THE ROLE OF PARROTS IN THE EPIZOOTIOLOGY OF NEWCASTLE DISEASE

BY

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THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

Department of Microbiology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy - Trichur

1981

DECLARATION

I hereby declare that this thesis entitled "THE ROLE OF PARROTS IN THE EPIZOOTIOLOGY OF RAT CASTLE DISEASE" 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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C RTIFICATE

Certified that this thesis entitled "THE ROLE OF PARROTS IN THE PHOTOLOGY OF THE CASTLE [illegible]" is a record of research work done independantly by Sri.V.Vijayan, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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ACKNOWLEDGEMENTS

I wish to express my deep sense of gratitude to:

Dr.(Mrs) S.Sulochana, Ph.D.(Guelph), Associate Professor, Department of Microbiology, Chairman of the Advisory Committee for her inspiring guidance and constant encouragement throughout the work.

Dr. P.K. Abdulla, Professor, Department of Microbiology, member of the Advisory Committee for his valuable advice and generous help for this study.

Dr.A.Rajan, Professor, Department of Pathology and Dr.A.Ramakrishnan, Professor, Department of Poultry Science, for their valuable help and advice at various stages of this work, as members of the Advisory Committee.

Dr.P.G.Nair, former Dean and Dr.M.Krishnan Nair, Dean, Faculty of Veterinary and Animal Sciences, for the facilities provided for the research work.

The members of the staff of the Microbiology Department for their esteemed help and assistance.

The Director of Animal Husbandry, Kerala State, for deputing me for this study.

Dr.N.Divakaran Nair and Dr. [illegible] for the help rendered.

Mr. [illegible]karan Nair for putting this manuscript into neat type.

[illegible] VIJAYAN

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INTRODUCTION

INTRODUCTION

New Castle disease (ND) is the most important disease among poultry causing the greatest economic loss. The disease not only adversely affects the economy and growth of the Poultry Industry, but also endangers the future development programmes. Due to its highly contageous character the disease continues to receive considerable attention even today.

In spite of the fact that there are effective and efficient vaccines to control the disease, attempts made to eradicate this disease have not so far been successful. Several epizootiologic features have been attributed to the partial failure of eradication. Although it was thought to be primarily a disease of gallinaceous birds, New Castle disease virus (NDV) has been reported to have a wide range of susceptible hosts. (Baczynski,1960). This would probably explain the sudden re-appearance of the infection in countries which had already controlled the disease for a long time, and the outbreaks in places where it had never occured before.

The factors that influence the spread of NDV are the marked survivability, invasive property(the ability of the virus to infect a variety of avian hosts) and airborn

nature of infection. Palmer and Trainer (1971) have given a very comprehensive list of the species of birds that could be infected with the virus. A vast majority of wild birds are susceptible to ND, although it varies with the species of birds and the strain of the virus involved. A perusal of the available literature revealed that over 60 species of birds are susceptible and the virus was isolated and identified from more than 20 species (Scott and Tinmill,1960).

The sources of infection to these birds are poorly understood. Wild free flying birds caught in the viscinity of poultry farms were found to be infected with NDV (Lancaster and Alexander,1975); this suggested that they might have picked up the infection from affected chicken. Sudden re-appearance of the original classical type of the virulent ND in South East Asia and South America and later on in the Near East, indicated that NDV can spread rapidly across continents and Oceans (Hanson,1973). Although the exact cause of spread of these type of ND was not identified, the possible role of free-flying birds, and the captive and cage birds moving in international trade, in the spread of the disease cannot be ruled out (Lancaster,1977).

Many reports from 1930 onwards recorded the incidence of ND in birds belonging to the order Psittaciformes. Among Psittaciformes, the natural disease has been recorded

virtually in all families and subfamilies, and cases of spontaneous infection often resulting in severe disease and death have been demonstrated in 31 species. Parrots when infected with ND were reported to show symptoms that similate ND in chicken. The recovered birds can act as chronic carriers excreting the virus, in their oral and/or cloacal secretions, and possibly could serve as sources of exposure to domestic fowl. (Erickson, et al.1978).

Although isolations of ND have been made from parrots in USA (Hanson,1973); Kenya (Scott and Tinmill,1960); West Germany (Hatchenhorfer and Luthgen,1971); Austria, (Grausgruber,1972); Guatemala (Matzer and Wehota,1971); Switzerland (Ehrsam,et al. 1975); U.K.(Gordon,1974) and Australia (Hawes and Grimes,1978),No report has so far been made on similar isolation in India.

Pet birds including parrots are being exported from India to various parts of the world and Huydam in Holland (1952) reported isolation of NDV from imported Indian parrots. Kerala also contributes considerably in the export of parrots to outside countries. The incidence and magnitude of prevalence of ND among parrots have not been investigated in our state before. Since parrots have been established as a carrier of NDV it was felt worthwhile to

undertake a study on the incidence, susceptability, mode and duration of virus excretion by Indian Parrots (Psittacula Krameri). Further, the study may help to gather more information on the role of parrots in the epizootiology of ND, which in turn may help in the eradication and control of the disease.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

New Castle disease (ND) is a contageous viral disease primarily of avian species, which can be subclinical or fatal with systemic, nervous, respiratory or gastrointestinal involvements. The disease first occured in and around Batavia on the Island of Jawa in Indonesia (Kranevald,1926). Simultaneously report on the occurance of this disease came from a poultry farm near New Castle of Tyne, England (Doyle,1927). Subsequently this disease was reported from most of the countries.

In 1927 this disease was recognised at Ranikhet in Kumaon hills by Edwards (1928). Cooper (1930) studied the disease in detail in India and gave the name Ranikhet Disease (RD). The antigenic identity of ND and RD using cross immunity tests were also studied by Cooper (1930). The disease occured in various forms which ranged from inapparent to fulminatin fatal one, where the mortality went upto 100 per cent (Hanson,1975). Less lethal forms of the disease were also reported to cause major economic loss, crippling impaired growth and poor feed utilisation among surviving birds (Berg et al.1947).

A variety of gallinaceous and non-gallinaceous birds were reported to be susceptible to natural as well as

experimental NDV infections. The virus was isolated from starling (Gillespie,et al.1950); Wild Pheasants (Cponik and Sitkovic, 1972); Quails (Higgins and Long,1968); Patridges (Galli and Cassi,1968); Sparrows (Abdul Majeed et al.1965); Pegions (Ulbrich and Coden,1965; Sulochana,1981); doves (Kraneveld and Wansjoer (1950); Crows (Haddow,1941, Sulochana,et al.1981); Owl (Ingalls et al.1951) and ducks and Swans (Acolin,1947; Sulochana and Nair,1979).

Wild birds have been incriminated as disseminators of NDV in India (Sahai,1937), in Middle Congo (Brandly et al.1946), Great Britain (Callender,1958), Ceylon (Crawford,1931) and Philippines (Parinas,1930). Many species of free-flying birds were also been infected artificially with NDV (Gustafson and Moses,1953). The reports of Schaff,(1974), Annon,(1975), Lancaster,(1977) and Vickers and Henson (1978), also indicated that NDV has a wide range of susceptible avian hosts.

Since there was excretion of NDV in the faeces of several species of wild birds Mastula (1951) and Gustafson and Moses (1953) thought that these birds could contaminate the farms very easily through their faeces. They also stated that the peculiar behavioural pattern of these birds also helped them to disseminate NDV and they played an

important role in the epizootiology of this disease. Makey (1967) observed an outbreak of ND among pheasants in Hungary and the clinical symptoms and the postmortem findings recorded by him were similar to that found in fowls. Spenik and Sitkovia (1972) reported that the virus strains isolated from pheasants and fowls in an outbreak of the disease were similar in antigenic structure but differed in virulence.

Sparrows were described as carriers by Gustafson and Moses (1953). Arias et al.(1978) isolated NDV from a sparrow hawk. These authors observed nervous signs of clonic spasms and opisthotonous in sparrows occurring 48 hours following aerosol infection. Cavrini and Cabassi (1960) observed nervous symptoms in peacock, parrots and guinea fowls during an epizootic. Nervous signs characterised by torticollis and opisthotonous were also observed in a naturally infected owl by Ingalls, et al.(1951). On the other hand an Osprey from which NDV was isolated did not show any symptoms (Puydam,1952), so also Shags and Cormorants(MacPherson,1956).

Although intra cardial or per oral exposure of crows to this virus did not produce any clinical symptoms, and subcutaneous inoculation produced only depression.

Intracerebral inoculation caused death due to nervous system involvement (Karstad, et al.1959). Sulochana, et al. (1981) also reported absence of any clinical disease in crows either by oral or intranasal inoculation.

Patridges infected intramuscularly with NDV manifested depression followed by paralytic symptoms of legs and wings (Thompson,1956). Pigeons also developed nervous signs on exposure, characterised by torticollis, in-coordination, paralysis, tremors and respiratory distress. (Walker et al.1954). Pheasants, pigeons and wild birds were severely infected during an outbreak and these birds were responsible for the spread of the disease throughout England. (Alexander,(1970)& Keymer and Dawson (1971) isolated NDV from a sick Kestrel. Castillo, (1972) reported an outbreak of ND among captive exotic birds and they were thought to be responsible for outbreaks of ND in United States (Grass,1972).

The role of wild birds, semi-domestic and exotic birds in the epizootiology of ND in Southern California during 1972-73 was evaluated by Pearson and McCann,(1975). Out of the 9446 free-flying wild birds examined, Velogenic viscerotropic NDV (VVNDV) was isolated from 0.04 per cent

of birds. They had also isolated AVNDV from 0.76 per cent of 4367 semi-domestic birds and 1.01 per cent of 3780 exotic birds examined. The authors have further observed that sparrows and crows were the only free-flying wild birds infected, and psittacines, pittas and toucans accounted 92 per cent of the AVNDV isolations made. They also isolated native NDV from free-flying wild (0.29 per cent), semi-domestic (1.65 per cent) and exotic birds (0.19 per cent). Narthedal, et al.(1973) observed that the source of infection for two consecutive outbreaks that occured in 1955 and 1962 in Denmark was from the newly introduced pullets and imported wild birds. Pearson, et al.(1975) reported that out of the 127 exotic birds submitted for importation, NDV was isolated from 29 birds, by the chicken embryo inoculation of tracheal/cloacal swabs. These isolates were found to be pathogenic to domestic poultry. Chu et al.(1976) isolated 11 velogenic NDV from 44 birds of prey that died in captivity in United Kingdom. First report on the isolation of NDV from wild birds in U.S.A. was made by Lvov, et al.(1977). They made 15 isolations either from the tracheal or cloacal washings or from viscera of 477 wild birds.

