Assiut Vet. Med. J. Vol. 44 No. 87, October 2000

Animal Health Res. Inst.
Assiut Lab.

PIGEON MYCOPLASMOSIS: CHARACTERIZATION OF ISOLATES AND PATHOLOGICAL MANIFESTATIONS

(With 6 Tables and 18 Figures)

By
FATMA A. MOUSTAFA

(Received at 30/9/2000)

ميكوبلازما الحمام : توصيف المعزولات ودراسة باثولوجية

فاطمة عبد المجيد مصطفى

تمت هذه الدراسة على أنواع وأعمار مختلفة من الحمام البلدى والحمام الزاجل وحمام الكينج من مزرعة كلية الزراعة جامعة أسيوط. وكانت محاولات العزل الأولى للميكوبلازما من طيور سليمة ظاهريا وأخرى تعانى من أعراض تنفسية. وقد أخذت مسحات من البلعوم والقصبة الهوائية وكان إجمالى عدد العترات المعزولة ٣٢ عترة من ٨٩ حالة. وتم تقسيم العترات المعزولة بيوكيميائيا وسيرولوجيا بإستخدام إختبار المانع للنمو إلى ٢٣ عترة م.كولمبينم، ٧ عترة م.كولمبورال، ٢ عترة م.كولمبينزال. ولتحديد ضراوة العترات المعزولة تم حقن حمام عمر ٤ أسابيع فى الأكياس الهوائية الشمالية وكانت فترة الملاحظة ١٠ أيام. وقد تم ذبح الحمام بعد هذه الفترة. وقد تم وصف ومناقشة الأعراض الاكلينيكية والنفوق الذى حدث وخاصة فى المجموعة المحقونة بم. كولمبيورال. وبعد ذلك تم عزل الميكروبات المحقونة من الطيور الحية والمذبوحة. كذلك تم وصف ومناقشة التغيرات الباثولوجية المرئية والتى كانت عبارة عن زيادة فى سمك الأكياس الهوائية مع تفاوت فى درجتها. تم وصف ومناقشة التغيرات الباثولوجية الميكروسكوبية فى الأكياس الهوائية والرئتين والحنجرة والكبد والبنكرياس. أجرى اختبار حساسية العترات المعزولة ضد ٨ من المضادات الحيوية الشائعة معمليا وقد وجد أن جميع العترات حساسة لكل من اللنكوميسن والجنتاميسن وسبكتينوميسن والتيلوزين والنيومايسين وغير حساسة للستربتومايسين، تتراسيكلين، والكلورمفينكول.

SUMMARY

In this study mycoplasma was isolated from 89 living pigeons including racing and king pigeons from Faculty of Agriculture, poulty farm and also from local breads at Assiut Governorate. Swabs for culturing were obtained from oropharynx, trachea and cloaca of clinically healthy birds

as well as from these showing sings of mild respiratory disease. A total of 32 (36%) isolates were recovered from examined birds. Based on biochemical as well as serological tests, the isolates were 23 (71.9%) mycoplasma columbinum, 7(21.9%) M.columboral and 2 (6.4%) M.columbinasal. For detection of the virulence of isolated strains, thirty-4 weeks old pigeons were inoculated with 0.5 ml of two-day subculture mycoplasma strains in the left abdominal air sac. The inoculated organism was reisolated from living and slaughtered pigeons. Macromorphological and histopathological studies reaveld pathognomanic changes in the air sacs, trachea, lung, liver, bursa, of Fabricius, spleen and cloaca. The recovered strains were tested against the avilable antibiotics by the in-vitro sensitivity test, where all strains were sensitive to lincomycin, gentamycin, spectinomycin, tylosin, neomycin and resistant to streptomycin, tetra cycline and chloramphincol.

