SEROPREVALENCE AND PATHOGENICITY OF EGG DROP SYNDROME' 1976 VIRUS IN CHICKENS

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

(FACULTY OF VETERINARY SCIENCE)

PUSA (SAMASTIPUR)

BY

Jitendra Kumar Singh

In partial fulfilment of the requirements

FOR THE DEGREE OF

Master of Veterinary Science

(MICROBIOLOGY)

ST GRADUATE DEPARTMENT OF VETERINARY MICROBIOLOGY

BIHAR VETERINARY COLLEGE

PATNA-800014

1993

STUDIES ON MICROBIOTHERAPY OF RUMINAL METABOLIC DISORDERS IN BOVINE

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DEDICATED

TO

MY FATHER

SHRI RAJESHWAR PRASAD SINGH

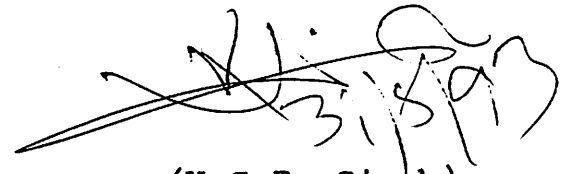
Dr. K. C. P. Singh
Assistant Professor

Department of Veterinary Microbiology
Rajendra Agricultural University
Bihar Veterinary College, Patna-14

CERTIFICATE - I

This is to certify that the thesis entitled **"SEROPREVALENCE AND PATHOGENICITY OF EGG DROP SYNDROME'1976 VIRUS IN CHICKENS"**, submitted in partial fulfilment of the requirements for the degree of **"Master of Veterinary Science (Microbiology)"** of the Faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar, is the record of bonafide research carried out by **Dr. Jitendra Kumar Singh** under my supervision and guidance. No part of the thesis has been submitted for any other Degree or Diploma.

It is further certified that such help or informations received during the course of this investigation and preparation of the thesis have been duly acknowledged.



(K.C.P. Singh)

CERTIFICATE - II

We, the undersigned, members of the Advisory Committee of **Dr. Jitendra Kumar Singh** a candidate for the degree of **Master of Veterinary Science** with major in **Microbiology** have gone through the manuscript of the thesis and agree that the thesis entitled **"SEROPREVALENCE AND PATHOGENICITY OF EGG DROP SYNDROME' 1976 VIRUS IN CHICKENS"** may be submitted by **Dr. Jitendra Kumar Singh** in partial fulfilment of the requirements for the Degree.

(K.C.P. Singh)
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1. Dr. C.B. Prasad
2. Dr. Md. Murtuza
3. Dr. L.N. Prasad 31-5-93
4. Dr. Basant Kumar Sinha

CERTIFICATE - III

This is to certify that the thesis entitled **"SEROPREVALENCE AND PATHOGENICITY OF EGG DROP SYNDROME'1976 VIRUS IN CHICKENS"**, submitted in partial fulfilment of the requirements for the degree of **Master of Veterinary Science (Microbiology)** of the Faculty of Post-Graduate Studies, Rajendra Agricultural University, Bihar was examined and approved on ..... 1993.

29/7/93

(K.C.P. Singh)
CHAIRMAN
ADVISORY COMMITTEE

R. G. Dhawedkar 20.7.93
(R. G. Dhawedkar)
EXTERNAL EXAMINER.

MEMBERS OF THE ADVISORY COMMITTEE :

1. Dr. C.B. Prasad
2. Dr. Md. Murtuza
3. Dr. L.N. Prasad 20.7.93
4. Dr. Basant Kumar Sinha 29/7/93

Dean, Post-Graduate Studies

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Dean, Post-Graduate Studies

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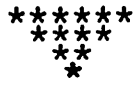
Jitendra Kumar Singh.

(Jitendra Kumar Singh)

Patna

/ / 1993.

C O N T E N T S



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LIST OF ABBREVIATIONS

A/F	Allantoic fluid
Amer.	American
ANOVA	Analysis of variance
Biol.	Biological
CELO	Chicken embryo lethal orphan
Clin.	Clinical
Co.	Company
Contd.	Continued
°C	degree centigrade
DNA	Deoxyribonucleic acid
Dr.	Doctor
Ed.(s)	Editor(s)
edn.	Edition
EDS	Egg Drop Syndrome
EDS'76	Egg Drop Syndrome'1976
Fig.	Figure(s)
g	gram
HA	Haemagglutination
H and E	Haematoxylin and Eosin
HI	Haemagglutination Inhibition
IB	Infectious Bronchitis
Ind.	Indian
I/O	Intraocularly
Inst.	Institute
J.	Journal
lb.	pound
Med.	Medical
mm	millimetre
MP (M.P.)	Madhya Pradesh
M/S	Messors
M.V.Sc.	Master of Veterinary Science
Natl.	National
No.	Number
no.	number

p.	page
Pathol.	Pathology
PBS	Phosphate Buffer Saline
Ph.D.	Doctor of Philosophy
PI	Post inoculation
Poult.	Poultry
pp.	pages
Pvt.	Private
Q.	Quarterly
RBC	Red Blood Cell
Rec.	Record
Rev.	Review
RIR	Rhode Island Red
Sc.	Science
Sci.	Science
SE	Standard Errors
SGOT.	Serum glutamate oxaloacetate transaminase
SGPT	Serum glutamate pyruvate transaminase
Sl.	Serial
SLE	Shell-less eggs
Tierarzt.	Tierarztliche
VB	Veterinary Bulletin
Vet.	Veterinary
viz.	(videlicet) namely
VN	Virus neutralization
WLH	White Leghorn

*

CHAPTER - I

INTRODUCTION

INTRODUCTION

The drop in egg production in laying hens is considered to be of multiple etiological origins of both infectious and non-infectious nature. Amongst the infectious diseases affecting egg production, Egg Drop Syndrome '1976 (EDS'76) virus is considered as the most serious threat to poultry industry world-over. Since the report by Van Eck et al. (1976) the disease has been reported from several parts of the world. This disease is caused by haemagglutinating double stranded DNA virus of the genus Aviadenovirus and is characterized by sudden drop in egg production, laying of thin shell, soft shell, shell-less, crack, misshapen, small size and discoloured eggs. In India, the occurrence of this virus has been confirmed in several states like Karnataka, Andhra Pradesh, Punjab and Madhya Pradesh (Sukumar and Babu, 1986; Shakya and Dhawedkar, 1991). However, there appears to be no report available on the prevalence of EDS'76 in Bihar.

The clinical signs associated with this viral infection are limited to drop in egg production and laying of abnormal eggs. However, several factors, such as type and nature of the virus strain and the virus titre in the inoculum on the one hand, and age, breed and susceptibility of birds on the other may affect the clinical manifestations of this virus in natural host. This necessitates frequent monitoring of the virus in respect of its effect on egg production and production of egg abnormalities in laying hens.

There are varying reports on production of gross and histopathological changes in various organs including ovary and oviduct of chickens. It has also been postulated that the virus produces marked histopathological changes in the uterus, as characterized by degeneration and desquamation of mucosal epithelial cells and atrophy of uterine glands which are largely responsible for causation of egg shell aberration in the EDS'76 virus infected laying hens Taniguchi et al., 1981). Further studies on the pathological changes produced by the virus in the reproductive tract of susceptible birds may be helpful in arriving at logical conclusion. Besides, studies on the pathogenicity of EDS'76 virus in laying hens may also be considered necessary to define the precise role of the virus.

Amongst the abnormalities observed in eggs laid by EDS'76 virus infected hens, shell formation disorders, as characterized by thin shell, soft shell, or shell-less eggs are of great economic importance. The egg shell mainly consists of calcium carbonate.Besides calcium and carbonate , the blood acid base balance is also important in shell formation. The blood acid base balance is maintained by electrolytes, amino acids and proteins. Essential electrolytes in this respect include calcium, magnesium, chloride,sodium , potassium and phosphate. The concentration of glucose as well as the activity of enzymes, alkaline phosphatase and transaminases also have significant bearing on egg shell

formation. Alkaline phosphatase plays a role in calcium phosphorus metabolism. The liver is main source of egg yolk lipoproteins and phosvitins and the enzyme, transaminase activity. Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) are indicative of liver cell damage in laying hens (Ibrahim et al., 1980). However, there is scanty literature on effect of EDS'76 virus on serum biochemical profile of laying hens (Van Eck and Vertommen, 1984). Accordingly, it would be pertinent to ascertain serum biochemical profiles of laying hens with particular reference to the constituents having role in egg shell formation.

Therefore, the present work has been undertaken with the following objectives :

1. To study the seroprevalence of Egg Drop Syndrome'1976 virus in different poultry farms in Bihar.

2. To study the effect of EDS'76 virus on egg production and egg quality in chickens.

3. To study the pathological changes in the female genital tracts of EDS'76 virus infected chickens.

4. To study the serum biochemical profile of EDS'76 virus infected chickens.

CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

McCraken and McFerran (1978) recorded changes following oral infection of fowl with haemagglutinating adenovirus. The first changes were observed 7 days after inoculation when loss of egg shell pigmentation occurred. Colour patterns did not return to normal until 25 days after inoculation. Thining of egg shells was first seen one day after the loss of pigmentation, and by 3 days after inoculation soft shelled and shell-less eggs were laid. From 13 to 16 days after inoculation 25-40 per cent of the eggs laid were either shell-less, soft shelled or very thin shelled. Most of the birds were laying normal eggs by 24 days post inoculation. In spite of the marked egg shell changes, the overall daily egg production remained at approximately 80 per cent during the 17 weeks experimental period.

McFerran et al. (1978) described the etiology and characteristic feature of depressed egg production syndrome, now called Egg Drop Syndrome'1976 (EDS'76) in Northern Ireland. This syndrome is caused by a haemagglutinating adenovirus and its characteristic feature included sudden drop in egg production and production of misshapen, discoloured, shell-less or soft shell eggs. They isolated the causative virus from the oviduct, upper respiratory tract and the faeces of the affected birds.

Meulemans et al. (1978) undertook seroepidemiological survey of laying poultry flocks in Belgium. Eleven of the 17

flocks (65%) showed a drop in egg production by 3-32 per cent and had antibodies against 127 strain of EDS'76 virus. These birds produced more than 2-30 per cent soft shelled or shell-less eggs. Antibodies against 127 strain were not detected in flocks with normal egg production. Role of infectious bronchitis (IB) virus in causing drop in egg production was also emphasized.

Bennejean et al. (1979) isolated EDS'76 virus from the laying hens showing drop in the number of eggs and deterioration of shell quality including shell-less eggs. The postmortem findings revealed only discrete lymphoid infiltration of the uterus. Experimental reproduction of the disease was also reported.

Fehervari et al. (1979) reported studies on egg drop syndrome in a large laying flock in Hungary. The condition was characterized by diarrhoea at the start of laying period, followed by decreased egg production and formation of incompletely calcified deformed eggs. There was no gross lesions. Histologically, there was an inflammatory reaction in the propria of the uterus which commenced with oedema and degeneration of the calcium secreting glands, followed by mononuclear cell infiltration and the formation of lymphoid follicles. Electron microscopic studies of the content of small intestine and caecum revealed adenovirus that was identical morphologically and in haemagglutinating activity to BC 14 strain of EDS'76.

Meulemans et al. (1979) reported the seroprevalence of EDS'76 virus in 7 weeks old broilers. However, they failed to detect any correlation between the presence of antibodies to EDS'76 virus and a specific disease. The role of infected broiler flocks in dessimination of infective agent to laying chickens was not ruled out.

Darbyshire and Peters (1980) conducted studies on EDS'76 virus (strain D 61) in laying chickens. Chickens of different ages were infected orally. All birds produced eggs with aberrant shells in varying numbers but internal quality of the eggs was not affected. There were no other constitutional defects and no obvious clinical signs throughout the period of this study. Fertility and hatchability remained unaffected. The total daily egg production of 19 weeks old laying chickens was 15 to 20 per cent lower than that of uninfected controls of the same age. The total daily egg production of older chickens was unaltered except for approximately 2 weeks after infection at 26 weeks of age and for 5 weeks at 47 weeks of age. The highest number of eggs with abnormal shells was laid by chickens infected at 33 weeks of age (upto 38%) or 47 weeks of age (upto 40%). Infective virus was recovered from the livers of chicks immediately after hatching from eggs laid by hens 7 to 11 days after infection. Neutralizing HI and precipitating antibodies to EDS'76 virus developed within 6 days of infection and were associated with IgG

immunoglobulins. Chicks hatched from eggs of infected hens possessed maternal antibodies with a half life of 3 days. The maternal antibodies conferred a passive immunity to challenge for approximately first 4 weeks of life.

Kaleta et al. (1980) reported HI antibodies to EDS'76 virus in 68 of 232 samples from domestic ducks, 15 of 33 wild ducks and 47 of 161 domestic geese.However, pigeons, pheasants, and quail were negative for antibodies to EDS'76 virus.

Macpherson (1980) studied 213 broiler breeder flocks and found the prevalence of antibodies to EDS'76 virus in only 51 flocks by HI test.

Malkinson and Weisman (1980) reported the prevalence of antibodies to EDS'76 virus in domesticated and wild birds. They found HI antibodies to EDS'76 virus in 8 of 316 fowl serum samples from 13 flocks. Seropositive reactors were also detected in Pekin and Muscory duck flocks that were in direct or indirect contact with Pekin ducks. However, no seropositive reactors could be detected in wild palm doves, water fowl, turkeys and goose belonging to the Zoological department.

Van Eck (1980) demonstrated vertical transmission of egg drop syndrome'1976 virus in fowl. Eggs of infected hens were incubated weekly after disinfection with formalin gas. These eggs were homogenized after 18 days of incubation and fed to adult hens housed in isolators. These

birds became positive to antibodies against EDS'76 virus suggesting egg transmission of the virus. It was further suggested that egg transmission occurred as a result of viraemia.

Zanella et al. (1980) conducted studies on the etiopathology and epidemiology of EDS'76 virus in Italy. They isolated a viral strain E-77 from cloacal swab of hens affected with depressed egg production in chick embryo liver cells. This virus strain was indistinguishable from EDS'76 virus strain (127, BC 14 and 3877) and was stable over wide range of pH, resistant to ether, chloroform and temperature of 50°C. The virus produced cytopathogenic effect (CPE) in chick embryo liver cells and duck embryo fibroblast. It killed duck embryos in 7-10 days. The virus could be recovered from the intestine and bursa fabricii following experimental infection of day old chicks with EDS'76 virus through intraocular or intracloacal route.

