

Dept. of Biological Sciences,
Quaid-i-Azam Univ., Islamabad, Pakistan,
Head of Dept. Dr. T. Javed.

**PERSISTANCE AND MORPHOPATHOLOGICAL STUDIES ON
INFECTIOUS BRONCHITIS IN CHICKENS IN PAKISTAN**
(With 4 Tables & 7 Figs.)

By
T. JAVED; M. SIDDIQUE* and A. HAMEED
(Received at 28/10/1990)

دراسات على انتشار مرض الالتهاب الشعبي المعدي
في الدجاج بباكستان ووصف المصروف التشريحية والهستوباثولوجية للمرض
طارق جاويد ، محمد صديق ، عبدالحמיד

خلال فترة زمنية (عام) عمل استبيان لعدد ٢٢ قطع دواجن بحالات مرضية مختلفة
لدراسة معدل انتشار المشاكل التنفسية المتعددة، فمن بين ال ٢٢ المستبين وجد أن
١٨ (٨١.٨١٪) كانت ايجابية لمرض الالتهاب الشعبي المعدي . سجلت أعلى معدلات
لحدوث الإصابة (٣٥.٧١٪) في عمر ١ - ٥ أسابيع وتم تسجيل أعلى معدلات حدوث بنسبة
٦٦.٦٦٪ في موسم الشتاء. وتمت دراسة الصفة التشريحية والصورة الهستوباثولوجية لمرض
الالتهاب الشعبي المعدي وتركزت الإصابة في الحنجرة ، الشعب الهوائية ، الجيوب الأنفية
القنطرة الهوائية ، الرئة ، قناة انبيض ، الكلية والاكياس الهوائية.

SUMMARY

During one year period 432 chicken flocks with variable disease conditions were surveyed for the prevalence of various respiratory problems. Among 432 flock surveyed 18(4.16%) were positive for IB. Regarding agewise incidence maximum (35.71%) infection was recorded in 1-5 week age group. Seasonwise maximum (66.66%) incidence was recorded in winter season. Maximum IBV was isolated from lungs, trachea and kidneys. IBV affected embryos showed curling and dwarfing alongwith severe haemorrhages on the egg shell membrane. Different physico-chemical parameters of eggs from hens infected with IB were significantly different from normal eggs. Studies of gross and histopathological alterations in different visceral organs of chickens affected with infectious bronchitis were studied. In IB trachea, larynx, lungs, oviduct and kidneys were the target organs.

* Department of Microbiology, University of Agricultural Faisalabad.

T. JAVED, et al.

INTRODUCTION

Viral diseases of respiratory tract, like infectious bronchitis, newcastle disease and infectious laryngotracheitis have greater economic significance (TARIQ, et al. 1989). Clinical manifestations and gross pathological changes in these diseases are very similar, thereby there is difficulty in differential diagnosis. Direct air born transmission is the most usual method of spread. Infectious bronchitis is egg borne and infection is transmitted to the next generation causing serious implications in hatcheries (LUKERT, 1980).

Avian infectious bronchitis was first reported by SCHALK and HAWN (1931). DELAPLANE and stuart (1939) reported the disease for the first time in growing and adult birds. During 1940's infectious bronchitis was a serious respiratory disease of laying flocks and caused marked losses in egg production. Now the disease is prevalent throughout the world, (LUKERT, 1980). Prodicularly in Italy (MARCHI and ZANELLA, 1984), Japan (SAWAGUCHI, et al. 1984), Gera (EISENGAITEN, 1964). Sudan (GAFFAR ELAMIN, et al. 1986), India (MALIK and VERMA, 1969), Poland (KAREZEWSKI and CAKALA, 1967), Pakistan (JAVED and SIDDIQUE, 1990), Turkey (SAYIM, et al. 1988) and Yugoslavia (VELHNER, et al. 1988).

Infectious brouchitis is highly contagious acute infection of upper respiratory tract affecting brids mostly upto 3 months of age (ROSZKOWSKI, et al. 1986). Infection may however, persist in some birds for many months and outbreaks may occur under any stress conditions (GAFFAR ELAMIN, 1986). There may be aberrant development of the oviduct with partial or complete failure of the development of oviduct resulting in reduction in egg production and poor quality eggs (JAVED and SIDDIQUE, 1990).