Key words: *Pigeon Mycoplasmosis.*

INTRODUCTION

Respiratory problems appear to be common in pigeons especially in racing pigeons and are difficult to diagnose, prevent and treat (Keymer et al., 1984). Although pigeon herpes virus (PHV1) infection (Vindevogel and pastoret, 1981) and lentogenic strains of newcastle disease virus (Vindevogel et al., 1982) also cause respiratory signs, but the role of mycoplasmas in pigeons is debatable. So mycoplasmosis is regarded as a clinical entity by many pigeon breeders and some veterinarians (Schrag et al., 1974). Many strains of mycoplasma columbinum and M. columborale have been isolated from the trachea and oropharynx of healthy feral pigeons in Japan (Shimizu et al., 1978). Sinclair (1980) isolated M.columbinasale from pigeons showing respiratory disease. Jordan *et al.*, (1981) isolated M.columbinum and M.columborale from respiratory tract and oesophagus of apparently healthy feral pigeons in Britain. Macowan et al. (1981) isolated M. columborale from pigeons in Britain also and demonstrated the pathogenicity of the organism by infecting pathogen-free chickes. Keymer et al. (1984) isolated mycoplasma columbinum, M. columboral and M.columbinasale from live and dead racing pigeons. The oropharynx, nasal sinuses, brain, lungs and air sacs were the site of isolation. Nagatoma, H. et al. (1997) isolated mycoplasmas from the

oropharynxes of 60 fantails pigeons under natural conditions. Mycoplasma columbinum, M. columboral and M. columbinasale were isolated from 28 (46.7%), 22 (36.7%) and 1 (1.7%) of 60 oropharynxes, respectively. Howse and Jordan (1983), tried to treat a group of racing pigeons naturally infected with mycoplasma columboral and M.columbinum by tiamulin hydrogen fumarate in drinking water for 35 days. They found that tiamulin was not able to eradicate mycoplasmas after a prolonged course of treatment. Reece et al. (1986) isolated mycoplasma columbinum, M.columboralc, M.synoviae and M.gallinarum from conjunctivae, tracheas and airsacs. Tylosin has shown efficacey for treatment the mycoplasmas. This work was directed to cover the investigations on the local distribution of pigeon mycoplasmosis in the area of Assiut province, determination of the pathogenicity of isolated strains in pigeons and study of the in-vitro sensitivity of the isolated strains to antimycoplasmal agents available in the field.

MATERIALS and METHODS

1- Pigeons:

Materials were collected to cover different ages of living racing pigeons and king pigeons from the poultry farm of Agriculture College-Assiut University and local breed from a private farm. Both clinically healthy birds and those showing signs of mild respiratory disease were included. Some of the birds showed varing amounts of mucus at the back of their throats.

2- Sites of Swabbing:

The oropharynx, trachea and cloaca of each bird were swabbed. Swabs were Sown onto mycoplasma medium.

3- Mycoplasma medium:

Mycoplasma broth and agar media were prepared as described by Yoder (1980) which composed of brain Heart infusion broth or agar (Difco), 20% Fresh Horse serum, 5% yeast extract, 2% thallium acetale, penicillin G.Sodium 1000 μ/ml and its pH was adjusted to 7.8.

4- Specific antisera:

M.columbinum standared antisera (MMP1), M. columboral (MMP4) and M. columbinasale (694) were kindly supplied by Prof. Dr. Adel - Mohamed Soliman Dept. of Poultry Diseases, Fac. Vet. Med. Assiut University.

5- Pathogenicity test:

40 one-month old pigeons were divided into 4 equal groups, these pigeons were proved to be free from mycoplasma infection by

bacteriological and serological examination. 3 groups were used for experiment. The other group was used as a control. Each pigeon was inoculated with 0.5 ml of broth inculture of 10^8 colony forming units inoculated into the left abdominal airsac of each bird. All groups were kept in cages in separate places under the same enviromental conditions. The observation period continued for 10 days after inoculation. The clinical signs were observed and recorded. The birds of all groups were sacrificed and examined for microscopic lesions.

6- Sensitivity of the isolated strains against antimycoplasmal agents:

This experiment was done to determine the more effective antimycoplasmal drugs avilable in the field. The isolated strains were used for this test and brain Heart infusion media agar, where broth culture of strain with known colony forming unit (10^8/ml) were cultured by runing drop technique. Plates were incubated at 37°C in moist candal jar for 3-4 days, then examined microscopically. The results were expressed by the method of Clyde. (1964).

