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INFECTIOUS BURSAL DISEASE IN PIGEON AND CHICKEN

(With 3 Tables and 16 Figures)

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مرض التهاب الغدة الفابريسية في الحمام والدجاج

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تم عزل فيروسين من الحمام والتأكد منهم بالاختبارات المختلفة وتم استعمالهم في عدوى كل من الحمام والدجاج واستخدم الاختبار الترسيبي والاختبار المناعي المشع والاختبار اليروكسيداز والمناعي للتأكد من وجود الأنتيجين في الأنسجة . تم عمل فحص الدم وكذا الهيستوباثولوجي كما تم عمل الـ Dott ELISA على السيرم.

SUMMARY

Two infectious bursal disease viral isolate were isolated from pigeon characterised and used experimentally to infect both chickens and pigeons. Agar gel precipitation immunofluorescence and immunoperoxidase tests were used for detection of antigen in 38 tissues. Heamatological and histopathological picture were described. Dott ELISA was used for detection of antibody in sera in 96 serum sample.

Key words: *Infectious bursal disease in pigeon and chicken*

INTRODUCTION

Infectious bursal disease (IBD) is either acute contagious viral disease or with subclinical course.

Allan et al. (1973) and Gaimborne (1978) reported on an experimental infection in Turkey with sterotype 1 IBD virus. The existence stereotype infects both Turkeys and chickens was firstly recorded by Mcferran et al. (1980).

Natural infection of ducks has been recorded by Mc Nulty et al. 1979 and Mcferran et al 1980. Experimental infection of ducks, revealed their resistance to infection. McFerran et al., 1980; Yamado et al., 1982; Eddy., 1990; Okoye et al., 1990 and Khafagy et al. (1995).

Vindvogol (1979) and Fritzche et al., 1981 stated that pigeons resistant to IBD. Yamada et al. (982) failed to induce clinical sings in SPF ducks inoculated with IBDV of chicken origin and the inoculated ducks respond only by developing neutralizing and precipitating antibodies.

Vindvogol (1979) failed to infect 4-week-old pigeons with IBDV from chickens. The viral antigen could not be detected in bursa and the serological tests were negative. Natural infections of turkeys and ducks have been recorded basing on serologic evidence and isolation of IBDV from these species Page et al., 1978, Mc Nalty at al., 1979, Johnson et al., 1980, McForron et al., 1980; Perlman and Heller, 1981).

Louzis et al. (1979) recorded an outbreak of natural IBD in artificially reared pheasants with mortality of 2-80%.

Hirose and Hirai (1976) found no antibodies against IBDV in egg yolk from quail, ducks, geese, bantame and pigeons. Nawothe et al. (1978) detected no serologic evidence of IBDV infection in turkeys guinea fowl and some wild avian species.

Ezeifeka et al. (1992) examined sera from wild and domestic birds for ND and IBD by H I and AGPT, ten of sera were positive for ND and only (58.3%) had antibody against IBD virus.

Maidugue et al. (1992) studied the prevalence of infectious bursal disease in endogenous chickens and pigeons. The auther stated that non of the pigeon sera was found positive by agar gel precipitation test.

Khaphagy et al. (1995) failed to induce the clinical disease in ducks and pigeons. The ducks responded serologically by developing antibodies while pigeon failed.

MATERIAL and METHODS

Agar gel precipitation test:

The test was carried out to the method described by Anon 1971 1.2 agarose was dissolved in phosphate buffered saline in 8.5% sodium chloride and adjusted to 7.2 pH. The medium was poured in petridishes. After solidification wells were done. The known reference antisera were put in the central well and surround by tested Anigen and the opposite were done with sera. The petridish's were put in humid chamber at 37°C and periodically examined during 3 days, for the prescence of specific lines.

Sera:

Reference antiserum was kindly applied by Dr. Sabry Infectious bursal disease in pigeon and chicken.

