birds and wild

life. Almost all species of birds are susceptible to this viral infection and many of them are believed to act as silent carriers of the disease. There are reports of isolation of the virus from most of the species of birds such as turkeys (Gray et al. 1954); pigeons (Hilbrich, 1972; Erickson et al. 1980); sparrows (Gustafson and Moses, 1953); doves (Magid et al. 1965); pheasants (Makay, 1967); guinea fowls (Ballarini, 1964); peacock and peafowl (Tsiroyannis et al. 1971; Sokkar and Refaie, 1967); parrots (Cullen et al. 1974); crows (Sulochana et al. 1981 b); ravens (Danchev, 1970); king penguin (Krauss et al. 1963); quails (Higgins and Wong, 1968) and ostrich (Corrado, 1966). Wild free-flying birds caught in the vicinity of poultry farms were found to be infected with NDV (Lancaster, 1977) and such birds can act as the focus of infection to the susceptible birds. This would explain the sudden reappearance of the disease in areas which are free of ND.

Eventhough ducks are considered to be resistant to NDV infection (Asplin, 1947) there are reports of isolation of the virus from both normal and ailing birds (Rosenberger, 1974; Higgins, 1971). Some of the isolates were highly pathogenic to chicken (Sulochana et al. 1981 a) while

others were of low virulence (Rosenberger et al. 1975). The ducks can pick up NDV infection, remain normal, and transmit the same to chicks and other susceptible birds.

The migratory and foraging pattern of waterfowls provide ideal conditions for transmission of viruses from them to domestic poultry or even man and other animals. The nonmigratory ducks will contaminate the waterway system and thus help in transmission of the virus (Bahl et al. 1977). Since the duck breeders transport the birds to different places according to harvesting seasons, the silent carriers among them can spread the virus to different parts of the country and this will give the effect of migratory birds in disease transmission. The desi breeds of ducks need constant access to water there by contaminating it with the excreta and other discharges.

Ducks are shown to respond serologically to NDV and isolations have been made from both normal (Bahl et al.1977) and diseased ducks (Kingston et al. 1978). According to some workers (Sarma et al. 1977) ducks are susceptible to experimental ND, while others report their resistance by experimental transmission (Sriraman et al. 1980).

During the middle of 1976, outbreak of an acute disease characterized mostly by respiratory and nervous

symptoms occurred in ducks in Kerala. Detailed investigations carried out in the department of Microbiology (Nair, 1978) showed that the disease was due to duck plague virus and could be controlled by vaccinating the birds against duck plague. At present duck plague vaccination is carried out as a routine in Kerala. Eventhen there are reports of the occurrence of a disease of similar nature in vaccinated as well as unvaccinated flocks from different parts of the state. Moreover Sulochana and Nair (1979) and Sulochana et al. (1981 a) have isolated NDV from ducks showing respiratory/alimentary tract affections. They have also reported that one of the isolates was highly pathogenic to eight week-old chicken (Sulochana et al. 1981 a). However, the actual role of NDV in causing the disease condition of the above nature has not been established.

In the above circumstances it was felt worthwhile to take up a study on the incidence of ND among duck population in Kerala by serological survey and virus isolation trials. The susceptibility, type of symptoms and lesions, duration, mode of virus excretion and antibody response were studied by employing ducklings of two different age groups. The role of ducks in the transmission of ND to

susceptible chicks and the possible infection of ducks from infected chickens were also investigated.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Newcastle disease (ND) is a highly contagious disease which affects mainly chicken and guinea fowl. It can also affect a wide range of domestic as well as wild birds. The family Anatidae which includes ducks, geese and swans, collectively called as waterfowls, although generally refractory, can pick up infection, leading to heavy mortality or the birds become chronic carriers of the virus. Since the first report of ND in ducks by Doyle (1927) several reports describing either the susceptibility or resistance of these birds to NDV infection have been published.

Natural Infection of Ducks and other Waterfowls

Susceptibility.

Farinas (1930) and Picard (1934) observed that ducks were susceptible to ND and many of them died during NDV epizootics. Naturally occurring cases of ND in ducks with clinical symptoms leading to death were also reported by Albiston and Gorrie (1942) and Beaudette (1943). However, Albiston and Gorrie (1942) and Beaudette (1943) considered ducks to be resistant to ND as they could not recover the virus from dead birds. Similar reports were also made by Kaschula *et al.* (1946) and Nobindro (1946). Moine (1950)

and Bush (1954) reported naturally occurring cases of ND in ducks and geese and recorded a mortality rate of up to 100 per cent. Newcastle disease virus was isolated from six of the nine outbreaks that occurred in Hong Kong (Higgins, 1971). All the outbreaks were acute affecting mainly young birds and characterized by rapid spread, high morbidity and mortality. The symptoms observed were anorexia, diarrhoea, oculo-nasal discharge and paraplegia. When affecting laying flock a drop in egg production was also noticed. It was also found that most of the outbreaks occurred during the cool dry season. None of the affected birds had any specific haemagglutination inhibition (HI) antibodies. The probable source of the virus was thought to be either from the neighbouring duck farm or chicken farm. Higgins (1972) could identify 819 outbreaks of ND affecting ducks, geese, quails, pigeons and some birds of prey during a period of three years. The acute disease among Alabio and Entok ducks in Indonesia described by Kingston et al. (1978) commenced as sudden death of ducklings of two weeks of age and persisted for six weeks. The symptoms shown by the birds were anorexia, watery eyes and depression followed by death. The postmortem lesions were not marked. The virus isolated from brain,

kidney, liver, trachea, intestine, proventriculus and lungs of dead and sick birds were of mesogenic type and pathogenic to three-day-old ducklings. The authors claimed that their isolates were distinct from that of chicken NDV as they failed to agglutinate goose erythrocytes. Newcastle disease virus isolations were also made from ducks and ducklings showing respiratory/alimentary tract affections (Sulochana and Nair, 1979; Sulochana et al. 1981 a). The birds from which the isolations were made had anorexia and respiratory distress followed by sudden death. They had also stated that one of their isolates was highly pathogenic to eight week-old chicken, but the role of this strain of NDV in producing the disease condition was not confirmed.

Resistance.

Cooper (1931) reported that ducks could be infected by artificial inoculation but the natural infection was very rare. Dobson (1939) found that ducks were resistant to both natural and experimental infection with NDV. According to Asplin (1947) NDV infection in ducks and geese were very mild and could be detected only by an increase in HI antibody titre. Attempts to isolate the virus from liver, spleen and bone marrow were also unsuccessful.

Mild and asymptomatic form of the disease were also observed by Khan and Huq (1963) and Gaudry et al. (1970). Resistance of ducks to ND was also reported by Berthelon and Tournut (1949) and Forbes and Row (1966).

Geese and swans were also reported to be either resistant (Beaudette, 1943; Nobindro, 1946; Asplin, 1947; Lancaster, 1963) or susceptible (Moine, 1950; Bush, 1954). Raszewska (1966) isolated two velogenic strains of NDV from dead goslings aged 11-14 days, which were highly pathogenic to goslings of a few days of age. Weidenmuller (1972) described a natural outbreak of ND in geese following contact with infected fowls. The symptoms described were inappetence, diarrhoea and somnolence, but no respiratory symptoms. He found that out of the 29 geese which were in contact with the infected fowls, eight had severe symptoms and five of them died. Newcastle disease was recognized in all cases by postmortem lesions, virus isolation from brain or by serological tests.

Experimental Infection

Ducks and Geese.

Cooper (1931) infected the ducks experimentally and recovered the virus, but Dobson (1939) found that the ducks

were resistant to both natural and experimental infections. Iyer (1945) conducted experimental infection of mature ducks and eight week-old ducklings by subcutaneous inoculation of a virus suspension containing one million minimum lethal dose (MLD) per ml. Varying doses of primary and secondary infections at different intervals were tried but none of them produced reaction. Infection by contact also failed to produce any reaction. The emulsion of liver and spleen collected from infected birds was incapable of infecting susceptible chicken. He also found that the serum samples were negative for any virus neutralizing antibodies and suggested that ducks and ducklings were resistant to NDV infection.

Asplin (1947) could infect ducks and geese artificially with NDV and observed virus excretion from day three onwards. However, he considered ducks to be more resistant than fowls as no symptoms of the disease could be observed in the experimentally infected birds. Teklinska et al. (1956) found that experimentally infected ducks and geese commenced to excrete the virus in three to six days and their droppings were infective to hens even after 15 days of infection. Although the excretion of virus by geese was only of short duration the authors

opined that this factor should be considered in prophylactic measures against ND. The virus was found to persist in the intestine of experimentally infected duck for a period of six months (Winmill and Haig, 1961), and the virus had a particular tropism to the intestine compared to other tissues and organs (Marek et al. 1967).

Friend and Trainer (1972) experimentally infected mallards with NDV. Only large intravenous doses of NDV could produce clinical disease and/or death. They were of opinion that the death was due to virus toxicity rather than an infectious process. The clinical manifestations of the disease were suggestive of nervous system involvement. They isolated virus mainly from the brain and in a few cases from lung, liver and spleen, but no evidence of excretion of infectious virus was observed. They also found that the antibody response which appeared in three to four days persisted at diagnostic levels even up to the fortieth day.

Kontrimavichus and Akulov (1973) studied experimental ND in goslings aged 18-26 days by intramuscular inoculation. Five to seven days following inoculation the birds developed acute respiratory and nervous symptoms ranging from complete paralysis to tremors or circling movements. Mortality was

variable, reaching 100 per cent in younger goslings. Postmortem lesions were catarrhal pneumonia, enteritis, oedema of the brain and haemorrhages and microscopic necrotic foci in the internal organs. Virus was found in the excreta by five to eleven days after inoculation. Haemagglutination inhibition antibodies were also detected in goslings infected with virulent or attenuated strains of NDV. Spalatin and Hanson (1975) exposed Canada geese to experimental NDV infection. About 20 per cent of the infected geese developed clinical signs seven days after intravenous exposure from which NDV could be recovered from the brain and spleen. There was no specific gross lesions in the brain. No viraemia was also detected in any of the exposed goose and none of them was found to excrete the virus through the cloaca. Serum samples collected from these birds had shown that virus neutralizing (VN) and HI antibodies were present in the sera from day eight onwards and it persisted through 184 days following exposure. The goose having preexposure antibody showed a rapid rise following infection and reached a higher titre than the other geese.

Experimental infection of ducklings with RDV was carried out by Sarma et al. (1977). Week-old ducklings

were infected either by drinking water, intranasal, intraocular or subcutaneous route. Eleven out of 24 ducklings exposed to infection by different routes died and virus isolations were made from their tissues. All ducklings infected intramuscularly with the virus showed only a rise in antibody titre. The sera of recovered ducklings also had HI antibodies ranging from 1:4 to 1:32. They concluded that ducklings were susceptible to infection through drinking water, and intranasal/intraocular inoculation of virulent virus, but were relatively resistant to infection by subcutaneous route. Chang (1976) tried to attenuate NDV by continuous passage in ducks. He found that a velogenic strain of NDV could be passed serially in ducks for only three to five passages and to obtain 32 passages the virus had to be propagated at intervals in chick embryos. Virus of the 16th and 32nd duck passage showed little attenuation, and could still be considered velogenic.

Kingston et al. (1978) could produce ND in three day-old ducklings with a mesogenic strain of NDV isolated from an acute disease in Indonesian ducks. He could also recover viruses from all the experimentally infected ducklings. Sriraman et al. (1980) experimentally infected

nondescript ducklings with velogenic RDV by intranasal, intraocular or oral routes at the rate of 0.2 ml per bird. Although one each from intraocular and oral group died by day three and four respectively they neither had any specific lesions of ND nor their tissues were positive for NDV antigens by immunoperoxidase test. They also found that the ducklings that showed mild symptoms such as slight serous nasal discharge and glueing of the eye recovered and became normal by the sixth day.

Duck and Goose embryos.

The first report on the propagation of NDV in duck embryos was by Collier and Dinger (1950). Novilla and Navarro (1970) in their preliminary study on the identification and classification of NDV strains obtained from field outbreaks in Philippines used embryonated duck eggs for titration of five lentogenic and two mesogenic strains of NDV. Sarma et al. (1977) while conducting experimental infection of ducklings with NDV tested the ability of the virus to multiply in duck embryos by yolk-sac or allantoic routes of seven and 11 day embryos respectively. The duck embryos were found to support virus multiplication as indicated by embryo mortality, lesions produced and considerable rise of haemagglutination titre. The mortality pattern,

either by allantoic or yolk-sac route, was almost similar but the presence of virus could be demonstrated only in the allantoic fluid.

Kontrimavichus and Akulov (1973) found out the susceptibility of 10 day-old goose embryos to NDV by allantoic inoculation with virulent (T-61) and attenuated (H, Lasota) strains. Goose embryos inoculated allantoically with T-61 strain died after 24-72 hours, those with H strain after 44-64 hours and Lasota strain after six to 10 days suggesting that goose embryos were susceptible to NDV. Spalatin and Hanson (1975) infected goose embryos to find out their susceptibility by allantoic sac inoculation. They employed a lentogenic strain (B_1) and a velogenic strain (Texas-GB) for inoculation into goose embryos of two different age groups — 14 and 24 days old. Both B_1 and Texas-GB strains were rapidly fatal to 14 day old goose embryos. Neither B_1 nor Texas - GB was able to kill all goose embryos that were 24 days old. The hatchability of both infected and uninfected eggs was about 50 per cent.