Antibiotic discs used were:

Spectinomycin (100ug), streptomycin (10ug), lincomycin (20ug), tylosin, (100ug), gentamycin (10ug), neomycin (30ug), tetracyclin (30ug.), chloramphenicol (30 ug).

7- For the hisopathological studies:

Specimens from air-sacs, lungs. traches, liver, spleen, bursa of fabricius and cloaca were taken and fixed in neutral buffer formalin and embedded in paraffin blocks. Sections in thickness of 4-6m. were done and stained by haematoxilin and eoisin. Liver sections staind were also by gram stain and PSA. Bancroft, D. and Stevens, A. (1982).

8- Isolation of mycoplasma:

The collected samples were cultured as described by Sabry and Ahmed (1975). Each swab sample was put into 5 ml brain heart infusion broth then incubated at 37°C for 3-days, from which 0.02 ml was inoculated and streaked on brain heart infusion agar. The agar plate was incubated at 37°C in moist candle jar under reduced oxygen tension. The plates were observed daily from the 3rd to the 10 th day postincubation by dissecting microscop. In case of mycoplasma growth on agar plates a single colony was picked up with an agar-block and transplanted into fresh liquid medium and the growth was checked by regular plating of inoculated sample. After purification the isolated strains were identified with:

a) **Biochemical tests** which include Glucose fermentation and arginin utilization. Media of glucose or arginine were inoculated with

Mycoplasma suspected isolates and incubated at 37°C for three days. The positive results appeared as a change in the colour or change in pH of inoculated medium. In case of glucose positive results, the colour changed to orange or yellow but arginine changed to dark red or violet colour. This recomended by Freundt et al. (1979).

b) **Serological identification** by growth inhibition test which recomended by Clyde, (1964). The test was done by inoculation of agar plates with broth culture using running drop technique. Filter paper disc soaked in 0.02 ml of antiserum. Dried at 37°C then placed on the middle of the plate. Plates were incubated at 37°C in moist anaerobic candle jar for 3 days. The absence of growth in the presence of specific antiserum was recorded.

RESULTS

1- Microbilogical studies:

32 isolates were recovered from 89 examined pigeons (Table 1-2), so according to the biochemical patterns. 32 recovered isolates of Mycoplasma were classified into two groups (Table 3). Group I : isolates did ferment glucose but not split arginine (Glucose + ve and Arginine - ve). Group II isolates did not ferement glucose but split arginine (Glucose -ve and Arginine +ve). According to the results of the growth inhibition test which considered positive if the inhibitory zone was more than 2mm. The recovered 32 isolates were classified serologically into M.columbinum 23, M.columborale 7 and M. columbinasale 2 (Table 4).

Results of the pathoginicity of the isolated strains during the observation period. No mortalities were observed in groups inoculated with M.columbinum group (B) and group (C) which inoculated with M.columbinasale while 2 birds died in group (A) which inoculated with M.columborale. Birds in the infected groups appeared clinically normal except three birds in group (A) have slight respiratory symptoms.

Macromorphological studies: sacrified birds showed air sacculitis which was sever in some cases and extended to the peritoneum. Affected air sacs were thickened, opaque, and flecked with numerous yellowish white foci up to 1 mm in diamter, congestion of the lung. Neumerous necrotic foci and streaks of haemorrhages were observed in the liver in group (A). The inoculated mycoplasma were reisolated from respiratory organs only of living, slaughtered and died birds (Table 5).

II- Morphopathological studies:

Histopathological changes of variable degree was observed in different organs of the experimental birds. However the most sever changes were seen in the liver of pigeon experimentaly infected with M.columboral.

The pathological changes of the lung in mildly affected cases groups (B & C). Consists of: Congestion, prevascular lymphoid cell reaction and proliferation of peribroncheal lymphoid aggreagation (Fig. 1). This proliferation is manifested by increase amount of mytotic figers. In severly affected cases group (A) the lung showed alviolar macrophage cell reaction along infiltration with hetrophil cells. Necrotic change were sometime evedent in this nemonic area (Fig. 2).