Pierson and Pfow (1975) in their report on the surveillance of ND in USA have observed that 24 of the

100 birds offered for entry harboured VVND. They further stated that the isolations were made mostly from psittacine birds, falcons and penguins brought from Australia. Utterback and Schwartz (1973) observed that the major factor in the dissemination of VVND in Southern California was the movement of infected domestic or exotic birds. These authors studied in detail the role of free-flying wild birds and came to the conclusion that free-flying wild birds did not play an important role in the dissemination of VVND. They were able to infect the crows and sparrows only with heavy inoculum and this lead to the death of the birds, apparently without shedding the virus. The possibility of vaccinating turkeys and pheasants was also discussed by them.

Vickers and Hanson (1979) studied the effects of NDV infection in three species of wild birds (Red-winged black bird, African cover finch and Sandhill Crane) and found that only the crane continued to excrete the virus for periods varying from weeks to months. Antibodies to ND in high titres were detected in 71 of the 97 captive falcons destined for export to Europe from Nigeria (Okot,1979).

Psittacines, in general were considered to be responsible for the introduction of virulent NDV to domestic poultry flocks in a number of countries.

As early as 1930, Farinas mentioned unnamed species of parrots among possible carriers of ND to philippines. Cooper (1930) studied the susceptibility of the Indian green parakeet (Psittacula Krameri) to parenteral administration of the virus, and observed that the birds were highly susceptible with nervous, respiratory and conjunctival symptoms. Rivers and Schlvenliker (1932) observed a virus disease in parrots and parakeets differing from Psittacosis. Huydam (1952) in Holland, isolated NDV from the spleen of sick parakeets which arrived from India.

During an enizootic in Kenya in 1955 grey parrots brought from Belgian Congo were among the species clinically affected (Scott et al.1956) . Eight of the nine birds died of ND and the disease was characterised mainly by respiratory symptoms. There was no nervous system involvement. Postmortem lesions were also not marked except for diffuse congestion of the proventriculus. Enteritis and Tracheitis were observed in one case each. Although the remaining birds were protected by vaccination with an inactivated vaccine, there was no development of HI antibodies. A neurogenic strain of NDV which was highly pathogenic to grey parrots was isolated from the dead birds. However, the spread of the disease among parrots was slow (Scott and Hinmill,1963).

Despite the non-excreting carrier state, psittacines have been responsible for the introduction of virulent ND to domestic poultry flocks in a number of countries such as Kenya, (Scott and Hamill,1960) east Germany (Watchendorfer and Luthgen,1971), Austria (Grausgruber,1972), USA (Walker et al,1973), Great Britian (Allan,1974), South Africa (Francis,1974) and Canada (Lancaster,1974).

Allan (1968) isolated a haemagglutinating agent from an African Grey parrot and from a Malabar Blue ring parakeet, which was later on identified as a virulent strain of NDV. In both the cases there were no specific lesions.

Luthgen and Watchendorfe (1970) recorded deaths among three consignments of parrots imported to West Germany from Brazil. The quarantined affected birds showed clinical signs of injury to central nervous system. Viral agents identified by HI test and gel-precipitation tests as NDV were isolated from the brain of dead parrots by chick embryo inoculation. Watzer and DeMota (1971) described an outbreak of ND in Amazon parrots (Amazona Achricephala) in captivity. These authors also isolated neurotropic strain of NDV from 22 parrots captured from

Guatemala. The virus was found to be pathogenic to original hosts as well as for 10 day old chicks.

Francis and Revelli (1971) described an outbreak of a disease in an aviary which shipped parrots and parakeets to a number of countries in Europe and U. S. A. This disease spread among domestic poultry causing very high mortality and a virulent strain of NDV was isolated by chick embryo inoculation. Grausgruber, (1972) reported ND in Rose-ringed and Blossom headed parakeets imported from India to Austria from which the virus could spread to poultry. He found that the Alexandrian parakeet imported from Columbia and Grey parrots shipped from Belgium were also associated with the spread of ND to poultry.

Outbreaks of ND had occured repeatedly in East Germany among psittacine birds imported from South East Asia and South America. All the viral isolates were reported to be neurotropic and virulent to poultry (Batchendorfer and Luthgen,1971). Mastulich, (1974), Walker et al.(1973) and Gorden (1974) reported occurrence of exotic ND in United States in August 1970. The virus was isolated from many places and the source of these outbreaks was traced to be the imported pet birds including parrots. While studying the epizootiology of VVND in Southern California, Utterback

and Schwartz (1973) found that parrots develop an overt and often fatal infection with MVNDV. The authors made an extensive study on the role of free flying wild birds, and exotic birds in the dissemination of this virus. Since virus isolations were also made from parrots imported from other countries, the authors were of opinion that the viral sensitivity of the various species of parrots offered for importation has to be investigated for implementing effective quarantine measures.

In his review on the role of parrots in many of the outbreaks in United States during 1970-71 Francis (1973) stated that free movement of parrots that were imported from Paraguay, played an important role in the spread of ND. Cullen et al.(1974) have described incidence of ND combined with psittacosis in a consignment of Amazon parrots arrived at Heathrow Airport, on their way to Japan from South America. Ten sick birds subjected to detailed examination at the Central Veterinary Laboratory, Weybridge had enteritis, enlargement of liver, distended and flabby heart and thickened airsacs with purulent material. Virulent strains of NDV were isolated from six of these birds. Gordon,(1974) provided indisputable evidence to prove that the disastrous outbreak of Asiatic ND on the West Coast of America was originated from imported

psittacines.

In 1974 Cavill was able to isolate a highly virulent strain of NDV from a green cheeked parrot, which was suffering from an acute illness characterised by anorexia, adypsia, and abnormally soft droppings and died within 24 hours. The author thought that the parrot picked up infection from cockatoos which was imported to U.K. from Switzerland.

An overt and fatal infection of parrots with NDV was reported by Hanson (1973). The isolates were found to be infective for sentinal chickens by contact with these sick and dying parrots. Thream, et al. (1975) in Switzerland reported that the outbreak of ND among widely separated poultry flock in 1973 was associated with purchase of parrots imported from South America. The authors further stated that these birds were subjected to 8 weeks quarantine and the quarantined parrots were caged along with pullets susceptible to ND. It was possible for them to isolate the virus from all the dead birds. NDV was also isolated from parrots imported from Columbia, Bolivia and Indonesia. The clinical picture of ND in parrots was variable with symptoms of apathy, ruffled plumage, diarrhoea, emaciation and in protracted cases central nervous system disorders. The lesions were

catarrhal enteritis with petechiae or fibrinous deposits in the abdominal air sacs. All the isolates from parrots were velogenic. Pearson et al.(1975) and Pierson and Pfow (1975) were also able to isolate VVNDV from parrots.

Pathogenecity of virulent strain of NDV in parrots was studied by Dugue and Satupinan (1976). Out of the three routes,(oral, intramuscular and intranasal) intramuscular route of inoculation was found to be most effective in infecting parrots. They observed nervous and respiratory symptoms within 3-5 days. A rise in antibody titre was noticed from day 10 onwards till the 60th day. Histopathological lesions were not significant except for haemorrhage and oedema in the lung and tracheal mucosa and haemorrhage and perivascular infiltration in the brain. Virus was also re-isolated from the viscera of the experimentally infected birds. It was possible to protect the parrots from virulent NDV by vaccination with LaSota strain.

Experimentally infected parrots were found to excrete the virus for one year (Erickson,1977). Serum neutralisation and HI tests, with sera from these birds were also suggestive of prolonged carrier state. NDV strain re-isolated from the experimentally infected psittacines were passaged through budgerigars and parrots

(Erickson et al.1978). They could see that on back passages in chicken 15 of the 19 had potential virulence for chicken. The remaining 4 isolates produced large red plaques characteristic of highly virulent strain(VV) . These authors also found that the NDV isolates showed little change in parameters originally evaluated for the pet bird isolates, used for the back passage studies. But the psittacine isolate was found to have slowly changed to a relatively avirulent strain on passage in pet birds and reversion did not occur during the chicken back passages. The authors concluded that parrots would all be expected to become infected within 2 weeks after direct or contact exposure and more than one third of the birds would die within 3-5 days of VVNDV.

Dawes and Srimes (1978) isolated a lentogenic strain of NDV from an exotic parrots illegally introduced to Australia. This virus produced a severe respiratory disease in day old chicks by intranasal, and oral routes and by contact with infected birds. However, no harmful effects were noticed when this virus was given orogenally or intravenously to 6 week old chicken.

Hitchner and Hiraf (1978) reported the isolation of NDV from parrots died of diarrhoea. On necropsy the liver and spleen were found to be enlarged. The authors

in a study on the growth characteristics of the psittacine viruses in chicken embryo have stated that out of the eight virus isolates passaged through yolk sac, allantoic and chorioallantoic membrane routes, only one of the re-isolates agglutinated chicken RBC. The authors also observed that the peak virus concentration was attained in about 72 hours. Onunkwo and Momot (1980) reported the isolation of NDV from an African Grey parrot in Nigeria, which succumbed to the disease in three days. The authors isolated a highly virulent strain of NDV from a pooled sample of lung and proventriculus from the infected birds. The authors concluded that the parrots picked up infection from chicken and the isolation indicated the role of wild birds in the spread of NDV in Nigeria.

MATERIALS AND METHODS

MATERIALS AND METHODS

Incidence of New Castle Disease in Parrots

The incidence of NDV infection in parrots was determined by screening birds either for haemagglutination-inhibition (HI) antibodies or by virus isolation.