Cook and Darbyshire (1981) demonstrated loss of egg weight and change in internal egg quality following experimental infection of day old chicks. They did not find any effect of experimental infection of day old chicks on egg production as well as egg shell quality. However, lateral transmission of the virus was demonstrated throughout the rearing period in such experimentally infected chickens.

Firth et al. (1981) described an outbreak of egg drop syndrome in broiler poultry and reported the prevalence of antibodies to 127 strain of EDS'76 virus in 102 of 106 serum samples by HI test. The isolation of the virus was also reported from the affected flock. The disease was characterized by delayed onset of laying, a lower peak in egg production and drop in egg production shortly after reaching peak production.

Lohr et al. (1981) undertook studies on distribution of EDS'76 virus antibodies in various flocks of fowls, ducks, geese and gulls by HI test. HI antibodies were found in only one flock of fowl, 7 goose flocks and 4 duck flocks. All gulls were negative.

Ng et al. (1981) conducted a serological survey of 30 adult poultry flocks on 18 farms and found that 25 flocks on 15 farms had haemagglutination inhibition antibodies to aviadenovirus strain EDS'76. The clinical disease syndrome was either a failure to attain peak egg production,or laying eggs with loss in shell colour and soft-shelled and shell-less eggs with or without a fall in egg production around a peak of 20-50 per cent. The depression in production lasted usually about 10 weeks. Field trials conducted with two commercial oil adjuvant vaccines showed that they were effective in protecting against natural exposure.

Redmann et al. (1981) confirmed the prevalence of EDS'76 virus antibody in 14 commercial laying flocks by

HI test. They reported horizontal spread of the virus and no resistance developed with age.

Taniguchi et al. (1981) conducted studies on pathological changes in Rhode Island Red laying hens inoculated with JPA-1 strain of EDS'76 virus and examined pathologically 1, 3, 5, 7, 10, 14, 21, 28 and 80 days post inoculation. Eggs with a discoloured or soft shell or no shell were laid over a period from 10-24 days PI. Egg production failed transiently in some affected birds after 13 days PI. At autopsy, the uterus revealed remarkable oedema and swelling of the mucosal folds together with the deposition of whitish exudate 14 days PI. Microscopically, intranuclear inclusion bodies were found in the epithelial cells of the uterus, isthmus and vaginal gland region 10 and 14 days PI. There were also severe degeneration and desquamation of the epithelial cells, atrophy of the uterine glands and remarkable infiltration of heterophils, lymphocytes and plasmacytes accompanied with extensive oedema. Twenty one days after inoculation, lymphoid follicles were formed in the mucosal fold of some part of the oviduct. Electron microscopic examination revealed various amounts of virus particles mainly in the nuclei of the epithelial cells on the mucosa and in the exudate, and also in the cytoplasm of macrophages in the uterus.

Van Eck et al. (1981) conducted serological examination of flocks of fowl during the year 1980 and did not

detect HI antibodies to EDS'76 virus in adult broiler and laying flocks. The sharp decrease in the incidence of EDS'76 virus infection was probably due to large scale vaccination in 1978 and 1979.

Yamaguchi et al. (1981a) reported the prevalence of EDS'76 virus in 14 broiler breeding flocks in 2 farms in Japan. The haemagglutinating adenovirus indistinguishable from BC 14 strain of EDS'76 virus was isolated from cloacal swabs and uterus of laying hens. The virus was stable against organic solvents and at pH 3.0. The infectivity of the virus remained unaffected at 50°C and was completely destroyed at 60°C. Egg production fell suddenly when the hens were 30 to 55 weeks of age and depression lasted 3 to 7 weeks. The fall in egg production ranged from 6 to 25 per cent. Depressed egg production was accompanied by laying of shell-less, soft-shelled and thin-shelled eggs associated with loss of egg-shell pigment. All 57 serum samples collected after onset of the fall in egg production had HI antibody to EDS'76 virus and the titre ranged from 1:16 to 1:512. However, the serum samples collected before the onset of fall in egg production were negative for HI antibody.

Yamaguchi et al. (1981b) studied the pathogenicity of JPA-1 strain of EDS'76 virus in laying hens. Rhode Island Red laying hens that lacked HI antibody were inoculated orally with this virus and infected birds were observed for 80 days. Inoculated hens laid abnormal eggs

such as shell-less, soft-shell and cracked eggs and those with loss of pigmentation from 8 days PI. Fifteen out of 16 hens laid abnormal eggs. The egg production rate fell from 94 to 50 per cent between 13 and 16 days PI.When the abnormal eggs were excluded, egg production was 17 per cent only. These birds recovered slowly reaching 67 per cent production by the end of experiment.

Zsak and Kisary (1981) demonstrated the biological and physicochemical properties of the Hungarian strain of EDS'76 virus (B8/78). This strain was propagated in allantoic cavity of embryonated duck eggs and in chick embryo liver cell cultures. The virus could equally be propagated in cell cultures of fowl and goose origins. Viral replication was characterized by intranuclear inclusion bodies and by the immunofluorescent positivity of the affected cells. The thermal and pH sensitivity of the virus was comparable to those described for FAV-1 (CELO virus).

Al-Hilly et al. (1982) conducted studies on the prevalence of antibodies to EDS'76 virus in poultry farms in Iraq by employing HI test using EDS'76 virus strain 127 antigen. Out of 2444 serum sample from 13 laying and breeding flocks 29.3 per cent were positive for H I antibodies to EDS'76 virus. No positive reactors were present in two flocks. In the remainder flocks seropositivity ranged from 9 to 45 per cent. HI antibody titres were ranging between 1:32 and 1:256.

Bartha et al. (1982) reported that EDS'76 virus existed in the poultry population even before 1975. They demonstrated HI antibodies in sera from domestic duck(60%), wild ducks (59%), geese (70%) and herring gulls (20%). They suggested that it is primarily a duck adenovirus.

Gylstorff and Rolf (1982) experimentally infected 60 laying hens with 127 strain of EDS'76 virus by oral route and studied the histopathological changes for 29 days post infection. The histopathological changes revealed inflammatory infiltration with lymphocytes, small amounts of heterophils, granulocytes, plasma cells and macrophages. The inflammation was most evident in the uterus followed by infundibulum and vagina. There was almost no reaction in magnum and isthmus. Atrophy of the surfaces and glandular epithelium in the uterus was observed and sometimes glandular cells were even degenerated. In all parts of the oviduct, oedema and follicles of lymphocytes were present in variable frequency. The first lesions were seen 5 days after inoculation and inflammation reached its maximum between 11th and 13th day. Inflammation did not entirely disappear within 29 days.

Heffels et al. (1982) reported absence of any clinical signs or gross lesions in susceptible chicks of various ages as well as in adult cocks experimentally infected with 127 strain of EDS'76 virus. However, virus persisted in internal organs and the rate of excretion of

viruses by infected chickens declined rapidly with age. The virus was detected in the organs of chicken upto 5 weeks after infection and in faeces upto 2 weeks. In adult birds the virus persisted in tissues for about 3 weeks and was excreted with faeces for only about a week after infection.

Kaleta et al. (1982) reported the relative efficacy of HI test and virus neutralization (VN) test for detection of antibodies to EDS'76 virus in wild birds. The VN test was found to be more sensitive than HI test.

Loupal et al. (1982) conducted studies on effects of 127 strain of EDS'76 virus in experimentally infected laying hens through oral route. The drop in egg production ranged from 10.7 to 13 per cent. The infected hens laid smaller, shell-less and shape-less eggs. Antibodies to EDS'76 virus was first detected on 7th day post inoculation and all the infected hens developed antibody.

Picault et al. (1982) studied the susceptibility of 6 commercial varieties of laying hens to EDS'76 virus and reported white Leghorn (WLH) more resistant to the virus than four lines of Rhode Island Red (RIR) as determined by the proportion of soft shelled eggs laid after infection.

Rhee et al. (1982) reported the prevalence of antibodies to EDS'76 virus (strain BC 14) and found 14.1 per cent positive reactors in breeding poultry farms and 78 per cent positivity in commercial laying flocks in

Korea. The syndrome was characterized by watery diarrhoea, depigmented egg shells and production of cracked, soft shelled and shell-less eggs. They reported average drop in egg production of 21 per cent and recovery to normal level took 4 to 6 weeks.

Van Eck (1982) demonstrated the vertical transmission of strain BC 14 of EDS'76 virus by the presence of HI antibodies in chicks. These chicks were hatched out of the eggs laid by hens already infected with BC 14 strain of EDS'76 conjunctively. The egg production of the birds derived from infected hens was subnormal between 28 and 38 weeks of age. Most serologically positive birds resisted challenge infection with strain BC 14 virus. Chicks hatched from the eggs of hens infected 3-4 weeks earlier possessed maternal antibodies but the fertility and hatchability of eggs having normal shells were not impaired.

Zsak et al. (1982) studied the effects of experimental EDS'76 virus (strain B8/78) infection in young and laying geese. Of the 19 large flocks of geese 15 were serologically positive and 65 to 100 per cent of serum samples collected from each flocks were positive with HI titres ranging from 1:8 and 1:256. All the progeny of the geese with HI antibodies had maternally derived antibodies. Oral infection of 8, 19, and 29 goslings with B8/78 strain of EDS'76 virus failed to produce any clinical sign of the disease and there was no mortality. The infected birds

developed antibody and shed the virus in faeces. No histopathological lesions were noted. There was no effect on egg production as well as in internal egg quality. They suggested that when investigating the origins of EDS'76 virus infection not only duck but also geese should be considerd as a potential source of infection.

Githkopoulos et al. (1983) reported antibodies in titres above 1:8 to aviadenovirus strain EDS'76 in 72 of 465 (15.5%) serum samples. Positive results were obtained from 12 of 106 (11.3%) layer flocks and 1 of 4 (25%) duck flocks but none of 21 broiler and 4 turkey flocks.

Van Eck et al. (1983) did not observe any clinical signs of the disease following experimental infection of 33 weeks old layers fowl with EDS'76 virus. There was drop in production of normal shell eggs from 87 to 49 per cent within 3 weeks. However, total eggs production, weight of normal shell eggs and internal egg quality were not affected. There was breed difference in abnormal shelled egg problem following infection and vaccination or virus infection of birds at 17 weeks of age was successful in preventing adverse effects from subsequent challenge. The adverse effects of EDS'76 virus in chickens were more severe when the birds were concurrently infected with avian infectious bronchitis (IB) virus.

Mohanty et al. (1984) conducted a serological survey of EDS'76 virus in different parts of the country over

a period of 4 years. Twenty three of 25 flocks (92%) had HI antibodies to aviadenovirus strain 127. The HI antibodies titres ranged from 1:2 to 1:64 in different flocks tested and the incidence varied from 9.37 to 100 per cent. Of the 25 flocks, 19 experienced drop in egg production. The production losses in these farms ranged from 22 to 64 per cent. In most of the flocks, the drop commenced soon after peak production and the trend continued for 3 to 10 weeks. Following recovery the egg production remained at a lower level than the previous peak. On analysing the egg production records, it was observed that the trend of increase in production after the drop had close similarity with the rise of egg production before peak production.

Rozhdestvenskii (1984) studied the effect of various inactivating agents on EDS'76 virus (strain B8/78). It was observed that aviadenovirus strain B8/78 propagated in duck embryos was inactivated by exposure to 60-70°C for 10-40 min., by 0.05-1.0 per cent formaldehyde solution for 24-48 hours or by ethyleneimine (0.01-0.3%) for 3-72 hours at 37°C. Exposure to 0.2 per cent ethyleneimine for 3 hours was the best method of inactivation to preserve intact the haemagglutinating activity of the virus.

Van Eck and Vertommen (1984) reported biochemical changes in blood and uterine fluid of hens following experimental EDS'76 virus infection through conjunctival route. There was no change in the pH of oviduct mucosa in comparison with infected hens. There was an increase in

sodium and decrease in phosphorus, potassium, calcium, magnesium and glucose in oviduct fluid from hen laying soft-shell and shell-less eggs. It was concluded that functional disorder responsible for abnormal eggs took place in superficial epithelial cells of the oviduct, and were probably initiated by abnormal sodium exchange.

Bartha and Meszaros (1985) infected the laying hens by oral route with strain KT/80 of EDS'76 virus isolated from ducks showing symptoms of egg drop syndrome. They observed that the effect produced by the virus was similar to that of strain 127 of EDS'76 virus.

Gupta et al. (1985) in an attempt to study the seroprevalence of "Egg Drop Syndrome'76" virus in Haryana studied a total of 508 serum samples of poultry by employing haemagglutination inhibition test. The overall incidence of the disease was reported to be 30.9 per cent with antibody titres ranging from 1:4 to 1:64. The per cent prevalence of EDS'76 virus positive reactors was 43.4 per cent in broiler breeding females, 35.5 per cent broiler breeding males, 33.9 per cent in commercial broilers, 26.3 per cent in breeding layers and 23.0 per cent in commercial layers.

Lu et al. (1985) isolated haemagglutinating adenovirus from cloacal swabs and faeces of laying hens from outbreaks of EDS'76 virus in Tiwan. The birds between 24 to 41 weeks of age showed sudden drop in egg production and the depression lasted 4 to 12 weeks. The production was reduced by 6-25 per cent. Depressed egg production was accompanied by laying of

shell-less, soft-shelled and thin-shelled eggs associated with loss of egg shell pigment. The virus isolate was indistinguishable from JPA-1 strain of EDS'76 virus.

Adair et al. (1986) reported the comparative efficacy of HI, ELISA, serum neutralization test, fluorescent antibody technique and Agar gel precipitation test for detection of EDS'76 virus antibodies and found that only HI or serum neutralization test should be used for seroepidemiological studies of EDS'76 virus.

Borzemska et al. (1986) reported the prevalence of HI antibodies to EDS'76 virus in 72 to 99 per cent of laying hens. Incubation of 120 eggs from hens experimentally infected with 127 strain of EDS'76 virus yielded 20 per cent of chicks having micropthalmia and 11-59 per cent of chicks having various abnormalities of yolk sac, liver, kidneys and heart. In flocks naturally infected with the virus, hatchability was reduced by 4 per cent.