The air sacs in mildly affected cases showed hyperplasia of lymphoid aggreagation (Fig. 3). Odema of the submucosa and congestion of the blood vessels (Fig. 4). This in group (B&C). In severly affected cases group (A), the air sacs showed necrosis & discumation of the lining epithelium. The submucosal layer were grealty thickned by mononuclear cell infiltration and fibrin depositon (Fig. 5).

Trachea from group (A) showing excessive mucous secreation which adher to the epithelial. Increase the amount of goblet cell and submucosal odema. The trachea from group (B&C) showed no pathological changes.

The liver from pigeons inoculated with M.columboral (group A). showed a significant pathological change in the form of multiple granulomas (Fig. 6). This granuloma consists of necrotic center surrounded by a zone of giant cells followed by lymphoid cells (Fig. 7). A bundent aggreagation of esinophil cells were demonstrated among these lymphoid cells, by application of PAS stain on these granuloma no mycotic infection could be demonstrated (Fig. 8). However by using gram stain, gram -ve bacterial colonies could be demonstrated in these granuloma (Fig. 9). The hepatic cells were distorted, atrophied and showed necrobiotic changes (Fig. 10-11). The liver from group (A & B) showed focal area of lymphoid cell reaction along with mild degenerative change of the hepatic cells (Fig. 12).

The lymphoid follicles of the bursa of Fabricius showed different degree of lymphoid cell proliferation and increase population of lymphocyt. Mylotic figures are sometimes observed.

Spleen from group (A) showed proliferation of reticuloendothelial system cells (Fig. 13). The spleen from all infected group showed increase amount of lymphoid cells population (Fig. 14).

Hetrophil cells were sometime evedent among the proliferating lymphocyt (Fig. 15).

The cloaca in pigeon from group (A) showed hyperplastic change of lymphoid aggreagation (Fig. 16). In addetion the mycosal epithelium of the cloaca is heavly infiltrated with hetrophil cells (Fig. 17). The pigeon from group (A&B) the proliferation of lymphoid aggreagation was the main feature (Fig. 18).

In vitro-sensitivity of the isolated strains to antimycoplasmal agents showed that all strains were sensitive to lincomycin, gentamycin and spectinomycin but slightly sensitive to tylosin and neomycin. On the other hand they were resistance to streptomycin, tetracycline and chloramphenicol (Table 6).

Table 1: Recovery rate of mycoplasma from pigeons.

Materials	No. of swabs	No. positive	Percentage
Racing pigeons	60	20	33.3
King pigeons	9	8	88.9
Local bread	20	4	20
Total	**89**	**32**	**36**

Table 2: Site of mycoplasma isolation.

No. positive	Site of isolation		
	Oropharynx	Trachea	Cloaca
20	13	7	-
8	5	3	-
4	-	4	-

Table 3: Biochemical properties of isolated strains

Biochemical test	Species		
	M.columbinum	M.clumborale	M.columbinasale
Glucose	-	+	-
Arginine	+	-	+

Table 4: Serological properties of isolated strains.

Species	Refernce strains	Number	percentage
M.columbinum	MMP1	23	71.9
M.columborale	MMP4	7	21.9
M. columbinasale	694	2	6.3
		32	

Table 5: Results of experimently infected pigeons with the isolated strains and reisolation.

Strain	Rout	No of inoculated birds	No. of deaths	Reisolation	
				Respiratory organs	Other organs
CM	Left Abdominal Air sac	10	-	8	-
Cr		10	2	8	-
Cs		10	-	7	-

Table 6: Results in vitro sensitivity test of mycoplasma species to different antibiotics.