Serological Survey

Collection of blood

The technique described by Beard and Brugh (1977) was followed (Nobuto paper strip). Blood samples were collected on filter paper strips of 20 x 40 mm size, cut from Whatman filter paper No.1. A total of 103 samples (Palghat 9, Trichur 41, Trivandrum 12, Quilon 21, and Calicut 20) were collected. At the time of collection, the wing veins of the birds were punctured with a hypodermic needle and a large drop of blood was absorbed on one end of the paper strip. When saturated with blood, the strip was placed in penicillin vials and dried at room temperature and brought to the laboratory.

Elution procedure

The paper strip containing blood was cut with scissors into small pieces and soaked in 0.5 ml normal saline. The pieces were agitated well and kept at 4°C

overnight for complete elution. The next day, the paper strips were squeezed with a Pasteur pipette and the elute was subjected to HT test.

Virus

The strain of RDV used for challenging vaccinated birds at the Veterinary Biological Institute, Palode, was employed for all experimental studies and haemagglutination inhibition tests. On receipt in the laboratory, the virus was passaged once in 10-day embryonated eggs and the allantoic fluid collected from these infected embryos was stored at -20°C in small aliquots for further use.

Chicken RBC

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

Haemagglutination (HA) test

The stock virus was first titrated by haemagglutination test before it was used for haemagglutination inhibition test.

Two-fold dilutions of the infected allantoic fluid

were made in physiological saline in haemagglutination plates. To these dilutions in each well, an equal quantity of 0.5 per cent washed chicken RBC was added and mixed well. Simultaneous saline and RBC controls were also made. Then the plates were incubated at room temperature for 30-45 minutes and readings were taken after the controls had settled.

Haemagglutination-inhibition test (H.I.test)

After determining the haemagglutination titre of the stock virus the required volume of 8 HA unit was prepared by diluting the virus in normal saline. Serial double fold dilutions of (0.2 ml each) the paper strip elutes were made in normal saline in Perspex Haemagglutination plates and each of these dilutions were mixed with 0.2 ml of the 8 HA unit of the virus and incubated at room temperature for 30 minutes. Following incubation, 0.4 ml of 0.5 per cent suspension of chicken RBC was added to each well and mixed. Simultaneous RBC and virus controls were set up side by side. The readings were taken after incubation for 30 minutes at room temperature. For the calculation of titre of ND antibodies, the eluted serum sample was considered equivalent to 1:10 dilution.

Virus Isolation

Collection and processing of cloacal and throat swabs

Seventy cloacal and fortytwo throat swabs were collected from the Common Indian Parrot(Psittacula-Krameri) seen in different parts of the State. The details of source of parrots are given in table 1. Soon after collection, the swabs were soaked in 1.5 ml tryptose phosphate broth containing 1000 I.U. of penicillin and 1000 microgram of streptomycin per ml (TPB-A) and stored at -20°C until used.

At the time of chick embryo inoculation the swabs were thawed at room temperature and squeezed for about five times with a sterile pipette. The fluid so separated from the swabs were collected and centrifuged at 1000g for about 10-15 minutes. The supernatent was collected and incubated at 37°C for 1 hour and inoculated into the allantoic cavity of 10 day embryonated eggs.

Collection and processing of tissues

Tissues such as liver, spleen, lungs and brain were collected under sterile conditions from the dead parrots and from those that were given experimental infection. These tissues were immediately transferred to vials containing

TPB-A and stored at -20°C. At the time of chick embryo inoculation the tissues were processed by emulsifying in a Tenbrock tissue grinder and making a 10-15 per cent suspension. It was then centrifuged at 1000 g for 10-20 minutes. The clear supernatent, separated and incubated at 37°C for 1 hour formed the inoculum for chick embryo.

Tryptose phosphate broth (TPB)

The powder was rehydrated by dissolving 3gm in 100ml distilled water by heating. It was then sterilised by autoclaving at 15 pounds for 30 minutes and antibiotics such as penicillin (500 I.U./ml) and streptomycin (500 microgram/ml) were added (TPB-A).

Chick embryos

Ten day embryonated eggs were obtained from the University Poultry Farm, Mannuthy.

New Castle disease antiserum

Collected from chicken that were vaccinated with NDV-K strain and later on challenged with virulent NDV and found solidly immune.

Chick embryo inoculation

Allantoic cavity inoculation (Bishai, et al. 1974).

The ten day embryonated eggs were candled and the

air cell and head of the embryos were marked. After disinfecting this region with tincture of iodine and drilling a hole, 0.2 ml of the inoculum was introduced into the allantoic cavity using a sterile tuberculin syringe and 22 gauge needle. The hole was then sealed with melted paraffin and the eggs incubated at 36-37°C in an upright position. Control eggs were similarly treated except that 0.2 ml of sterile normal saline replaced the virus inoculum. All the eggs were candled daily. The embryos that died after 24 hours and those alive after seven days were transferred to the refrigerator. Negative samples were passaged two more times before they were considered as negative.

Collection of allantoic fluid

Eggs, pre-chilled for 6 hours at 4°C were disinfected at the air cell region with alcohol. The shell at this point was cut and flipped open and the shell membrane and chorioallantoic membrane were sheared off with a sterile forceps. The allantoic fluid was then collected using a Pasteur pipette in small labelled vials and stored at -20°C for further studies. The haemagglutinating activity of each sample was tested simultaneously by mixing equal quantity of the allantoic fluid and 0.5 per cent chicken RBC. Positive samples were subjected to HI test employing

known ND serum to determine its specificity.

Experimental Infection Studies

Parrots

The common Indian green parrots (age not assessable) were purchased from a local pet-bird dealer. On arrival in the laboratory they were kept under observation for a week during which they were tested for the presence of any ND antibodies or virus as described previously.

Chicks

Day old unvaccinated chicken received from the University Poultry Farm, Mannuthy, were reared in the Laboratory to the required age before they were used for experimental purposes.

Virus

Three strains of NDV differing in their virulence were used for experimental infections.

a) Strain of NDV used for challenging vaccinated birds
b) Mesogenic strain (Komorov)
c) Lentogenic (F1 strain)

All these strains of the virus were received from Veterinary Biological Institute, Palode. Since the degree

of virulence of the first strain was not indicated, it was subjected to various tests to determine its virulence.

Mean death time

Serial ten-fold dilutions of the virus was made upto 10^{-9} and the last three dilutions were inoculated into the allantoic cavity of the ten day old embryonated eggs. Eight eggs were used for inoculation of each dilution of the virus and incubated. Candling was done at every eight hours and the results were recorded. The mean death time was calculated from these results as described in poultry biologics (1963).

Intracerebral pathogenicity Index (ICPI)

Ten, day-old unvaccinated chicks were inoculated into the cerebral cortex, with 0.1 ml of a 1 in 10 dilution of the virus, using a 25 gauge needle. To another five, day-old chicks 0.1 ml of sterile normal saline was given intracerebrally, as controls. The birds were then housed separately and observed for any development of symptoms or deaths till the 8th day. The ICPI was calculated by using the factor '0' for normal, one for signs of disease and '2' for death. The sum total of all the factors was divided by the total number of observations (Hanson 1975).

Intravenous pathogenecity index (IVPI)

Eight week old unvaccinated chicks were inoculated with 0.1 ml of the 1:10 dilution of the virus subcutaneously. Control birds were simultaneously inoculated with 0.1 ml of sterile normal saline by the same route. Then the birds were housed separately and watched daily for symptoms, paralysis and death till the 10th day. The IVPI was calculated by using the factor '0' for normal, one for signs of disease and two for paralysis and 3 for deaths. The sum total of all the factors was divided by the number of observations (Poultry Biologics, 1963).

Hank's Balanced Salt Solution (HBSS),(Cunningham,1966)

The required pH (7.2) was obtained by adding 7.5 per cent sodium bicarbonate solution.

Growth medium

Hank's Balanced Salt Solution supplemented with 0.5 per cent lactalbumin hydrolysate, 0.15 per cent yeast extract and 5-8 per cent calf serum constituted the growth medium. Antibotics at the rate of 200 I.U. of penicillin, 200 micrograms of streptomycin and 50 units of Mycostatin per ml were also added.

Tissue culture maintainence medium

It was prepared as above, except that the serum concentration was brought down to 2 per cent.

Calf serum

Blood collected from bull calves of about one to one and a half years of age, was allowed to clot in a slanting position and refrigerated overnight for easy separation of serum. The serum thus separated was transferred to a sterile flask, inactivated at 56°C for half an hour, filtered through seitz filter pads, and stored at -20°C until used.

Calcium magnesium free buffer (CMF-PBS)

Prepared as described by Cunningham,(1966).

7.5 per cent sodium bicarbonate solution

Prepared as per the method described by Rishai,et al. (1974).

Trypsin

A stock solution of 5 per cent trypsin (1:250 difco) was prepared in CMF-PBS, sterilised by filtration through seitz filer pads, distributed in 10 ml quantities and stored at -20°C. The working solution was prepared by diluting the stock solution with CMF PBS to give a final concentration of 0.25 per cent.

Antibiotic solution

A stock solution of Benzyl penicillin and dihydrostreptomycin sulphate was prepared in sterile distilled water and preserved at -20°C. The concentration of these antibiotic solution was adjusted in such a way that when 1 ml of this mixture was added to 100ml, a final concentration of 200 I.U. of penicillin and 200 micrograms of streptomycin per ml was obtained.

Mycostatin

Stock solution to contain 5000 units per ml in sterile distilled water and stored at -20°C.