Keeping in view the importance of this disease of the respiratory tract, this project has been designed to study the incidence, clinical findings, gross and histopathological details of infectious bronchitis.

MATERIAL and METHODS

Survey:

Various poultry farms in and around Faisalabad, Pakistan were surveyed and the birds showing clinical respiratory distress were selected to study the incidence of IB by isolation, serology, and pathology of Infectious Bronchitis (IB). A total of 432 flocks were taken for survey. These flocks consist of 135 broiler flocks, 7 broiler breeder flocks and 290 layer flocks. Details about the history of flock and managemental conditions were recorded. Serum samples, eggs, respiratory exudates and tissues from sick and dead birds were collected for further studies.

INFECTIOUS BRONCHITIS IN PAKISTAN

Serology:

Among serological studies lyophilized infectious bronchitis antigen prepared from strain H-52 was obtained with the courtesy of centre of Research and Bio-preparation, volutar, pasteur Institute, Bucharest, Romania. Antigen was propagated in embryonated chicken eggs of 9-11 days. Haemagglutination with chicken R.B.Cs. Trypsin induced infectious bronchitis haemagglutination test was performed (KAHRAMAN, 1988). Indirect haemagglutination Inhibition (IHI) test, Agar gel ppt test (WITTER, 1962), Micro ppt test (BAUDITZ, 1963) were main confirmatory tools by using IB positive antisera.

Virus Isolation:

Respiratory exudates and triturated tissue samples were inoculated for isolation of virus and study the embryonic changes.

Gross and Histopathological Studies:

Different visceral organs from birds confirmed through different tests as IB affected were examined for the gross and histopathological studies. Selected pieces of 5-6 mm size from various morbid organs were fixed in 10 per cent formalin solution. The fixed tissue pieces were dehydrated through ascending grades of ethyl alcohol, cleared in xylol, infiltrated in melted paraffin for 12 hours and tissue blocks were prepared. Section of 5-7 μ m thickness were cut with rotary microtome with disposable microtome knife (SABRI, *et al.* 1986).

Physico-Chemical parameters:

Physio-chemical parameters affected and that of normal hens were included, weights of egg, shell, albumin and yolk according to ZUBAIR (1985). Specific gravity of the eggs was recorded with the help of gravitometer, surface tension of the albumin with "Due Novy", torsion balance. Viscosity of albumin was done with the viscometer (FAZAL and ALI, 1984). Total proteins and cholesterol contents of eggs from hens suffering from IB and normal eggs were determined.

RESULTS

Are presented in tables 1-4 and figures 1-7.

DISCUSSION

During the one year period a total of 432 chicken flocks with variable disease conditions were surveyed for the prevalence of IB, out of them 135 (31.25%) broiler flocks and 7(1.62%) broiler breeder flocks. Antibodies against infectious bronchitis

T. JAVED, et al.

were determined by indirect haemagglutination inhibition, microprecipitation and agar gel precipitation tests. Out of 432 flocks surveyed, 18(4.16%) flocks were positive for antibodies against IB. Among 290 layer flocks 10(3.44%) were sero positive for IB. Similarly in broiler flocks incidence of IB was 5.18 percent. Out of 7 breeder flocks surveyed, only one was positive for infectious bronchitis. GAFFAR ELAMIN, et al. (1986) reported 33 per cent prevalence of infectious bronchitis in Sudan. Incidence of IB in different types of birds is summarised in Table I.

Regarding agewise incidence of infectious bronchitis, 11(22.00%) flocks were of 1-5 weeks age; 5(35.71%) of layers and 6(17.14%) of broilers. Among the age group of 6-10 weeks, only 1(6.25%) broiler flock was positive (Table II). Only one (3.5%) layer flock was positive inbetween the age of 11-20 weeks and 3(12.50%) between 21-40 weeks. In age group 41-60 weeks one of layer and chicken breeder flocks (each) was positive. Among the areawise incidence of infectious bronchitis, it varied from place to place. Prevalence of particular viral diseases in particular areas has documented by MALIK and VERMA (1969).

Incidence of infectious bronchitis influenced by the change in seasons. Maximum incidence was recorded in winter season (66.66%), followed by 17.24 per cent in spring season, while it was lower in summer (3.44%) and autumn (11.11%). In layers it was maximum (20.0%) in broiler in winter season (JAVED and SIDDIQUE, 1987).