Antibiotic discs	Concentrations	Species		
		CM	Cr	Cs
Lincomycin	20 ug	+++	+++	+++
Gentamycin	10 ug	+++	+++	+++
Spectinomycin	100 ug	+++	+++	++
Tylosin	100 ug	++	++	++
Neomycin	30 ug	+	+	+
Streptomycin	10 ug	-	-	-
Tetracycline	30 ug	-	-	-
Chloramphenicol	30 ug	-	-	-

(-) Resist , (+) Week , (++) Moderatly , (+++) Strong sensitive

DISCUSSION

Mycoplasma infection accounts for major economic losses to the poultry industry due to downgrading of meat, reduced feed utilization, egg production efficiency and increased medication costs. Pigeons respiratory problems appear to be commen especially in racing pigeons and are difficult to controled Keymer et al. (1984). Out of 89 swab samples 32 isolates were recovered from racing pigeons, king pigeons and local bread. Our results were similar to those obtained by Nagatomo et al. (1997) and Keymer et al. (1984). The isolates were identified as 23 isolates M.columbinum, 7 isolates M.columboral and 2 isolates M.columbinasale. The growth inhibition test was used to identify the isolates obtained during this study. This test recommended by Kleven (1975) and Soliman (1984).

The experimental infection of pigeons with the isolated strains showed that M.columorale was more pathogenic than other types which

couses deaths for 2 birds from experimental group. Similar result was observed by Macowan (1981). The postmortem lesions were varied from mild to moderat in all groups except the liver in a group "A", the lesion was severe. The air-sacs showed turbidity, thickning and opaque. The lungs were congested and streaks of haemorrhages were observed in the liver. These findings were in close a greement with these observed by Macowan (1981).

Micromorphological studies of different organs from pigeons infected wtih M.columbinum and Columbinasale revealed increase amount of lymphoid cell population in these organs including liver, spleen, lung, air-sacs, bursa of Fabricuis and cloaca. Along with this a mild degenarative change of hepatocyt and few hetrophil cell reaction. This result indicated that these strains provoke stimulating effect on the immune system of these birds. However in pigeons inoculated with M.columboral showed sever histopathological changes in the liver, air-sacs, lung and cloaca. Such result proved that this strain are more pathogenic than the preceding two. Moreover the appearance of granulomatus hepatitis in birds inoculated with M.columboral rolled the fact of lowering resistant and the secondary infection.

In the available litratures experimental infection of pigeons by different strains of Mycoplasma were not recorded. Only few work dealing with experimental infection in ducks by Soliman (1984). El-Fbeedy et al. (1982) in turkey and Macowan et al. (1981) in chicken. They observed a pathological changes more or less similar to those obtained by us in pigeon. In vitro sensitivity test of the isolated strains to antimycoplasmal agents showed that the strains were sensitive to lincomycin, gentamycin and spectinomycin, and tylosin. these results go hand in hand with Soliman (1984) and Sinclair (1980). On the other hand the isolates revealed resistance to streptomycin, tetracycline and chloramphenicol. This were in agreement with Soliman (1984).

According to our results obtained in this work we can concluded that M.columboral is more pathogenic for pigeon than M.columbinum and Columbinasale. It can induce sever histopathological changes in the liver, air-sacs, lung and cloaca. moreover the experimental infection of these strains lower the resistant of pigeons and can lead to secondary infection. Experimental infection with M.columbinum and M.columbinasale stimulat the immun system and lead to increase cellular immunity. This manifested by wild spread proliferation of lymphoid cells in diffenent organs.

REFERENCES

Bancroft, D. and Stevens, A. (1982): Theory and practice of histological techniques. 2nd Edn. Churchill Livingstone (Edinburgh, London, Melbourne).

Clyde, W.A. Jr (1964): Mycoplasma species identification based upon growth inhibition by specific antisera. J. Immunol : 92: 958-965.

El-Ebeedy, A.A., Easa, M. El. S., Sabry, M.Z., Hafez, M.A., Ammar, A.M. and Amal Rashwan (1982): Pathological changes in air-sacs and lungs of tufkey poults after experimental inoculation with different isolates of M.meleagridis. J. Egypt. Vet. Med. Assoc., 42 (4): 91-100.

Freundt, E.A. Erno, II and Lemcke, R.M. (1979): Identification of Mycoplasmas Methods of Microbiol., Vol. 13 Academic press.