Chicken embryo fibroblast cultures

Ten-day old embryonated chicken eggs were candled for viability and the air cell and head of the embryos were marked. After disinfecting with alcohol the air cell region was cut with care employing sterile scissors. The embryos were transferred to a Petri dish containing CMF-PBS with 200 I.U. of penicillin, 200 micrograms of streptomycin and 50 units of mycostatin per ml. The head, limbs and viscera of the embryos were removed and the rest was minced well with scissors and washed twice with CMF-PBS. The minced tissues were then transferred to a trypsinisation flask, to which pre-warmed (37°C) 0.25 per cent trypsin solution was

added at the rate of 25 ml per embryo. A tiflon coated magnetic stirring bar was introduced and stirred on a magnetic stirrer for 3 minutes. The supernatant was poured off and washed with fresh pre-warmed trypsin to remove any cytotoxic factors, if present. Fresh trypsin was again added and stirred for 20-30 minutes. This dispersed cell suspension was filtered through a sterile double layered muslin cloth. The filtrate was centrifuged at 100 g for 5 minutes, discarded the supernatent and resuspended in growth medium. The process of washing was repeated twice. At the end the cells were resuspended in growth medium, to get a final concentration of 5×10^{-5} cells per ml, and seeded into tissue culture tubes containing cover slips in 1 ml quantity. The tubes were then incubated at 37°C in a slanting position.

Cytopathic effects (CPE)

Tubes with satisfactory monolayers were selected, poured off the growth medium and the cell layer washed with maintainence medium. To each of these tubes 0.2 ml of a 1:100 dilution of the virus was inoculated and incubated at 37°C for one hour, to facilitate absorption. Following this absorption period, the inoculum was poured off, washed again with maintainence medium and incubated at 37°C. Control tubes were similarly treated except that 0.2 ml of

M.M. replaced the virus inoculum. At 24 hour intervals, the tubes were examined under the microscope for cytopathic effects. Coverslip cultures inoculated with the virus were stained either by Giemsa or Haemotoxylin and Eosin, after fixation with methanol or formosaline.

Experimental infection - 1

Twenty parrots which were pre-tested for the presence of ND antibody or virus were divided into three batches. Batch A and B consisted of seven birds each and Batch C six. The first two batches were inoculated with 0.1 ml of undiluted virus in the form of infected allantoic fluid by intraocular and intranasal routes respectively. The third batch of birds was kept as uninfected controls. All the three batches were kept in separate cages to avoid cross-contamination. The birds were examined daily for the development of any symptoms or deaths. Cloacal and throat swabs were collected from all the 20 birds from day 4 onwards for virus isolation trials. Spleen, liver, lung and brain of dead birds were also collected separately and preserved in maintainence medium with antibiotics for virus isolation. For histopathological examination they were fixed in 10 per cent formalin.

Contact infection

The possibility of infected parrots transmitting the

disease to susceptible chicken was studied by keeping unprotected week-old chicks (Six each) along with Batch A and B. Cloacal and throat swabs were also collected from these birds. Antibody response to NDV was studied by collecting blood at weekly intervals till the 6th week.

Experimental infection - II

Thirty six parrots divided into six group of six each for further infectivity studies. The first two groups (I and II) were infected with the virulent NDV diluted 1:100 by intranasal and subcutaneous routes respectively. The dose for each bird was 0.1 ml (Table 9 and 10).

The third group of six birds received 0.25 ml each of 1 in 50 dilution of Komorov strain of the virus by the subcutaneous route. One vial of Ranikhet disease vaccine for day old chicks (R1) diluted to 10 ml was given to fourth batch of parrots at the rate of one drop each into the nostrils and eye.

The fifth group of parrots were left unintected along with five, 5-week-old unprotected chicks that were infected with a 1 in 10^6 dilution of the virulent NDV at the rate of 0.1 ml per bird by subcutaneous route.

The reamining 6th group of 6 parrots was kept as

uninfected controls. All these groups were kept in separate pens with stone walls in between, so that no cross contamination occured between groups.

Contact infection

The possible spread of NDV from infected parrots, through different routes was also studied by keeping unprotected 5 week old chicks (five each) along with group 1 and 2.

All the birds were examined daily for the development of symptoms or deaths. Cloacal and throat swabs were collected from all the birds from day three onwards. Tissues such as liver, spleen, lung and brain were collected in TPB-A for virus isolations. Pieces of all the above tissues were fixed in 10 per cent formalin for histopatholo ical examination.

Challenge with virulent NDV

Parrots that survived infection with lentogenic strain of NDV, and all the chicks that were kept for contact infection studies, but did not show any manifestations of ND were challenged with 0.1 ml of a 1:100 dilution of the virulent strain by subcutaneous inoculation. All the birds were examined daily and on death, they were

examined for the presence of any specific lesions. Tissues such as liver, spleen, lung and brain were also collected from these birds for virus isolation trials.

The cloacal and throat swabs and tissues collected from all experimental birds were subjected to virus isolation trials as described previously.

RESULTS

RESULTS

Incidence of ND in Parrots

Serological survey

Out of 103 blood samples collected from parrots from various parts of the state, only 16.5 per cent were found to possess HI antibodies against ND (Table 1). The HI titres ranged from 20 to 160.

Virus isolation

Seventy cloacal and fortytwo throat swabs collected from parrots inhabiting in different parts of Kerala (Table 2) were screened for NDV by inoculation into the allantoic cavity of ten-day embryonated eggs. Allantoic fluid collected from none of the inoculated embryos gave any HA with chicken RBC. All the samples were found to be negative even after three blind passages.

Pathotyping of the Virus

Mean death time (MDT)

The mean death time at terminal dilutions was calculated to be 57 hours (Table 3). The dead embryos showed haemorrhage at the suboccipital region and all over

the skin. The abdominal region of the embryo was oedematous.

Intracerebral pathogenecity index (ICPI)

Inoculation of 1:10 dilution of the virus into the cerebral cortex of ten, day-old chicks caused death of all the chicks by day six (Table 4). The index was calculated to be 1.60 .

Intravenous pathogenecity index (IVPI)

All the chicks that were given 0.1 ml of 1:10 dilution of the virus subcutaneously died by the fourth day, and IVPI was found to be 1.56 (Table 5).

Cytopathic effects in chick embryo fibroblasts (CPE)

Satisfactory monolayers of chicken embryo fibroblasts were obtained 24 hours following seeding. The cells were more or less spindle shaped with acidophilic cytoplasm which contained few vacuoles and granules. The nucleus was centrally placed, more or less oval in shape with one or more nucleoli (Fig.1).

Morphological changes of the infected cells appeared by fortyeight hours and were characterized by rounding of cells. The changes were first observed along

the periphery of the monolayers. By about seventytwo to ninetysix hours, syncytium formation by the affected cells was also a characteristic features. By about ninetysix hours most of the cells got detached from the glass surface (Fig.2). Coverslip cultures taken at different intervals to study the CPE by staining with Haematoxylin and Eosin showed cytoplasmic granulation and eosinophilic intracytoplasmic inclusion bodies.

Experimental infection with undiluted virulent strain of IBV

Intraocular infection

All the seven parrots that received undiluted virulent virus intraocularly, showed symptoms of inapetance, conjunctivitis, respiratory distress, drooping wings, leg paralysis and diarrhoea, from day two onwards. Mortality was observed from the third day and all the parrots died by the sixth day of infection (Table 6). Virus isolation was possible from the throat swabs from day three while the cloacal swabs became positive only on day five and six. Virus was isolated from the spleen of all the seven birds, while isolation from liver was possible only from four. The number of birds that gave positive isolation from the lung and brain were six and two respectively.

Intranasal infection

The seven parrots given undiluted virus intranasally showed the same symptoms described above from day two onwards. Death was noticed from day three onwards and all the birds were dead by day six (Table 7).

Virus excretion from the throat swabs was possible from day three onwards and that from the cloacal swabs was only from day four.

Virus was isolated from the spleen of all the seven birds while only 4 isolations was made from the liver. The number of isolations made from lung and brain were six and two respectively.

Contact infection

All the unprotected chicks kept along with the infected parrots excreted the virus through cloacal and tracheal routes from day ten onwards and showed HI antibodies from day twelve onwards (Table 8). The HI titres ranged from 10 to 160, initially and later on the titre was found to be decreasing. By the sixth week, only three chicks showed an antibody titre upto ten and only one chick was positive for viral excretion through the cloacal route. The control parrots were normal even after the experiment.

Experimental infection with various strains of NDV

Batch I

In the six parrots that were given 0.1 ml of 1:100 dilution of the virulent virus subcutaneously, symptoms of inappetence, leg and wing paralysis, diarrhoea and depression were seen from day two. Four of the six birds died by the third day and the remaining two by the fourth day (Table 9). Virus was excreted through the cloacal route by all the birds on days three and four, while only one bird excreted the virus by the tracheal route on day three. Isolation of virus was possible from the spleen of all the six parrots, from the liver of four birds and from the lungs and brains of two birds each.

Batch II

The group of six parrots that received 0.1 ml of 1:100 dilution of the virulent virus by intranasal route also showed the same symptoms described above by day two onwards. One of them died on the second day itself while the others died during days three to five (Table 10). Examination of the cloacal and tracheal swabs showed that virus was excreted by the tracheal route in all the birds, while only four birds excreted the virus through cloaca. Virus was isolated from the spleen and lung of all the six

parrots, while isolations from liver and brain were possible from only two and one respectively.

Batch III

All the six birds inoculated subcutaneously with Komorov strain of NDV showed symptoms of ruffled plumage, diarrhoea inappettance and leg and wing paralysis from day four. Four birds died on the fifth day and the remaining two died on the sixth and seventh days. Examination of the cloacal and throat swabs showed that virus was excreted through the cloacal route by all the six parrots while only two birds excreted the virus on day five by the tracheal route. Virus was isolated from the spleen and liver of all the six birds, while isolations were possible only from the lungs of three and brains of two birds (Table 11).

Batch IV

The birds that received lentogenic strain of NDV(F1) by intranasal and ocular routes remained healthy and did not show any apparent symptoms until two weeks' post exposure. Cloacal and throat swabs screened for virus excretion showed that four out of the six birds excreted the virus by both routes for a period of fifteen days.

Haemaggelutination inhibition antibody titres were 1:20. On challenge with the virulent strain after day fifteen, all of them showed symptoms of ND from day four onwards. Mortality was noticed from day six and all were dead by day 8(Table 12). All the birds excreted the virus by the cloacal route while only two excreted the virus through the throat. Virus was isolated from the spleen of all the birds while isolations were obtained only from the liver of five, brain of four and lungs of three parrots (Table 12).