Isolation from 18 positive flocks was confirmed by different serological tests. Lungs, tracheas and kidneys were collected and preserved in glycerine. Two hundred and seventy three samples of lungs, trachea and kidneys were taken for isolation of infectious bronchitis virus. Out of total samples inoculated, 17 were positive for IB giving an isolation incidence of 6.22 per cent (Table III). Out of these samples, 10 lungs (10.90%) 5 tracheas (5.0%) and 2 kidneys (2.19%) were positive. Maximum (6.97%) isolation was from layers followed by broiler (5.95 per cent).

Infective materials confirmed by isolation were injected into 10-12 days old embryonated hen eggs and subsequently incubated. Curling and dwarfing of the embryos was very typical in most of the cases after the 1st pasage. (Fig. 1). Severe haemorrhages on the shell membrane were very common in many cases (Fig. 2). Lungs showed pneumonic changes, liver revealed typical necrotic foci (JAMIL, et al. 1986).

Infectious bronchitis in most of the flocks started as respiratory distress, difficult breathing with stretched neck, rattling sounds, sneezing, ocular and nasal discharge and these symptoms were rapidly followed by dullness and in ultimate stages development of diarrhoea and high quantity of uric acid alongwith drop in production with mis-shapen and thin shelled eggs (Fig. 3 and 4). Morbidity was in more than 25 per cent of the flocks up to 100% while mortality varied from 3-8 per cent. Daily mortality in most of the flocks varied from 02 to 5.91 per cent. (GAFFAR ELAMIN, 1986)

INFECTIOUS BRONCHITIS IN PAKISTAN

reported 25 per cent mortality in chicken. Recovery in most of the flocks took 2 weeks. Drop in egg production was recorded (12.84 to 37.28 per cent). Egg production came to pre infection level after 3 weeks (CAVANAGH, 1988).

Different physico-chemical parameters of eggs from hens infected with infectious bronchitis were studied. Affected flocks laid 0.5 to 1 per cent abnormal mis shapen and disfigured eggs. Approximately 50 per cent mis-shapen eggs were thin shelled and cracked easily (Fig. 5). Physico-chemical parameters including total weight, weight of shell, weight of albumin, weight of yolk, specific gravity, viscosity, surface tension, total proteins and cholesterol were compared with those of eggs from normal hens (Table V). All the physical and chemical parameters were lower in IB affected eggs as compared to normal eggs and difference was statistically highly significant. Albumin of infectious bronchitis affected eggs have spreading nature (Fig. 6) and poor storage quality (CAVANAGH, 1988).

In IB affected birds, trachea and bronchi were severely inflammed with excessive mucous in sinuses and in tracheal lumen. The lungs appeared congested and pneumonic. Some of the birds showed cloudiness of the thoracic air sacs and sinusitis. Urate crystals were also seen in some ureters. Bursa of fabricuis showed haemorrhagic spots in a few cases. These findings are in line with the findings of CHANDRA, et al. (1981).

Glandular depletion of the internal surface of the oviduct especially that of isthmus was recorded in laying birds. This may be the most important factor of mis-shapen, disfigured and irregular eggs as also reported by HAGEN and BRUNER (1961) and JAVED and SIDDIQUE (1990).

In IB affected larynx and trachea cellular infiltration with plasma cells, histiocytes and lymphocytes were the main histological lesions recorded. Vascular congestion, oedema and hyperplasia of the epithelium were also common (Fig. 7). These findings are almost in line with that of GOUFFAX, et al. (1977) and DUCATELLE, et al. (1984).

Infectious bronchitis positive lungs showed pneumonic changes, leukocytic infiltration and slight exudation. Bronchitis was common lesion observed. These findings are identical with those reported by DUCATELLE, et al. (1984).

In IB affected birds kidneys showed inflammatory changes, tubules appeared distended with crystals of uric acid. These observations are close to the findings of CHANDRA, et al. (1981) and SIDDIQUE, et al. (1987).

The picture might be aggravated by the presence of other disease. Precise study might be done with SPF.