Virus isolation:

This was done in 10-day-old embryanated chicken egg by Chorio Allantoic membrane inoculation as well as in chicken embryo fibroblast cell culture, using tissue homogenate as inocula; 3 blind passages were carried out to detect IBDV.

Thermostability test:

Isolates in the form of tissue cultures were subjected to 3 cycles of freezing and thawing and centrifuged, distributed in tubes of 1 ml. Per tubes, incubated in water path at 56°C for 5, 10, 15, 30, hours, 2 hour minutes and an and two fours and samples were checked for infectivity by tissue culture inoculation.

Sensitivity to ether and chloroform:

Ether sensitivity was carried out according to the method described by Andrews and horstman (1949).

Chloro form sensitivity technique used was that described by Fieldman and Wong (1961).

Haemagglutination activity:

The isolates were tested for HA activity against chicken, duck, goat, rat, mice, guinea pig, and rabbit erythrocytes according to Anon (1971).

Neuturalizatim test:

This was carried out in chicken embryo fibroblasts (CEF) in micrometer plates.

Reference serum was triturated (serial dilution of serum constant amount of antigen, 100 $TCID_{50}$.

Serial ten fold dilutions of antigen constant amount of triturated reference serum diluted 1:10 in MEM, after 30 minutes 0.05 ml. Of CEF added each plate, the plate were incubated at 37°C in Co_2 incubator for 3 days, suspension was checked daily for neutralization indexes were calculated.

Tris buffer, pH 7.8 (Ed & David, 1988):

Tris base	6.55 gm.
Sodium chloride	11.68 gm.
* Adjust pH to 7.8 using 1 N HCl.	
Distilled water to	1 Liter.

Dot ELISA Blocking Buffer:

3% NFDM in Tris.

Dot ELISA Diluting Buffer:

0.5% NFDM in Tris containing 0.05% tween 20.

Dot ELISA washing Buffer:

4 Tris containing 0.05% tween 20.

Substrate solution:

4 - Chloro - 1 - naphthol.	30 mg.
Pure methanol.	10 mg.

* Keep in dark brown bottles at 4°C for not more then 2 weeks.

Experimintal design:

Group of pigeon was inoculated with isolates and chickens were kept incontact other group of chicken and was infected pigeon were kept in contact after 24-48 hours 4, 12, 28 day 25% from each group were scarified and post mortem from lesion histopathology slids from immune phlosenscence, imumoperoxidase, electron microscope were done from kidney and bursa showing lesion. Serum examined with dott elisa.

Indirect Immunoperoxidase test (I-IP):

Indirect in immunoperoxidase test (p)

The basic technique by Hyera et al. (1987) was adopted with some modification.

1- Paraffin section slides were prepared.
2- The slides were overlaid with 1% H_2O_2 in PBS' Van Duejin (1957).
3- The slides were then thoroughly washed with PBS three times 5 minute each to remove the remaining H_2O_2.
4 - Slides were over lead in suitable volume of serum and incubated at 37% °C in humid shamber.
5- Slides were then washed three times with PBS 10 minute each.

6- Slides were again overlead with suitable volume of antichicken peroxidase conjugate diluted as recommended in PBS and incubated at 37°C in humid chamber for hour.
7- The slides were washed as in step 5.
8- The slides were finally over laid with substrate working solution for 20 min. to allow development ment of the color.
9- The reaction was stopped by washing with PBS.
10- The slides were dried and examined under ordinary light microscope. Cells showing brown coloration of cytoplasm were considered positive.

8 - Fluorescent conjugated anti chicken gamma globulin:

For use of indirect fluorescent antibody technique conjugated normal chicken gamma globulin was provided by Animal Health Institute (Dr Afaf. Amin).

Preparation of slides, fixation and staining:

Paraffin slides were prepared from experimentally infected pigeon and chickens bursas, livers, spleens, kidney, thymus, and tissue culture

The smears were mounted with hyperimmune sera and incubated in moist chamber at 37°C for 30 - 45 minutes.