Marcial et al. (1986) reported the presence of HI antibodies to EDS'76 virus in commercial poultry breeding flocks. Only 2 of the flocks found positive for antibodies to EDS'76 virus had egg drop syndrome.

Sukumar and Babu (1986) in an attempt to study the egg drop syndrome in chicken between 18-36 weeks of age detected HI antibodies to EDS'76 virus strain 127 in 51 (6.53%) of 781 serum samples from various parts of Andhra Pradesh. The HI titres of the antibodies ranged from 0.6 - 1.8/0.05 ml.

Friederichs et al. (1987) studied the effects of EDS'76 virus in day old chicks and 56 weeks old laying hens. The virus showed an affinity for the reproductive organs and induced acute tissue lesions in the oviduct of adult hens. Infection of day old chicks resulted in the persistence of virus during the rearing period. At point of lay virus replication increased again resulting in typical lesions in the oviduct.

Higashihara et al. (1987) studied the experimental infection in laying chickens with EDS'76 virus (strain H 162). Depressed and abnormal egg production was observed for 3 days or longer in 17 of 18 brown layers (BC strain) hen and 10 of 17 white layers (WL) hens. The abnormal egg production began 8.8 and 12.2 days after infection of brown layer and white layer hens respectively. Abnormal egg production was much less frequent in the WL hens than in the BC hens. Abnormal eggs included shell-less, soft shelled, thin shelled and discoloured eggs. No eggs of abnormal internal quality or shape were observed. Egg production was depressed in the WL hens but only little depression was observed in BC hens.

Moorthy et al. (1987) conducted an experimental study in chicken using 127 strain of EDS'76 virus. Histopathological, haematological and biochemical studies were made in chicks and growers for 3-15 weeks of age. The outstanding histopathological changes were haemorrhages and lymphoid aggregates in the lung, liver, kidney and proventriculus. The lesions in the reproductive tracts were not significant except

for mild fibrosis and atrophy in the oviduct and occasional haemorrhages in the ovary. Among the haematological and biochemical parameters, the serum inorganic phosphorus level of the infect chicks were significantly lower than those of control chicks ($P < 0.01$). Values of other parameters like erythrocytes count, the packed cell volume, leucocyte count and serum calcium were not significantly different from those of controls.

Reddy and Raghavan (1987) conducted studies into the incidence of EDS'76 virus in poultry and duck flocks. Out of 770 serum samples from 16 flocks of WLH chickens experiencing drop in egg production, an overall incidence of 27.3 per cent was recorded. Birds between 44-60 weeks of age showed the highest incidence of EDS'76 virus antibodies compared to 20,28,35 and 36 weeks old birds. A similar study conducted in 5 flocks of ducks reared in a breeding farm revealed an overall incidence of 37.6 per cent with titre range of 1.0-2.5. The viral antibodies were present in ducks of different age groups from 20-52 weeks.

Sharma (1987) studied the seroprevalence of EDS'76 viruses by employing HI test in various duck and poultry flocks in and around Jabalpur. Out of 745 serum samples tested from layers and broilers, 128 (17.2%) were found positive for HI antibodies to EDS'76 virus. The broiler had higher (25.2%) prevalence rate than layers (10.9%). Among different age groups of birds maximum positive reactors (23.3%) were in layers of 33 weeks of age and (46%) among broilers of 9 week of age group. He

also reported 37 (31.6%) of 117 duck eggs positive for egg yolk antibodies to EDS'76 virus.

Fomina et al. (1988) reported application of immunoenzyme test for detection of antibodies to aviadenovirus strain EDS'76. They demonstrated the greater sensitivity of immunoenzyme assay by comparing results for 30 serum samples with those of the HI test.

Szeleszczuk (1988) studied the effects of EDS'76 virus on egg shells of 120 eggs produced by Rhode Island Red hen in the second week of clinical EDS'76. Twenty per cent showed moderate and 41.6 per cent severe changes in the shell, while 29.4 per cent had a normal appearance. Chemical analysis of egg shells showed the levels of calcium, magnesium, sodium, iron, copper and zinc significantly lower and potassium significantly higher than the egg shells from healthy controls.

Holmes et al. (1989) vaccinated a group of 18 weeks old commercial pullets intramuscularly with a trivalent vaccine containing inactivated EDS'76 virus (strain 127). Newcastle disease virus and infectious bronchitis virus. It was observed that all the birds responded serologically when 28 weeks old and laying at 90 per cent production. The vaccinated birds and a control group of unvaccinated birds were challanged intranasally and orally with EDS'76 virus. The vaccinated hens continued to lay normally with good quality brown eggs but the egg production of unvaccinated birds fell by 60 per cent and soft-shelled eggs, eggs with poor shell quality and white eggs were laid.

Shakya and Dhawedkar (1991a) reported the seroprevalence of EDS'76 virus in Jabalpur area of Madhya Pradesh. A total of 147 out of 1024 serum samples were found positive with overall prevalence rate of 14.35 per cent. The incidence of the disease in commercial layer and broiler birds was 9.94 and 24.51 per cent respectively. Highest positive reactors (22.41%) were encountered in chickens of 5 to 10 weeks of age while the adult birds between 21 to 30 weeks of age showed the lowest (4.34%) positivity.

Shakya and Dhawedkar (1991b) failed to adopt EDS'76 virus in embryonated chicken eggs even after 4 to 5 passages. They concluded that EDS'76 virus is refraractory to growth in growing chicken embryos.

Rao et al. (1992) conducted seroepidemiological survey in Andhra Pradesh to know the status of EDS'76 virus infection in chicken populations of the state. Of the 2115 pooled serum samples tested, 390(18.4%) had specific HI antibodies to EDS'76 virus. The antibody titres of positive serum samples ranged from $\log_2$ 3.00 to $\log_2$ 7.00. The districtwise seropositives ranged from 0.88 to 50.98 per cent. A total of 181 pooled serum samples from ducks were also screened for the presence of EDS'76 virus antibodies employing micro HI test and 61 (33.15%) were found positive.

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CHAPTER - III

MATERIALS AND METHODS

MATERIALS AND METHODS

MATERIALS :

Virus :

The egg drop syndrome'1976 (strain 127) was obtained from the Department of Veterinary Microbiology, College of Veterinary Sciences and Animal Husbandry, Jabalpur (M.P.), through the courtesy of Dr. R.G. Dhawedkar, Professor of Microbiology. Infected allantoic fluid of growing duck eggs was used as antigen during the period of this experiment.

Antiserum :

The reference antiserum against EDS'76 virus (strain 127) was obtained from M/S Alved Biological Pvt. Ltd., Madras.

Antiserum against EDS'76 virus (strain 127) was also raised in this laboratory and used during the present investigation as and when required.

Chicken Red Blood Cell :

0.8 per cent suspension of chicken RBC in phosphate buffer saline (PBS) was used for haemagglutination test.

Composition of Phosphate Buffer Saline (PBS) pH 7.2 :

Potassium chloride (Kcl)	-	0.2 g
Potassium dihydrogen phosphate (KH_2PO_4)	-	0.2 g
Sodium chloride (Nacl)	-	8.0 g
Disodium hydrogen phosphate (Na_2HPO_4 $2H_2O$)	-	1.44 g
Distilled water	-	1000 ml

The solution was autoclaved at 15 lb pressure for 15 minutes and stored at 4°C.

Alsever's Solution :

Dextrose	-	2.05 g
Sodium citrate	-	0.90 g
Sodium chloride	-	0.42 g
Citric acid	-	0.055 g
Distilled water	-	100 ml

It was sterilized by autoclaving at 10 lb pressure for 15 minutes.

Red Blood Cell (RBC) Suspension :

Two adult chickens were used as donors of blood and 1.0 to 1.5 ml of blood was collected from each bird in Alsever's solution (one part to one part of blood). The blood was pooled and mixed with equal quantity of PBS and centrifuged at 500 rpm for 10 minutes. The supernatant was removed and packed cells were washed three times with PBS. Finally 0.8 per cent RBC suspension was made in fresh PBS and stored at refrigerator temperature which could be used for four days after which fresh suspension was prepared.

Embryonated Duck Eggs :

10-11 days old duck eggs obtained from flocks free from EDS'76 virus antibody were used for propagation of

the virus and production of virus antigen.

Serum Samples :

A total of 645 serum samples were collected from different poultry farms spread throughout the state. The samples were collected from different strains of commercial layers and broilers chicken aged between 5-26 weeks. Both apparently healthy as well as birds with history of sudden fall in egg production were screened for the presence of antibodies to EDS'76 virus.

METHODS :

Propagation of Viruses :

10 days old embryonated duck eggs were inoculated with 0.2 ml of virus suspension via allantoic sac route. The virus inoculum contained 128 HA unit of virus per 0.05 ml and Kanamycin to final concentration of 50 mg per ml of the virus suspension. After inoculation eggs were incubated at 38°C in egg incubator and candled daily. The mortality if any within 24 hours was considered as non-specific. The observation on the activity of embryos (normal, sluggish or inert) were also made. After 5 days of incubation the eggs were chilled at 4°C for 8-12 hours. Allantoic fluid (A/F) from individual embryo was collected and tested for HA activity by spot HA test. The A/F found positive was centrifuged and the supernatant was collected in sterile vials and stored in deep freeze at -20°C.

Production of Hyperimmune Serum :

The hyperimmune sera against EDS'76 (strain 127) were raised in 15 weeks old apparently healthy WLH cockerels free from EDS'76 virus antibodies. A total of 4 cockerels were used for this purposes.

Each cockerel was given 0.2 ml of EDS'76 virus orally in the form of allantoic fluid having virus activity of 512 HA units per 0.05 ml and collected at third passage level in this laboratory. The second dose of the antigen was given after 12 days. These birds were test bled on 27th day after first dose and the HI activity of the serum was determined. All the birds showing high HI titre were finally bled next day and their sera were separated, pooled and stored at -20°C after determining their HI titre for future use.

Collection of Serum Samples from Chickens :

The blood was collected from wing vein with 5 ml sterilized syringe using 22 gause needles. From each bird two to three ml of blood was drawn and immediately transferred to sterilized test tubes which were kept in slanting position and the blood was allowed to clot. After four to five hours at room temperature, the serum was collected in a sterilized Laxbro vials of two ml capacity. All the serum samples were inactivated at 56°C for 30 minutes and stored at -10°C until processed.

Spot HA Test :

0.1 ml of harvested allantoic fluid was placed on a glass slide . To this fluid was added 0.1 ml of a 5 per cent suspension of chicken RBC. The two were mixed with a toothpick and the result was observed within a minute.

Haemagglutination Test :

The HA test was carried out by microtitration technique according to the procedure described by Beard (1980). To perform HA using 0.05 ml volume, a two fold serial dilutions of virus material were made in PBS, except in control well in which only 0.05 ml PBS was added. In next step 0.05 ml of 0.8 per cent RBC suspension was added to all the wells. A known positive control was also included. The plate was swirlled gently for mixing and uniform distribution of erythrocytes and left at room temperature for 40 minutes. The EDS'76 virus produced a diffused sheet of agglutinated RBC covering the bottom of the wells. Negative and control wells showed circumscribed compact button at the bottom. The HA pattern was read with the aid of reading mirror and result of HA titre was recorded as reciprocal of the highest dilution showing complete agglutination of erythrocytes and expressed as $\log_2$/0.05 ml. To detect the presence of virus in test material spot HA was carried out using 5 per cent RBC suspension.

Haemagglutination Inhibition (HI) Test :

The HI test was performed by microtitration technique as per the method suggested by Beard (1980). This test was carried out by beta technique (constant antigen and diluted serum). As suggested by McFerran et al. (1978), four HA units of virus antigen and 0.8 per cent chicken RBC suspension were used in the test.

Using 0.025 ml volume of test serum, a two fold serial dilutions were made in PBS. In each test a known positive and negative serum samples were included as controls. To each serum dilution 0.025 ml (4 HA units) of virus antigen was added and after reaction time of 30 minutes at room temperature, 0.05 ml of 0.8 per cent RBC suspension was added to each well containing serum virus mixture. The plate was shaken gently, and left at room temperature. The results were read after 40 minutes. The reciprocal of the highest serum dilution showing complete inhibition of haemagglutination was taken as the HI titre.

Effect of EDS'76 Virus on Egg Production and Egg Quality :

To study the effect of EDS'76 virus on egg production and quality of egg, 27 weeks old 50 WLH laying hens free from antibodies to EDS'76 virus were taken. These birds were divided into two groups, experimental and control, each comprising of 25 hens. The birds of the experimental and control groups were maintained separately in two different rooms and provided the

same nutritional and managerial cares. Their feeding and watering arrangements were made separately so as to avoid any possibility of spread of infection to control groups. The daily egg production of the birds of the two groups were recorded for 7 days. At 28 weeks of age birds in the experimental group was given 0.2 ml of allantoic fluid suspension containing 1024 HA units of EDS'76 virus (strain 127) at fourth passage level in duck embryo by intraocular (IO) route. The birds in the control group received 0.2 ml of PBS through the same route. The plan of experiment has been depicted in table 1. The birds were observed for a period of 40 days PI for clinical manifestations. The daily egg production was recorded and mean egg production of each group was calculated every 10 days. Eggs were examined and faults recorded. The eggs were analysed for various qualitative changes such as shell-less, thin shelled, cracked and small size eggs. For grading thin shell egg (TSE) the minimum egg shell thickness (0.30 mm) of normal eggs laid before inoculation was taken as the standard and below this thickness, the eggs were graded as TSE. The weight and volume of the eggs were also taken. Finally the eggs were broken and shell thickness of all eggs except shell-less was measured with the help of a slide caliper. Mean egg shell thickness, egg weight and egg volume of each group were calculated every 10 days.

Table 1 : Showing plan of experiment to study the effects of EDS'76 virus on egg production, egg quality, pathogenicity and serum biochemical profile in chickens.

Sl. No.	Group	Treatment	Route	Dose	No. of birds	Age in weeks	Parameters studied	Remarks
1.	Experimental	EDS'76 virus	I/O	0.2 ml (1024 HA units)	25	28	i) Egg production and egg quality.	i) Data collected in respect of daily egg production, distribution of egg changes, egg weight, eggs volume and egg shell thickness were analysed. Mean values for various parameters for every 10 days upto 40 days PI were also determined.
							ii) Pathogenicity.	ii) 5 randomly selected birds from each group sacrificed 10, 20, 30 and 40 days PI for studying gross and histopathological changes in various organs.
							iii) Serum biochemical profile.	iii) Birds selected for sacrifice for pathogenicity study as mentioned above were bled separately and serum samples collected for determination of various biochemical constituents.
2.	Control	PBS	I/O	0.2 ml	25	28	As above.	As above.