REFERENCES

- Bauditz, T. (1963): Microprecipitation test for the diagnosis of avian infectious bronchitis. *Dtsch. Tierarztl. Wschr.*, 70: 605-607.
- Cavanagh, D. (1988): International symposium on infectious bronchitis. *Poultry international*, 27 (12): 32-36.
- Chandra, M.; S. Balwant and S. Gurkirpal (1981): Pathology of experimental nephrosis - nephritis in chicks induced by infectious bronchitis virus. *Indian J. Exp. Biol.*, 19: 508-510.
- Delaplane, J.P. and H.O. Stuarts (1939): Studies of infectious bronchitis in Rhode Island. *Agri. Exp. Station. Bull.*, 273.
- Ducatelle, R.G.; Meulemans, W. Coussement and J. Hoorens (1984): Aetiopathology of the fowl trachea early after inoculation with H 52 infectious bronchitis virus. *Zbl. Vet. Med. B.*, 31 (2): 151-160.
- Eisengaiten, H.J. (1964): Study of incidence and distribution of avian infectious bronchitis in Gera. *Microbiol. Vet. Med.*, 19: 771-773.
- Fazal, I.H. and M. Ali (1984): *Physical chemistry* 2nd ed. M.R. Brothers Urdu Bazar Lahore, Pakistan.
- Gaffar-Elamin, M.A.; A.K. Elmubark and H. Elsayed (1986): The isolation of infectious bronchitis virus from a disease outbreak in chickens in Eastern Sudan. *Bulletin of Animal Health and production in Africa*, 34 (3): 181-183.
- Gouffax, M.; H. Vinderogel; G. Meulemans; A. Dewaele and P. Halen (1977): Element du diagnostic histopathologique differential des principale affection respiratoires de la povie, *Avian pathol.*, 6: 61-76.
- Hagen, W.A. and D.W. Bruner (1961): Avian infectious bronchitis. *Infectious Diseases of Domestic Animals*, 4th ed. comstock Pub. Associates, N.Y. 955.
- Jamil, A.; M. Ashfaq; M. Irfan and A. Haq (1986): Incidence of infectious bronchitis in poultry. *Pakistan Vet. J.* 6 (1): 19-20.
- Javed, T. and M. Siddique (1987): Prevalence, diagnosis and pathology of various poultry diseases prevalent in Punjab. *Proceeding of 1st Inter Provincial Seminar on Animal Health and Production*, VRI, NWFP, Pakistan.
- Javed, T. and M. Siddique (1990): Comparative pathological studies on Newcastle disease, infectious bronchitis and infectious Laryngotracheitis in chickens. *Pakistan Vet. J.* 10 (4): 155-165.
- Kahraman, M. (1988): Haemagglutination-inhibition titres in mice infected with the Hitchner B Strain of Newcastle disease virus. *Etlik Veteriner Mikrobiyoloji Dergisi*. 6 (2): 77-82.
- Karezewski, W. and A. Cakala (1967): Serological studies on the spread of avian infectious bronchitis in Poland. *Medycyna Wet.* 23: 475-480.
- Lukert, P.D. (1980): In isolation and identification of avian pathogens by S.B. Hitchner, pp. 70-72. Creative printing Company Inc., 2011 East Main Street, Endwell, New York 13760, USA.