Howse, J.N. and Jordan, F.T.W (1983): Treatment of racing pigeons naturally infected with Mycoplasma columborale and M.Columbinum. veterinary record, 112: 324-326.

Jordan, F.T.W., Howse, J.N., Adams, M.P. and Fatunmbi, O.O. (1981): The isolation of Mycoplasma columbinum and M.columborale from feral pigeons. Veterinary record, 109:450.

Keymer, I.F., Leach, R.H., Clarke, R.A., Bardsify, M.E. and Mcintyre, R.R. (1984): Isolation of Mycoplasma spp. from Racing pigeons (Columba livia). Avian pathol. B : 65-74.

Kleven, S.H. (1975): Antibody response to Avian Mycoplasmas. Amer. J. Vet. Res., 36 : 563-565.

Macowan, K.J. Jones, H.G.R., Randall, C.J. and Jordan. F.T.W. (1981): Mycoplasma columborale in a respiratory condition of pigeons and experimental air sacculitis of chickens. Veterinary record, 109:562.

Nagatomo, H., Kato, H., Shimizu, T. and Katayama, B.C. (1997): Isolation of Mycoplasmas from fantail pigeons. J.Vet. Med. Sci. 59 (6) : 461-462.

Reece, R.H., Ireland, L., and Scott, P.C. (1986): Mycoplasmosis in racing pigeons. Australian veterinary journal. Vol. 63: 166-167.

Sabry, M.Z. and Ahmed, A.A. (1975): Evaluation of cultural procedure for the primary isolation of Mycoplasma from female genitalia of farm animals J. Egypt. Vet. Med. Ass., 35: 18-34.

Schrag, L., Enz, H. and Klette, H. (1974): In: Healthy pigeons. Recognition, prevention and treatment of the major pigeon diseases, pp. 57-72. Hengerberg. West Germany. Verlagl. Schober.

Shimizu, T., Erno, H. and Nagatomo, H. (1978): Isolation and characterization of Mycoplasma columbinum and Mycoplasma columborale, two new species from pigeons. International jornal of systemic bacteriology, 28: 538-546.

Sinclair, D.V. (1980): Respiratory disease in pigeons. Veterinary record, 106 : 466-467.

Soliman, A.M. (1984): Further investigation on duck Mycoplasmosis in Upper Egypt. pH. D., Thesis; Fac. Vet. Med. Assiut University.

Vindevogel, H. and Pastoret, P.P (1981): Pathogenesis of pigeon Herpes virus infection. Journal of Comparative Pathology, 91 :415-426.

Vindevogel, H., Thiry, E., Pastoret, P.P. and Meulemans, G. (1982): Lentogenic strains of Newcastle, disease virus in pigeons. Veterinary record, 110 : 497-499.

Yoder, H.W. Jr. (1980): Mcoplasmosis. in isolation and identification of Avian pathogens, 2nd., S.B. Hitchner; C.S. Domermuth; H.G. Puchase and J.E. Williams, Eds. American Association of Avian pathologists college station, Texas. P. 40-42.

LEGENDS FOR FIGURES

Fig. 1: Lung showed lymphoid cell reaction. H&E 10X.
Fig. 2: Lung showed pnemonic area associated with necrotic changes. H&E 10X.
Fig. 3: Air-Sacs showed lymphoid cell reaction. H&E. 25X
Fig. 4: Air-Sacs showed oedema of submucosa and congestion of the blood vesseles. H&E. 10X.
Fig. 5: Air-Sacs showed fibrin depostion mononuclear cell reaction, necrosis and desqumation of the epithelium H&E. 40X.
Fig. 6: Liver showed multiple granuloma. H&E. 4X.
Fig. 7: Showed structure of the granuloma. H&E. 10X.
Fig. 8: Showed granuloma with PAS stain. 25X.
Fig. 9: Showed granuloma with gram negative bacterial colonies. H&E. 10X.
Fig. 10: Showed necrobiosis of the hepatocyte. H&E. 10X.
Fig. 11: Showed atrophy, necrosis and extensive cellular reaction of the liver. H&E. 10X.
Fig. 12: Liver showed focal area of lymphoid cell reactions H&E. 40X.
Fig. 13: Spleen showed proliferation of reticuloendothelial system cells H&E 40X..