Role of Waterfowls in Epizootiology of Newcastle Disease

Virus Isolation.

Reid (1961) screened the birds that were imported to

Great Britain for the presence of NDV infection. Random samples of tissues, mainly portions of skin, from the carcases were tested by inoculating into developing chick embryos. He could isolate the virus from 11 per cent of the ducks and 6.9 per cent of the geese. Rosenberger (1974) collected cloacal and tracheal swabs from 159 birds including blue and green winged teal, mallard, black ducks, wood ducks and Canada geese and isolated 15 haemagglutinating agents of which nine were identified as NDV by HI test. Pearson and McCann (1975) while investigating the role of indigenous, wild, semidomestic and exotic birds in the epizootiology of VVND in Southern California found that ducks, pheasants, peafowl, pigeons and doves were infected with VVND virus. Domestic NDV was also isolated from 1.65 per cent of semidomestic birds and suggested that free-flying wild ducks and doves were potential carriers of NDV over long distances. Nine VVND and 17 non VVND - NDV were isolated from 1,679 ducks. These authors suspected water-borne infection in waterfowls. They also found that NDV was highly antigenic for mallards, though there was no evidence for the multiplication of the virus. During a study on the epizootiology of ND, Spalatin and Hanson (1975) isolated and characterized four lentogenic strains of NDV in California. These strains failed to induce apparent

disease in chickens, and to plaque on chick embryo fibroblast without additives, and had characteristics similar to that of the four isolates from migratory geese of the Atlantic flyway. These isolates differed from the chicken lentogenic strains in the thermostability of haemagglutinins. Isolation of lentogenic strains of NDV from migratory waterfowls in U.S.A was also made by Rosenberger et al. (1974) and they suggested that clinically normal ducks could carry mild strains of NDV. Rosenberger et al.(1975) isolated four strains of NDV from Canada geese in the Atlantic migratory route, all of which were lentogenic, with an embryo mean death time greater than 100 hours and nonpathogenic to day-old and three week-old chickens. The haemagglutinin of two of the isolates was stable at 56°C for 15 minutes, one for 30 minutes and the other for at least two hours. Plaques were also produced by all the isolates in chicken embryo fibroblasts and they differed in many respects from the currently used commercial vaccine strains. The possibility of captive waterfowls acting as carriers of NDV was also suspected by Humphrey (1976). Bahl et al. (1977) isolated three lentogenic NDV from 184 tracheal swabs of healthy migratory mallard ducks in the Mississippi flyway during their investigation into

the epizootiology of influenza in Minnesota. They were of the opinion that the migratory and foraging patterns of waterfowls could provide ideal conditions for transmission of viruses from migratory waterfowls to domestic poultry or even man and other animals. They also thought that nonmigratory ducks would contaminate the waterway system and thus help in the transmission of the virus. Avirulent strains of NDV indistinguishable from the chicken isolates of Australia and Northern Ireland and some turkey isolates in United States were isolated from migratory waterfowls in North America. However, these isolates differed from the avirulent thermostable chicken viruses of United States and United Kingdom. Shortridge and Alexander (1978) screened apparently healthy ducks, geese and fowls at a poultry dressing plant in Hong Kong. Tracheal and cloacal swabs were collected at random on a weekly basis from November, 1975 to June, 1977. Fifty-five isolates reacting specifically with reference NDV antisera in the HI test were isolated from the trachea or cloaca of 2,046 individual birds. More isolations were made from the cloaca than the trachea of ducks whereas equal numbers were obtained from both sites in geese. Although NDV was isolated throughout the year, approximately 70 per cent of

the isolations were made during the winter months of November to March. The authors also noticed that the birds from which the isolations were made showed no evidence of illness. However two isolates from ducks (D10/75 and D12/75) were highly pathogenic to chicken and eluted rapidly from chicken erythrocytes while those of low virulence did so slowly. All the isolates were heat labile. The authors suggested that the birds were either asymptomatic carriers or had only recently been infected and that signs of disease were yet to appear. A less virulent strain of NDV, different from other viruses of low virulence, in the heat resistance of the haemagglutinin at 56°C was isolated from a wild mallard duck (Alexander et al. 1979). Although the authors were unable to determine the origin of the duck — whether migratory or resident — they concluded that nonvaccinal NDV of low virulence could be present in wild birds of Great Britain. Shortridge (1980) sampled domestic poultry on a weekly or fortnightly basis from November, 1977 to October, 1978 and isolated two hundred and sixteen haemagglutinating agents from the trachea and cloaca of 2,844 individual ducks, geese and fowls. The isolations of NDV made from 1,061 tracheal and 605 cloacal swabs of ducks were 15 and 16 respectively, while from geese, 156 tracheal and 125 cloacal swabs gave one and

eight isolations. They found the pattern of isolation as cyclical and seasonal.

Contact Infection.

Nobindro (1946) reported that fowls of all breeds were susceptible to NDV by natural infection, but ducks and geese appeared to be resistant. He opined that ducks and geese could not pick up infection by contact from infected fowls since these birds kept in a common pen together with ailing birds had failed to contract the infection. Crowther (1952) was also unable to get any field evidence to say that ducks and geese were associated with the spread of ND. Report of an outbreak of ND following the feeding of uncooked goose viscera (Heller, 1957) and the fact that virus could be recovered from the intestinal contents of ducks (Winmill and Haig, 1961) suggested that these birds could play a role in the transmission of the disease. Evidence were also provided by Marthedal et al. (1963) to substantiate that ducks and geese were associated with transmission of ND. The role of ducks in the transmission of ND as a mild asymptomatic carrier was also reported by Khan and Hug (1963). Vrtiak (1958) described the epidemiology of ND in Eastern Slovakia, where the incidence was highest in spring and early summer. Newcastle disease virus was isolated from

48 of the 265 samples which included brain, spleen and lung tissue. He suspected ducks as major source of infection as they could be latent carriers of the disease. Lancaster (1963) in his review on the modes of spread of ND opined that ducks and geese were more resistant to this disease but they might play a part in its dissemination. Lancaster and Alexander (1975) suspected wild birds and poultry including ducks as important in the local spread of the disease. However, Lancaster (1977) in his review on the geographical incidence and epizootiology stated that he could observe clinical symptoms and antibody in both domestic as well as wild waterfowls. Lancaster also observed that the infection persisted longer in the intestine than in other organs and that wild life particularly waterfowls are indispensable in the spread of the disease. He also found that they play a very important role in the transmission of virulent virus to poultry.

Weidenmuller (1972) reported an outbreak of ND in a flock of geese which was in contact with infected fowls. Newcastle disease was diagnosed in the geese from postmortem lesions, isolation of the virus from brain and serological tests. This indicated that geese could get contact infection from fowls. Spalatin and Hanson (1975) isolated four

strains of the virus from migratory ducks. They were lentogenic but differed from lentogenic strains prevalent in chicken being thermostable. From their observations they opined that wild waterfowls neither got infection from domestic poultry nor transmitted the disease to poultry. Sarma et al. (1977) in their studies on experimental infection of ducklings with NDV showed that ducklings could transmit the disease to contact chickens.

Antibody Response.

Haemagglutination inhibition antibodies in the sera of ducks having sub clinical infection of ND was reported by Asplin in 1947. During a serological survey on the incidence of ND in ducks Vrtiak (1968) had shown that 16.7 per cent of the serum samples had HI antibody titre of 1/32 and higher, while Ahmed et al. (1968) found only 3.3 per cent of a total of 690 pooled serum samples positive for HI antibodies to ND, with titres ranging from 10 to 40. Friend and Trainer (1970) screened a total of 200 adult female and 35 adult male mallards from three commercial game farms in Wisconsin and Illinois for the presence of HI antibodies. Eighty from group one and nine from group two had antibodies to NDV, but only six of the 106 mallard ducklings had a positive HI titre. Higgins (1971) was

unable to demonstrate any antibodies in the sera of ducks belonging to an affected flock in Hong Kong. He concluded that ND vaccines are nonimmunogenic in this species. Shortridge and Alexander (1978) screened apparently healthy ducks, geese and fowls at a poultry dressing plant in Hong Kong. Blood samples for serum antibody assay were collected at random on a weekly basis from November, 1975 to June, 1977. Among the 100 ducks and geese examined five ducks and 31 geese had HI antibody. The titres ranged from 10 to 160 in ducks and 10-120 in geese. The authors did not observe any difference in seropositivity or titre levels in birds originating from Hong Kong and People's Republic of China.

Newcastle disease antibodies were also detected in the sera of other waterfowls such as teals, geese, mallards and swans. During their survey for viral antibodies Hore et al. (1973) trapped 168 grey teals between June and July of 1972. They could detect antibodies to NDV in 16 per cent of ducklings in Gippsland and 10 per cent in Victoria. Although these two flocks were well separated they showed a similar pattern in the HI titre, suggesting that the virus was wide spread at least in one species of waterfowls. Bradshaw and Trainer (1966) could detect ND antibodies in wild migratory geese and mallards. Palmer and Trainer (1970) tested 3,010 serum

samples from migratory and nonmigratory Canada geese and 31 per cent of them were found to show HI antibodies ranging from 1:20 to 1:128. About one per cent of the goslings were also reactors. These authors also reported that the number of reactors did not differ consistently between migratory and nonmigratory or between sexes.

Serological survey on the incidence of ND in inter-continental migratory birds showed that two of the six genera (Anas and Fulica) had HI antibodies in their sera (Chandra et al. 1973). Evidences were also there that these birds visited the continents of Europe, Asia, North Africa and North America which made the authors to think that these birds could act as potential transmitters of ND between continents. During an epornitic of velogenic viscerotropic ND in Southern California Pearson and McCann (1975) collected free-flying wild birds, captive and free-ranging semidomestic birds and exotic birds from a quarantine area to determine their role in the epizootiology of the disease. Haemagglutination inhibition antibodies against domestic NDV was demonstrated in 8.28 per cent of 2,004 semidomestic sera tested. Among the semidomestic birds waterfowl had the highest frequency of HI. Spalatin and Hanson (1975) conducted serologic survey of waterfowl of

Mississippi flyway. Mallard ducks and wild geese were trapped-live and the sera tested for the presence of HI and VN antibodies to NDV. Newcastle disease virus reactors were found in both the groups obtained at all the four collecting sites. Out of 483 birds tested 77 (16%) showed positive response of 1:10 and above. The VN test was also positive having a titre of 100 or greater. Egg yolk specimens from 36 eggs of domestic geese and seven eggs of Canada geese were examined for the presence of antibodies to NDV. More than 50 per cent of the eggs tested from both domestic and Canada geese had neutralizing antibodies. Antibody response of goslings infected experimentally showed that about 50 per cent of them had antibodies immediately after hatching but disappeared by the 4th week.

Friend and Trainer (1970) collected sera from one hundred swans from five captive flocks in Michigan and forty-nine of them were found to have HI antibodies of 1:20 or above.

MATERIALS AND METHODS

MATERIALS AND METHODS

Natural Infection of Ducks with Newcastle Disease Virus

Ducks.

Desi as well as Khaki campbell cross-bred ducks from different parts of the State suffering from respiratory/ alimentary tract affections and also normal ones were screened for the virus infection by virus isolation and/or antibody titration.

Virus Isolation.

Tryptose phosphate broth (Difco).

This was prepared according to the instructions given by the suppliers. Antibiotics were added to get a final concentration of 1000 IU of benzyl penicillin and 1000 micrograms of streptomycin per ml of the medium (TPB -A).

Specimens for virus isolation.

Cloacal and throat swabs.

Cloacal and throat swabs collected from 75 desi ducks were brought to the laboratory, soaked in TPB-A and stored at -20°C until used for inoculation into developing chick embryos.

Tissues.

When dead birds only were available, tissues such as

liver, lung, spleen and brain were collected under aseptic conditions and preserved at -20°C in TPB-A.

Processing of specimens for inoculation into developing chick embryos.

Cloacal and throat swabs.

Swabs soaked in TPB-A and stored at -20°C were allowed to thaw at room temperature. These swabs were then squeezed well with a sterile pipette for two minutes and centrifuged at 1000 G for 15-20 minutes at 4°C. The clear supernatant fluid was collected, incubated at 37°C for one hour and inoculated into the allantoic cavity of 10 day embryonated eggs.

Tissues.

At the time of use the tissues were emulsified in TPB-A, with the help of a Tenbroeck tissue grinder, to get a 10-15 per cent (W/v) suspension. This tissue homogenate was centrifuged at 1000 G for 15-20 minutes to remove the coarse particles. The clear supernatant was then incubated at 37°C for one hour and then inoculated into 10 day embryonated eggs by allantoic route.

Chicken embryos.

Ten day-old embryonated eggs were received from the University Poultry Farm, Mannuthy.

Chicken embryo inoculation (Rovozzo and Burke, 1973).

After determining the viability of the embryo, the air cell region and the position of the embryo were marked. The air cell region was sterilized with tincture of iodine and a hole was made, using a driller, about one centimetre away from the margin of the air cell. The sample to be tested was inoculated into the allantoic cavity at the rate of 0.2 ml per embryo with a tuberculin syringe and a 20 - gauge needle. Two eggs were used for each sample. The hole was then sealed with melted paraffin and incubated at 37°C with the broad end up. Care was always taken to provide 55-60 per cent of humidity (Hoskins, 1967) in the incubator. All the eggs were candled daily and the embryos died within 24 hours of inoculation were discarded. The embryos that died after 24 hours and those still alive after the fifth day were transferred to the refrigerator for chilling.

Harvesting of Allantoic fluid.