INFECTIOUS BRONCHITIS IN PAKISTAN

- Malik, B.S. and K.C. Verma (1969): Co-existence of antibodies against CRD, LTD and IB on poultry farms of Uttar Pradesh, Andhra Pradesh. *Avian Diseases.*, 13: 695-699.
- Marchi, A. and D. Zanella (1984): Avian infectious bronchitis, isolation of a new sero-type and its distribution. *Chinese Veterinaria*, 107 (3/6): 121-125.
- Roszkowski, J.E.; Karpinska, W.; Kozaczynski (1986): Identification of H 120 and H 52 strains of infectious bronchitis virus by histopathological method. *Bulletin of the Veterinary Institute in Pulawy* 28/29 (1-4): 77-81.
- Sabri, M.A.; T. Javed; M.A. Chishti and G. Hur (1986): Use of disposable knife in routine microtomy. *Pakistan Vet. J.*, 6 (3): 129-130.
- Sawaguchi, K.; S. Iwaki; K. Mikazaki; S. Vachida; S. Aoyama; N. Takahashi; Y. Iritani; T. Kitabataki and K. Kawasaki (1984): Survey of respiratory diseases among chicken flocks in Japan for avian infectious bronchitis, *Jap. J. Vet. Sci.*, 45 (4): 767-774.
- Sayim, Y.; A. Akman and H. Girgin (1988): Isolation and epidemiological studies on infectious bronchitis, infectious laryngo tracheitis, infectious bursal disease, egg drop syndrome 76, avian encephalomyelitis and adeno-virus infections of chicken in the Ankara region.
- Schalk, A.F. and M.C. Hawn (1931): An apparently new respiratory disease of baby chicks. *JVAMA*, 78: 413-422.
- Siddique, M.; T. Javed and M.A. Sabri (1987): Incidence and pathology of various poultry diseases prevalent in Faisalabad and surrounding districts. *Pakistan Vet. J.*, 7 (4): 148-154.
- Tariq, N.; T. Javed and M. Siddique (1989): Prevalence of respiratory problems in chickens in Faisalabad and surrounding districts. *Pakistan Vet. J.*, 9 (4): 162-164.
- Velhner, M.; J. Nikolovski and X. domi (1988): The health situation and disease problems in poultry production in Serbia in the period 1985-87. *Godine Peradarstvo*. 23 (7-8): 198-202.
- Witter, R.L. (1962): The diagnosis of infectious bronchitis of chicken by agar gel precipitation test. *Avian Diseases*, 6: 478-492.
- Zubair, S. (1985): Comparative study on the meat quality trials of desi breed, of chicken vs Lyallpur Silver Black (LSB), Plymouth Rock and White Leg Horn. Ph.D. thesis, Faculty of Animal Husbandry U.A. Faisalabad, Pakistan.

T. JAVED, et al.

Table (1)
Incidence of infectious bronchitis among flocks with respiratory
problems in different type of chickens

TYPE OF FLOCK	NUMBER OF FLOCKS			PERCENTAGE	
	Total surveyed	With resp. problem	IB positive	Out of surveyed	Out of resp.
Layer	290	85	10	3.44	11.76
Broiler	135	51	7	5.18	13.72
B.Breeder	7	4	1	14.28	25.00
Total	432	140	18	4.16	12.85

Table (2)
Number (Percentage) of flocks positive for infectious bronchitis
in different age groups

TYPE OF FLOCKS	AGE IN WEEKS					TOTAL (%age)
	1-5	6-10	11-20	21-40	41-60	
Layer	5 (35.71)	-	1 (3.57)	3 (12.50)	1 (25.00)	10 (11.76)
Broiler	6 (17.14)	1 (6.25)	-	-	-	7 (13.72)
B.Breeder	-	-	-	-	1 (50.00)	1 (25.00)
Total	11	1	1	3	2	18
%age	(22.00)	(3.12)	(3.57)	(12.50)	(33.33)	(12.85)

INFECTIOUS BRONCHITIS IN PAKISTAN

Table (3)
Organwise isolation of infectious bronchitis virus from different tissues
of layers, broilers and B. breeder

TYPE OF FLOCK	SOURCE OF SAMPLE	TOTAL SAMPLES	NO. OF POSITIVE SAMPLES	NO. OF TOTAL POSITIVE SAMPLES	PERCENTAGE OF POSITIVE SAMPLES
LAYER	Lungs	43	5		
	Trachea	43	3	9	6.97
	Kidney	43	1		
BROILER	Lungs	29	3		
	Trachea	28	1	5	5.35
	Kidney	28	1		
B. BREEDER	Lungs	20	2		
	Trachea	20	1	3	5.00
	Kidney	20	0		
TOTAL		273	17	17	6.22

Table (4)
Different physico chemical parameters of normal and infectious
bronchitis affected chicken eggs