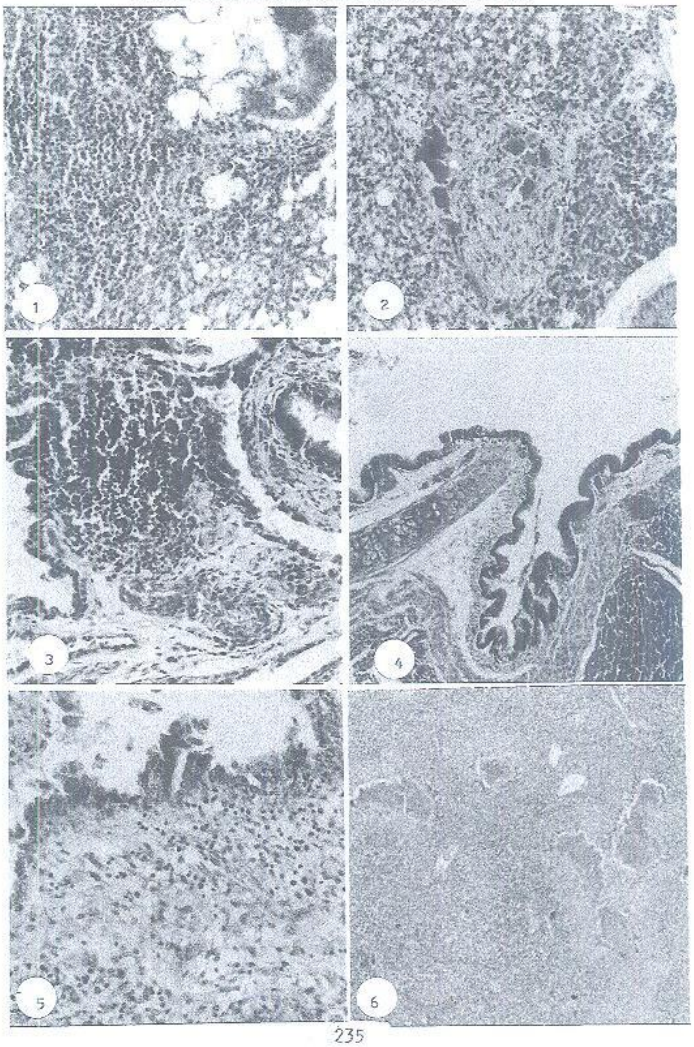
Fig. 14: Spleen showed increased amount of lymphoid cells population. H&E 40X.