The slides were gently washed 3 times in PBS (pH7.5) dried and mounted with conjugated gamma globulin and incubated again in moist chamber at 37°C for 30 - 45 minutes. The proposed slides were washed three times in PBS dried and conesed with 3% Evan's blue counter stain for few seconds.

After washing and drying the slides were examined in fluorescent microscope.

Dot-immunobinding assay (Dot-ELISA):

The tchnique was carried out according to (Hawkes et al., 1982) for detection of bovine parvovirus antigen.

a) Sheet preparation:

A nitrocellulose binding filter (Gelman Sciences, Biotrace, NT) was overlaid on the top of 96%- well microtitre plate, where light finger pressing was practiced on the paper giving depressions corresponding to the individual wells of the underlying plate (Handing of filter should be with gloves and forceps), The filter paper was then washed 5 minutes by gentle aggitation in distilled water and left to dry at room temperature.

b) Dotting:
3-5 µl of antigen were dotted on the circular depression of the membrane filter and left to dry at room temperature to enhance the binding of viral proteins. The membrane was then washed for 5 minutes with TBS on shaker.

c) Blocking:
This step was devoted for blocking non specific protein binding sites on the membrane. The membrane was immersed in blocking buffer and incubated at room temperature for 1 hour with slight shaking each 15 minutes.

d) Primary incubation:
The blocked membrane was flooded with 10 ml of serum. The membrane was washed with 3 changes of washing buffer, 10 minutes interval on a rapid speed shaker (to remove unbound antibodies).

e) Second blocking:
The membrane was immersed in a blocking for 15 minutes.

f) Secondary incubation:
The membrane was immersed with lgG peroxidase conjugate diluted 1:500 in the blocking, then incubated at room temperature for 2 hours on a slow speed shaker.

g) Washing:
The membrane was then washed for 30 minutes with 3 changes of washing buffer, 10 minutes interval on a rapid speed shaker.

h) Development:
The membrane was immersed working solution and in room temperature at room temperature in a dark place. Blue dots (positive colouration) were developed within 10-15 minutes on the membrane. The enzyme reaction was stopped by washing the membrane several times under running tap water and finally with distilled water distilled water and then dried and kept in a dark place. Negative reaction showed no colour on the membrane.

I - Light microscopy:
Tissue specimens from the bursa of fabricius, spleen, kidney, liver, thymus, thyroid and harderian glands were fixed in 10% neutral buffered formalin, dehydrated in ascending grades of ethyl alcohol, cleared in methyl benzoate and embedded in paraffin. Tissue sections of 5-7 u were stained with hematoxylin and eosin for light microscopical examination.

2 - **Electron microscopy:**
Sample from the bursa of Fabricius and kidneys were fixed in 5% Cacodylate buffered glutaraldehyde, post fixed in 2% osmic acid, dehydrated and embedded in epon. Semithin sections were stained with tolouidine blue and the ultrathin sections were contrasted uranylacetate and lead citrate and examined with Joel EM 100 CX II at 60 kV.

12- Determination of total protion (A.O.A.C, 1990).

13- Determination of total fat content (ISO,1973).

14- Determination of total moisture content (ISO,1973).

RESULTS

Isolate: In the present work 2 isolate were obtained from pigeon which showed reddness of Bursa.

Bursae was examined by Agar gel precipitation test and inoculated in embryos (CAM) showed oedema, congested blood vessels in legs and wings, liver, was pale and parboiled heart. Passage in chicken embry fibro blasts indicating cytapathic effect (agregation and rounding of 40% cell line) started from first passage.

Showing all aggregation, rounding of 40% of cells.

Heat resistance

Isolates were resistant to 56°C for 2 hours

Effect of chloroform and ether on virus

Isolate	Titer of isolate		
	Before	Ether	Chloroform
2	4.75	4.75	4.75
3	6.75	6.75	6.75

Heamagglutination:

Isolate did not show haemagglutination with chicken, duck, mice, rat, guineapig, sheep and rabbit RBCs.