Total weight (gms)	Shell weight (gms)	Weight of Albumin (gms)	Weight of yolk (gms)	Sp. Gravity	Surface tension (dyne/cm ²)	Proteins (gms/100 gms)	Cholesterol (gms/100gm)								
X ₁	X ₂	X ₁	X ₂	X ₁	X ₂	X ₁	X ₂								
53.7156	59.3279	9.0967	6.4673	34.5255	32.2027	25.4162	18.3050	1.045	1.035	63.50	53.45	12.50	11.50	10.50	8.50
53.7354	63.2157	8.9596	6.2534	35.5720	31.3702	23.4056	21.2849	1.043	1.032	64.15	52.92	12.27	11.40	9.30	8.30
53.7244	59.3135	7.6995	6.4657	33.5255	31.8998	24.1251	19.2213	1.050	1.034	63.95	53.25	11.90	11.50	8.50	7.30
59.7182	64.0005	7.7215	5.2375	36.1521	32.7754	19.2213	21.6109	1.047	1.031	54.25	52.75	12.30	11.70	11.05	5.50
62.7135	54.3435	9.3125	7.2451	25.9137	31.2756	22.1314	18.6937	1.048	1.040	65.07	53.12	12.20	11.50	10.80	9.10
53.3596	62.3739	8.9852	5.9739	34.7325	32.1082	25.4152	22.2675	1.050	1.040	64.36	54.03	12.50	11.40	10.30	10.10
53.5634	59.3379	7.6125	6.5321	34.5342	31.2043	24.7516	17.3316	1.048	1.036	62.58	52.92	12.60	11.25	10.12	9.30
55.5012	63.0549	9.5510	6.2570	35.5297	31.4333	25.5152	21.2132	1.050	1.040	63.59	54.12	12.80	12.00	10.60	8.70
59.3036	59.2571	8.9756	5.9895	32.1920	30.9147	20.0193	21.0756	1.045	1.031	56.12	53.65	12.20	10.50	10.70	5.30
52.7193	59.3053	9.0021	6.4683	34.5913	30.6985	23.0132	19.2469	1.048	1.034	55.65	52.95	13.00	11.35	9.30	7.30
X	63.3622	3.7015	6.3999	34.0369	31.5783	23.3025	19.3255	1.0474	1.0353	64.339	53.296	12.427	11.91	10.11	7.50

X₁. NORMAL VALUES
X₂. INFECTIOUS BRONCHITIS AFFECTED.

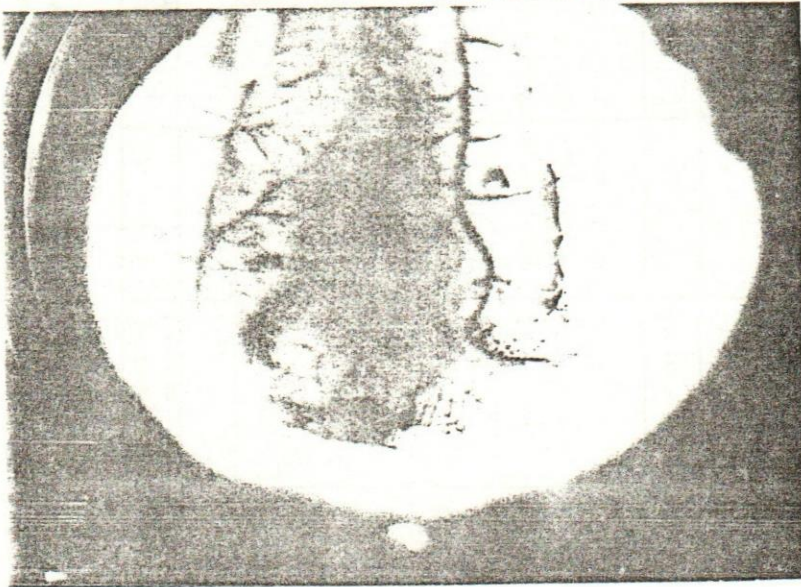
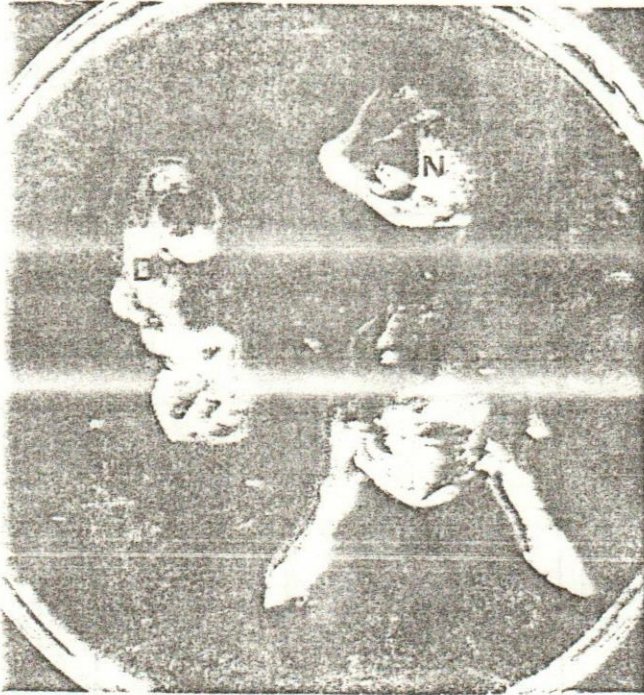


Fig. (1): Normal (N) and IBV (D) inoculated embryo showing dwarfism and stunted growth.
Fig. (2): Haemorrhages on shell membrane in IBV inoculated embryonated egg.