The air cell region of the chilled egg was sterilized with alcohol. The shell at this region was removed with a sterile scissors, the shell membrane and chorio allantoic membrane were separated and the allantoic fluid was collected with separate sterile Pasteur pipettes for each sample. The

fluid from the two eggs inoculated with the same sample was pooled and were tested for haemagglutinating activity by mixing equal quantity of allantoic fluid and a 0.5 per cent chicken RBC. When agglutination was observed with a particular sample it was then titrated by the method described below. Always a sample was considered negative only after three blind passages.

Haemagglutination test (Poultry Biologics, 1963).

Serial double fold dilutions of the virus were made in normal saline solution, mixed with equal quantity of 0.5 per cent suspension of chicken RBC and incubated at room temperature for 30 minutes. Simultaneously, RBC controls were made by mixing RBC suspension with equal quantity of normal saline. The results were read after the controls had settled.

Chicken erythrocytes.

Blood collected in Alsever's solution was washed three times with normal saline and suspended in the same solution to get a final concentration of 0.5 per cent RBC.

Haemagglutination Inhibition Test.

The specific identity of a haemagglutinating agent as NDV was determined by HI test using known ND positive serum.

Newcastle Disease Virus Antiserum.

Sera collected from birds hyperimmunized with NDV were inactivated at 56°C for 30 minutes to destroy the nonspecific agglutinins and were used as ND antiserum.

Haemagglutination Inhibition Procedure (Poultry Biologics, 1963).

The beta method of HI test was employed. Serial double fold dilutions of the preinactivated sera were made in normal saline (0.2 ml) and were then mixed with equal quantity of 8 HA units (0.2 ml) of the virus. Incubated at room temperature for 30 minutes. Equal quantity (0.4 ml) of 0.5 per cent suspension of chicken RBC was added and mixed well. Simultaneously, RBC and virus controls were made, and the readings were taken after 30 minutes of incubation at room temperature.

Detection of Haemagglutination Inhibition Antibodies.

Serum samples.

Blood collected from the wing vein of 226 birds belonging to various parts of Kerala (Table 1) was allowed to clot at room temperature. After about 30 minutes the clot was disturbed with a glass rod for easy separation of the sera and kept at 4°C. Next day, the serum was collected,

inactivated at 56°C for 30 minutes and stored at -20°C until used for titration of HI antibodies.

Newcastle Disease Virus.

A strain of NDV used for challenging vaccinated birds received from the Veterinary Biological Institute, Palode was used for HI tests.

Experimental Infection Studies

Virus.

The pathogenicity of the above strain of ND virus was studied by mean death time, intracerebral pathogenicity in day-old chicks, intravenous pathogenicity in eight week-old chicks and cytopathic effects in chicken embryo fibroblasts before it was used for experimental infection studies.

Chicks.

Day-old unvaccinated chicks were received from the University Poultry Farm, Mannuthy. The two and eight week-old chicks required for the study were obtained by rearing these chicks under controlled conditions in the laboratory.

Equine Erythrocytes.

Equine blood collected in Alsever's solution was washed three times in normal saline and used as a 0.5 per cent suspension.

Hanks' Balanced Salt Solution (HBSS) (Cunningham, 1966).

The pH was adjusted to 7.6 to 7.8 using sodium bicarbonate.

Tissue culture Growth Medium.

Hanks' balanced salt solution was supplemented with 0.5 per cent lactalbumin hydrolysate, 0.15 per cent yeast extract and five to eight per cent calf serum. Antibiotics at the rate of 200 IU of penicillin and 200 micrograms of Streptomycin per ml along with 50 units of mycostatin per ml were also added.

Maintenance Medium.

Same as above except that no serum was added.

Calcium Magnesium free Buffer (CMF - PBS).

Prepared as described by Cunningham, 1966.

7.5 per cent Sodium Bicarbonate.

Prepared as per the method given by Bishai et al. (1974).

Trypsin (0.25%).

A five per cent stock solution of 1:250 Difco trypsin was prepared in CMF-PBS, sterilized by filtration through Seitz filter pads, distributed in small quantities and stored at -20°C. The working solution of 0.25 per cent was prepared at the time of use by diluting the stock solution with CMF-PBS.

Antibiotic Solution.

A stock solution of sodium penicillin and dihydrostreptomycin sulphate, was prepared in sterile distilled water and stored at -20°C. The concentrations of these antibiotics were decided in such a way that when one ml of this mixture was added to 100 ml, a final concentration of 200 IU of penicillin and 200 micrograms of streptomycin per ml was obtained.

Mycostatin.

A stock solution to contain 5,000 units per ml was prepared in sterile distilled water and stored at -20°C.

Calf serum.

Blood collected from young crossbred calves was allowed to clot in a slanting position, for easy separation of serum. The separated serum was transferred to a sterile flask, inactivated at 56°C for 30 minutes, sterilized by filtration through Seitz filter pads, and stored at -20°C until used.

Ducklings.

Ducklings of two age groups were used for this study. The first set of experimental infection studies were conducted in eight week-old ducklings while the second set was in week-old ones. All of them were purchased from a local duck

breeder in Trichur. The ducklings were kept under observation for a minimum of three days. Before inoculation they were tested for the presence of ND antibodies or virus.

Newcastle Disease Virus titration in embryonated eggs (Hoskins, 1967).

Serial ten-fold dilutions of the virus was made in TPB-A, from 10^{-1} to 10^{-11}. The diluted virus (0.1 ml) was inoculated into the allantoic cavity of 10 day embryonated eggs as described earlier, employing three eggs per dilution. Post inoculation incubation and examination of eggs were as described previously. Fifty per cent embryo lethal dose (ELD_{50}) was calculated as per the method described by Reed and Muench (1938).

Mean Death Time.

Serial ten-fold dilutions of the virus ranging from 10^{-1} to 10^{-9} were prepared. The last four dilutions were inoculated into the allantoic cavity of 10 day embryonated eggs, using eight eggs per dilution. The eggs were incubated as described earlier. Candling was done at eight hour intervals and the observations were recorded. From this the mean death time was calculated using the formula given in Poultry Biologics (1963).

Intracerebral Pathogenicity Index in day-old chicks (ICPI) (Hanson, 1975).

Ten, day-old unvaccinated chicks were inoculated with 0.1 ml of a 1:10 dilution of the virus, into the cerebral cortex using a 25 gauge needle. To five control chicks 0.1 ml of sterile saline was given. The chicks were housed separately and observed daily until death. The survival index was based on time of death and calculated by using the factor zero for normal, one for diseased and two for dead. The resulting sum was divided by the number of observations.

Intravenous Pathogenicity Index in eight week-old chickens (IVPI).

Eight week-old unvaccinated chicks were inoculated subcutaneously with 1:10 dilution of the virus in the form of infected allantoic fluid. Control birds were given 0.1 ml of sterile TPB. The chicks were observed daily until death. The factor for each observation was, zero for normal, one for signs, two for paralysis and three for death. IVPI was then calculated as described in Poultry Biologics (1963).

Haemagglutination with Equine Erythrocytes.

The ability of the virus to produce agglutination of equine RBC was detected by the technique as described with chicken RBC replacing it with the former.

Chicken Embryo Fibroblast cultures (Cunningham, 1966).

The air cell region of 10 to 11 day embryonated eggs was sterilized by mopping it with 70 per cent alcohol. The egg shell at this region was cut opened with sterile scissors. After separating the shell membrane and Chorio allantoic membrane (CAM) the embryo was transferred into a Petridish containing CMF-PBS, with 200 IU of penicillin, 200 micrograms of streptomycin and 50 units of mycostatin per ml. The head, limb and visceral organs were removed by grasping with a sterile forceps. The embryos were transferred to a wide mouthed flask, minced into pieces of about 1-2 mm size and washed twice with CMF - PBS and once with CMF-PBS containing 0.25 per cent trypsin.

The minced tissues were transferred to a trypsinization flask containing teflon coated magnetic stirrer, added ten volumes of prewarmed 0.25 per cent trypsin in CMF-PBS and stirred over a magnetic stirrer for three to five minutes. The supernatant was discarded and washed with 0.25 per cent trypsin in CMF-PBS. Fresh trypsin solution was added and stirred for 20-30 minutes. The cell sediment was resuspended in growth medium and recentrifuged. Likewise washing of the cells was repeated twice and finally resuspended in growth medium to get a final concentration of 5×10^5 cells per ml.

Chicken Embryo Fibroblast cultures (Cunningham, 1966).

The air cell region of 10 to 11 day embryonated eggs was sterilized by mopping it with 70 per cent alcohol. The egg shell at this region was cut opened with sterile scissors. After separating the shell membrane and Chorio allantoic membrane (CAM) the embryo was transferred into a Petridish containing CMF-PBS, with 200 IU of penicillin, 200 micrograms of streptomycin and 50 units of mycostatin per ml. The head, limb and visceral organs were removed by grasping with a sterile forceps. The embryos were transferred to a wide mouthed flask, minced into pieces of about 1-2 mm size and washed twice with CMF - PBS and once with CMF-PBS containing 0.25 per cent trypsin.

The minced tissues were transferred to a trypsinization flask containing teflon coated magnetic stirrer, added ten volumes of prewarmed 0.25 per cent trypsin in CMF-PBS and stirred over a magnetic stirrer for three to five minutes. The supernatant was discarded and washed with 0.25 per cent trypsin in CMF-PBS. Fresh trypsin solution was added and stirred for 20-30 minutes. The cell sediment was resuspended in growth medium and recentrifuged. Likewise washing of the cells was repeated twice and finally resuspended in growth medium to get a final concentration of 5×10^5 cells per ml.

One ml of diluted cell suspension containing 5 x 10^5 cells was transferred into test tubes containing coverslips and was corked tightly. The tubes were incubated at 37°C in a slanting position. A satisfactory monolayer was obtained on the next day and this was used for virus inoculation.

Inoculation.

Tubes with confluent monolayers were selected, poured off the growth medium and washed twice with 1-2 ml of serum free maintenance medium. Newcastle disease virus diluted 1 : 100 was then inoculated on to the monolayer culture at the rate of 0.1 ml per tube, and incubated at 37°C for one hour for adsorption. After the adsorption period 0.9 ml of maintenance medium was added and incubated at 37°C. Control tubes were similarly treated except that virus inoculum was replaced by the same quantity of maintenance medium. The tubes were examined under microscope everyday for the presence of cytopathic changes.

When cytopathic changes were observed the medium was poured off, washed the coverslips thrice with phosphate buffered saline, fixed with formol saline and stained with haematoxylin and eosin.

Infection of Eight week-old Ducklings.

Twenty-three, eight week-old ducklings were screened

for the presence of ND antibodies and virus as described previously. Three of them were found to possess antibodies, and were discarded. The remaining 20 ducklings were divided into three batches. The first two batches containing seven each and the third six. Th the first batch, 0.2 ml of the undiluted virus was inoculated by intranasal route (Table 8), while second batch was infected by swabbing over the conjunctiva (Table 9). The third batch of six ducklings served as uninfected control. All the three batches were kept in separate pens and adequate care was taken to avoid cross contamination. They were examined everyday for the presence of any symptoms suggestive of ND. Both cloacal and throat swabs were collected on third, fifth and seventh day post infection and thereafter at weekly intervals till the sixth week. Serum samples were also collected from second to sixth week post infection to determine any rise in HI antibody titres.

Contact transmission.

To find out the ability of the infected ducks to transmit the virus to chicken, four chicks of two weeks of age were housed with each group of ducklings from fourth day of infection. Swabs and sera were collected from these birds also to find out the evidence of infection.

Infection of week-old Ducklings.

Thirty, one week-old ducklings were divided into six groups of five ducklings each (Table 10). All the ducklings were tested and found negative for both antibodies and virus. The virus was diluted at the rate of 1:10 before inoculation. To the first group 0.2 ml of the virus was administered both by intranasal and intraocular routes. To the second group 0.2 ml of the virus was given by intramuscular route, and the third group received 0.2 ml of the virus subcutaneously. The fourth batch was kept in a room in which NDV (Velogenic strain of NDV isolated from a crow) infected birds were kept till the previous day of transfer of ducklings. All the ducklings were examined daily for the development of any abnormal symptoms. Swabs and sera were collected at intervals as mentioned above.

Contact transmission.

Unvaccinated two week-old chickens (six each)were housed along with the first three groups of ducklings on the day of infection itself. To study the possibility of contact infection of ducklings from infected chickens, six chickens were infected through intranasal route and were kept with the fifth batch of uninfected ducklings. The 6th batch of five ducklings served as uninfected controls. In both the experiments

adequate care was taken to avoid cross contaminations between groups. Swabs and sera were collected at regular intervals as mentioned above.

Collection of tissues.

From dead ducklings and chickens pieces of liver, lung, spleen and brain were collected for virus isolation trials. Tissues were also collected and fixed in 10 per cent formalin for histopathological studies.

Processing of swabs and tissues were done as described previously. Chick embryo inoculation, harvesting of the allantoic fluid, testing for haemagglutinating agent and specific identification by HI test were done as before.

Histopathological examination of tissue.

The formalin fixed tissues were processed, embedded in paraffin, sections of three micron thickness were cut and stained with haematoxylin and eosin (Luna, 1968).

RESULTS

RESULTS

Natural Infection of Ducks with Newcastle Disease Virus

Virus Isolation.