Neutralization index

Virus	Neutralization index
2	2.50
3	1.5

Pathogenically:

After 4 days of experimental infection the pigeons appeared sick, decreased in weight and showed diarrhea. On post mortum examination, the kidneys were swollen, the bursae were congested and with peticheal hemorrhages one case the spleen appeared dark in colour and of mosiac appearance.

Infected chicken showed decrease in weight and whitish diarrhea and the post mortorm examination the liver were streaked with heamorhage, kidneys-showed nephrosis (Fig. 16) and ureters were filled with urates bursae were slightly congested and were positive on immune peroxidase and immune fluorscence reactions.

At 12 days post infection all pigeon showed ruffeld feather (Fig. 13) post mortem examination the kidney were increased in size nephrosis with peticeal heamorhage, ureter filled with urates and the Bursa were slightly increased in size and showed peticael heamorhages.

At 28 day post infection the liver were streaked with heamorhages, kidney were enlarged. Bursal membrane exudate was noticted in one case but the peticael hemorrhage were consistant in the other cases. The chickens put with the infected pigeon showed streakes of heamorhages on the outer surface of the thigh (Fig. 15) and the bursa were oedoematus with viscid material. The infected chicken showed congested liver and peticeal heamorhage in the inner searface of the thigh. The pigeon put with them showed congested dark liver with peticael heamorhages, purple spleen and enlarged and purple kidney. At 28 day post infection the swollen showed peticeal heamorrhages sowllen and bursal membrane exudate were constant finding.

Immune fluorescent (Fig. 7-9) and immune peroxides (Fig. 6, 14) were positive for bursa spleen thymus, kidney in two and four day as from chicken and pigeon.

Bursa, thymus, spleen, kidney of pigeon and chicken are positive at 12 day.

Bursa are and thymus until 28 day also dudenum showed +ve by IP and FA. But bursa and spleen only positive by agar gel.

All serum were +ve with dott ELISA in all experimental bird except control (Fig. 11).

Hematology:

At 4 day white blood corpuscle and red blood corpuscle are increased in infected pigeon than control but at 12 day white rBc and red blood corpuscle are increased in control pigeon than diseased bird and

also red blood corpuscle but in chicken white blood corpusle and red blood corpuscle are increased in chicken put with diseased pigeon than control.

Infected chicken large lymphocyte are more than non infected, hetrophile also increased esonophile and basphile increased in infected bird.

In pigeon infected (Lymphocyte were less than non infected but monocyte were increased.

Hemoglobin percentage were decrease in infected pigeon and chiken.

Experimental infection:

Histopathology:

Histopathological examination of bursae revealed cystic formation and interfollicler tissue showed oedema (Fig. 6). Kidneys showed degenerative changes in the epithelium of proximal convoluted tubule (Fig. 10) hyalinization and desquamation of epith cells (Fig. 1, 2) liver showed diffuse fatty change and congestion of central vien. Spleen showed depletion of lymphocyte in the white pulp.

Thymous showed, lymphocytic showed depletion (Fig. 3) and thyroid showing heamorhages and lymphocytic infiltration (Fig. 4).

Electron microscopy:

Transmission of clumbing electron micrograph of bursa showed clumping of chromatin material, and cytoplasm contain the lysosome.

Table (3)

Sample No.	Protein %	Fat %	Moisture
1	18.0	4.8	74.6
2	17.6	5.1	74.5
3	18.2	5.0	74.8
4	17.5	4.8	75.0
5	18.1	5.3	74.7
6	17.4	4.9	75.8
7	18.0	4.6	74.9
8	17.2	4.9	76.0
9	17.5	405	74.9
10	17.8	4.6	75.4
11	17.7	408	76.1
12	18.6	4.5	75.4

* 5, 6, 11, 12 non infected.

1 - 4 infected pigeon at 4 days.

7 - 10 infected pigeon at 12 days.