Pathogenicity Study :

The plan of experiment as shown in table 1 was followed. Five birds from each group were randomly selected and sacrificed at 10 days interval for 40 days PI. These birds were examined for gross pathological changes in the various organs. For histopathological studies, the different parts of oviduct, ovary, pieces of liver, spleen and lungs were collected and preserved in 10 per cent formal saline solution. The tissues were processed by conventional paraffin embedding method and the section of 5-6 microns were cut and stained by haematoxylin and Eosin method (Drury and Wellington, 1980).

Effect on Serum Biochemical Profile :

To study the effect of EDS'76 virus on the biochemical profile of serum of chickens the experimental plan shown in table 1 was followed. The blood samples from 5 birds per group were collected at 10 days interval for 40 days post inoculation. For this purpose the birds randomly selected for sacrifice for pathogenicity study were bled by puncturing wing vein before sacrifice. The blood was collected separately without adding any anticoagulant and allowed to clot. The serum was collected in sterile vials separately and processed for determination of the level of different biochemical constituents as described below:

1. Estimation of Calcium :

The serum calcium was determined as per the method of Clark and Collip (1925).

2. **Estimation of Sodium and Potassium :**

The estimation of serum sodium and serum potassium were carried out by Flame photometer as described by Oser (1979).

3. **Estimation of Total Proteins :**

The total serum proteins was determined following the method of Reinhold (1953).

4. **Estimation of Total Cholesterol :**

The estimation of serum total cholesterol was done by the method of Zlatkis et al. (1953).

5. **Estimation of Inorganic Phosphorus :**

The serum inorganic phosphorus was estimated as per the method of Fiske and Subbarow (1925).

6. **Estimation of Alkaline Phosphatase :**

The serum alkaline phosphatase activity was determined as per Bodansky's modified method as described by Kind and King (1954).

7. **Determination of Transaminase Activity :**

The serum transaminase activity was estimated by the method of Reitman and Frankel (1957).

Statistical Analysis :

Mean values and their standard errors (SE) were calculated. The analysis of variance (ANOVA) was performed as per Snedecor and Cochran (1967).

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CHAPTER - IV

RESULTS

CHAPTER - IV

RESULTS

RESULTS

SEROPREVALENCE :

To study the prevalence of EDS'76 virus infection in different poultry farms in Bihar, the serum samples collected were subjected to haemagglutination inhibition test using reference EDS'76 virus antigen (strain 127). A total of 164 out of 645 serum samples collected from 39 flocks of 10 poultry farms were positive for HI antibodies to EDS'76 virus, with an overall incidence of 25.42 per cent (Table 2).

The flockwise incidence of EDS'76 virus antibodies ranged from 10.00 to 46.66 per cent. The farmwise incidence was the highest (37.5%) in farm 'A' and the lowest (16.92%) in farm 'E' (Table 2).

The age prevalence of HI antibodies to EDS'76 virus was highest in chickens between an age group of 5-9 weeks and lowest in chickens of 25 weeks and above age groups (Table 3). The breedwise incidence of antibodies to EDS'76 virus was 32.85 per cent in commercial broiler chickens, whereas the incidence was only 16.77 per cent in commercial layer birds (Table 4).

The HI titres of positive serum samples (164) varied from 1:4 to 1:512 (2 $\log_2$ to 8 $\log_2$/0.025 ml). In broilers the HI titres varied from 1:4 to 1:512 whereas in layers the titres varied from 1:4 to 1:64. The flockwise geometric mean of HI antibodies titres ranged from 2.0990 to 5.5920 (Table 5). The overall geometric mean of antibodies titres was 4.3905.

Table 2 : Showing farmwise incidence of HI antibodies to EDS'76 virus.

Sl. No.	Farm	Flock	Breed, egg type/ meat type	Age in weeks	No. of serum* samples tested	No. of serum samples postive	Per cent incidence
1	2	3	4	5	6	7	8
1	A	1	Broiler	7	22	8	36.36
		2	Broiler	9	18	7	38.88
			Total :		40	15	37.50
2	B	1	Broiler	8	15	7	46.66
		2	Broiler	7	18	5	27.77
		3	Layer	26	22	3	13.63
		4	Broiler	6	14	4	28.57
			Total :		69	19	27.53
3	C	1	Broiler	10	20	7	35.00
		2	Layer	13	21	3	14.28
		3	Layer	16	17	5	29.41
		4	Broiler	5	23	8	34.78
			Total :		81	23	28.39
4	D	1	Layer	22	23	8	34.78
		2	Layer	21	22	3	13.63
		3	Broiler	5	15	3	20.00
		4	Broiler	6	20	7	35.00
		5	Layer	25	18	3	16.66
			Total :		98	24	24.48

Table 2 : Contd.

1	2	3	4	5	6	7	8
8	H	1	Broiler	9	18	7	38.88
		2	Layer	22	12	2	16.66
		3	Broiler	7	10	3	30.00
			Total :		40	12	30.00
9	I	* 1	Broiler	8	22	8	36.36
		2	Layer	24	20	3	15.00
		3	Broiler	6	23	8	34.78
		4	Layer	19	17	3	17.64
		5	Layer	26	22	3	13.63
		6	Broiler	9	14	4	28.57
			Total :		118	29	24.57
10	J	1	Broiler	8	7	2	28.57
		2	Broiler	9	10	3	30.00
		3	Layer	22	8	1	12.50
		4	Layer	24	7	1	14.28
			Total :		32	7	21.87
Grand Total (Sl.No. 1 to 10)					645	164	25.42

* Serum sample from five birds were pooled to constitute one sample.

Table 3 : Showing agewise prevalence of HI antibodies to EDS'76 virus.

Sl. No.	Age group in weeks	No. of serum samples tested	No. of serum samples positive	Per cent prevalence
1	5-9	327	107	32.72
2	10-14	41	10	24.39
3	15-19	34	8	23.52
4	20-24	154	27	17.53
5	25 and above	89	12	13.48
	Total :	645	164	25.42

Table 4 : Showing prevalence of HI antibodies to EDS'76 virus in egg type (layers) and meat type (Broilers) birds.

Sl. No.	Breed	No. of serum samples tested	No. of serum samples positive	Per cent prevalence
1	Layer	298	50	16.77
2	Broiler	347	114	32.85
	Total :	645	164	25.42

Table 5 : Showing flockwise HI antibodies titres to EDS'76 virus.

Sl. No.	Farm	Flock No.	Breed	Age in week	No. of positive serum samples	HI antibody titre	Geometric mean of HI antibody titre
1	2	3	4	5	6	7	8
1	A	1	Broiler	7	8	1:4 - 1:64	3.7927
		2	Broiler	9	7	1:4 - 1:16	2.2944
2	B	1	Broiler	8	7	1:8 - 1:512	4.5887
		2	Broiler	7	5	1:4 - 1:8	2.3445
		3	Layer	26	3	1:4 - 1:16	2.0990
		4	Broiler	6	4	1:8 - 1:256	3.6923
3	C	1	Broiler	10	7	1:4 - 1:64	3.1907
		2	Layer	13	3	1:4 - 1:16	2.7960
		3	Layer	16	5	1:4 - 1:8	2.1940
		4	Broiler	5	8	1:4 - 1:32	3.2910
4	D	1	Layer	22	8	1:8 - 1:64	4.4883
		2	Layer	21	3	1:16	4.3880
		3	Broiler	5	3	1:4 - 1:16	2.4950
		4	Layer	25	3	1:8 - 1:32	3.4917
		5	Broiler	6	7	1:4 - 1:16	3.5920
5	E	1	Broiler	8	3	1:32 - 1:64	5.5920
		2	Layer	24	3	1:16 - 1:32	4.8930
		3	Broiler	9	2	1:8 - 1:16	3.9465
		4	Layer	21	3	1:8 - 1:32	3.1940

Table 5 : Contd.

1	2	3	4	5	6	7	8
6	F	1	Broiler	8	3	1:16 - 1:64	4.8930
		2	Layer	25	1	1:32	5.0000
		3	Broiler	7	3	1:16 - 1:64	4.8930
7	G	1	Broiler	6	7	1:32 - 1:128	5.0870
		2	Layer	24	3	1:8 - 1:32	3.3445
		3	Layer	26	2	1:32	5.000
		4	Broiler	8	5	1:16 - 1:128	4.1405
8	H	1	Broiler	9	7	1:8 - 1:16	3.188
		2	Layer	22	2	1:8	3.00
		3	Broiler	7	3	1:8 - 1:16	3.4950
9	I	1	Broiler	8	8	1:32 - 1:128	5.5853
		2	Layer	24	3	1:16 - 1:32	4.0435
		3	Broiler	6	8	1:8 - 1:32	3.1907
		4	Layer	19	3	1:8 - 1:16	3.4950
		5	Layer	26	3	1:4 - 1:16	2.7960
		6	Broiler	9	4	1:16 - 1:32	4.4415
10	J	1	Broiler	8	2	1:8 - 1:16	3.9465
		2	Broiler	9	3	1:16 - 1:32	5.0435
		3	Layer	22	1	1:16	4.000
		4	Layer	24	1	1:16	4.000

Overall Geometric Mean = 4.3905

EFFECT OF EDS'76 VIRUS ON EGG PRODUCTION AND EGG QUALITY :

Clinical Signs and Egg Production :

The clinical symptoms observed in the infected hens were mild to severe diarrhoea between 9th and 10th days PI. Most of the birds recovered within a day or two. However, some of the birds remained dull during the entire period of experiments. No abnormal respiratory symptoms were noticed in these birds. All the birds in the infected group developed HI antibodies to EDS'76 virus 9 days PI and remained positive for HI antibodies during the period of this experiment. The uninoculated control group of birds remained healthy and were negative for HI antibodies to EDS'76 virus throughout 40 days experimental period.

The virus produced the disease syndrome which was characterized by drop in egg production, laying of abnormal eggs viz, shell-less eggs (SLE), thin shell eggs (TSE) cracked eggs or small size eggs. Different types of abnormal eggs laid by EDS'76 virus infected hens are shown in figure 2-5. The results of daily egg production are shown in table 6. The depressed egg production was observed from 10th days PI and continued till 22 days PI (Fig. 1). Thereafter, there was improvement in the egg production trend and the production reached 80 per cent by 40th day PI. The per cent decline in egg production varied from 13.0 to 21.5 per cent between 11 and 40 days post inoculation (Table 7). The rate of decline in egg production was maximum (21.5%) during 11 to 20 days PI followed by 21-30 days PI (17.33%) and 31-40 days PI (13.00%). The absolute egg production in infected group was 74.86