Cloacal and throat swabs and tissues collected from 76 ducks reared in different parts of Kerala were inoculated into the allantoic cavity of 10 day embryonated eggs for the isolation of newcastle disease virus. Out of the 151 samples screened (from 76 ducks), eleven showed positive haemagglutination (HA) by the first passage itself and the HA was inhibited by specific ND antiserum. All the other samples were negative even after three blind passages (Table 1).

Antibody Response.

A total of 226 serum samples of ducks from different parts of the State were screened for the presence of HI antibodies employing a strain of NDV received from the Veterinary Biological Institute, Palode. Thirty-four samples had an HI titre of 1:20 and above accounting to 15.04 per cent. The serum titres and number of reactors with each titre are given in table 2. All the remaining samples had titres lower than twenty. The ducks from which the virus isolations were made were negative for any HI antibody.

Experimental Infection Studies

Virus characteristics.

The strain of NDV used for challenging birds vaccinated with test vaccine, received from the VBI, Palode, was used for all the experiments during this study. The virus on receipt in the laboratory was characterized before it was used. The original virus had an HA titre of 1 : 640 and was inhibited by specific NDV antisera. The results of various tests of characterization are presented in table 3.

Embryo Lethal Dose $_{50}$.

The ELD_{50} of the virus in developing chick embryos was $10^{-8.75}$ as determined by Reed and Muench method (1938) (Table 4). All the dead embryos had lesions characteristic of NDV infection such as haemorrhage in the occipital region, interdigital space and under aspect of the abdomen.

Mean Death Time.

The mean death time of the minimum lethal dose was calculated (Table 5) to be 53 hours as per the method given in Poultry Biologics (1963). The rate of death was as follows: one died at 40 hours of incubation, two at 48 hours, four at 56 hours and one at 64 hours. All the dead embryos showed specific lesions of ND as described earlier.

Intracerebral Pathogenicity Index (ICPI).

Ten, day-old chicks inoculated intracerebrally with 1 : 10 dilution of the virus were observed daily for symptoms or death. Two of them were found dead on the next day. Four chicks showed symptoms of drowsiness, paralysis of wing, diarrhoea, inappetence and were in sleeping posture. Four chicks were normal. On the second day of infection, six chicks died and one of the remaining showed the above symptoms and died on the third day. The other chick remained normal till the fourth day. On fifth day it showed nervous symptoms like convulsion, turning the head to one side and respiratory distress and died on the sixth day. The ICPI was calculated to be 1.67 (Table 6). The control chicks inoculated intracerebrally with normal saline, were normal and active during all these days.

Intravenous Pathogenicity Index (IVPI).

Intravenous pathogenicity index was tested in six, eight week-old chickens. All birds were normal on the day following infection, but showed symptoms of ND on the next day. The symptoms included drowsiness, dropping wings, white diarrhoea and inappetence. No paralysis was observed. Three of them died the next day while other three continued in the same stage. They also died on the fourth day. From

the data IVPI was calculated to be 1.5 (Table 7). Control chickens did not show any abnormal symptoms.

Haemagglutination with Equine Erythrocytes.

The virus did not produce agglutination of equine erythrocytes.

Cytopathic effect (CPE).

A confluent monolayer of chicken embryo fibroblast was formed 24 hours following seeding. The morphology of the cells were of fibroblastic type. They were spindle shaped and in areas of high density were oriented with their long axis parallel to one another (Fig. 1.). The cytopathic effect of NDV was studied by inoculating 1 : 100 dilution of the virus in 0.2 ml quantities as described previously. Cytopathic effect began to appear from 48 hours following infection and was characterized by initial granularity of the cytoplasm. The affected cells showed a tendency to get rounded up and by 96 hours gradual disintegration of the monolayer occurred (Fig.2). Giant cells containing large number of nuclei were also seen. Eosinophilic intracytoplasmic inclusions were also a feature in cells stained with haematoxylin and eosin. None of the above changes were noticed in the control cultures.

Infection of Eight week-old Ducklings.

Intranasal inoculation.

All the seven ducklings that received intranasal inoculation showed symptoms suggestive of ND. The symptoms manifested were droopiness, general depression, inappetence and white diarrhoea (Fig. 3). However, all of them became normal and active by seventh day. No other abnormal changes were noticed during the period of six weeks of observation. The swabs collected at intervals detailed earlier were tested by chick embryo inoculation and all were found to excrete the virus from third till the seventh day post infection. After the seventh day all of them became negative and no virus could be isolated during the remaining period of the experiment. The sera collected at intervals from these birds showed a steady increase in titre up to the fourth week and remained steady during the next two weeks. The titre ranged from 10-160 (Table 8).

Intraocular inoculation

All the seven birds infected by the intraocular route showed the same symptoms and recovered by the fifth day. The birds were found to excrete the virus either through the oral or cloacal route as evidenced by virus isolations from the throat or/and cloacal swabs taken at intervals. The

virus excretion started from day three and lasted till the fifth day (Table 9). Out of these seven birds, two excreted the virus by throat alone, one by cloaca alone and four by both the routes. Antibody titres also showed a steady increase which ranged from 10-320. All the ducklings were active and normal for the rest of the experimental period.

Contact Transmission to Chicken.

To study the ability of the ducklings to transmit the disease to chicken, four, two-week old chickens were housed with each of the two groups of experimental ducklings. On the third day following exposure one bird showed symptoms of general depression, white diarrhoea and paralysis of the wings. All of them died by seventh day. On postmortem examination the proventriculus and caecal tonsils showed slight peticheal haemorrhages. Inoculation of processed tissues into the allantoic cavity of 10 day embryonated eggs resulted in the isolation of virus from one chick in each group.

None of the control ducklings showed any symptoms of ND. Attempts to isolate the virus and to detect serum antibodies by haemagglutination inhibition test were also unsuccessful.

Experimental Infection of week-old Ducklings.

Intranasal/Intraocular inoculation.

The first batch of five ducklings inoculated by the

intranasal/intraocular route showed no abnormal symptoms up to the seventh day. On the eighth day three birds showed the general symptoms of ND including swelling and glueing of the eyelids, nasolachrymal discharge, paralysis of legs and respiratory distress. The symptoms were suggestive of pneumoencephalitis. Death commenced from tenth day post-infection and continued up to the 25th day. All the five ducklings died (Table 11) and on postmortem examination no specific lesions were found in any of the visceral organs or brain. Out of the five birds virus isolations were made from four. The HA activity of these isolates was inhibited with specific ND antisera. Sera collected from these birds had only titres below 10.

Six unprotected chickens that were housed with the ducklings to find its ability to transmit the disease did not show any symptoms even after one month and the sera from these chickens were also negative for any HI antibodies. On challenge with virulent NDV all these contact birds showed typical symptoms and died by day four.

Intramuscular inoculation.

One of the five ducklings showed symptoms of central nervous system involvement by the 5th day. The pneumo-encephalitic symptoms included paralysis of legs, inability

to feed properly, and in later stages the bird sat on its breast with head down and turned to one side (Fig. 4 & 5). Death occurred on the seventh day. The other birds in the group showed symptoms of nervous system involvement but not as severe as in the first case. Postmortem examination showed no lesions in the digestive tract. The liver and lungs were congested in some cases. Virus was isolated from the lung and spleen of the bird that showed marked symptoms (Table 12). Swabs taken at regular intervals were also positive for the virus. Sera from none of the birds showed any HI antibodies.

The incontact chickens showed no abnormal symptoms (Fig. 6) or any rise in antibody titre. All of them died on challenge with the virulent virus with typical symptoms and lesions of ND.

Subcutaneous inoculation.

Nervous symptoms showed by the above two batches of ducklings were also seen with this group of birds that received subcutaneous inoculation. The first death occurred on the tenth day following infection which continued up to the sixteenth day. The virus was isolated from the cloacal swabs of one duckling from day seven onwards. Postmortem examination of the dead ducklings did not show any marked

lesions except that the liver was pale in three cases. No HI antibody could be detected in any of the birds (Table 13).

Four of the six incontact chicken died between first and second week after infection. Before death they showed droopiness, off feed, paralysis of legs and wings, dullness with a tendency to stand in a sleeping posture with the head drawn towards the body, soiled vent, white diarrhoea and in advanced stages fowls lay on the floor with their beak turned down. Postmortem examination showed lesions like peticheal haemorrhages in proventriculus, caecal tonsils and intestine. Liver, lung and spleen showed slight congestion. Intestinal contents were very little. Virus could be isolated from all the four cases. The remaining two chickens were normal and possessed no antibody. On challenge all of them died with typical lesions of ND.

Indirect infection.

In the fourth group all the birds placed in the contaminated coop, died. The symptoms were as mentioned earlier and death occurred more rapidly. On postmortem examination no specific lesions could be seen except slight congestion of visceral organs (Table 14).

Contact infection of Ducklings.

The fifth batch of five ducklings were kept along with intranasally infected chickens. All the exposed chickens died by third day showing specific symptoms and lesions of ND. The contact ducklings started showing symptoms on sixth day post-infection. The symptoms included general depression, inappetence, slight respiratory distress and profuse diarrhoea. They were inactive and drooping. They started dying on eighth day post infection and continued up to twentieth day. The postmortem lesions included slight congestion of all the organs. Most of them showed yellowish liver with streaks of congestion. Inoculation to embryonated eggs resulted in isolation of virus from all five cases. Sera collected on the second week had an antibody titre of 1:20 (Table 15).

Control ducklings.

The control ducklings were active and normal during the entire experiment period and showed no antibody in their system.

Histopathological examination.

The changes observed in tissues were as follows. In the liver moderate congestion of all the vessels and sinusoids were present. Focal areas of degeneration, necrosis and

fatty change were also observed. The lung showed congestion and were infiltrated with neutrophils and mononuclear cells. Accumulation of oedematous fluid was also seen in focal areas. In spleen there was moderate congestion of vessels and depletion of lymphocytes. Neuronal degeneration and moderate gliosis were the changes observed in the brain.

Table 1. Screening of Ducks for Virus and Antibody

Place of collection	No. of samples collected	Virus isolation		Percentage of isolation	Serum samples tested	HI positive samples.	Range of antibody titre	Percentage of birds showing antibody
		T*	C**					
Irinjalakuda	24	2	2	16.67	12	2	20	16.67
Mudikode	33	1	2	9.09	17	8	20-64	47.06
Mukkattukara	76	1	3	5.26	38	8	20-160	21.05
Quilon	18	0	0	0	9	-	-	-
Mannuthy	0	-	-	-	150	16	20-40	10.67
Total	151	4	7	7.28	226	34	20-160	15.04

* Throat swabs

** Cloacal swabs

Table 2. Serum Titres and Number of Reactors

Antibody titre	Number of birds showing reaction	Percentage
160	1	0.44
64	1	0.44
40	8	3.54
20	24	10.62
Below 20	192	84.96

Table 3. Characterization of Newcastle Disease Virus

Characteristics	Results
Embryo lethal dose $_{50}$	$10^{-8.75}$/0.2 ml
Mean death time	53 hours
Intracerebral Pathogenicity index	1.67
Intravenous pathogenicity index	1.5
Haemagglutination with equine erythrocytes	Negative
Cytopathic effects	Present

Table 4. ELD_{50} of the Challenge Virus

Dilution	No.of eggs	No. of eggs positive	No.of eggs negative	Cumulative values		Ratio positive	Percentage positive
				Positive	Negative.		
10^{-1}	3	3	0	25	0	25/25	100
10^{-2}	3	3	0	22	0	22/22	100
10^{-3}	3	3	0	19	0	19/19	100
10^{-4}	3	3	0	16	0	16/16	100
10^{-5}	3	3	0	13	0	13/13	100
10^{-6}	3	3	0	10	0	10/10	100
10^{-7}	3	3	0	7	0	7/7	100
10^{-8}	3	3	0	4	0	4/4	100 *
10^{-9}	3	1	2	1	2	1/3	33.3
10^{-10}	3	0	3	0	5	0/5	0

* Fifty per cent infectivity between dilution eight and nine

$$\text{Proportionate distance} = \frac{100 - 50}{100 - 33.3}$$

$$= \frac{50}{66.7} = 0.75$$

$$ELD_{50} = 10^{-8} + 0.75 = 10^{-8.75}/0.2 \text{ ml}$$

Table 5. Mean Death Time of the minimum lethal dose

Dilution / Death in hours	10^{-6}	10^{-7}	10^{-8}	10^{-9}
8	0	0	0	0
16	0	0	0	0
24	0	0	0	0
32	1	0	0	0
40	3	1	1	0
48	4	2	2	0
56	o	4	0	0
64	0	1	0	0
72	0	0	0	0

Mean Death time of the minimum lethal dose $\bar{0}_{\bar{0}}$

$$= \frac{(40x1) + (48x2) + (56x4) + (64x1)}{8}$$

$$= \frac{424}{8} = 53 \text{ hours}$$

Table 6. Intracerebral Pathogenicity Index

Days Observations	1	2	3	4	5	6	Sum	Factor	Sum X Factor
Death	2	8	9	9	9	10	47	2	94
Signs	4	1	0	0	1	0	6	1	6
Normal	4	1	1	1	0	0	7	0	0
Total	-	-	-	-	-	-	60	-	100

$$ICPI = \frac{100}{60} = 1.67$$

Table 7. Intravenous Pathogenicity Index

Days Observations	1	2	3	4	Sum	Factor	Sum X Factor
Death	0	0	3	6	9	3	27
Paralysis	0	0	0	0	0	2	0
Signs	0	6	3	0	9	1	9
Normal	6	0	0	0	6	0	0
Total	-	-	-	-	24	-	36

$$IVPI = \frac{36}{24} = 1.5$$

Table 8. Intranasal Inoculation of eight week-old Ducklings

No. of birds	Intervals of observation (days)	No.of birds showing symptoms	Symptoms	Antibody titre	Virus excretion	
					T*	C**
7	3	7	Droopiness, general depression, inappetence and white diarrhoea	-	6	7
7	5	4	"	-	4	6
7	7	-	Normal	-	-	1
7	14	-	"	10-80	-	-
7	21	-	"	20-80	-	-
7	28	-	"	40-160	-	-
7	35	-	"	40-160	-	-
7	42	-	"	40-160	-	-