DISCUSSION

Isolation of infectious bursal disease in pigeon made by egg inoculation though Corrio allantoic membrane (CAM) and this is the best route recorded by Hitchner (1970), two isolate was obtained, passaged in tissue culture fibrobast. Examination revealed rounding, cell aggregation, Cho et al. (1979) the isolates resisted chloroform and ether the virus was stable at 56 four 2 hours. Chicken, ducks, sheep, mice, rat, rabbit and guinea pig erthrocytes were not agglutinate with virus and this agree with Ahlam (1989) Zanati (1982) and Onunkwo (1975) and Mousa (1986).

Pigeon infected intra ocularly after Cheville 1967 pigeon showed clinical maninfestation. The clinical symptoms were watery whitish diarrhea and ruffled ferather, similar symputoms were reported in chicken by Casgrove (1962), Ahlam (1989) by Mousa and Bayoumi, 1984.

The post mortem lesion included heomorhagic streaks on outer surfac of the thigh and shank. Liver showed diffuse subcapsular redened areas, kidney were swollen and occasionally showed peticheal hemorrhages and uretes were filled with urates. The bursa of Fabrictus was oedematus and inner surface revealed peticheal heamorhages and this lesion was recorded by Mohamed (1983) in addition peticheal hemorrhages were observed by Lensing (1969).

The virus was detected IFA from bursa and kidney thymous at 2, 4, 12 and 28 days. Valde's et al. detected virus kidney and bursa 2 days in bursa until 10 days. Astruball and Gialleti (1971) observed fluorescence from 36-240 hours in spleen and 48-72 hin lirn Fadly and Nazerian (1983) detected the virus by FA at bursa at 1-4 days spleen at 1-2 days Muller et al. (1979) used immune fluorescence to monitor theearly virus propagation after oral infection IBDV was first detected 4 hours PI in macrophages and lymphoid cells of the deucum and one hour later in similar alls of the duodenum and jejunum. Virus passed to the liver and was phagocytised by Kapher cells, which showed fluorescence five hour later. From the liver the virus reached the bur where massive repulication took plea and specific fluorescence was detected from 11 hour after infection.

Ide (1975) revealed that bursa were positive for FA 5 to 6 day.

Immune peroxides IP method are pased on the use of horse redish peroxides as marker to visualize antigen sat in cellular levels in the variety of specimens including monolayer cell smears, imprints, etospins, cryostat section and even paraffin section.

IBD lesions were positive cells were numerous and present in cortex and medulla of many follicles.

In this work IBD were detected in methonl fixed paraffin embed tissue sample was possitive+ 2, 4, 12, and 28 days kidney and bursa after infection. Aleksic Kovaeevic et al. (1999) used the same method with paraffin embeded tissue sample virus was detected 10 days after infection. An intensive exprission of IBDV antigen. The auther record that immune histochimecal reaction was veryweek 10 day after infection but in this work the reaction were positive untile 28 day in bursa showing that giving positive byagargel precipitation test.

All slide give positive result in IP give positive in FA the antigen detect by FA and immune peroxides were more positive than agar gel precipitation test and this agree with Aleksic - Kovecer et al. (1999).

Inoue et al. (1994) recorded IBDV in Hymus at 2-7 day but here dtetected antil 12 day.

Hemoglobin percentage were decreased in infected pigeon and chicken. White blood corpuscle are increased in diseased case and also red plood car puscle and thes agree with Mohamed (1983).

Infected chicken large lymphocyte are more than non. Infected, hetrophile also increased esonophile and basopil inerased in infected bird. In pigeon infected (Lymphocyte were less than non infected only mocyted present in infected pigeon and these result are agree with (Mohamed, 1983 and Jantosovica et al., 1998).

Examination of serum of 4, 12, 28 days reveled positive by dott elisa, Pastami (1980) recorded the appearance of ppt antibodies 4 and increased at 10 days and decreased at 18 days by agar gel precipitation test and Ahlame recorded PP and; bodies from 4 to 10 days in Turkey infected by turkey and chicken isolate and positive on 10-21 days infected by reference.