INFECTIOUS BRONCHITIS IN PAKISTAN

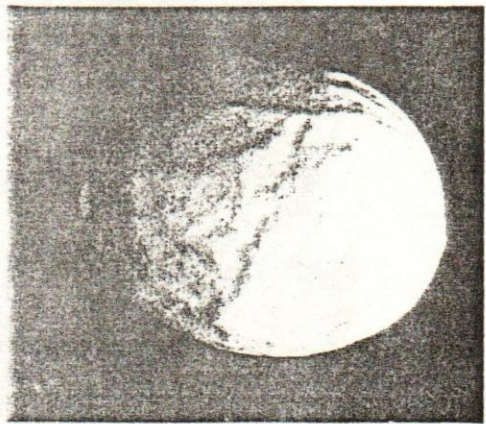
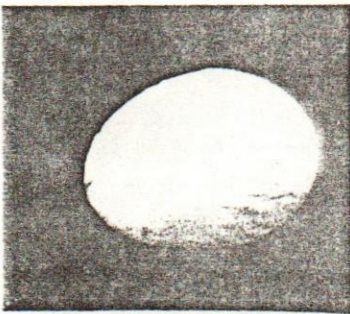
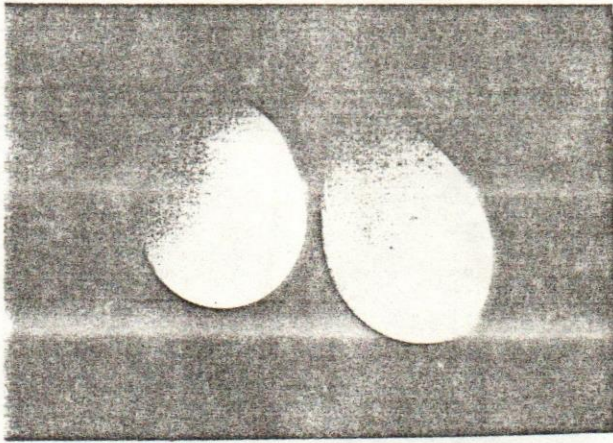


Fig. (3): Mis-Shapen eggs from IB effected layer.

Fig. (4): Thin shwelled egg from IB affected layer.

Fig. (5): Crackes, ridges and groves present on the egg shell from IB affected hen.

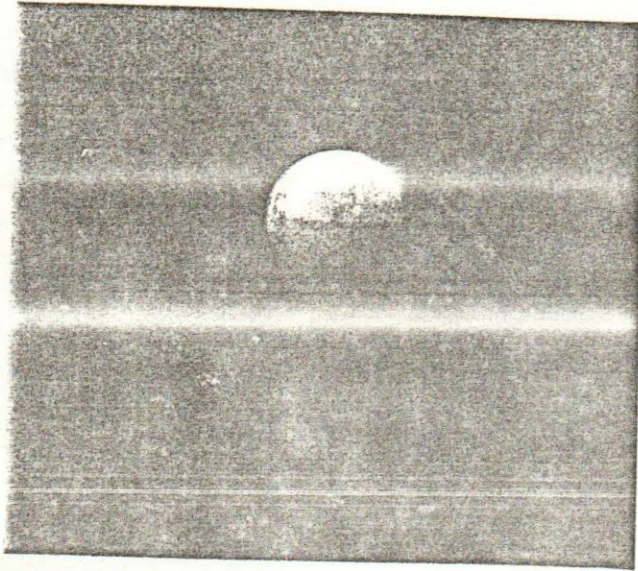


Fig. (6): Watery albumin in IB affected egg.

Fig. (7): Trachea showing leukocytic infiltration (L) sloughing of mucosa (S) and haemorrhages (H).