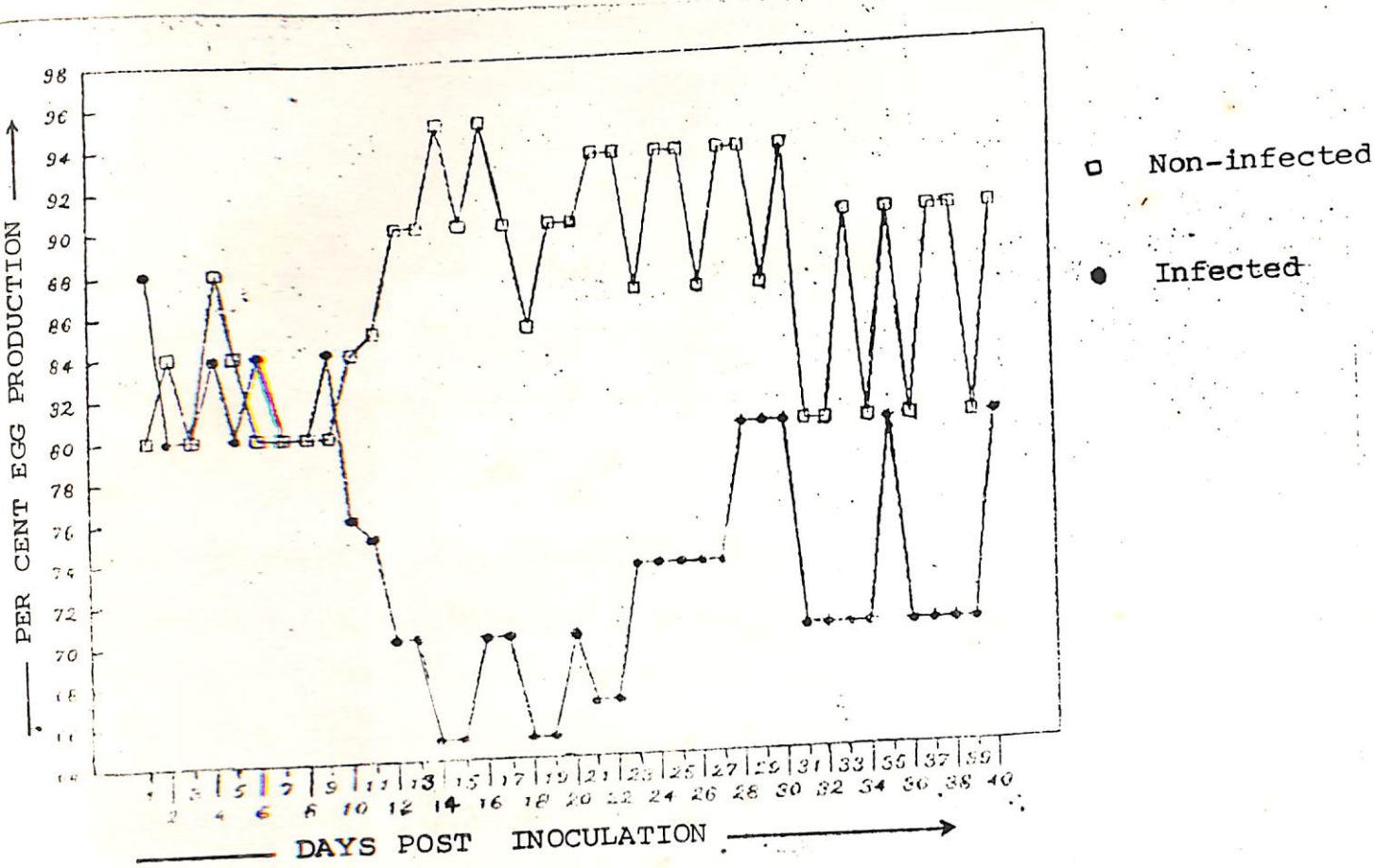
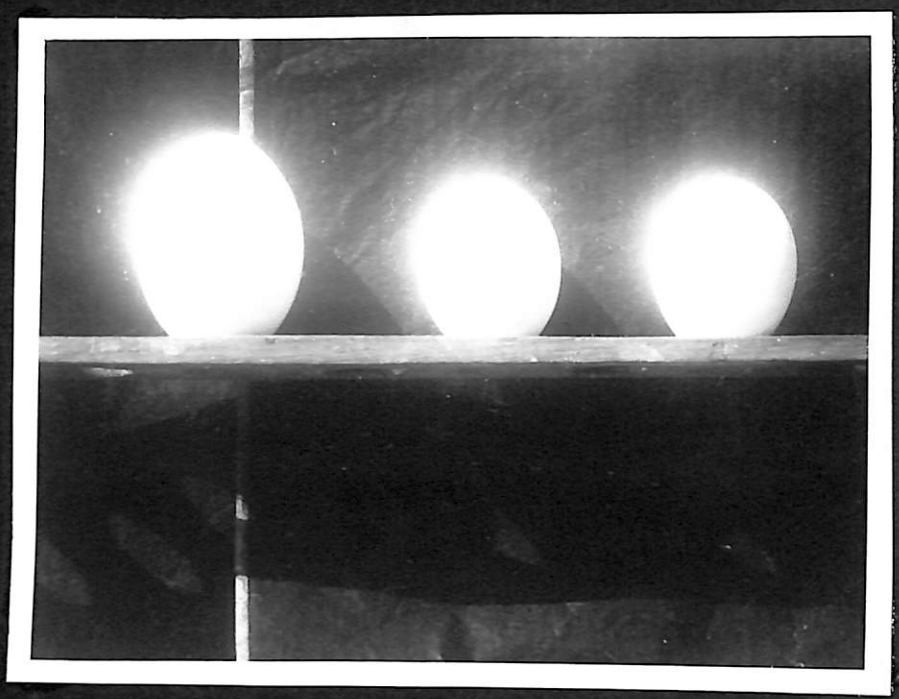
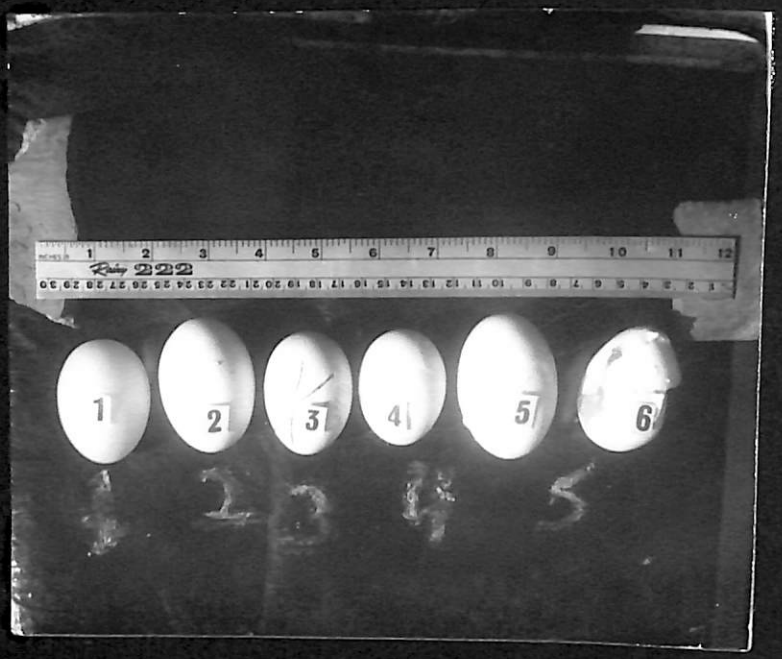


Fig. 1 : Showing daily egg production in EDS'76 virus-infected and non-infected chickens.

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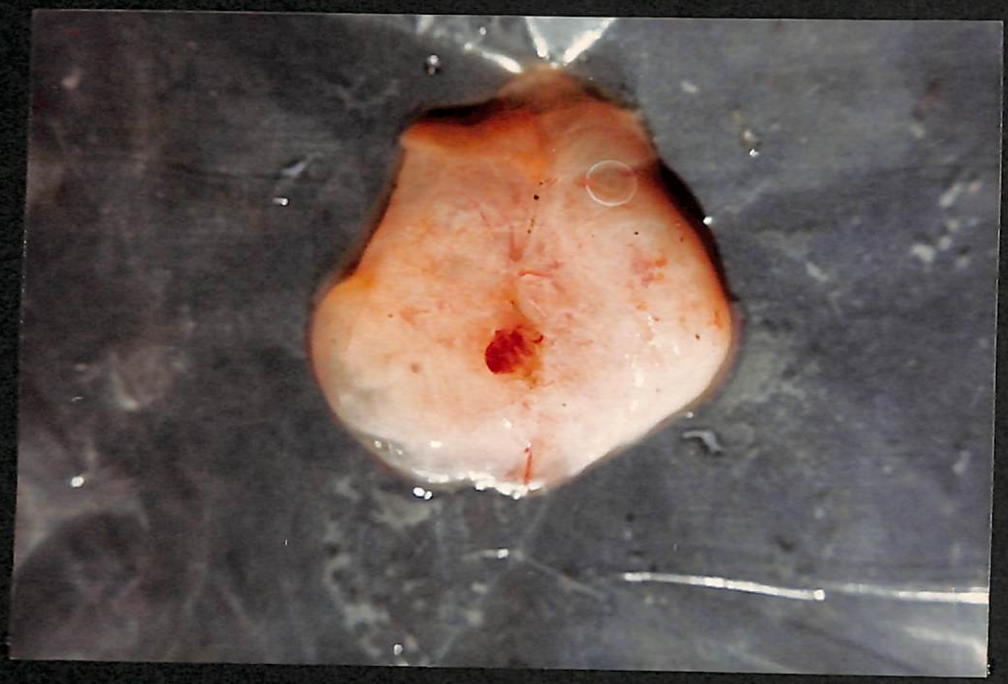

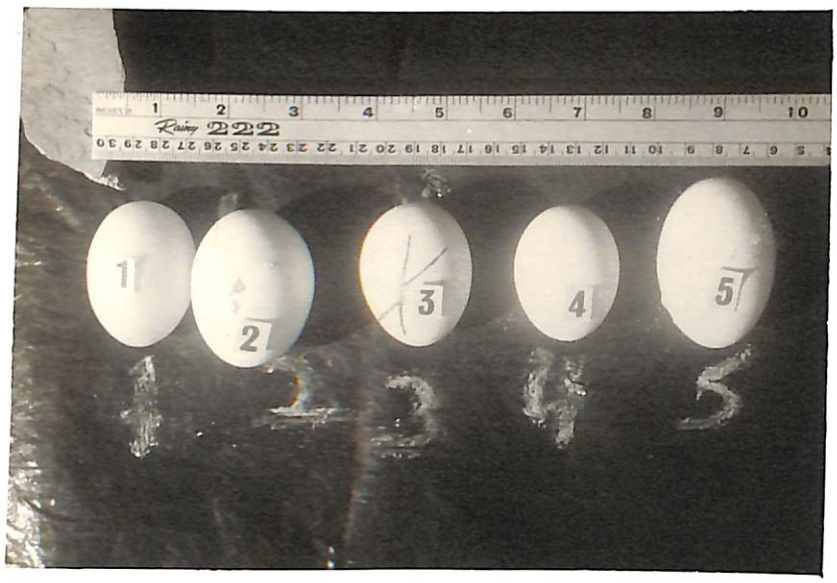
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Table 6 : Showing daily egg production in EDS'76 virus infected and non-infected chickens.

Days PI	Infected						Control					
	Total no.of eggs	Normal eggs	Abnormal eggs				Total no.of eggs	Normal eggs	Abnormal eggs			
			TSE	SLE	Crack eggs	Small eggs			TSE	SLE	Crack eggs	Small eggs
1	2	3	4	5	6	7	8	9	10	11	12	13
1	22	22	-	-	-	-	20	20	-	-	-	-
2	20	20	-	-	-	-	21	21	-	-	-	-
3	20	20	-	-	-	-	20	20	-	-	-	-
4	21	21	-	-	-	-	22	22	-	-	-	-
5	20	20	-	-	-	-	21	21	-	-	-	-
6	21	21	-	-	-	-	20	20	-	-	-	-
7	20	20	-	-	-	-	20	20	-	-	-	-
8	20	20	-	-	-	-	20	20	-	-	-	-
9	21	18	1	-	1	1	21	20	-	-	-	1
10	19	16	2	-	1	-	21	21	-	-	-	-
11	14	10	1	-	2	1	17	17	-	-	-	-
12	15	11	3	-	1	-	18	18	-	-	-	-
13	14	6	3	-	3	2	18	18	-	-	-	-
14	13	7	2	-	2	2	19	19	-	-	-	-
15	13	6	2	-	2	3	18	17	-	-	-	1
16	14	8	1	2	3	-	19	19	-	-	-	-
17	14	8	1	2	2	1	18	18	-	-	-	-
18	13	6	1	3	2	1	17	16	-	-	-	1
19	13	5	1	3	2	2	18	17	-	-	-	1

Table 6 : Showing daily egg production in EDS'76 virus infected and non-infected chickens.

Days PI	Infected						Control					
	Total no.of eggs	Normal eggs	Abnormal eggs				Total no.of eggs	Normal eggs	Abnormal eggs			
			TSE	SLE	Crack eggs	Small eggs			TSE	SLE	Crack eggs	Small eggs
1	2	3	4	5	6	7	8	9	10	11	12	13
1	22	22	-	-	-	-	20	20	-	-	-	-
2	20	20	-	-	-	-	21	21	-	-	-	-
3	20	20	-	-	-	-	20	20	-	-	-	-
4	21	21	-	-	-	-	22	22	-	-	-	-
5	20	20	-	-	-	-	21	21	-	-	-	-
6	21	21	-	-	-	-	20	20	-	-	-	-
7	20	20	-	-	-	-	20	20	-	-	-	-
8	20	20	-	-	-	-	20	20	-	-	-	-
9	21	18	1	-	1	1	21	20	-	-	-	1
10	19	16	2	-	1	-	21	21	-	-	-	-
11	14	10	1	-	2	1	17	17	-	-	-	-
12	15	11	3	-	1	-	18	18	-	-	-	-
13	14	6	3	-	3	2	18	18	-	-	-	-
14	13	7	2	-	2	2	19	19	-	-	-	-
15	13	6	2	-	2	3	18	17	-	-	-	1
16	14	8	1	2	3	-	19	19	-	-	-	-
17	14	8	1	2	2	1	18	18	-	-	-	-
18	13	6	1	3	2	1	17	16	-	-	-	1
19	13	5	1	3	2	2	18	17	-	-	-	1

Table 6 : Contd.

1	2	3	4	5	6	7	8	9	10	11	12	13
20	14	7	2	2	1	2	18	17	-	-	-	1
21	10	8	1	-	1	-	14	13	-	-	-	1
22	10	7	2	-	1	-	14	14	-	-	-	-
23	11	8	1	-	1	1	13	13	-	-	-	-
24	11	8	-	-	1	2	14	14	-	-	-	-
25	10	8	1	-	1	-	14	14	-	-	-	-
26	11	9	1	-	1	-	13	13	-	-	-	-
27	11	9	-	-	1	1	14	14	-	-	-	-
28	12	11	-	-	1	-	14	14	-	-	-	-
29	12	12	-	-	-	-	13	13	-	-	-	-
30	13	13	-	-	-	-	14	14	-	-	-	-
31	7	7	-	-	-	-	8	8	-	-	-	-
32	6	6	-	-	-	-	8	8	-	-	-	-
33	8	8	-	-	-	-	9	9	-	-	-	-
34	7	7	-	-	-	-	8	8	-	-	-	-
35	7	7	-	-	-	-	9	9	-	-	-	-
36	7	7	-	-	-	-	8	8	-	-	-	-
37	8	8	-	-	-	-	9	9	-	-	-	-
38	8	8	-	-	-	-	9	9	-	-	-	-
39	6	6	-	-	-	-	8	8	-	-	-	-
40	8	8	-	-	-	-	9	9	-	-	-	-
Total :	524	437	26	12	30	19	607	601	-	-	-	6

Table 7 : Showing effect of EDS'76 virus on egg production and egg quality in different periods.

Periods days PI	No.of hens	Infected No. of eggs			Control No. of eggs			Per cent decline (8-5)
		Normal	Abnormal	Total	Normal	Abnormal	Total	
1	2	3	4	5	6	7	8	9
1-10	25	198 (97.05%)*	6 (2.94%)	204 (81.60%)	204 (99.51%)	1 (0.49%)	205 (82.00%)	0.40%
11-20	20	74 (54.01%)	63 (45.98%)	137 (68.5%)	176 (97.78%)	4 (2.22%)	180 (90.0%)	21.50%
21-30	15	93 (83.78%)	18 (16.21%)	111 (74.00%)	136 (99.27%)	1 (0.73%)	137 (91.33%)	17.33%
31-40	10	72 (110.00%)	0 (0.00%)	72 (72.00%)	85 (100.00%)	0 (0.00%)	85 (85.00%)	13.0%
Total :		437 (83.4%)	87 (16.6%)	524 (74.86%)**	601 (99.01%)	6 (0.99%)	607 (86.71%)	11.85

* Figures in the parentheses indicate values in per cent.

** On 40 hen days basis.

per cent as against 86.71 per cent in uninfected control hens (Table 7). The over all decline in egg production was 11.85 per cent.