The post mortem lesion was heamorhage in the outer surface of thigh. Kidney increased in size, congestion renal parynchema appeared this agree with Boushra (1982). Multiple focal aggregation spleen showed general depletion and loss of demarcation of the germinal center and this agree with Cheville (1967) Mohamed, 1983 and Ahlam et al. (1997).

Bursa showing lymphocytic depletion and increased inter follicular tissue and connective bands between the follicles were oedematous these reported by (Helmboldt and Garner, 1964 and Cheville, 1979; Mohamed 1983 and Ahlam et al., 1997; Neeber et al., 1999 and Inoue et al., 1994).

Lymphoid necrosis of the thymous cortical Lymphocytic condensation and depletion in the thymous and this agree with Inoue et al., 1994.

Protien, fat and moisture conten in normal and infected bird are normal.

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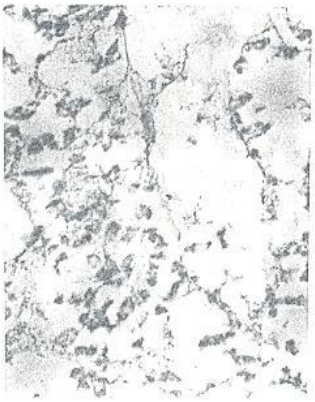

Fig. 1: Kidney showing nacrobiotic changes and lymphocytic infiltration (H & E 40x).

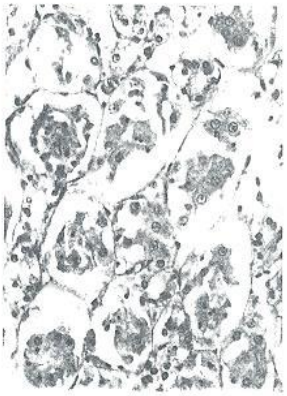

Fig. 2: Kidney showing desquamation and Necrobictic changes (H & E 40x).

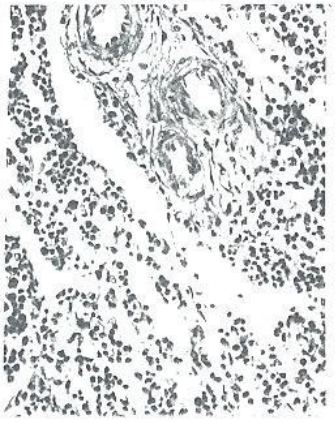

Fig. 3: Thymous showing hemorrhage, Lymphocytic depletion (H & E 40x).

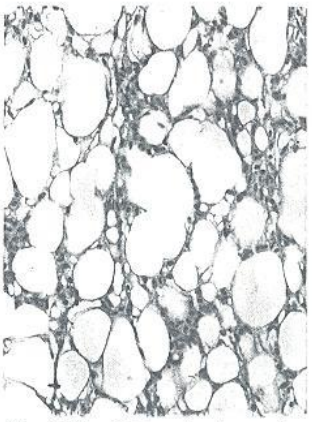

Fig. 4: Thyroid showing hemorrhages and Lymphocyic infiltration (H & E 40x).

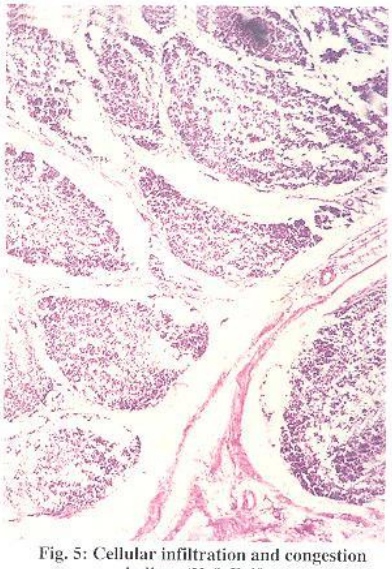

Fig. 5: Cellular infiltration and congestion in liver (H & E 40x)