* Throat swabs

** Cloacal swabs

Table 9. Intraocular inoculation of Eight week-old Ducklings

No. of birds	Intervals of observation (days)	No. of birds showing symptoms	Symptoms	Antibody titre	Virus excretion	
					T*	C**
7	3	7	Droopiness, depression, inappetence, white diarrhoea and ocular discharge.	-	6	5
7	5	1	"	-	1	4
7	7	-	Normal	-	-	-
7	14	-	"	10-40	-	-
7	21	-	"	40-80	-	-
7	28	-	"	80-320	-	-
7	35	-	"	80-320	-	-
7	42	-	"	80-320	-	-

* Throat swabs

** Cloacal swabs

Table 11. Results of Intranasal/intraocular inoculation of week-old Ducklings

No.of birds	Intervals of observations (days)	No.of birds showing symptoms	Symptoms.	No.of birds died	Antibody titre	Virus isolation		
						T*	C**	Tissue
5	3	-	-	-	-	-	-	-
5	5	-	-	-	-	-	-	-
5	7	-	-	-	-	-	-	-
5	8	3	Swelling and glueing of eyelids, nasolachrymal discharge, paralysis of legs and respiratory distress	-	-	-	2	-
4	10	2	"	1	-	-	-	1
2	12	1	"	2	-	-	-	2
2	14	1	"	-	-	-	-	-
1	21	1	"	1	-	-	-	-
0	25	-	-	1	-	-	-	1

* Throat swabs

** Cloacal swabs

Table 12. Results of Intramuscular inoculation of week-old Ducklings

No.of birds	Intervals of observation (days)	No. of birds showing symptoms	Symptoms	No. of birds died	Antibody titre	Virus isolation		
						T*	C**	Tissue
5	3	-	-	-	-	-	-	-
5	5	1	Pneumoencephalitic symptoms included paralysis of legs, inability to feed properly, in later stages the bird sat on its breast with head down and turned to one side	-	-	1	-	-
4	7	2	"	1	-	1	-	Lung & spleen
2	9	1	"	2	-	-	-	1
1	13	1	"	1	-	-	-	1
1	14	1	"	-	-	-	-	-
0	18	-	-	1	-	-	-	-

* Throat swabs

** Cloacal swabs

Table 13. Results of Subcutaneous inoculation of week-old Ducklings

No.of birds	Intervals of observation (days)	No. of birds showing symptoms	Symptoms	No. of birds died	Antibody titre	Virus isolation		
						T*	C**	Tissue
5	3	-	-	-	-	-	-	-
5	5	-	-	-	-	-	-	-
5	7	2	Paralysis of legs, profuse diarrhoea, off feed and respiratory distress	-	-	-	1	-
5	9	3	"	-	-	-	-	-
4	10	2	"	1	-	-	-	1
2	11	1	"	2	-	-	-	1
2	14	2	"	-	-	-	-	-
0	16	-	-	2	-	-	-	1

* Throat swab

** Cloacal swab

Table 14. Indirect infection of week-old Ducklings

No.of birds	Intervals of observation (days)	No. of birds showing symptoms	Symptoms	No. of birds died	Antibody titre	Virus isolation		
						T*	C**	Tissue
5	3	-	-	-	-	-	-	-
5	5	-	-	-	-	-	-	-
5	7	4	Swelling and glueing of the eyelids, paralysis of legs and wings, inappetence and profuse diarrhoea		-	-	-	-
2	9	2	"	3	-	-	-	2
0	11	-	-	2	-	-	-	-

* Throat swab

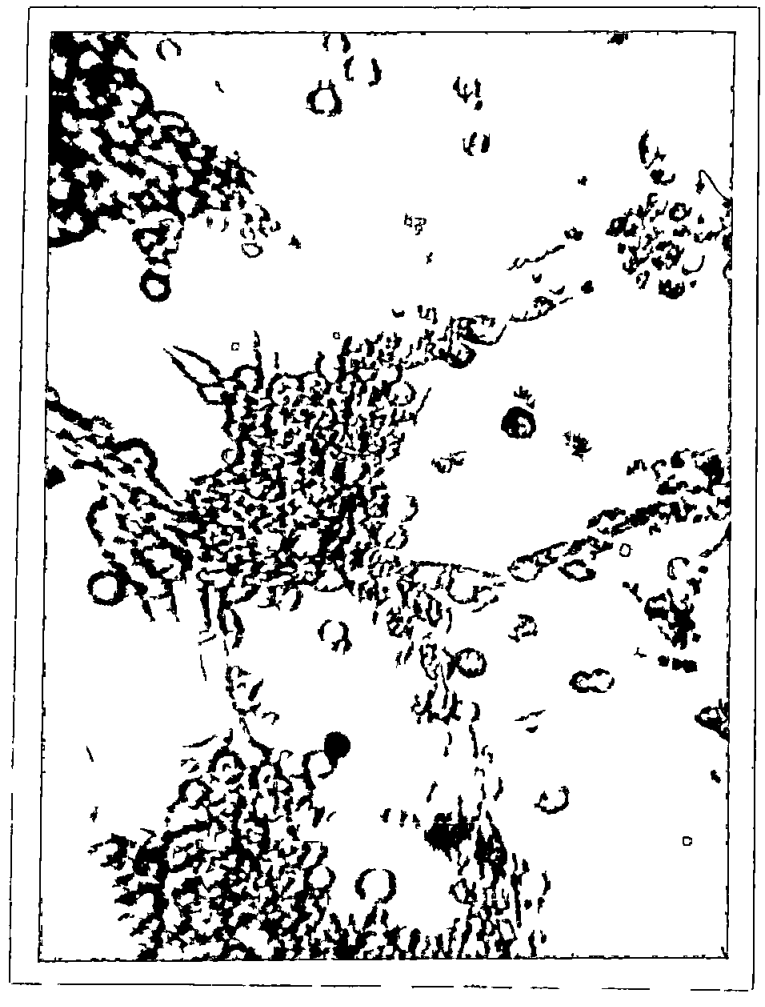
** Cloacal swab

Table 15. Infection among week-old Ducklings kept in contact with Intranasally infected Chicken

No.of birds	Intervals of observation (days)	No. of birds showing symptoms	Symptoms	No.of birds died	Antibody titre	Virus isolation		
						T*	C**	Tissue
5	3	-	-	-	-	-	-	-
5	5	-	-	-	-	-	-	-
5	6	1	General depression, inappetence, slight respiratory distress and profuse diarrhoea	-	-	-	-	-
5	7	2	"	-	-	-	-	-
4	8	1	"	1	-	-	-	1
4	10	2	"	-	-	-	-	-
3	12	2	"	1	-	-	-	1
3	14	3	"	-	1:20	-	-	-
2	17	2	"	1	-	-	-	1
0	20	-	-	2	-	-	-	2

* Throat swab

** Cloacal swab



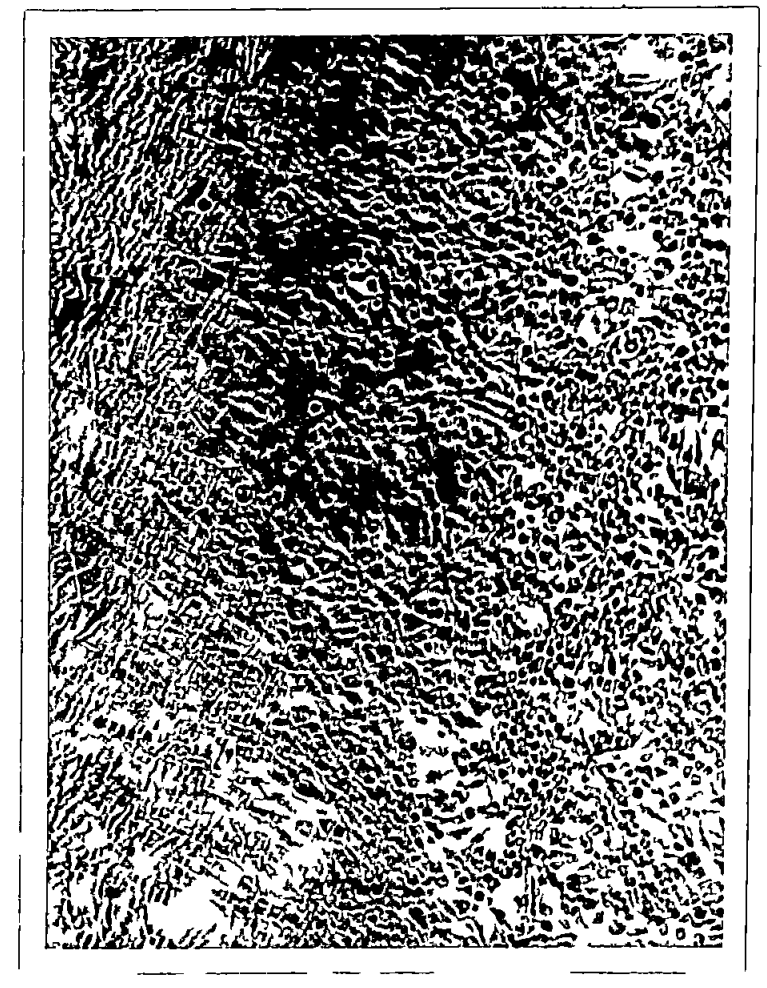
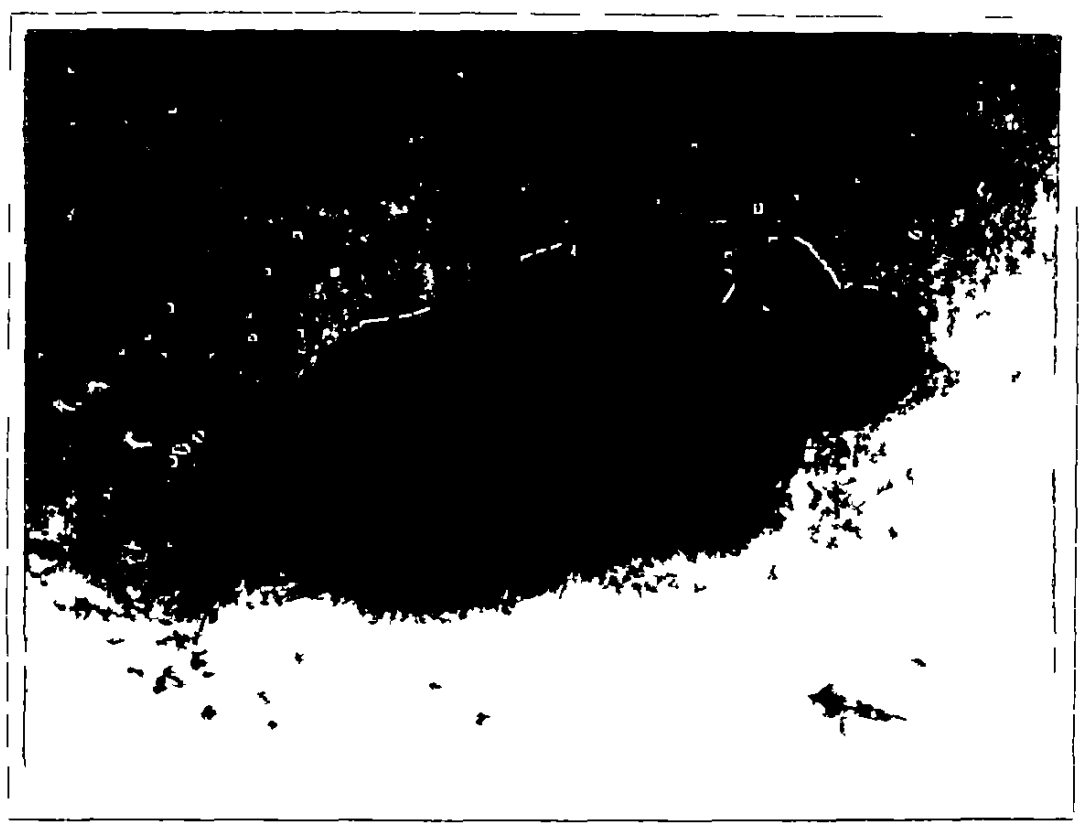
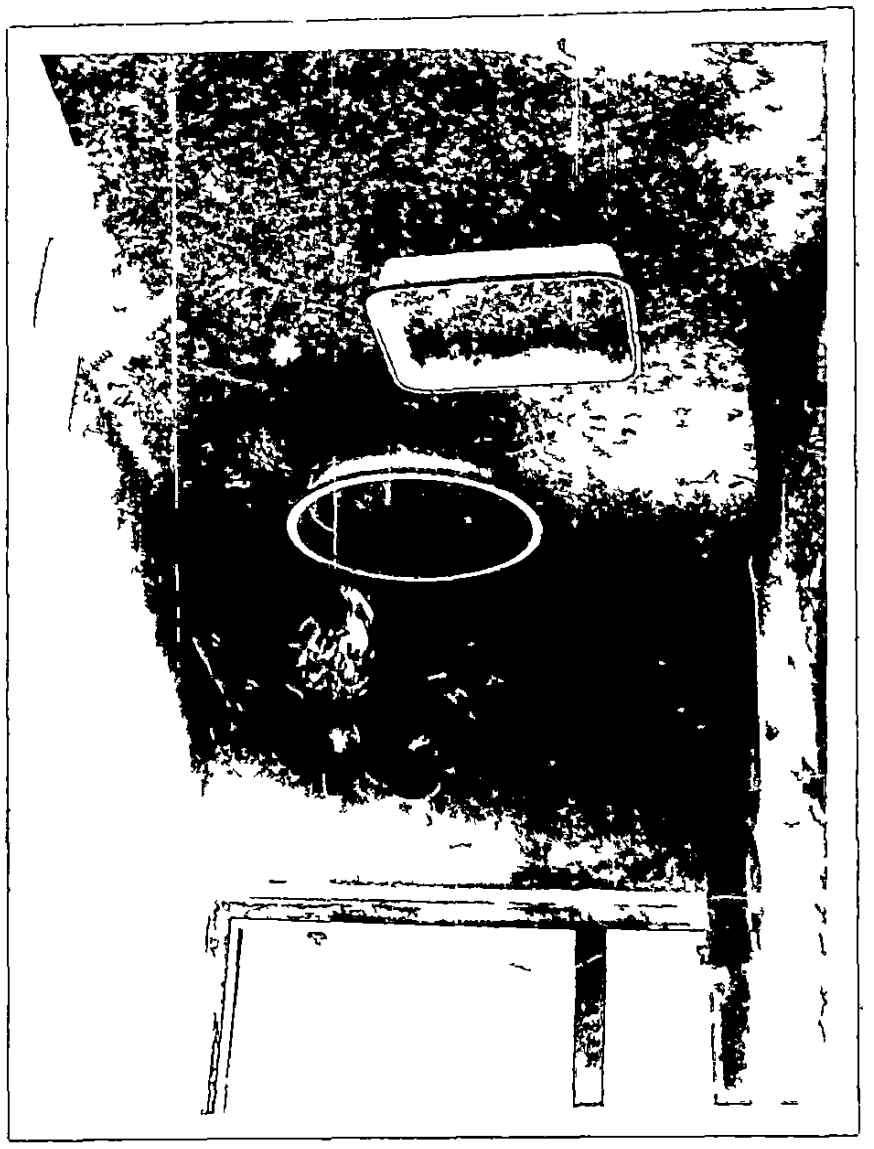
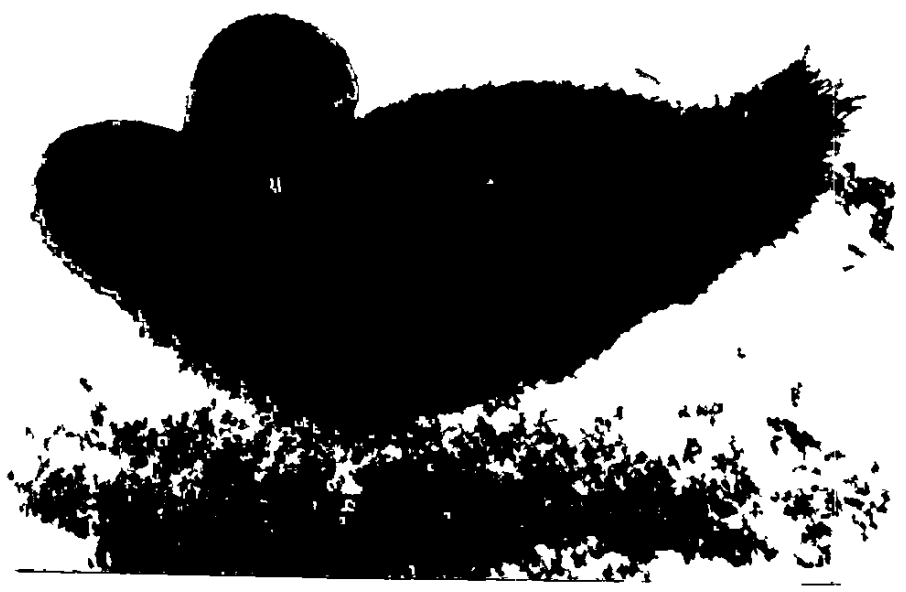