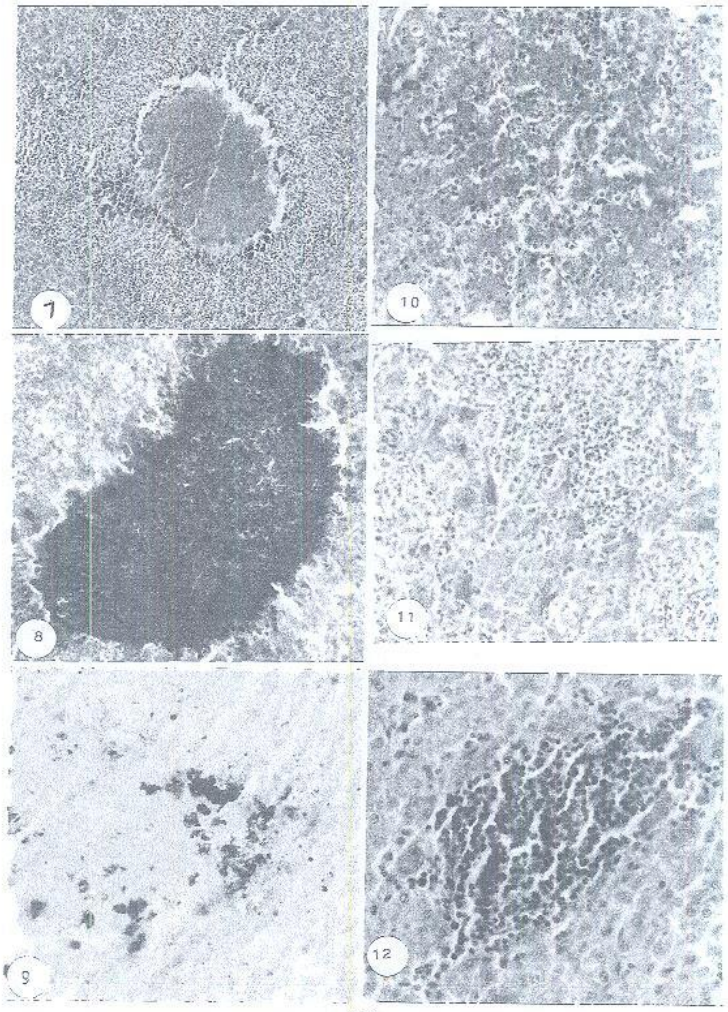
Fig. 15: Spleen showed some heterophil among the proliferating lymphoid cells. H&E. 40X.

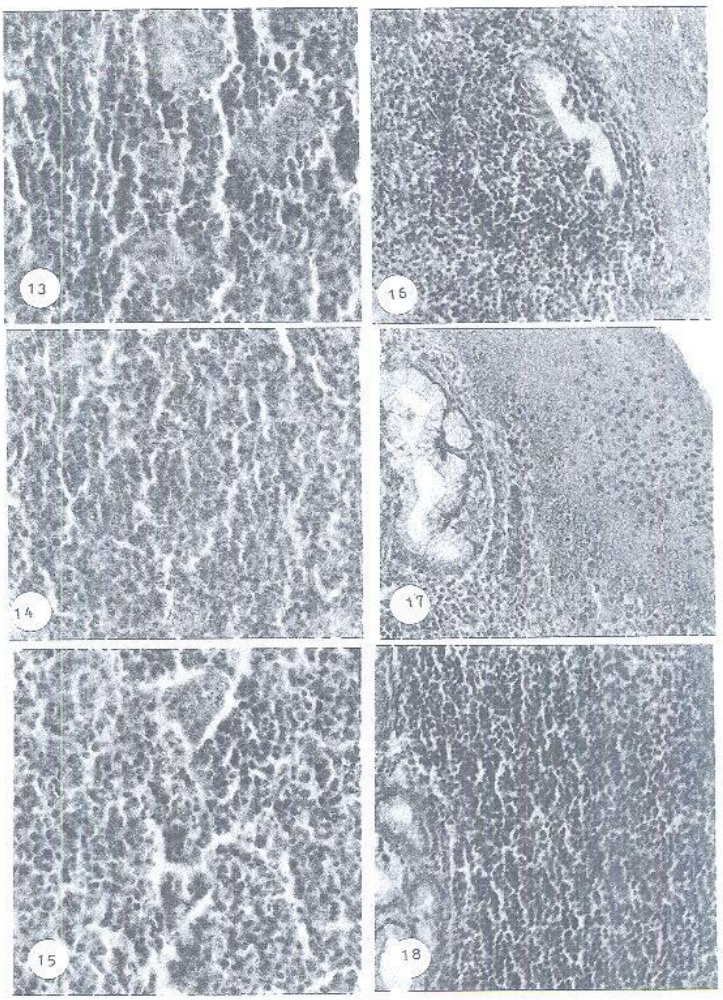
Fig. 16: Showed hyperplasia of lymphoid aggreagation of the cloaca. H&E. 25X.

Fig. 17: Showed hetrophil cells reaction in the mucosal epithelial of cloaca. H&E. 25X.

Fig. 18: Showed proliferation of the lymphoid aggreagation. H&E. 25X.


1
2
3
4
5
6


7
10
8
11
9
12


13
16
14
17
15
18