Batch V

Six uninfected parrots were kept along with chicks infected with virulent virus. The chicks showed symptoms of diarrhoea, torticollis, wing drooping, inappettence and died from day three to five. The contact parrots showed symptoms of inappettence, conjunctivitis, diarrhoea and drooping of wings from day six onwards. Three parrots died on the seventh day, one on day eight and the remaining two on day nine (Table 13). Examination of the cloacal and tracheal swabs showed that virus was excreted through the throat by all the parrots, while only three excreted the virus through the cloacal route. Virus was isolated from the spleen and lung of all the birds while isolation was

possible only from the liver of four and brain of two.

Contact infection

The five-week old chicks kept along with Batch I and 2 of the infected parrots, did not show any symptoms of the disease. But their cloacal and throat swabs were positive for virus from day 10 and the number of positive cases diminished gradually and by the seventh week none of the chicks excreted the virus. The sera showed the presence of ND antibodies from day 12 and the titre ranged between ten and eighty (Table 14). The control parrots were normal even after the experiment.

Challenge with virulent NDV

The chicks kept for contact infection studies, when challenged with the virulent virus, showed symptoms from day two and died on day three and four. These chicks on necropsy showed specific lesions of ND like petechial haemorrhage in the proventriculus, petechial haemorrhage and necrosis of the caecal tonsils and haemorrhagic lesions in the intestinal tract.

There was no marked gross lesions in infected parrots except slight enlargement of the liver and spleen and haemorrhage below the skullcap. Microscopically liver

showed diffuse engorgement of sinusoids, focal areas of degeneration and necrosis of the hepatic cells (Fig.3). There was isolated foci of haemorrhage with scattered lymphoid infiltration. Spleen showed congestion, areas of haemorrhage, multiple necrotic foci and congestion of sinusoids (Fig.4). Lungs showed congestion and focal areas of non-suppurative pneumonia (Fig.5). Brain had congestion, degeneration of cells and gliosis (Fig.6).

Table 1.

Results of screening of blood samples of parrots for new castle disease antibodies

Areas	No.of parrots	HI test positive	HI test negative	Percentage positive	Range of titre
Calicut	20	-	20	-	-
Palghat	9	2	7	22.22	20-80
Trichur	41	-	41	-	-
Quilon	21	8	13	38.09	20-160
Trivandrum	12	7	5	58.33	20-160
Total	103	17	86	16.50	

Table 2

Results of examination of cloacal and throat swab of parrots for NDV isolation

Areas	No.of swabs C	No.of swabs T	C +	T +	Percentage of positive T	Percentage of positive C
Calicut	10	6	-	-	-	-
Palghat	6	4	-	-	-	-
Trichur	36	22	-	-	-	-
Quilon	12	10	-	-	-	-
Trivandrum	6	-	-	-	-	-
Total	70	42	-	-	-	-

C = cloacal swab
T = throat swab

Table 3

Mean death time of the strains of NDV received from VSI, Palode

Dilution of virus	Examination for deaths at intervals in hours									
	8	16	24	32	40	48	56	64	72	80
10^{-7}	-	-	-	-	2	2	3	1	-	-
10^{-8}	-	-	-	-	-	3	2	2	1	-
10^{-9}	-	-	-	-	-	-	-	-	-	-

Mean death time = $\frac{\text{Sum of hours at which embryos died}}{\text{Total number died}}$

$= \frac{(3\times48)+(2\times56)+(2\times64)+(1\times72)}{8} = 57$

Table 4

Intracerebral pathogenecity index of the strain of NDV received from VSI, Palole

Days	1	2	3	4	5	6	7	Total	Factor	Sum of factor
Death	-	5	7	8	9	9	10	48	2	96
Signs	8	4	2	1	1	1	-	17	1	17
Normal	2	1	1	1	-	-	-	5	-	-
								70		113

Intracerebral pathogenecity index = $\frac{113}{70} = 1.6$

Table 5

Intravenous pathogenecity index of NDV received from VRI, Palode

Days	1	2	3	4	5	6	Total	Factor	Sum of factor
Death	-	-	3	4	6	-	13	3	39
Signs	-	3	3	2	-	-	8	1	8
Paralysis	-	-	-	-	-	-	-	2	-
Normal	6	3	-	-	-	-	9	-	-
							30		47

Intravenous pathogenecity index $= \frac{47}{30} = 1.56$

Table 6

Experiment 1

Results of intraocular infection of parrots with undiluted virulent virus

Observations in days	Symptoms	No.of birds dead	Virus isolation					
			Throat swabs	Cloacal swabs	Spleen	Liver	Lung	Brain
1	-	-	-	-	-	-	-	-
2	Inappetence difficult respiration, conjunctivitis, wing droop	-	-	-	-	-	-	-
3	Do and paralysis of legs and wings diarrhoea	1	1	-	1	-	1	-
4	Do	3	3	-	3	1	2	2
5	Do	2	2	2	2	2	2	-
6	Do	1	1	1	1	1	1	-

Table 7

Experiment 1

Results of intranasal infection of parrots with undiluted virulent virus

Observations in days	Symptoms	No.of birds dead	Virus isolation					
			Throat swabs	Cloacal swabs	Spleen	Liver	Lung	Brain
1	-	-	-	-	-	-	-	-
2	Inappettance, respiratory distress, conjunctivitis diarrhoea	-	-	-	-	-	-	-
3	Do with paralytic symptoms	2	2	-	2	-	2	-
4	Do	2	2	1	2	1	2	1
5	Do	1	1	1	1	1	1	-
6	Do	2	2	2	2	2	2	1

Table 8

Experiment 1

Contact infection studies of chicks

Observations in days	Batch I*				Batch II**			
	Cloacal swabs positive	Throat swabs positive	No.of birds with HI antibodies	Range of HI titre	Cloacal swab positive	Throat swab positive	No.of birds with HI antibodies	Range of HI titre
10	5	6	-	-	4	6	-	-
12	6	6	6	20-160	6	6	6	20-160
17	6	4	6	10-80	6	5	6	10-80
25	4	2	6	10-40	5	3	6	10-40
32	2	1	4	10-20	3	1	3	10-20
40	-	-	2	10	1	-	1	10

* Chicks kept along with parrots infected with unattenuated virulent strain of NDV by intraocular route

** Chicks kept along with parrots infected with unattenuated virulent strain of NDV by intranasal route

Table 9

Results of subcutaneous infection of parrots with virulent virus (1:100 dilution)

Observations in days	Symptoms	No.of birds dead	Virus isolation					
			Throat swabs	Cloacal swabs	Spleen	Liver	Lung	Brain
1	-	-	-	-	-	-	-	-
2	Inappetitance, diarrhoea, wing droop	-	-	-	-	-	-	-
3	Do with leg paralysis and depression	4	1	4	4	3	2	2
4	Do	2	-	2	2	1	-	-
5		-	-	-	-	-	-	-
6		-	-	-	-	-	-	-
7		-	-	-	-	-	-	-

Table 10

Results of intranasal infection of parrots with virulent virus (1:100 dilution)

Observations in days	Symptoms	No.of birds dead	Virus isolation					
			Throat swabs	Cloacal swabs	Spleen	Liver	Lung	Brain
1	-	-	-	-	-	-	-	-
2	Respiratory distress conjunctivitis inappetance difficulty to move	1	1	-	1	-	1	-
3	Do and paralysis of legs and wings	1	1	1	1	1	1	-
4	Do	3	3	3	3	1	3	1
5	Do	1	1	1	1	-	1	-

Table 11

Results of subcutaneous infection of parrots with Komorov strain of NDV

Observations in days	Symptoms	No.of birds dead	Virus isolation					
			Throat swabs	Cloacal swabs	Spleen	Liver	Lung	Brain
2	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-
4	Inappetence, diarrhoea wing droop with paralysis of head and neck	-	-	-	-	-	-	-
5	Do	4	2	4	4	4	3	2
6	Do	1	-	1	1	1	-	-
7	Do	1	-	1	1	1	-	-

Table 12

Results of subcutaneous infection with virulent virus (1:100 dilution) 2 weeks after inoculation with F1 vaccine

Observations in days	Symptoms	No. of birds dead	Virus isolation					
			Throat swabs	Cloacal swabs	Spleen	Liver	Lung	Brain
4	Inappetance, diarrhoea, wing droop and leg paralysis	-	-	-	-	-	-	-
5	Do	-	-	-	-	-	-	-
6	Do	2	1	2	2	2	1	2
7	Do	2	1	2	2	2	1	1
8	Do	2	-	2	2	1	1	1

Table 13

Results of contact infection of parrots from chicken

Observations in days	Symptoms	No.of birds dead	Virus isolation					
			Throat swabs	Cloacal swabs	Spleen	Liver	Lung	Brain
6	Inappettance, conjunctivitis, diarrhoea and wing droop	-	-	-	-	-	-	-
7	Do	3	3	1	3	2	3	-
8	Do	1	1	1	1	1	1	1
9	To	2	2	1	2	1	2	1

Table 14 Experiment II
Contact infection studies of 5 chicks each kept along with infected parrots (Batch I and II)

Observations in days	Batch I*				Batch II**			
	Cloacal swabs positive	Throat swabs positive	No.of birds with HI antibodies	Range of HI titre	Cloacal swabs positive	Throat swabs positive	No.of birds with HI antibodies	Range of HI titre
10	4	5	-	-	5	5	-	-
17	5	5	5	10-80	5	5	5	10-80
25	5	3	5	10-80	5	3	5	10-40
32	4	2	5	10-40	4	1	4	10-20
40	2	-	4	10-20	2	-	2	10
48	-	-	2	10	-	-	1	10

* Chicks kept with parrots infected with 1:100 dilution of the virulent virus by subcutaneous route