Egg Quality :

The daily production of different types of abnormal eggs has been shown in table 6. The laying of abnormal eggs was first noticed on 9th day PI and continued till 28th days PI. The laying of abnormal eggs calculated on 10 days basis for 40 days PI has been shown in table 7. The highest number of 63 abnormal eggs were laid during 11 to 20 days PI. No abnormal egg was laid during 31 to 40 days PI. The data on types of eggs abnormalities (Table 8) revealed the highest laying of cracked eggs (3 0) followed by laying of thin shelled eggs (26), small size eggs (19) and shell-less eggs (12). The over all laying of abnormal eggs during the period of experiment constituted 16.60 per cent (Table 7) which included 4.96 per cent thin shelled, 2.29 per cent shell-less eggs, 5.73 per cent cracked eggs and 3.62 per cent small size eggs. The different types of egg abnormalities has been shown in table 8. The data revealed thin shelled eggs 29.89 per cent, shell-less eggs 13.79 per cent, cracked eggs 34.48 per cent and small size eggs 21.83 per cent. Out of the total number of abnormal eggs laid by EDS'76 virus infected birds thin shelled and shell-less eggs constituted 43.68 per cent (Table 9). The thin shelled eggs had a rough, sandpaper like texture.

The uninfected control hens did not lay abnormal eggs except laying of a few small size eggs (Table 6).

Table 8 : Showing periodwise distribution of abnormal eggs laid by infected hens.

Periods days PI	No.of hens	TSE	SLE	Crack	Small	Total
1-10	25	3 (50.0%)	0 (0.00%)	2 (33.33%)	1 (16.67%)	6
11-20	20	17 (26.98%)	12 (19.04%)	20 (31.74%)	14 (22.22%)	63
21-30	15	6 (33.33%)	0 (0.00%)	8 (44.44%)	4 (22.22%)	18
31-40	10	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0
	Total :	26 (29.88%)	12 (13.79%)	30 (34.48%)	19 (21.83%)	87

Table 9 : Showing proportion of thin shelled and shell-less eggs incomparison with total no. of abnormal eggs laid by virus infected hens.

Periods days PI	No.of hens	No. of abnormal eggs laid	No.of TSE	No.of SLE	Sum of TSE & SLE
1-10	25	6	3 (50.00%)	0 (0.00%)	3 (50.00%)
11-20	20	63	17 (26.98%)	12 (19.04%)	29 (46.03%)
21-30	15	18	6 (33.33%)	0 (0.00%)	6 (33.33%)
31-40	10	0	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Total :	87	26 (29.88%)	12 (13.79%)	38 (43.68%)

The mean egg shell thickness of eggs laid by EDS'76 virus infected group of birds was relatively lower than the corresponding values for uninfected group of hens over all the periods (Table 10). However, the reduction in mean egg shell thickness of infected hens was highly significant between 21 and 30 day PI when compared with the corresponding values for uninfected control birds.

EDS'76 virus showed highly significant reduction of mean egg weight between 11 and 20 day PI as well as 21 and 30 day PI when compared with the mean egg weight of eggs laid by uninfected control birds (Table 11) for the corresponding periods.

Studies on influence of the virus on mean egg volume revealed relative decrease in mean egg volume of the eggs laid by infected birds over all the periods when compared with mean egg volume of eggs laid by uninfected control hens (Table 12). However, these values did not differ significantly.

PATHOGENICITY :

Gross Findings :

The hens sacrificed on 10th days PI had slightly atrophic ovary and oviduct. The uterus of one hen was found to be slightly oedematous whereas oviduct of two out of 5 infected hens were paler. Other visceral organs appeared almost normal except there was slight spleenomegaly. The ovary and oviduct of the hens which were sacrificed on 20th days PI were highly atrophic and the oviduct was markedly reduced in size. The lungs of 3 hens showed

Table 10 : Mean egg shell thickness of eggs laid by hens infected with EDS'76 virus and uninfected groups.

Periods days PI	No. of hens	Mean egg shell thickness (mm) ± S.E.	
		Infected	Control
1-10	25	0.3229 ± 0.0012 (204)[1]	0.3240 ± 0.0012 (205)
11-20	20	0.3086 ± 0.0000016 (125)[2]	0.2911 ± 0.0071 (180)
21-30	15	0.3162 ± 0.0000015 (111)**	0.3266 ± 0.0011 (137)
31-40	10	0.3303 ± 0.0000012 (72)	0.3322 ± 0.0013 (85)

1 Figures in the parentheses indicate no. of eggs laid during the periods.

2 No. of eggs laid during the period excluding 12 shell-less eggs.

** Indicates highly significant difference.

Table 11 : Mean weight of eggs laid by EDS'76 virus infected and uninfected laying hens.

Periods days PI	No. of hens	Mean egg weight (g) ± S.E.	
		Infected	Control
1-10	25	53.28 ± 0.414 (204)[1]	54.06 ± 0.386 (205)
11-20	20	50.65 ± 0.848 (137)**	54.40 ± 0.404 (180)
21-30	15	53.39 ± 0.506 (111)**	55.72 ± 0.416 (137)
31-40	10	56.11 ± 0.909 (72)	57.23 ± 0.398 (85)

1 Figures in the parentheses indicates no. of eggs.

** Indicates highly significant difference at 1% level ($P < 0.01$).

Table 12 : Mean egg volume of eggs laid by hens of EDS'76 virus infected and uninfected group.

Periods days PI	No. of hens	Mean egg volume (ml) ± S.E.	
		Infected	Control
1-10	25	50.65 ± 0.381 (204)[1]	50.84 ± 0.359 (205)
11-20	20	48.18 ± 0.489 (137)	51.20 ± 0.373 (180)
21-30	15	50.41 ± 0.477 (111)	52.31 ± 0.543 (137)
31-40	10	53.32 ± 0.507 (72)	53.81 ± 0.389 (85)

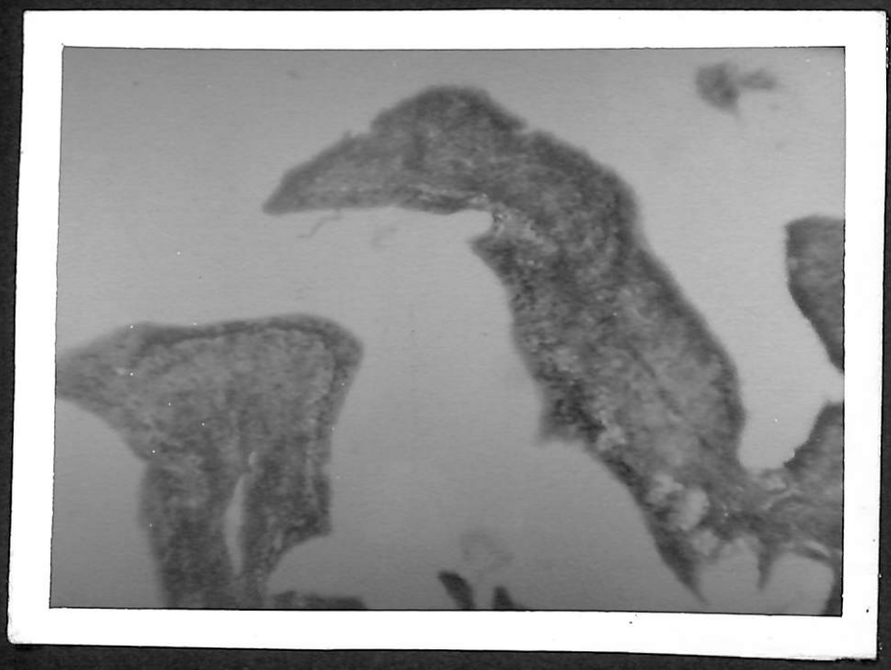
1 Figures in the parentheses indicate no. of eggs.

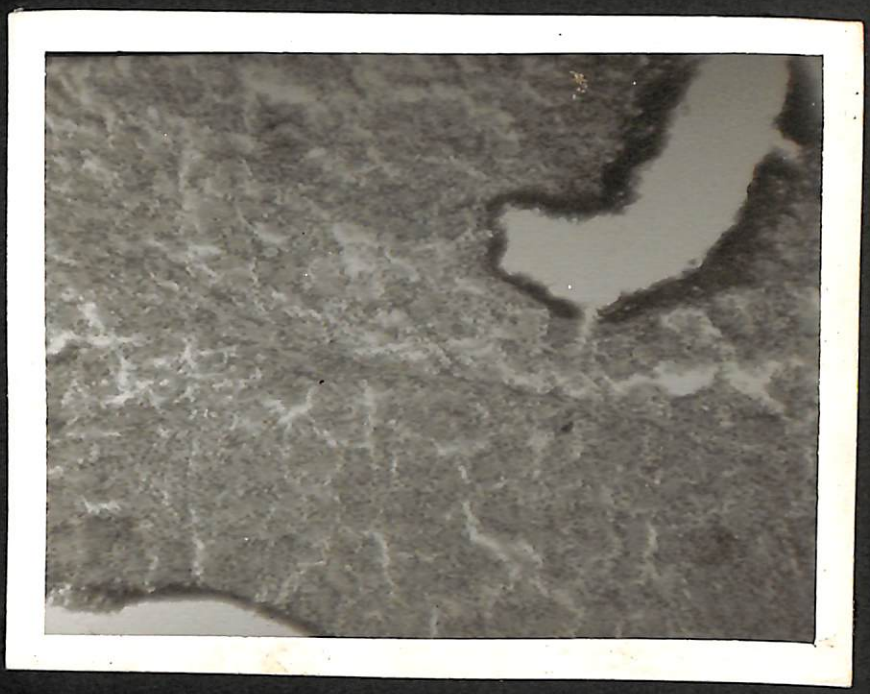
massive hyperaemia. The hen sacrificed on 30th and 40th days PI did not reveal marked changes in ovary, oviduct and other visceral organs.

No changes appeared in any organs of the control hens inoculated with PBS.

Microscopic Findings :

Histological changes were mostly noted in the oviduct and specially in uterus of the experimental birds sacrificed on 10th and 20th days PI. The lining epithelium of infundibulum was found to be desquamated. The lamina propria was infiltrated with mononuclear cells such as lymphocytes and macrophages (Fig. 6). Lymphocytic infiltration was seen in the mucosa of the oviduct and quite frequently in the uterus. Marked atrophy of the uterine glands were noticed in some hens (Fig. 7). In three of the hens, the uterus showed oedema of the mucosal folds. Lymphocytic infiltration appeared in the lamina propria and submucosa of the uterus of four hens sacrificed on 20th days PI. There was decrease in secretory glands and marked degenerative changes in the uterus of 2 hens. The uterine epithelial layer also showed desquamation in three of the hens and were sparsily infiltrated with heterophils and marcophages. Hydropic degeneration of glandular cells of magnum were also noticed in 2 hens. The lamina propria of oviduct of the hens which were sacrificed on 30th and 40th days PI showed mild to moderate infiltrations of macrophages and lymphocytes with occasional distribution of heterophils (Fig. 8).





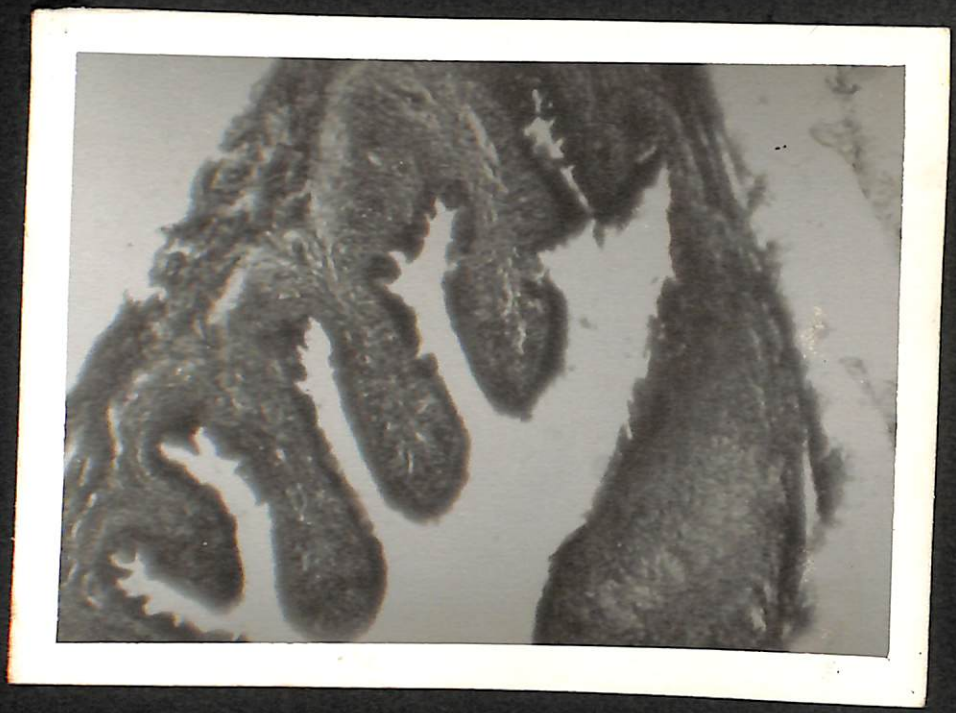
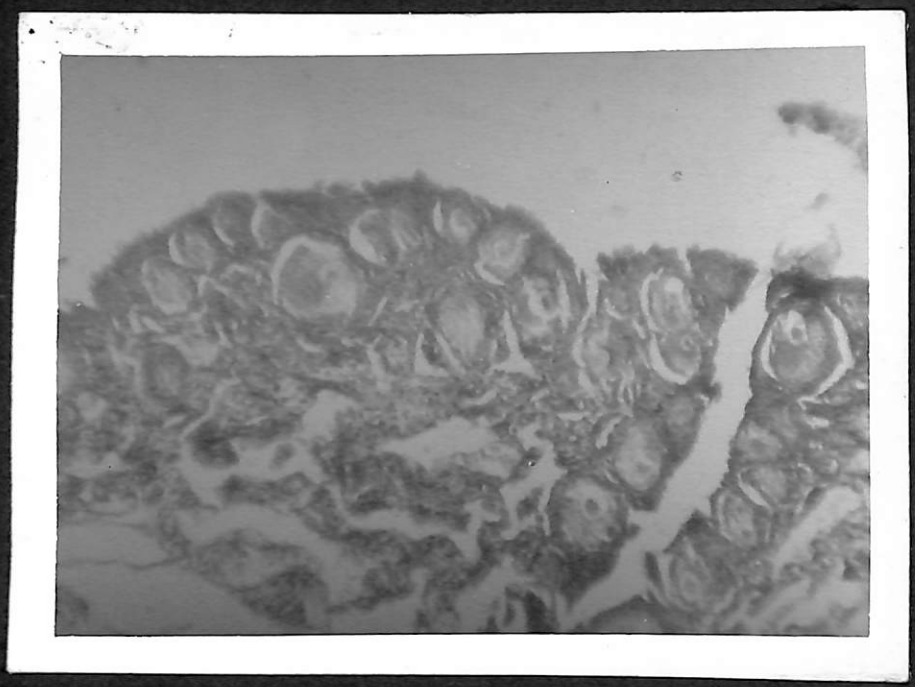
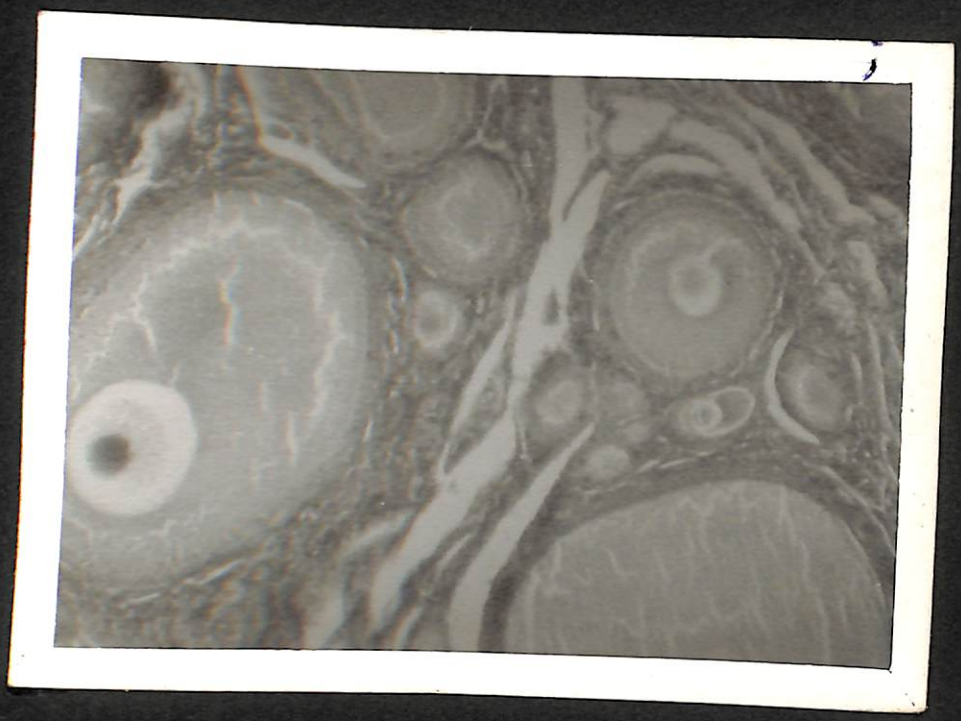