Table 10. Details of Experimental infection of week-old Ducklings

Batch	Route of inoculation	No. of ducks	Contact chicken
I	Intranasal/intra-ocular	5	Six uninfected chicken
II	Intramuscular	5	Six uninfected chicken
III	Subcutaneous	5	Six uninfected chicken
IV	Indirect infection	5	No chicken
V	Uninfected ducklings	5	Six intranasally infected chicken
VI	Control ducklings	5	No chicken







DISCUSSION

DISCUSSION

An infectious disease of a viral nature characterized mainly by respiratory and alimentary tract affections was causing heavy mortality to the duck population in Kerala for the last five years. Duck plague virus was isolated from these conditions (Nair, 1978) and control measures were adopted by vaccination. However, an absolute protection against this disease has not been obtained by vaccination. Meanwhile Sulochana and Nair (1979) and Sulochana et al. (1981 a) isolated strains of NDV from ducks having the same disease syndrome and found that one of the duck isolates was highly pathogenic to eight week-old chicken.

Natural Infection of Ducks with Newcastle Disease Virus

Virus Isolation.

The present investigation carried out to determine the incidence of ND among ducks in Kerala have shown that ducks could carry the virus and excrete them either through throat or cloaca. Out of a total of 151 throat and cloacal swabs collected from 76 ducks, comprising both diseased as well as clinically normal ones for virus isolation, eleven ducks were found to excrete the virus. Virus isolation could be made from 12.3 per cent of the diseased ducks as against 4.3 per cent of the clinically normal ducks indicating a higher

percentage of virus excretion by diseased birds. Eventhough, ducks are considered to be relatively resistant to NDV infection natural out breaks of this disease have been reported by Higgins (1971, 1972) and Kingston et al. (1978). Higgins (1971, 1972), Kingston et al. (1978), Sulochana and Nair (1979) and Sulochana et al. (1981 a) isolated NDV from diseased ducks which were highly pathogenic to chicken. In a study on the epizootiology of this disease Higgins (1971) came to a conclusion that ducks might have picked up infection from the neighbouring chicken farm. In Kerala, the farmers particularly in certain villages rear chicken and ducks together in the backyard. The duck breeders owning larger number of ducks transport them from place to place depending on the harvesting seasons and house their birds in the backyard of nearby houses. This practice enable them to pick up infection from the chicken. Moreover, chances of getting infection from the free-flying birds which have ready access to ducks also cannot be ruled out (Sulochana et al. 1981 b).

Brandly (1959) opined that cold weather could be a predisposing and exacerbating factor for ND. The outbreaks reported by Higgins (1971) also occurred during the cool dry season. Their isolations were mostly confined to young ducklings up to six weeks of age except in one case where the affected birds were mature ducks. But no such seasonal

incidence was reported in the disease condition that occurred in Kerala and the isolations were made from both young and adult ducks.

Isolation of NDV from normal waterfowls was made by Rosenberger (1974); Pearson and McCann (1975) and Shortridge and Alexander (1978). Rosenberger (1974) found that 2.3 per cent of the wild migratory birds screened by him excreted the virus. The rates of isolation of the virus by Shortridge and Alexander (1978) were 3.2 per cent and 3.3 per cent respectively from Hong Kong and People's Republic of China. In the present study 4.3 per cent of the normal ducks were found to excrete the virus either through the cloacal or throat or by both routes thus confirming the observation of the above authors. The major route of virus excretion was observed to be through cloaca since seven birds excreted the virus by this route while only four did so through oral route. Shortridge and Alexander (1978) also obtained twice as many isolations from the cloaca than from the trachea. These observations indicated that cloacal swabs are the ideal specimen for isolation of NDV from ducks.

Isolation of the virus from normal as well as diseased ducks and the demonstration of antibodies in their sera showed that ducks could get ND infection and excrete the virus

through cloacal and/or oral routes. The fact that no antibodies could be detected in birds that were excreting the virus and no isolations could be made from birds that were showing antibody titres suggest that ducks did not act as a persistent carrier of this virus. However, excretion of the virus by ducks which did not manifest any symptoms should be considered as an important factor in the epizootiology of the disease.

Screening for HI antibodies.

Haemagglutination inhibition test for the detection of antibodies to ND in the sera of 226 birds revealed that 15.04 per cent of them possessed HI antibodies. The sera collected from diseased flocks had a high titre. The percentage of reactors were also higher in this group. Out of the 29 ducks from the diseased flock 10 (34.5%) had antibodies. Presence of HI antibodies in the sera of ducks having subclinical infection of ND was reported by Asplin (1947). Antibodies were also demonstrated in the sera of birds from infected flocks by Vrtiak (1958) but Higgins (1971) was unable to demonstrate any antibodies in the sera of ducks belonging to the affected as well as those vaccinated with mesogenic strains in Hong Kong. So he considered that NDV is nonimmunogenic to ducks. Similar findings were also

reported by Iyer (1945). Shortridge and Alexander (1978) found detectable antibody titres in five per cent of duck and 31 per cent of goose sera. A similarity in the antibody response to NDV was also noticed among ducks in People's Republic of China and Hong Kong. They attributed the higher percentage of serological positivity to vaccination against ND by local farmers. Hore et al. (1973) detected antibodies to NDV in 16 per cent of the sera examined and the present findings are in agreement with this report. Antibodies were also detected in intercontinental migratory birds of the genus Anas (Chandra et al. 1973). Majority of the reports show that ducks can respond serologically to NDV. However, the observations made in this study also support the ability of ducks to respond serologically to NDV. The failure of Iyer (1945) and Higgins (1971) to demonstrate antibodies in the sera may be due to the fact that they might have collected the serum before antibodies developed in the system.

Experimental Infection Studies

Experimental infection of eight week-old ducklings.

Experimental infection of eight week-old ducklings with a virulent strain of NDV had shown that the infected birds excreted the virus through cloacal and tracheal routes

from day three onwards, but ceased to excrete any virus by day seven. A steady increase in antibody titre was also noticed in all infected birds by the second week. The pattern of virus excretion and antibody response were the same whether they were infected intranasally or intra-ocularly. The symptoms of general depression, diarrhoea, inappetence and oculonasal discharge noticed by Higgins (1971) and Sriraman et al. (1980) were also observed in the present study from the third day following infection. But Iyer (1945) failed to infect mature ducks and eight week-old ducklings with virulent NDV by any of the route and he considered ducks as resistant to infection. Asplin (1947) also could produce ND in ducks and geese by experimental infection. In contrast to the observations of Moine (1950), Bush (1954) and Higgins (1971) all the infected ducklings survived and became normal and active following the initial symptoms in the present investigation. Sriraman et al. (1980) also found that the ducklings recovered after an initial illness. Death of mallards following intravenous inoculation of NDV was not considered to be due to active infectious process but due to virus toxicity (Friend and Trainer, 1972).

The cloacal and throat swabs collected at regular intervals showed excretion of the virus from day three to seven. Virus

excretion from day three to four following experimental infection was also noticed by Asplin (1947). Teklinska et al. (1953) found that the droppings were infective even after 15 days of infection while, Winmill and Haig. (1961) could recover the virus six months after infection, and suggested that ducks could act as carriers of the virus.

During the present investigation virus excretion was noticed by eight week-old ducklings following experimental infection with a virulent strain of NDV for a period of three to four days. The cessation of virus excretion coincided with the development of specific antibody in the system. This definitely indicates that the symptoms shown by the experimentally infected birds were due to an infectious process by the virus and recovery occurred after the appearance of antibody. This suggests that ducks above eight weeks of age can pick up NDV but produce only a mild form of the disease which usually go unnoticed. Millen (1960) discussed the genetic background of the chicken influencing the HI titre and overcoming virulent ND without developing high HI titres and this might be applicable in the case of ducks also. Ability of ducks to respond to NDV was also shown by Sarma et al. (1977); Vrtiak (1958); Shortridge and Alexander (1978). But Iyer (1945) and Higgins (1971) were of the opinion that ducks will not respond serologically

to NDV as they could not demonstrate any virus neutralizing antibodies in the sera. A steady increase in HI titre was noticed by Sarma et al. (1977) in recovered ducklings, while Shortridge and Alexander (1978) could detect the same in clinically normal ducklings which were resistant to Newcastle disease.

The chicken kept in contact with the ducklings, died after showing specific symptoms and lesions of ND, and virus could be isolated from them. The symptoms and lesions were similar to those observed by Guha and Chatterjee (1950). This shows that infected ducklings can excrete the virus and contaminate the area and such virus can still retain their virulence to chicken. Little attenuation of virulent NDV by passage in ducks was also noticed by Chang (1976). This clearly indicate the role of ducks in the epizootiology of Newcastle disease.

Experimental infection of week-old ducklings.

Infection of one week-old ducklings resulted in the establishment of infection and death. The birds started to show eymptoms of pneumoencephalitis by fifth day of infection and were similar to those observed by Sarma et al. (1977). Week-old ducklings exposed to infection through all the routes died suggesting that they are more susceptible to

Newcastle disease than eight week-old ducklings. Sarma et al. (1977) observed that drinking water and intranasal routes of inoculation were more effective than parenteral routes while the present findings revealed that infection by subcutaneous, intramuscular, intranasal or intraocular routes were equally effective. The symptoms observed were the same as described by Sarma et al. (1977). Nervous symptoms following parenteral infection were a prominent feature in the infected birds as observed by Kontrimavichus and Akulov (1973) though their ducklings had more severe symptoms ranging from complete paralysis to tremors or circling movements.