** Chicks kept with parrots infected with 1:100 dilution of the virulent virus by intranasal route

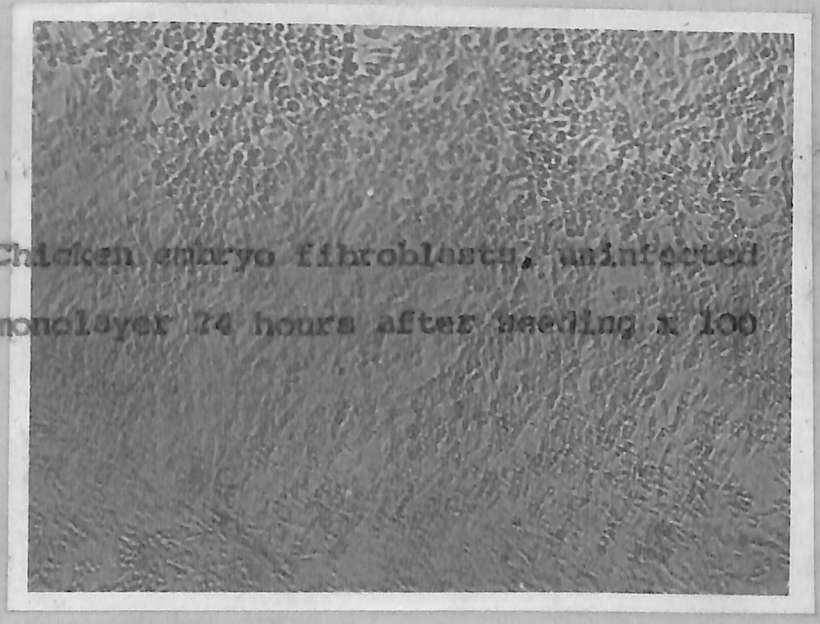


Figure 1. Chicken embryo fibroblasts, uninfected monolayer 24 hours after seeding x 100

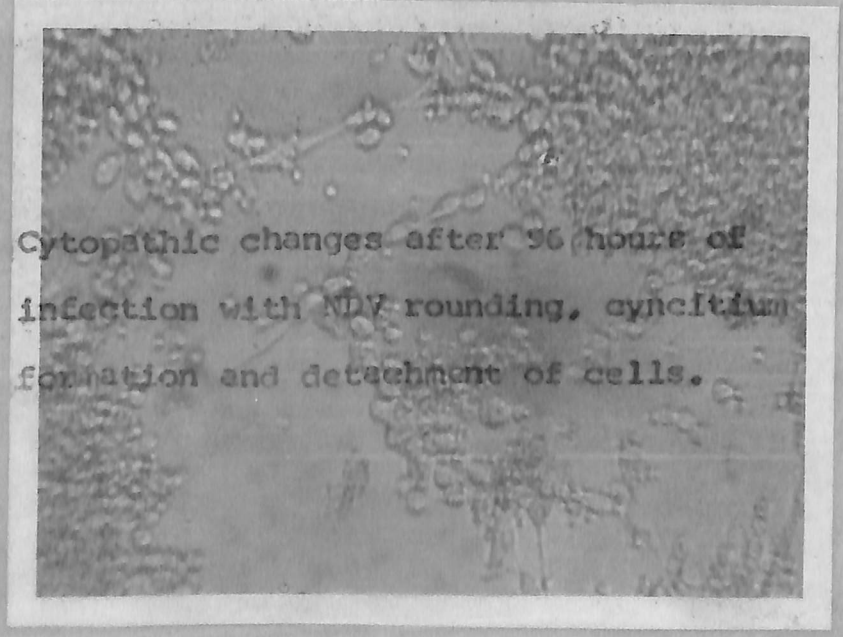
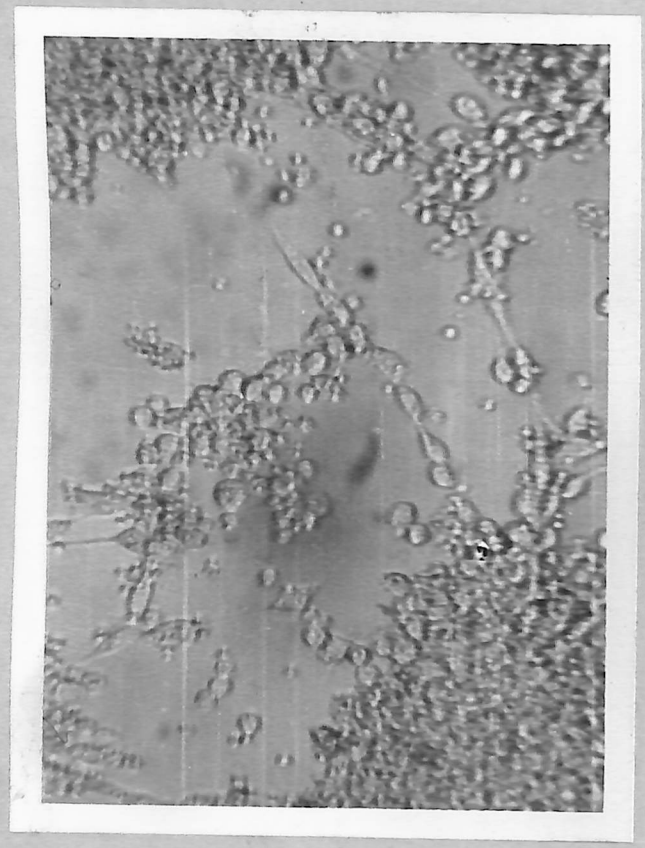


Figure 2. Cytopathic changes after 96 hours of infection with NDV rounding, cyncitium formation and detachment of cells.



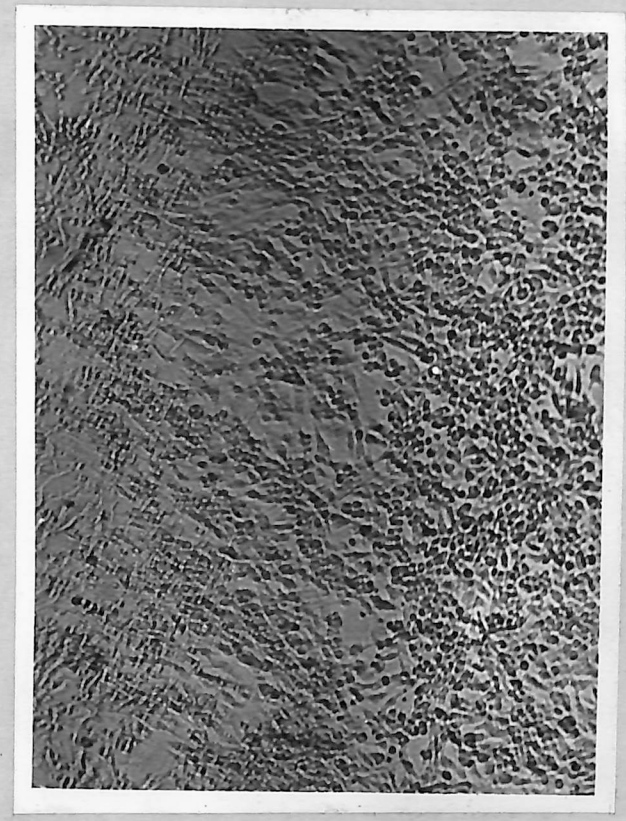


Figure 3. Focal areas of degeneration and necrosis of hepatic cells x 100.

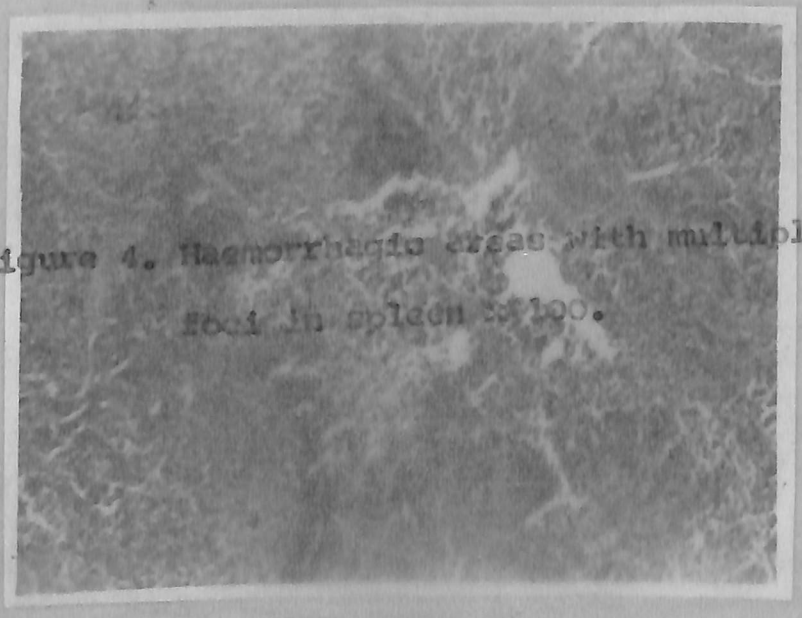


Figure 4. Haemorrhagic areas with multiple necrotic foci in spleen x 100.

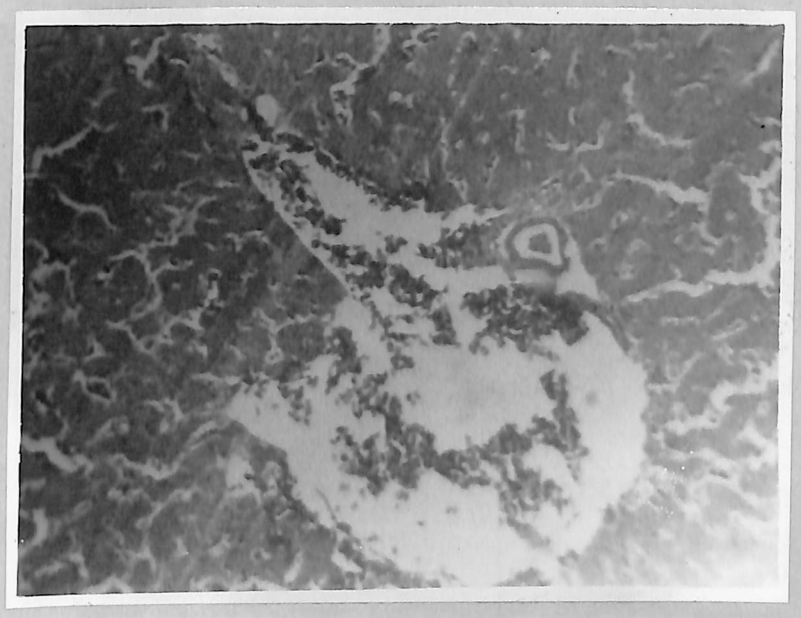


Figure 5. Focal areas of non-suppurative pneumonia in lungs x 100.