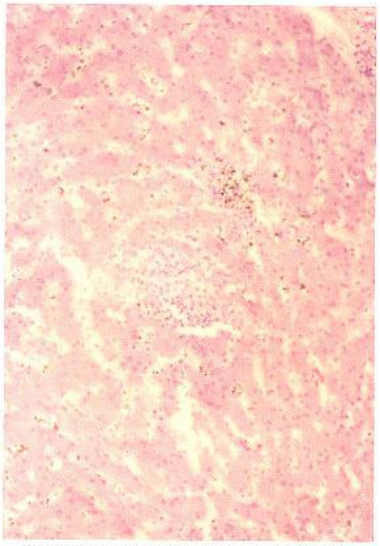

Fig. 6: Bursa showing lymphoid depletion and unter follicularodima.

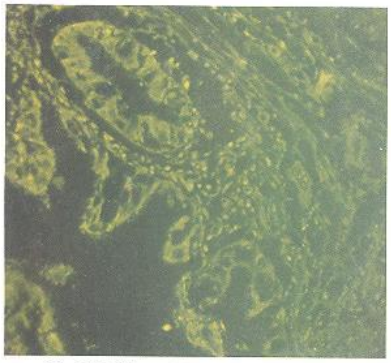

Fig. 7: Kidney with immumofluorcence.

Fig. 8: Bursa with immumofluorcence.

Fig. 9: Thymous with florescence.

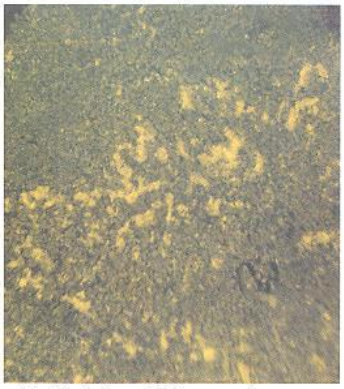

Fig. 10: Spleen with immumoflurcsence

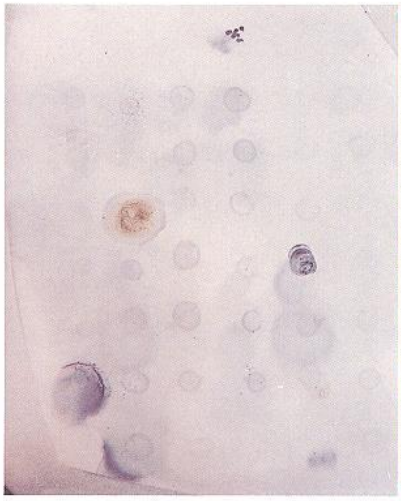

Fig. 11: Dott Elsi.

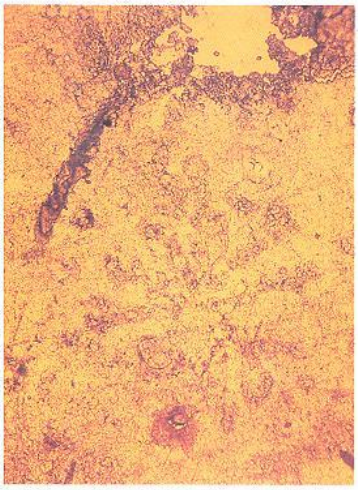

Fig. 12: Tlymous with immunobiroxidase

Fig. 13: Pigeon ruffled feather.

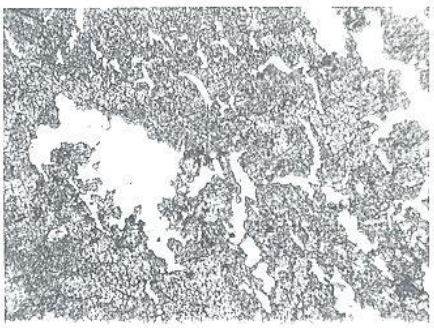

Fig. 14: Spleen with imunoperoxidus.

Fig. 15: Hemorrhage in thigh.

Fig. 16: Kidney in crease in size.