Sriraman et al. (1980) although observed symptoms such as dullness, serous nasal discharge and glueing of the eye, the birds did not succumb to the disease suggesting that they were resistant to ND. But screening the birds for antibody before infection had not been done by him and hence it could be possible that those birds might have possessed maternal antibodies which protected them from the disease.

Death of the ducklings occurred at varying intervals, from the ninth and seventeenth days as against 48-72 hours reported by Sarma et al. (1977) and the mortality was found to be 100 per cent. Kontrimavichus and Akulov (1973) also

observed development of symptoms in younger goslings by five to seven days after inoculation and 100 per cent mortality in two days after the onset. The postmortem lesions observed by them were catarrhal pneumonia, enteritis, oedema of the brain and haemorrhages and microscopic necrotic foci in the internal organs. The results presented here show that the lesions are confined to the visceral organs characterized by necrotic foci and fatty changes in the liver which are in agreement with that of Hanson (1972).

The blood collected at varying intervals did not show any HI activity. Sera of one bird in the incontact group showed HI activity of 1:20 after three weeks of infection, but died after a few days. Sarma et al. (1977) could demonstrate HI antibodies in the sera of recovered ducklings. Kontrimavichus and Akulov (1973) also demonstrated HI antibodies in the sera of goslings infected with virulent or attenuated strains of NDV. The failure to develop antibodies even after 20 days of infection as shown in tables 11 to 14 could probably be due to the inability of the ducklings to respond to the virus and not due to the failure to pick up infection as the virus could be isolated from these birds which subsequently died due to ND.

The chicks infected with NDV started dying by the

fourth day of infection showing specific symptoms and lesions of ND. This is in agreement with Guha and Chatterjee (1950). Four chicks kept in the subcutaneous group showed specific symptoms and lesions and died by the second week of infection. The remaining two chicks and the chicks kept in other two groups were normal and active for the rest of the period. But infection of the chicks in the subcutaneous group had made it clear that chicks could get contact infection from infected ducklings. Sarma et al. (1977) also proved that ducks could transmit the disease to contact chicken. The probable reason for the resistance of the other chicks could be due to the lack of sufficient exposure of the chicks to the virus. This was confirmed by conducting HI test and the serum was found to be negative for any antibodies. The birds on challenge with virulent virus died within four days showing specific symptoms and lesions of ND. The environmental temperature during the course of this experiment was high and this might have contributed to the failure of most of the contact chicks to pick up infection.

The reverse contact infection was also proved successful. All the ducklings in contact with the infected chickens died. Weidenmuller (1972) reported that geese could get contact

infection from diseased fowls which supports the possibility of ducks getting contact infection from infected chickens. In Hong Kong the disease outbreaks that occurred in duck farms were found to be originated either from the neighbouring duck farm or chicken farm (Higgins, 1971).

The virus was isolated from most of the fatal cases. Pooled samples of lung, liver, spleen and brain were used for isolation. Sarma et al. (1977) isolated the virus from brain and liver while Higgins (1971) from liver, lung and respiratory tract. Asplin (1947) could not isolate the virus from liver spleen or bone marrow. Weidenmuller (1972) isolated the virus from brain of infected geese.

The experimental infection of ducklings showed that ducklings of eight weeks are resistant to infection but they can support multiplication of the virus till antibodies develop in the system. Such birds will excreate the virus through cloacal and oral route which forms an important means of dissemination of the virus. The susceptible chicken kept in contact with them could get infection leading to death. Week-old ducklings were also susceptible to NDV, and unlike eight week-old ducklings they succumbed to the disease. These ducklings could also transmit the disease to chicken. The chances of ducks getting infection from infected chicken

were also proved by keeping uninfected ducklings along with the infected chickens.

Screening of ducks showed that virus could be excreted both by clinically normal as well as diseased ones. But none of the birds excreting the virus possessed any antibodies. Once the birds become positive for HI antibodies they ceased to excrete the virus in the case of both experimental and natural infection. Since the birds above eight weeks of age did not show significant symptoms even while they were excreting the virus they could be considered as inapparent carriers. Taking into consideration the nature and feeding habits of ducks this carrier state is of importance in the epizootiology of ND.

SUMMARY

SUMMARY

Lack of complete protection in ducks against the plague like disease in Kerala even after vaccination with duck plaque vaccine and the isolation of a highly virulent strain of NDV from similar cases in ducks led to the suspicion that NDV may also be associated with these conditions. Hence a detailed study was undertaken to find out the susceptibility of ducks to NDV.

A total of 151 cloacal and throat swabs collected from clinically normal and diseased ducks were screened for virus excretion by inoculation into the allantoic cavity of 10 day embryonated eggs. All the eleven haemagglutinating agents isolated from these materials were identified as Newcastle disease (ND) virus by haemagglutination inhibition test employing specific ND antiserum.

Sera collected from 226 birds revealed that 15.04 per cent of them carry HI antibodies, the titres ranging from 1:20 to 1:160. None of the birds that had detectable antibodies in their sera excreted the virus either through throat or cloaca.

The susceptibility of ducks to NDV was also studied by experimental infection of two age groups of ducklings. In the first experiment, eight week-old ducklings were infected either intranasally or intraocularly with a virulent strain

of NDV obtained from the VBI, Palode. The birds showed symptoms of general depression, inappetence, diarrhoea and respiratory distress within three days of infection but recovery occurred after the fifth day. All the ducklings excreted the virus either through cloaca or throat, till the seventh day of infection. Haemagglutination inhibition became demonstrable in the sera by the second week of infection, continued up to the fourth week and then it remained steady. The birds recovered and ceased to excrete the virus when antibody appeared in the serum.

The susceptible chicks which were housed along with infected ducklings died within seven days of contact and the virus could be isolated from the tissues of dead chickens.

In another experiment five batches of week-old ducklings were subjected to experimental infection by different methods. The methods of infection were subcutaneous, intramuscular, intranasal/intraocular, indirect infection and by contact with infected chicken. The virus used for infecting eight week-old ducklings was used for this experiment also. The birds showed symptoms of involvement of nervous system by fifth day and the first death occurred on the seventh day. Maximum number of death occurred between the ninth and seventeenth day. The birds showed a pneumoencephalitis form of the disease and all the

ducklings infected by different routes died. Postmortem examination showed only congestion of various organs. Virus could be isolated from pooled samples of the tissues from seventeen out of twenty-five cases. The chickens that were kept in contact with subcutaneously infected ducklings died with specific symptoms and lesions whereas those in contact with intramuscularly, intranasally/intraocularly infected ducklings survived without exhibiting any symptoms. Virus could be isolated from the dead chickens. The survived birds when challenged with virulent NDV succumbed to the infection with typical symptoms and lesions of ND.

It is concluded that ducklings below eight weeks of age are susceptible to NDV and they succumb to the disease, while those above eight weeks of age excrete the virus for a period of three to four days following infection, without showing any clinical disease. They responded serologically and the production of HI antibodies coincided with elimination of the virus from the system. This state of virus excretion without any clinical manifestation might be important in the epizootiology of Newcastle disease.

REFERENCES

REFERENCES

Ahmed, A.A.S., El-Sisi, M.A., El-Agroudi, M.A. and Zafer, S.A.W. (1968). A serological study of newcastle disease and avian adenovirus (CELO) infection in ducks. J. Egypt. Vet. Med. Ass. 28 : 117-123. (Vet. Bull. 1971. 41 : 4652).

Albiston, H.E. and Gorrie, C.J.R. (1942). Newcastle disease in Victoria. Aust. Vet. J. 18 : 75-79. Cited by Lancaster, J.E. (1977).

Alexander, D.J., Spackman, D., Allan, W.H. and Borland, L. (1979). Isolation of newcastle disease virus from a wild mallard duck. Vet. Rec. 105 : 328-329.

Asplin, F.D. (1947). Newcastle disease in ducks and geese. Vet. Rec. 59 : 621-624. Cited by Lancaster, J.E.(1963).

Bahl, A.K., Pomeroy, B.S., Mangundimedjo, S. and Easterday, B.C. (1977). Isolation of type A influenza and newcastle disease viruses from migratory waterfowl in the Mississippi flyway. J. Am. Vet. Med. Ass. 171 : 949-951.

Ballarini, G. (1964). Newcastle disease in guinea fowls. Nuova Vet. 40 : 299. (Vet. Bull. 1965. 35: 4609).

Beaudette, F.R. (1943). Proc. 47th. Ann. Meet. U S Livestock Sanit. Ass. 122. Cited by Lancaster, J.E. (1963).

Berthelon, M. and Tournut, J. (1949). Rev. Med. Vet. Lyon. et. Toulouse. 100 : 21. Cited by Lancaster, J.E.(1963).

Bishai, F., Blaskovic, P., Hamves, J.J., Iwaskata, S., Labzoffsky, N.A., Subrahmanyam, T.P. and Zalan, E.(1974). Virology Manual. Ministry of health, Ontario. p. 23.

Bradshaw, J.E. and Trainer, D.O. (1966). Some infectious diseases of waterfowls in the Mississippi flyway. J. Wildl. Mgmt. 30 : 570. Cited by Lancaster, J.E. (1977).

Brandly, C.A., Moses, H.E., Jones, E.E. and Jungherr, E.L. (1946). Epizootiology of newcastle disease of poultry. Am. J. Vet. Res. 7 : 243-249.

Bush, D.L. (1954). Observations on a newcastle disease enzootic in Haiti. J. Am. Vet. Med. Ass. 125 : 474-475.

Chandra, S., Kulshreshta, S.B., Mathur, B.B.L. and Kumar, S. (1973). A note on the serological evidence of newcastle (ranikhet) disease in intercontinental migratory birds. Indian J. Anim. Sci. 43 : 564-565.

Chang, H.C. (1976). An experiment on the attenuation of newcastle disease virus by continuous passage in ducks. Taiwan J. Vet. Med. Anim. Husb. 29 : 6-14. (Vet. Bull. 1978. 48: 159).

Collier, W.A. and Dinger, J.E. (1950). Research in newcastle disease 1940-1942. Docums. Indonesia Morbis. Trop. 2 : 189-192. Cited by Sarma, B.J.R., Rao, V.P., Murthy, K.G., Reddy, T.V. and Paragoanker, V.N.(1977).

Copper, H. (1931). Indian J. Vet. Sci. 1 : 107. Cited by Iyer, S.G. (1945).

Corrado, A. (1966). Studies on newcastle disease virus isolation from an ostrich. Zooprofilassi. 21 : 573-597. (Vet. Bull. 1967. 37 : 3746).

Crowther, R.W. (1952). Vet. Rec. 64 : 91. Cited by Lancaster, J.E. (1963).

Cullen, G.A., Allan, W.H. and Maff. (1974). Psittacosis and virulent newcastle disease virus in a consignment of parrots. Vet. Rec. 94 : 477-478.

Cunningham, C.H. (1966). A laboratory guide in virology. Burgess Publishing Company, Minneapolis, 6th ed. pp. 47-57 and 60.

Danchev, P. (1970). Newcastle disease in ravens (Corvus corax) as a source of infection for chicks. Vet. Sbir. Sof. 67 : 13-14. (Vet. Bull. 1971. 41 : 2314).

Dobson, N. (1939). Proc. 7th. Wlds. Poult. Cong. p. 250. Cited by Iyer, S.G.(1945).

Doyle, T.M. (1927). J. Comp. Path. 40 : 144. Cited by Hanson, R.P. (1972).

Erickson, G.A., Brugh, M. and Beard, C.W. (1980). Viscerotropic velogenic newcastle disease in pigeons-clinical disease and immunization. Avian Dis. 24 : 257-267. (Vet. Bull. 1980. 50 : 6528)

Farinas, E.C. (1930). Philipp. J. Agric. 1 : 311. Cited by Kingston, D.J., Dharsana, R. and Chavez, E.R. (1978).

Forbes, A.J. and Row, J.A. (1966). Newcastle disease in poultry. Aust. Vet. J. 42 : 138-139. (Vet. Bull. 1967. 37 : 1697 a and 1697 b).

Friend, M. and Trainer, D.O. (1970). Serological evidence of newcastle disease in captive mallards and swans. J. Wildl. Dis. 6 : 130-135. (Vet. Bull. 1971. 41 : 188).

Friend, M. and Trainer, D.O. (1972). Experimental newcastle disease studies in the mallard. Avian Dis. 16 : 706-713. (Vet. Bull. 1973. 43 : 711).

Gaudry, D., Precausta, P., Kunst, H. and Wemmenhore, R. (1970). Demonstration of infective agents in a flock of barbery ducks. c.r.hebd. seanc. Acad. Sci. 270 D : 237-238. Cited by Sarma, B.J.R., Rao, V.P., Murthy, K.G., Reddy, T.V. and Paragoanker, V.N.(1977).

Gray, J.E., Snoeyenbos, G.H. and Peck, H.A.(1954). Newcastle disease in turkeys - report of a field outbreak. J. Am. Vet. Med. Ass. 124 : 302-307.

Guha, S. and Chatterjee, S.N. (1950). Study of the symptoms and postmortem lesions in fowls experimentally infected with RDV. Indian Vet. J. 27: 69-73.

Gustafson, D.P. and Moses, H.E. (1953). The English sparrow as a natural carrier of newcastle disease virus. Am. J. Vet. Res. 14 : 581-585.