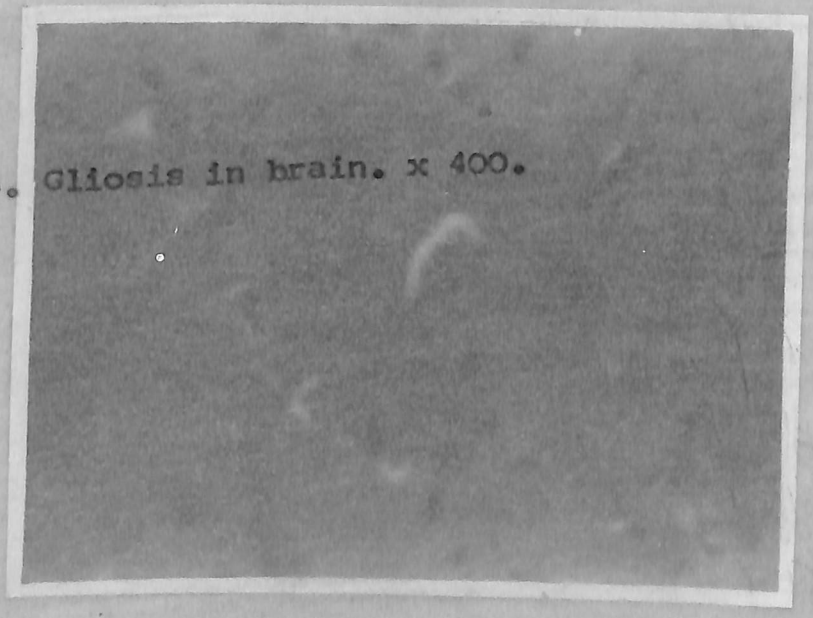
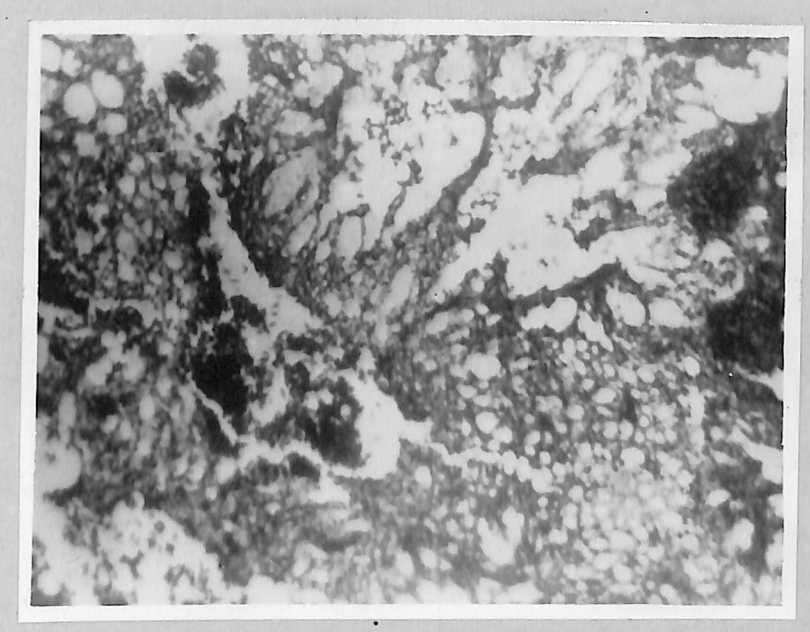
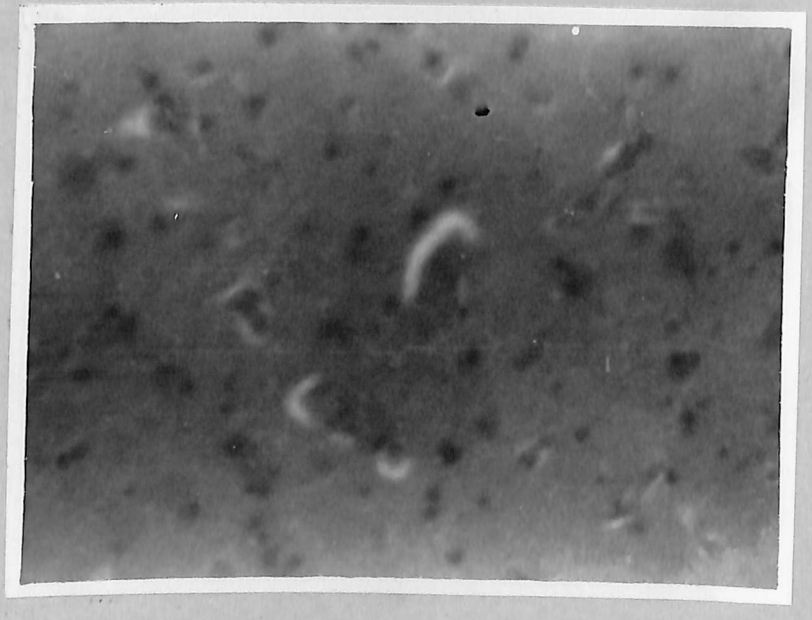


Figure 6. Gliosis in brain. x 400.





DISCUSSION

DISCUSSION

Serological survey

Blood samples collected on Nobuto paper strips from 103 parrots showed that 16.5 per cent of them possessed HI antibodies to ND ranging from 20 to 160. None of the samples collected from Trichur and Calicut Districts showed ND antibodies, while those from Trivandrum and Quilon Districts had a higher percentage of positive cases than those from Palghat District. The serum samples, which gave positive HI titres belonged to parrots kept as pets in households and allowed to mingle freely with domestic poultry and those housed in the Trivandrum Zoo, along with pigeons. This observation suggested that parrots might have picked up infection, either from poultry or from the pigeons. Vaccinated chicken was reported to excrete the virus and this could form a source of infection to susceptible contact birds(Farmer and Romeroy,1950). The possibility of pigeon becoming the source of infection to the parrots could not be ruled out as they were reported to act as inapparent carriers of N V for long periods (Ulbrich and Bolan,1965; Anon,1970; Kulachana,1981). Serological survey of various kinds of wild, domestic and semi-domestic birds showed that

3.9 per cent of the free flying wild birds possessed HI antibodies to ND (Pearson and McCann,1975). They also observed that 92 per cent of these positive cases were psittacine birds and pittas. Erickson,et al.(1977 a) also reported about three per cent of HI positive cases among parrots. The high percentage of positive cases observed in this study (16.5 per cent)might be due to the fact that the serum samples were obtained from birds, which had ready access to domestic poultry or to inapparent carriers of NDV. However, the carrier state of the pigeons in the Trivandrum Zoo was not investigated. Other species of birds such as pigeons, crows and mynas which could be readily infected with NDV (Sulochana,et al.1981) and have easy access to parrots, might also be involved in the infection of parrots.

These reports and the observations made in the present study suggested that parrots could respond serologically to NDV.

Virus isolation

Virus isolation trials made from 70 cloacal and 4 throat swabs were not successful. However, isolation of NDV from the cloacal and throat swabs of infected parrots were reported by Erickson (1977) and Erickson,et al.(1977a).

Zuydam (1952) isolated NDV from the spleen of a sick parakeet, while Scott et al.(1956) isolated a neurotropic strain of the virus from the tissues of a dead grey parrot. While reporting the isolation of a mesogenic strain of NDV from the spleen of an African Grey Parrot, Scott and Hinmill(1960) observed that despite its proven virulence, the spread of the disease through parrots was slow. Allan (1968), Luthgen and Batchendorfer (1970), Metzer and DeMota (1971) and Francis and Revelli (1971) also reported isolations from the tissues of dead parrots. Isolation of NDV from parrots died of acute infection contrasted from other psittacines was reported by Cavill (1974). Allan(1968) reported about the isolation of velogenic strains of NDV from an African Grey parrot and Malabar blue wing parakeet, which were apparently normal. An asymptomatic carrier State in parrots for nearly one year was reported by Erickson(1977), and Walker,et al.(1973). Lentogenic strains of NDV was also isolated from parrots (Paues and Grimes,1978).

It is possible that Indian parrots are highly susceptible to NDV but no carrier state exist in them. They might get infection from a virulent strain and succumb to the disease or the infection might be due to an avirulent strain which produces only a subclinical infection leading to antibody production. Once the parrots attain detectable

titres in the sera, self elimination of the virus might take place. This is in contrast to the report by Erickson, et al.(1977a) who observed that parrots could excrete the virus without any clinical symptoms for a period of one year.

Experimental infection with virulent strain of NDV

Experimental infection of parrots with a virulent strain of NDV by intranasal or intraocular route produced clinical disease manifested by anorexia, diarrhoea and paralysis. Similar observations were also made by Scott,et al.(1956); Allan (1968); Luthgen and Hatchendorfer (1970) and Thrsam,et al.(1975). The duration of illness of 3-6 days noticed in this study was also reported by Scott,et al.(1956). Symptoms following experimental infection of parrot with a velogenic strain of NDV described by Erickson,et al.(1977a)were also noticed during the present investigation except that the tremors of head and neck were not present. They have also observed that the parrots acted as carriers of NDV. The percentage of mortality reported by them was only 30 per cent as against the present observation of 100 per cent mortality in birds that received infection by various routes. The observations of McMill (1974) that the infection was acute in parrots

and died in 24 hours, and the report of Hanson (1973) that many parrots developed fatal infection with BFV from which virus could be readily isolated was in agreement with the present findings. The multiplication of the virus in the infected birds as evidenced by isolations from various tissues indicated that the death was due to establishment of infection and not due to any toxicity.