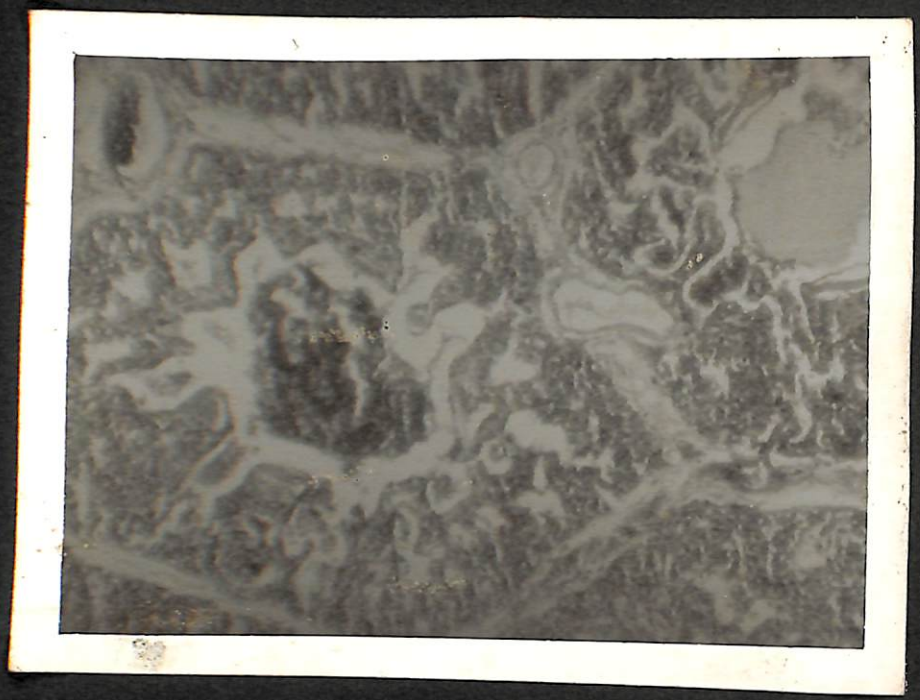
Table 13 : Showing serum biochemical profile of EDS'76 virus infected chickens.

Parameters	10 days PI		20 days PI		30 days PI		40 days PI	
	Infected	Control	Infected	Control	Infected	Control	Infected	Control
SGPT	13.14 ± 2.05	12.86 ± 2.12	13.00 ± 1.86	12.29 ± 1.93	12.71 ± 1.91	12.29 ± 1.93	12.71 ± 1.87	12.57 ± 2.02
SGOT	32.43 ± 1.07	31.57 ± 1.26	32.71 ± 1.30	32.00 ± 1.43	32.00 ± 1.25	31.43 ± 1.31	31.71 ± 1.78	31.57 ± 1.43
Calcium	19.44 ± 0.40*	20.87 ± 0.51	19.97 ± 0.34*	21.04 ± 0.47	20.32 ± 0.53	20.41 ± 0.54	19.75 ± 0.44	20.63 ± 0.42
Inorganic phosphate	6.52 ± 0.13*	7.01 ± 0.10	6.49 ± 0.13	6.98 ± 0.35	6.38 ± 0.46	6.82 ± 0.17	6.54 ± 0.26	6.83 ± 0.31
Sodium	141.04 ± 2.01	140.28 ± 2.37	141.08 ± 2.59	142.43 ± 2.36	140.71 ± 2.88	141.14 ± 2.27	139.84 ± 2.11	139.41 ± 2.23
Potassium	6.33 ± 0.22	6.24 ± 0.17	6.54 ± 0.16	6.30 ± 0.17	6.26 ± 0.24	6.28 ± 0.20	6.66 ± 0.15	6.44 ± 0.17
Total protein	5.41 ± 0.15	5.34 ± 0.15	5.46 ± 0.24	5.42 ± 0.14	5.17 ± 0.22	5.31 ± 0.14	5.46 ± 0.16	5.54 ± 0.13
Total Cholesterol	160.46 ± 3.88	179.67 ± 3.69	150.74 ± 3.69	148.93 ± 4.77	157.36 ± 3.08	155.53 ± 4.56	156.77 ± 3.52	153.92 ± 5.08
Serum alkaline phosphatase	16.78 ± 1.84	16.69 ± 1.39	21.28 ± 1.62	20.72 ± 1.88	20.59 ± 1.07	18.45 ± 1.70	20.89 ± 1.44	20.38 ± 1.72

* Indicate differed significantly at 5% level ($P < 0.05$).







The ovarian follicles appeared attretic in few cases when compared with the control hens (Fig. 9 and 10).

Changes of haemorrhagic pneumonia in the lung of hen sacrificed on 10 and 20 days PI were noticed (Fig. 11). No marked microscopic changes were noticed in the tissues section of the other organs included in the present study.

EFFECT OF EDS'76 VIRUS ON SERUM BIOCHEMICAL PROFILE :

Results of examination of blood to determine the level of different biochemical constituents in serum of EDS'76 virus infected hens are depicted in table 13. The results revealed no significant difference in the serum level of potassium, sodium, SGPT, SGOT, total cholesterol, total protein and serum alkaline phosphatase between EDS'76 virus infected birds and non-infected control birds in the period from 10 to 40 days after infection. In comparison with the non-infected control hens, the mean calcium concentration of blood serum of infected hens showed significant decrease 10 and 20 days PI. There was significant decrease in mean serum inorganic phosphate level of infected hens in comparison with the non-infected control birds at 10 days PI.

*

CHAPTER - V

DISCUSSION

DISCUSSION

Since the establishment of causative agent of egg drop syndrome'76 (EDS'76) in poultry from Netherlands by Van Eck et al. (1976), the disease has been reported from almost all parts of the world (McFerran et al., 1977; Baxendale, 1978; Meulemans et al., 1979; Zanella et al., 1980; Yamaguchi et al., 1981a; Marcial et al., 1986). In India, Mohanty et al.(1980) reported the occurrence of this disease for the first time. Subsequently, several workers have confirmed its existence in different parts of the country (Mohanty et al., 1984; Gupta et al., 1985; Sukumar and Babu, 1986; Reddy and Raghavan, 1987; Rao et al., 1992). Though, several instances of sudden drop in egg production associated with production of high percentage of soft shelled, thin-shelled, shell-less eggs, mis-shapen eggs, small size eggs, cracked shelled eggs or depigmented eggs have been noticed in a number of poultry farms in Bihar, the association of EDS'76 virus on such problems could not be ascertained earlier.

The seroprevalence studies conducted on 645 serum sample from 39 poultry flocks showed that overall prevalence of HI antibodies to EDS'76 virus was 25.42 per cent (Table 2). The present finding is in close agreement with those of Mohanty et al. (1984), and Reddy and Raghavan (1987). However, variations in seroprevalence of EDS'76 virus have been reported by several workers (Firth et al., 1981; Githkopoulos, 1983; Borzemska et al., 1986; Sukumar and Babu, 1986; Sharma, 1987; Shakya and Dhawedkar, 1991a). These variations in per cent prevalence of HI antibodies to EDS'76 virus may be attributed to method of

sampling, nature of infectious agent involved in a particular area, type of birds screened or the techniques employed.

The age prevalence of HI antibodies to EDS'76 virus was highest (32.72%) in chickens between an age group of 5-9 weeks. It is obvious that maternal antibodies to EDS'76 virus do not exist beyond 3 weeks of age (Gupta et al., 1985). Hence, the presence of HI antibodies to EDS'76 virus in 5-9 weeks of birds may be attributed to lateral spread of the virus and not due to vertical spread. Several workers have reported the highest seroprevalence of EDS'76 virus in 7 weeks old broiler birds (Meulemans et al., 1979; Sharma, 1987; Shakya and Dhawedkar, 1991a) which support the present observation. Such infected broilers flock might be the potential source of infection of EDS'76 virus to the susceptible flocks. The possibility of lateral spread of the disease has also been reported by Meulemans et al. (1979), and Cook and Darbyshire (1981). The incidence of antibodies to EDS'76 virus was lowest in chicken of 25 weeks and above age group. Sharma (1987), and Shakya and Dhawdkar (1991a) also reported relatively low incidence of EDS'76 virus antibodies in older birds. On the contrary several workers reported maximum prevalence of antibodies to EDS'76 virus in older birds (Reddy and Raghavan, 1987; Rhee et al., 1982; Borzemska et al., 1986).

The breed incidence of HI antibodies to EDS'76 virus was higher in commercial broilers (32.85%) and it was only 16.77 per cent in commercial layers. McFerran (1977) and Picault et al. (1982) reported increased susceptibility of broiler

breeders and heavy breeds of poultry to EDS'76 virus which probably explains the present findings. Sharma (1987), and Shakya and Dhawedkar (1991a) also reported higher incidence of antibodies to EDS'76 virus in commercial broilers which corroborate the present observation. The lower incidence of antibodies to EDS'76 virus in layers may be attributed to the fact that the layers included in the present studies had not attained peak production and were of the age group 26 weeks and below. It has been postulated that before attaining the age of peak production the virus remains in a quiescent state which gets reactivated when such birds come in peak production. McFerran (1979) observed that EDS'76 virus gets activated by stress of production and the birds in peak production exhibited apparent signs of infection mostly between 28 to 48 weeks of age.

Efforts to study the level of HI antibodies revealed that the HI antibodies titres ranged from 1:2 to 1:512. Similar observations were made by several workers (Yamaguchi et al., 1981a; Al-Hilly et al., 1982; Zsak et al., 1982; Mohanty et al., 1984; Gupta et al., 1985). The HI titres of the serum samples collected from selected broiler flocks ranged from 1:4 to 1:512 while the titres in the layer flocks varied from 1:4 to 1:64. The lower HI titres in layers may possibly be due to several factors including persistence of the virus in the infected birds in masked state without inciting any immune response and stage at which the serum samples were collected after infection.

However, further studies may be undertaken to arrive at definite conclusion.

The results of the present study demonstrated that EDS'76 is widespread in poultry flocks spread all over the state of Bihar. However, in none of the farms found positive for HI antibodies to EDS'76 virus any history of clinical signs of the disease were recorded. This in turn suggested wide spread prevalence of inapparent EDS'76 virus infections in the poultry flocks of the state. The occurrence of inapparent EDS'76 virus infection has also been reported by many other researchers (Rampin et al., 1978; Gupta et al., 1985; Shakya and Dhawedkar, 1991a).

McFerran et al. (1977) reported that HI test is simple, quick, and economical. The results are comparable to serum neutralization test. Accordingly, in the present study also HI test was used for the qualitative and quantitative studies of antibodies to EDS'76 virus. Adair et al. (1986) also advocated the application of HI test for seroepidemiological studies of EDS'76 virus.

There appears to be no consistency in appearance of clinical signs in EDS'76 virus infected chickens. However, clinical signs of transient diarrhoea, inappetance, and dullness in hens infected with EDS'76 virus have been reported both under experimental (Yamaguchi et al., 1981b) and field conditions (Van Eck et al., 1976; Fehervari et al., 1979; Rhee et al., 1982 and Higashihara et al., 1987). The observations

of these signs in infected birds in the present studies may be attributed to the virus replication in intestinal epithelial cells and some concomitant infection with intestinal microflora.