Hanson, R.P. (1972). Newcastle disease. In "Diseases of Poultry". (Hofstad, M.S., Calnek, B.W., Hemboldt, C.F., Reid, W.M. and Yoder, H.W. - Ed). The Iowa State University Press, Ames, 6th ed. pp. 634-636.

Hanson, R.P. (1975). Newcastle disease. In Isolation and Identification of Avian Pathogens. (Hitchner, S.B., Domermuth, C.H., Purchase, H.G. and Williams, J.E.-Ed). Arnold Printing Corporation, Ithaca, New York. pp. 160-175.

Heller, O. (1957). Mh. VetMed. 12 : 218. Cited by Lancaster, J.E. (1963).

Higgins, D.A. (1971). Nine disease outbreaks associated with myxoviruses among ducks in Hong Kong. Trop. Anim. Hlth. Prod. 3 : 232-240.

Higgins, D.A. (1972). Newcastle disease in Hong Kong. Agriculture Hong Kong. 1 : 1-72. (Vet. Bull. 1974. 44 : 3753).

Higgins, D.A. and Wong, F.S.F. (1968). Newcastle disease in a flock of Japanese quails. Vet. Rec. 83 : 437-440.

Hilbrich, P. (1972). Natural outbreak of newcastle disease in a pigeon flock. Deutsche Tierarztliche Wochenschrift. 79 : 181-184. (Vet. Bull. 1972. 42 : 4607).

Hore, D.E., Campbell, J. and Turner, A.J. (1973). A serological survey for viral antibodies in wild ducks. Aust. Vet. J. 49 : 238-239.

Hoskins, J.M. (1967). Virological procedures. Butterworths, London. pp. 171-176 and 93-94.

Humphrey, P.N. (1976). Problems of keeping ornamental waterfowl. J. Small. Anim. Pract. 17 : 607-616.

Iyer, S.G. (1945). The susceptibility of ducks to newcastle disease. Indian J. Vet. Sci. Anim. Husb. 15 : 165-166.

Kaschula, V.R., Canham, A.S., Diesel, A.M. and Coler, J.D.W.A. (1946). J.S. Afr. Vet. Med. Ass. 17 : 1. Cited by Lancaster, J.E.(1963).

Khan, M.Z. and Huq, M.M. (1963). Infectious respiratory diseases of poultry in Pakistan. Bulletin off international des Epizootics. 60 : 983-987. Cited by Higgins, D.A. (1971).

Kingston, D.J., Dharsana, R. and Chavez, E.R. (1978). Isolation of a mesogenic newcastle disease virus from an acute disease in Indonesian ducks. Trop. Anim. Hlth. Prod. 10 : 161-164.

Kontrimavichus, L.M. and Akulov, A.V. (1973). Experimental newcastle disease in goslings. Trudy. Vsesoyuznogo. Institute Eksperimental'noi Veterinarii. 41 : 233-242. (Vet. Bull. 1974. 44 : 140).

Kraneveld, F.C. (1926). Ned. Indisch. Bl. Diergeneesk. 38 : 448-450. Cited by Hanson, R.P. (1972).

Krauss, H., Paulick, C., Huchzermayer, F. and Gylstorff, I. (1963). Newcastle disease in a king Penguin. Dtsch. tierarztl. Wschr. 70 : 307 - 309. (Vet. Bull. 1964. 34 : 212).

Lancaster, J.E. (1963). Newcastle disease - modes of spread. Vet. Bull. 33 : 221 -226 and 279-282.

Lancaster, J.E. (1977). Newcastle disease - A review of the geographical incidence and epizootiology. Wlds. Poult. Sci. J. 33 : 155-164.

Lancaster, J.E. and Alexander, D.J. (1975). Newcastle disease virus and spread - a review of some of the literature. Monograph No.11. Canada Department of Agriculture. Ontario. (Vet. Bull.1975. 45 : 5598).

Luna, G.L. (1968). Manual of histologic staining methods of the Armed forces Institute of Pathology. McGraw - Hill Book Company, London, 3rd ed. pp. 1 - 37.

Magid, A.I.M., Mohamed, A.A.A. and El-Nassari, B.B. (1965). Newcastle disease in doves and sparrows in U A R. J. Arab. Vet. Med. Ass. 25 : 193-202. (Vet. Bull. 1966. 36 : 4357).

Makay, L. (1967). Newcastle disease among pheasants and its prevention. Magy. Allatrov. Lap. 22 : 528-532. (Vet. Bull. 1968. 38 : 2283).

Marek, K., Borgzemska, W. and Golnik, W. (1967). Carriership of toxic NDV in chicks immunized with anti - ND vaccine. Odbitka Z. Medycyny - Weteryaryjnej. 23 : 351. Cited by Lancaster, J.E. (1977).

Marthedal, H.E., Velling, G. and Settnes, O. (1963). Newcastle disease in Denmark (Preliminary report). Bull. Off. int. Epizoot. 60 : 939-945. (Vet. Bull. 1964. 34 : 2554).

Methods for examination of Poultry Biologics (1963). Publication No. 1038. National Academy of Science. National Research Council, Washington, D.S. pp. 35-59.

Millen, T.W. (1960). What makes an HI high? Poult. Sci. 39 : 1276.

Moine, G. (1950). Bull. Acad. Vet. Fr. 23 : 417. Cited by Lancaster, J.E. (1963).

Nair, G.K. (1978). An investigation on the aetiology of plague-like disease in ducks in Kerala. M.V.Sc. thesis. Department of Microbiology, College of Veterinary and Animal Sciences, Mannuthy.

Nobindro, U. (1946). Ranikhet disease in Assam province. Indian Vet. J. 22 : 135-137.

Novilla, M.N. and Navarro, J.A. (1970). A preliminary study on the identification and classification of NDV strains obtained from field outbreaks in the Philippines. Philipp. J. Vet. Med. 8 : 25-35. (Vet. Bull. 1971. 41 : 1198).

Palmer, S.F. and Trainer, D.O. (1970). Serologic evidence of NDV in Canada geese. Avian Dis. 14 : 494-502. (Vet. Bull. 1971. 41 : 1193).

Pearson, G.L. and McCann, M.K. (1975). The role of indigenous, wild, semidomestic and exotic birds in the epizootiology of velogenic viscerotropic newcastle disease in southern California, 1972-'73. J. Am. Vet. Med. Ass. 167 : 610-614.

Picard, W.K. (1934). Ztb. Bakt. 1 : 440. Cited by Iyer, S.G.(1945).

Raszewska, H. (1966). Newcastle disease in goslings. Bull. Vet. Inst. Pulawy. 10 : 124 -129. (Vet. Bull. 1968. 38 : 602).

Read, L.J. and Muench, H. (1938). A simple method of estimating 50 per cent end points. Am. J. Hyg. 27 : 493-497.

Reid, J. (1961). The control of newcastle disease in Great Britain. Brit. Vet. J. 117 : 275-288.

Rosenberger, J.K. (1974). Isolation of type A influenza and newcastle disease virus from migratory waterfowls in the Atlantic flyway. J. Am. Vet. Med. Ass. 165 : 741.

Rosenberger, J.K., Klopp, S. and Krauss, W.C. (1975). Characterization of newcastle disease virus isolated from migratory waterfowl in the Atlantic flyway. Avian Dis. 19 : 142-149. (Vet. Bull. 1975. 45 : 4358).

Rosenberger, J.K., Krauss, W.C. and Slemons, R.D. (1974). Isolation of newcastle disease and type A influenza viruses from migratory waterfowls in the Atlantic flyway. Avian Dis. 18 : 610-613. (Vet. Bull. 1975. 45 : 2450).

Rovozzo, G.C. and Burke, C.N. (1973). A manual of basic virological techniques. Prentice - Hall. Inc. Englewood cliffs, New Jersey. pp. 16-37.

Sarma, B.J.R., Rao, V.P., Murthy, K.G., Reddy, T.V. and Paragoanker, V.N. (1977). Experimental ranikhet disease in ducklings. Indian J. Anim. Sci. 47 :818-821.

Shortridge, K.F. (1980). Isolation of Ortho - and Paramyxoviruses from domestic poultry in Hong Kong between November 1977 and October 1978 and comparison with isolations made in the preceding two years. Res. Vet. Sci. 28 : 296-301.

Shortridge, K.F. and Alexander, D.J. (1978). Newcastle disease surveillance in Hong Kong on local and imported poultry. Res. Vet. Sci. 25 : 204-206.

Sokkar, I.M.H. and Refaie, E.M. (1967). A case of newcastle disease in a peafowl. Vet. Med. J. Giza. 13: 191-196. (Vet. Bull. 1968. 38 : 1425).

Spalatin, J. and Hanson, R.P. (1975). Epizootiology of newcastle disease in waterfowl. Avian Dis. 19 : 573-582.

Sriraman, P.K., Reddy, T.V. and Rao, P.R. (1980). Experimental ranikhet disease in ducklings. Indian J. Poult. Sci. 15 : 51-52.

Sulochana, S. and Nair, G.K.(1979). A note on the isolation of newcastle disease virus from a duckling. Kerala J. Vet. Sci. 10 : 337-339.

Sulochana, S., Pillai, R.M., Nair, G.K. and Abdulla, P.K. (1981 a). Characterization and pathogenicity of a strain of newcastle disease virus (NDV-D) isolated from ducks. Kerala J. Vet. Sci. 12 : 23-30.

Sulochana, S., Pillai, R.M., Nair, G.K. Sudharma, D. and Abdulla, P.K. (1981 b). Epizootiology of newcastle disease in Indian house crows (Corvus splendens splendens) Vet. Rec. (in press).

Teklinska, M., Cakalowa, A. and Karazewski, W. (1956). Geese as carriers of newcastle disease. Med. Vet. Warsovie. 12 : 21 -24. (Vet. Bull. 1956. 26 : 2310).

Tsiroyannis, E., Paschaleri, E., Parisis, E. and Lekkas, S. (1971). Newcastle disease in the peacock - clinical, pathological, histological and virological study. Kteniatrika Nea. 3 : 127-128. (Vet.Bull. 1972. 42 : 3897).

Vrtiak, J. (1958). Epidemiology of newcastle disease in eastern Slovakia. Sborn. ces. Akad. Zemedelsk. Ved. 3 : 437-448. (Vet. Bull. 1959 . 29 : 1787).

Weidenmuller, H. (1972). Newcastle disease in geese. Tierarztliche. Umschau. 27 : 280-282. (Vet. Bull. 1972. 42 : 6306).

Winmill, A.J. and Haig, D.A. (1961). Observation on newcastle disease in Kenya. Bull epiz. Dis. Afr. 9 : 365 -369. Cited by Iancaster, J.E. (1977).

SUSCEPTIBILITY OF DUCKS TO NEWCASTLE DISEASE VIRUS (NDV) AND THEIR ROLE IN THE TRANSMISSION OF THE DISEASE TO CHICKEN

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ABSTRACT OF A THESIS

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ABSTRACT

The susceptibility of ducks to Newcastle disease virus and their possible role in the epizootiology of this disease were investigated. Cloacal and throat swabs and sera were collected from clinically normal as well as diseased ducks from different parts of Kerala. The swabs were tested for virus excretion by inoculating into the allantoic cavity of 10 day embryonated chicken eggs and the sera were examined for the presence of haemagglutination inhibition antibodies. Eight and one week-old ducklings were infected experimentally by different routes or methods to find out their susceptibility to this virus. A virulent strain of the virus received from Veterinary Biological Institute, Palode was employed for all experimental infection studies. Two week-old chickens were used for determining contact transmission.

Inoculation of processed cloacal and throat swabs into the allantoic cavity of 10 day embryonated eggs resulted in the isolation of eleven haemagglutinating viruses from a total of 151 samples. The agglutination produced by these viruses were inhibited by specific ND antiserum, thereby confirming their identity as newcastle disease virus.

Out of a total of 226 serum samples collected from

ducks, 34 showed HI antibodies ranging from 1:20 to 1:160 in the titre.

Eight week-old ducklings infected intranasally and intraocularly showed symptoms of ND by third day of infection but recovered by the seventh day. All of them excreted the virus either through trachea, cloaca or by both the routes. Haemagglutination inhibition antibodies were demonstrable within two weeks of infection. The chickens kept in contact with the infected ducklings died after showing specific symptoms of newcastle disease. Postmortem examination revealed specific lesions and virus could be isolated from pooled tissue samples.

Week-old ducklings could successfully be infected by intranasal/intraocular, subcutaneous, intramuscular or by contact infection. All the ducklings exposed to infection died after showing symptoms of pneumoencephalitis and diarrhoea. Virus was isolated from seventeen out of twenty-five cases. Chickens placed in contact with subcutaneously infected ducklings died showing specific symptoms and lesions of ND and virus could be isolated from all the cases.

The eight week-old ducklings although showed a clinical infection they could eventually recover following the development of antibodies in the system. Hence ducklings

of eight weeks and above could be considered as resistant to Newcastle disease virus infection. On the contrary week-old ducklings readily succumbed to the infection indicating their greater susceptibility. Contact transmission to chickens was possible from both age group of ducklings. The isolation of virus from clinically normal and diseased ducks showed that ducks excrete the virus without showing any clinical symptoms. It is also possible that some ducklings may show symptoms of the disease, but the development of antibody in the system leads to complete elimination of the virus. The results and the observation of the present study indicate that ducks can play an important role in the epizootiology of newcastle disease by contracting infection from infected chicken or from other susceptible species and transmitting it to the incontact susceptible chicken.