Virus isolation trials employing the throat and cloacal swabs showed that the parrots infected by intranasal or intraocular routes excreted the virus first through the respiratory tract followed by cloaca. Throat swabs from these infected birds became positive for the virus by day three, while the cloacal swabs only by day five. The early appearance of the virus in the respiratory tract suggested that the virus first settled and multiplied at this site. In contrast, in cases of subcutaneous inoculation virus excretion initiated by the cloacal route.

Reisolation of the virus from the tissues were also possible from all the dead parrots. The percentage of isolations were, spleen, 100 per cent, lung 85.9 per cent, liver 59.1 per cent and brain 28.6 per cent. The isolation of the virus from the spleen of all the infected parrots was reported by Huydam (1957), Scott, et al.(1956) and Hirai,et al.(1979). Erickson,et al.(1977 b) could re-isolate

the virus from 83 per cent of the tissues of infected birds. There was no difference in the initiation and type of symptoms, mortality rate or in the rate of re-isolation of the virus, from parrots infected with undiluted or 1 in 100 dilution of the virulent virus.

Experimental infection with mesogenic(Komorov) strain of NDV

There was a slight prolongation in the incubation period, when the birds were infected with a mesogenic strain. The symptoms manifested were the same, but less pronounced. Mortality was noticed from day five and all the parrots died by day seven. Virus was isolated from the tissues of all the dead birds. This finding suggested that Indian parrots are highly susceptible not only to velogenic strains, but also to mesogenic strains of NDV.

Experimental infection with lentogenic(F)strain of N V

Eventhough symptoms were not evoked by intranasal or intraocular inoculation of a lentogenic strain of NDV (F1), there was multiplication of the virus in these parrots as evidenced by an increase in the HI antibody titre in the sera from day 10 onwards. Virus could be isolated from the throat swabs till the 15th day when they were challenged with the virulent virus. The antibody

titre that ranged from 20-40 in these birds were not sufficient enough to protect them against virulent strains and they succumbed to ND on challenge. In contrast to this observation, Dugue and Retupinan (1976) reported that they could control ND in parrots by vaccination with a lentogenic strain (Lasota). The low antibody titre that was obtained in the present study could probably be due to the low antigenicity of this strain to parrots. Isolation of a lentogenic strain of NDV from normal parrots which was non pathogenic to five week old chicks but caused severe respiratory disease in day old ones was reported by Dawes and Grimes (1978). This suggested that parrots could carry avirulent strains of NDV. The duration of the carrier state could not be assessed in the present study as the birds were challenged on day 15.

Uninfected parrots kept along with infected chicken picked up infection and died by day nine, after showing the symptoms of ND. Virus isolations were also made from the cloacal and throat swabs and from tissues of dead birds. Erickson, et al. (1977) also observed that parrots become infected within two weeks on direct or contact exposure and 30 per cent of them died within 3-5 days.

Though none of the chicken kept in contact with infected parrots showed any symptom of the disease, virus

isolation could be made from them and an increase in antibody titre was also noticed from day twelve. This could be due to a decrease in virulence of the virus by the passage in parrots, or the failure of this strain of virus to produce the infection by contact. As the parrots kept in contact with infected chicken died of ND and the contact chicken excreted the virus, and showed a slight increase in antibody titre, the decrease of virulence of the virus on passage in parrots as described by Erickson,et al.(1977 b) might be the reason for the absence of any clinical symptoms in the contact chicken.

The gross lesions of the infected parrots observed in this study were enlargement of liver and spleen and skullcap haemorrhage. Similar findings were reported by Francis (1973), Cullen,et al.(1974) and Erickson,et al.(1977a) The microscopical lesions like focal areas of degeneration and necrosis of the hepatic cells with isolated foci of haemorrhage, multiple necrotic foci and areas of haemorrhage in the spleen, congestion and focal areas of non-suppurative pneumonia in lungs and gliosis in the brain, were also observed by Cullen,et al.(1974), Erickson,et al.(1977a) and Kitchner and Hirai,(1979). On the other hand, the lesions observed by Francis (1973), Cavill (1974) and Ehrsam (1975) were haemorrhagic enteritis and tracheitis and petechiae and fibrinous deposition in

the air sacs.

Though the cloacal and throat swabs collected from parrots belonging to different parts of the State were negative for the virus, the detection of ND antibodies in 16.5 per cent of the parrots examined suggested that the Indian parrots could be infected with NDV.

Experimental infection with velogenic, mesogenic and lentogenic strains showed that parrots were highly susceptible to the velogenic and merogenic strains of ND and succumbed to the disease within a weeks' time, but were resistant to lentogenic strains. Although the parrots picked up infection with lentogenic strain, they did not produce antibody sufficient enough to protect them from the disease on challenge.

A carrier state that exists in parrots as reported by Erickson (1977) was not a feature of Indian parrots. However, parakeets imported from India were attributed to the disease outbreaks in chicken in Austria(Graungruber, 1972) and Holland (Huydum,1952). This could probably be due to the variation in viral sensitivity of various species of parrots (Utterback and Schwartz (1973). As the infected parrots succumb to the disease in a weeks time, the chances of dissemination of the virus could be prevented by quarantining them for a period not less than ten days.

SUMMARY

SUMMARY

Out of the 103 blood samples collected from parrots belonging to different parts of Kerala, 17 were found to possess ND antibodies with a titre ranging from 20-160. None of the samples collected from Trichur and Calicut Districts showed ND antibodies, while those from Trivandrum and Quilon had a higher percentage of positive cases than those from Palghat. The seventy cloacal and fortytwo throat swabs collected from these birds were found to be negative for the presence of any virus.

The experimental infection of parrots with diluted and undiluted virulent virus by various routes caused death of all the parrots within a week of infection. The clinical symptoms showed by these birds were inappetance wing and leg paralysis, conjunctivitis and diarrhoea. Virus could be isolated from the tissues of dead birds. The percentage of isolation from different tissues were spleen 100 per cent, lung 85.9 per cent, liver 59.1 per cent and brain 28.6 per cent.

The neurogenic strain of the virus (Komarov) was found to produce less severe symptoms, but cent - r cent mortality occured with an extended incubation period of five days. Virus isolation was possible from the cloacal and throat swabs of these birds before death.

Infection of parrots with a lentogenic strain of the virus (F1) neither produced symptoms nor caused deaths,but virus isolation was possible from a few of the parrots in their throat swabs, which lasted from day 10 to 15 of infection. Antibodies to ND were present in their sera from day 12 onwards, which declined gradually. These parrots succumbed on challenge with virulent virus, showing the same clinical symptoms.

Contact infection studies by housing uninfected parrots along with infected chicken, were effective as all the six parrots were dead by the nineth day of exposure to chicks. However housing of uninfected chicks along with parrots infected by various routes, was not effective, in transmitting the disease to chicken, as none of them developed any symptoms, though a low titre of antibodies could be demonstrated in them for a short period. Some chicks also showed virus excretion through the throat and cloacal routes for a few days, but succumbed to the disease when challenged with virulent virus.

Necropsy of dead parrots showed slight enlargement of spleen and liver. Microscopically degeneration and necrosis of the hepatic cells, multiple necrotic foci in the spleen and gliosis of brain were the lesions observed.

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THE ROLE OF PARROTS IN THE EPIZOOTIOLOGY OF NEWCASTLE DISEASE

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the
requirement for the degree

Master of Veterinary Science

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1981

ABSTRACT

The incidence, susceptibility, mode of infection and duration of excretion of the New Castle disease virus in the common Indian parrots (Psittacula Krameri) were studied in detail. The blood, cloacal and throat swabs of parrots, collected from different parts of the State were screened for the presence of ND antibodies and virus. Seventeen out of 103 blood samples were found to possess HI antibodies. The serum samples which gave positive HI titres belonged to parrots kept as pets in households and allowed to mingle freely with domestic poultry and those housed in Trivandrum Zoo along with pigeons. None of the 70 cloacal and 42 throat swabs were positive for the virus.

Experimental infection of parrots with undiluted virulent virus by the intranasal and intraocular routes and by the subcutaneous and intranasal routes with 1:100 dilution of the same virus gave almost the same results. All of them died within a weeks' time, after showing symptoms of inappettance, leg and wing paralysis and diarrhoea, from day two of infection. Virus could be isolated from the throat and cloacal swabs and also from the tissues of dead birds.

Chicks kept along with these infected parrots did not develop symptoms of ND, eventhough they excreted the virus, for a few days, and had a low titre of HI antibodies in their

sera. All the contact chicks died of ND with typical symptoms and lesions on challenge with the virulent virus. The parrots that received a mesogenic strain (Komorov) of the virus, also succumbed to the disease, but with less pronounced symptoms and with an extended incubation period.

The parrots that were infected with lentogenic strain of the virus (F1) did not develop symptoms of the disease. However multiplication of the virus occured in these birds as isolations could be made from cloacal and throat swabs and a slight increase in HI titre was noticed in the sera. However on challenge with a virulent strain of NDV, they showed symptoms of ND. All of them died within eight days and the virus could be isolated from them.

Contact infection of parrots from infected chicks were quite effective, as the parrots died with the same symptoms described above, almost within the same time as direct infection. Virus was also isolated from the tissues of the dead parrots.

The common Indian parrots were found to be highly susceptible to both velogenic and mesogenic strains of NDV, but they were resistant to the lentogenic strain. Uninfected chicks kept along with the parrots infected with virulent virus picked up the infection, and virus could be isolated from the cloacal and throat swabs of these chicks. They also showed an increase in the antibody titre. The failure to

produce clinical disease in chicken might be attributed to a decrease in virulence of the virus on passage in parrots. The carrier state with the lentogenic strain of the virus could not be assessed as they were challenged after 2 weeks.

Though a carrier role had been attributed to the parakeets imported from India, the parrots in this study were found to succumb to the disease within a week. This might be due to the variation in the susceptibility of various species of parrots to NDV. The chances of dissemination of the virus could be prevented by quarantining them for a period, not less than ten days.