As obvious from table 7 the absolute egg production of EDS'76 virus infected hens was 74.86 per cent as against 86.71 per cent in control birds for the same period. The overall decline in egg production of infected birds was 11.85 per cent when compared with uninoculated control hens. The drop in egg production observed in the present study is characteristic of EDS'76 virus infection and has been reported by number of workers (McFerran, 1978; Meulemans et al., 1978; Darbyshire and Peters, 1980; Yamaguchi et al., 1981a and 1981b; Loupal et al., 1982; Rhee et al., 1982; Van Eck, 1982; Mohanty et al.,1984 and Higashihara et al., 1987) both under natural and experimental conditions. On the contrary, McCraken and McFerran (1978), and Van Eck et al. (1983) did not observe the influence of virus on overall daily egg production under experimental conditions. The differences in respect of effect of virus on absolute egg production may be attributed to a number of factors including type of virus strain used and its virulence, infective titre of inoculum as well as age and breed of birds employed for the purpose. Darbyshire and Peters (1980), Yamaguchi et al. (1981a) and Loupal et al. (1982) reported 15 to 20, 6 to 25 and 10.7 to 13 per cent drop in egg production respectively. During the present experiment fall in egg production ranged from 13 to 21.5 per cent between 11 and 40

observation of Darbyshire and Peters (1980). The overall drop in egg production was 11.85 per cent against 21.0 per cent reported by Rhee et al. (1982). However, this difference in average drop in egg production is likely as the observations of Rhee et al. (1982) are based on their investigation under natural conditions.

In the infected group, egg production fell from 88 per cent to 65 per cent between 10 and 19 days PI. As obvious from Fig. 1 drop in egg production commenced from 10 day PI and continued till 22 day PI after which there were signs of improvement in daily egg production. Though there were signs of improvement in daily egg production, from 23 day PI till the end of experiment, it did not reach a level comparable to uninfected control group of the same age. Several workers have reported the trend of gradual recovery in daily egg production to normal after the drop (McFerran et al., 1978; Rhee et al., 1982). On the contrary, McFerran (1979) and Mohanty et al. (1984) opined that EDS'76 virus infected hens did not attain normal level of production and the egg production remained at lower level than the previous peak which corroborates the present finding. The laying of abnormal eggs in EDS'76 virus infected group commenced one day before the onset of fall in egg production (Table 6). The present findings corroborate the observations of McFerran et al. (1978) that laying of abnormal eggs occurred concurrent with or preceded the depression in production. Yamaguchi et al. (1981b) and Higashihara et al. (1987) reported laying of abnormal eggs from 8 and 12.2 days

PI respectively as against 9 day PI observed in the present studies. It may be seen from table 8 that the abnormal egg laid during the present experiment included shell-less (13.79%), thin shell (29.88%), crack shell (34.48%) and small size eggs (21.83%). It may be noted that laying of abnormal shell eggs such as shell-less and thin shell eggs are observed quite frequently (McCraken and McFerran, 1978; Darbyshire and Peters, 1980; Yamaguchi et al., 1981a and 1981b; Loupal et al., 1982 ; Rhee et al., 1982; Lu et al., 1985; Higashihara et al., 1987) . However, laying of crack egg (Yamaguchi et al., 1981b; Rhee et al., 1982) and small size egg (McFerran et al., 1978; Loupal et al., 1982) have also been reported on more than one occasion in EDS'76 virus infected chickens. Further, as apparent from Table 6 laying of small size egg was observed in both the groups, infected and uninfected. However, in infected group per cent production of small size egg, was higher. McFerran et al. (1978) also reported increased percentage of small eggs in infected group of hens. Out of a total number of abnormal eggs produced, shell-less and thin shell eggs together constituted 43.68 per cent (Table 9). On the other hand several workers have reported laying of abnormal shelled eggs ranging between 14.3 to 27.0 per cent (Yamaguchi et al., 1981b; Van Eck and Vertommen, 1984; Sharma, 1987) whereas, Szeleszozuk (1988) reported 70.6 per cent abnormally shelled egg in EDS'76 virus infected group. In the present studies the period of laying abnormal egg was 20 days which was in close agreement with the finding of McCraken and McFerran (1978). However, at least in

one case (Yamaguchi et al., 1981b) this period was found to be much longer than that in the present experiment. These differences may be due to several factors including susceptibility of the hens to the virus, virulence of virus strain and infective virus titre of the inoculum. The decrease in mean egg shell thickness (Table 10), mean egg weight (Table 11) and mean egg volume (Table 12) were mainly confounded over the period of laying abnormal eggs by virus infected hens. The present observations are in accordance with that of McCraken and McFerran (1978) and Sharma (1987). The present findings further confirm that egg shell thickness, egg weight and egg volume may form important criteria for evaluating the effects of EDS'76 virus on egg production and egg shell quality.

Studies into pathogenicity of EDS'76 virus were carried out in 28 week old WLH hens. The birds were infected by ocular route and the infection could be established easily. Various workers have reported increased susceptibility of adult hen for EDS'76 virus (Gylstorff and Rolf, 1982; Friederichs et al., 1987; Sharma, 1987) and appearance of marked clinical changes in hens just before or soon after peak production (McFerran et al., 1978; Firth et al., 1981; Mohanty et al., 1984). In the present experiment also the birds were infected when they were close to peak production.

Almost all workers have reported the presence of pathological lesions in the female reproductive tract of hens

as the most consistent feature of EDS'76 virus infection (Fehervari et al., 1979; Bennejean et al., 1979; Gylstorff and Rolf, 1982; Friederichs et al., 1987). In the present investigation also the lesions in reproductive tracts appeared to be the most prominent finding. Appearance of gross pathological lesions in experimental EDS'76 virus infection is rather controversial. Van Eck et al. (1978), Fehervari et al. (1979), Van Eck (1982) and Heffels et al. (1982) did not observe any gross lesions in EDS'76 virus infected susceptible chickens. However, gross lesions of mild to moderate in nature were detected in ovary, oviduct, and spleen of infected hens during the present study. Taniguchi et al. (1981) also reported gross lesions in ovary, oviduct and spleen of EDS'76 virus infected hens which bore close similarity with the present findings. The reasons for variations in pathological changes are not known, however, the possible role of type of virus strain and susceptibility of birds may not be ruled out.

In the present study histopathological changes in the infected hens were confined to uterus, infundibulum, magnum, ovaries and lungs. Uterine changes were characterized by marked degenerative changes, and desquamation of the epithelial cells, oedema of mucosal folds, decrease in secretory glands, atrophy of uterine glands and infiltration of heterophils, lymphocytes and macrophages. The histopathological changes were most evident in the uterus followed by infundibulum and magnum, and there was almost no reaction in isthmus and vagina. Van Eck et al. (1978), Taniguchi et al. (1981), and Gylstorff

and Rolf (1982) also observed prominent changes in uterus of infected birds. Further, it was observed that histopathological changes were highly prominent on 10 and 20 days PI, the period when the production of abnormal eggs and drop in egg production were most marked. This further confirms the observations of Taniguchi et al. (1981) that the abnormal egg production in EDS'76 virus infected laying hens may be largely due to secretory disturbances of shell materials induced by atrophy of the uterine glands and degeneration and desquamation of the mucosal epithelium. Yamaguchi et al. (1981b) furhter suggested that the virus exerts direct effect on the uterus so that the uterus is unable to form the shell properly. Van Eck and Vertommen (1984) also postulated that functional disturbances which account for shell aberrations following EDS'76 virus infection are located in the surface epithelial cells of the uterine mucosa. Moreover, concerted studies on blood parameters, biochemical profile of uterine fluid and pathogenicity trials in susceptible laying hens may prove helpful in providing precise aswswer on the causative factors associated with production of the abnormal shelled eggs in EDS'76 virus infected hens.

Any change in blood parameters of EDS'76 virus infected hens may be considered indicative of metabolic disturbances in vital organs. In the present study significant decrease in serum calcium level of infected hens was recorded on 10 and 20 days PI (Table 13). The serum inorganic phosphate level was also significantly lower on 10 days PI when compared with uninfected control birds for the same period. Moorthy et al. (1987) also

observed significant decrease in serum inorganic phosphate level of chicks after EDS'76 virus infection. However, Van Eck and Vertommen (1984) did not observed the effect of this virus on various blood parameters indicating absence of metabolic disturbances in the vital organs. In the present study also virus failed to exert any influence on the levels of potassium, sodium, SGPT, SGOT, total cholesterol, total protein and serum alkaline phosphatase when compared with the values for non infected control birds. The present study suggested the effect of virus on serum biochemical profiles of infected hens to a limited extent. However, a meaningful conclusion is possible after going in further research on this aspect.

Though, the reduction in the level of calcium was most marked on 10 and 20 days PI, the period during which maximum number of abnormal eggs specially TSE and SLE were laid, it would be advisable to plan further studies on the influence of virus on various blood parameters. The future research may also include parameters like biochemical analysis of uterine fluid of infected birds to establish linkage between egg shell abnormalities and level of various biochemical constituents of blood serum as well as uterine fluid.

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CHAPTER - VI

SUMMARY

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A total of 39 flocks from 10 poultry farms distributed in different parts of Bihar were surveyed to ascertain the seroprevalence of EDS'76 virus. In all 645 serum samples were collected. These serum samples were subjected to HI test employing reference strain of EDS'76 virus (strain 127) to determine the presence of virus antibodies. A total of 164 serum samples were positive for EDS'76 virus antibodies and the overall prevalence of the disease was 25.42 per cent (Table 2). The flockwise incidence of the disease ranged from 10.00 to 46.66 per cent. The farmwise incidence was highest (37.50%) in farm 'A' and lowest (16.92%) in farm 'E'.

The age prevalence of HI antibodies to EDS'76 virus was highest in chickens of 5 to 9 weeks whereas, adult birds of 25 weeks and above showed the lowest (13.48%) positivity. The per cent prevalence of seropositive reactors in birds of 10-14, 15-19 and 20-24 weeks of age were 24.39, 23.52 and 17.53 respectively (Table 3). The breedwise incidence of antibodies to EDS'76 virus was 32.85 per cent in commercial broiler chickens and only 16.77 per cent in commercial layer birds (Table 4).

The HI titres of positive serum samples varied from $2 \log_2 - 8 \log_2$. In broilers the HI titres varied from 1:4 to 1:512 whereas, in layers the titres varied from 1:4 to 1:64. The flockwise geometric mean of HI antibodies titres ranged from 2.0990 to 5.5920 and the overall geometric mean of antibodies titres was 4.3905 (Table 5).

The results of the present study demonstrated the widespread prevalence of EDS'76 virus in the state of Bihar. It was further concluded that the disease mostly occurred in inapparent form as in none of the serologically positive farms any history of clinical signs of the disease could be recorded.

Fifty WLH laying hens of 28 weeks old were experimentally infected with 1024 HA units of EDS'76 virus (strain 127) intraocularly. An equal number of uninfected laying birds of the same breed was also maintained to serve as control. These birds were observed for appearance of clinical signs till 40 days PI. The egg production and egg quality were recorded daily.

EDS'76 virus infected birds showed mild to severe diarrhoea between 9th and 10th days PI, and most of the birds recovered within a day or two.The birds in the control group did not reveal any symptoms.

There was reduced egg production in virus infected hens as compared with uninfected control birds. The absolute egg production in infected group was 74.86 per cent as against 86.71 per cent in control group (Table 7). The overall decline in egg production was 11.85 per cent. The drop in egg production was observed from 10 days PI and continued till 22 days PI and thereafter, there was improvement in egg production. The mean egg production on 10 days basis for 40 days PI revealed highest drop in egg production (21.5%) during 11 to 20 days PI followed by 21 to 30 days PI (17.33%) and 31 to 40 days PI (13.00%).

This virus also showed its effects on quality of eggs. The infected hens laid abnormal eggs which included TSE, SLE, crack or small size eggs. The laying of abnormal eggs was first noticed on 9th days PI and continued till 28th days PI (Table 6). Out of a total of 87 abnormal eggs laid 26(29.88%) were TSE, 12 (13.79%) were SLE, 30 (34.48%) were crack and 19 (21.83%) were small size eggs. The overall laying of abnormal eggs was 16.60 per cent (Table 7) which included 4.96 per cent TSE, 2.29 per cent SLE, 5.73 per cent crack eggs and 3.62 per cent small size eggs. It was further observed that TSE and SLE together constituted 43.68 per cent of the abnormal eggs laid. The uninfected control hens did not lay abnormal eggs except laying of a few small size eggs (Table 6).

The mean egg shell thickness of infected hens was significantly lower ($P < 0.01$) than the corresponding value for uninfected control birds between 21 and 30 days PI. These value did not differ significantly over other periods (Table 10). The mean egg weight of infected hens was significantly lower ($P < 0.01$) than the corresponding value for control birds between 11 and 20 days PI as well as 21 and 30 days PI (Table 11). There was no significant difference in mean egg volume of infected hens when compared with uninfected hens (Table 12). However, in view of the varying reports in respect of egg shell thickness, egg weight and egg volume, it is suggested that further studies may be undertaken before considering these parameters as criteria for evaluation of effect of EDS'76 virus in chickens.

The pathological changes produced by this virus were mostly confined to ovary and oviduct. Macroscopically the changes were characterized by atrophy of ovary and oviduct, hyperaemia of lungs and slight spleenomegaly on 10th and 20th days PI. Microscopically most conspicuous changes were noted in the oviduct and specially in the uterus of EDS'76 virus infected birds sacrificed on 10th and 20th days PI. There was oedema of mucosal folds, decrease in secretory glands and marked degenerative changes in the uterus of infected hens. Desquamation of epithelial layer of uterus, atrophy of uterine glands (Fig. 7) and lymphocytic infiltration in the uterine mucosa were other important histopathological changes. Infundibulum and magnum also revealed histopathological changes of varying degree. On 30th and 40th days PI lamina propria of oviduct of infected hens showed mild to moderate infiltrations of mononuclear cells with occasional distribution of heterophils (Fig. 8). The ovarian follicles appeared attretic. The lungs revealed changes of haemorrhagic pneumonia on 10th and 20th days PI (Fig. 11). No changes were noted in tissues sections of liver and spleen.

The results of studies on effect of EDS'76 virus on serum biochemical profile of laying hens showed significant decrease in the mean calcium level of infected hens on 10 and 20 days PI when compared with the corresponding values for control birds. The mean serum organic phosphate level of infected hens was significantly lower than uninfected control

birds at 10 days PI ($P < 0.05$). However, there was no significant difference in the serum level of potassium, sodium, SGPT, SGOT, total cholesterol, total proteins and serum alkaline phosphatase between infected birds and uninfected control birds. Nevertheless, it would be advisable to plan further research on the effects of virus on various serum biochemical constituents before establishing their correlations with egg shell changes as observed in EDS'76 virus infected hens.

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