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Avian Diagnostic Cytology

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Avian Diagnostic Cytology

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Abstract

Cytology is the study of cells and has an important role in avian medicine. Samples from 900 falcons and 110 bustards were sampled from the upper and lower alimentary tract; 1200 samples were collected from the upper respiratory tract of falcons and 200 bustards. Biopsy samples from the airsac were collected during anaesthesia which includes 600 falcons and 80 bustards. Conjunctiva and skin samples were examined from 100 falcons and 80 bustards; blood samples were taken from 1500 falcons and 400 bustards; aspirated fluids were collected from 16 falcons and 21 bustards. Autopsy samples also examined from the internal organs of 22 falcons and 13 bustards which includes liver, kidney, heart, spleen, small intestine and lungs. Cellular responses are categorised as inflammation, hyperplasia, metaplasia and neoplasia. Inflammatory responses were the most common type encountered in birds. Inflammation is due to aetiological infectious agents. Acute inflammation is primarily characterised by heterophils (more than 70% of inflammatory cells). Chronic active inflammation is characterised by a mixture of heterophils (50% of the inflammatory cells) and mononuclear cells including macrophages, lymphocytes and plasma cells. Chronic inflammation is marked by the presence of predominantly mononuclear cells (more than 50% of the inflammatory cells).

Arthritis is the one of the common synovial disease in falcons and bustards. Inflammatory cells, fibrin strands and bacteria are the findings in inflammatory conditions caused by *Mycoplasma* sp, *Staphylococcus aureus* and *Streptococcus* sp. Pox lesions are associated with Bollinger bodies in squamous cells. Gout is characterized by urate crystals. Conjunctivitis is the common conjunctiva disease in falcons and bustards caused by bacteria, pox virus, cryptosporidia and eye flukes. In cytology smears, numerous inflammatory cells noticed in association with bacteria, chlamydia and cryptosporidia. Pox lesions are associated with Bollinger bodies in squamous cells. Pericarditis and gout are the common cardiac diseases reported from birds. Pericarditis characterized by inflammatory cells and bacteria, while gout is characterized by urate crystals. Major renal diseases found in bustards and falcons were bacterial septicemia, septicemic pox, avian leucosis and gout. Reactive renal cells

were found in infectious diseases, while urate crystals are found in cases of gout. Hepatitis causes mortality in bustards and falcons, which is caused by mainly virus and bacteria. Inflammatory cells including heterophils and kupffer cells are characteristics of hepatitis. Non-inflammatory diseases such as fatty liver and amyloidosis are also commonly seen in captive birds. Spleenomegaly was found in association with bacterial septicemia, herpes, septicemic pox, avian leucosis, chlamydiosis and tuberculosis. Lymphoid cells are found in normal spleen imprints while reactive lymphoid cells and plasma cells are seen in reactive spleen. In septicemic cases, bacteria also present along with reactive cells. Bumble foot infections are common in captive falcons, while not seen in bustards.

Chapter 1: Introduction

Cytology is a branch of diagnostic medicine that deals with the study of cells. It is a useful diagnostic tool for the differential diagnosis of inflammatory diseases and neoplasia. Cytological studies can be performed on samples collected from discharges, lesions, scrapings, aspirated fluids and imprint smears derived from tissues. Cytology helps in the rapid diagnosis of diseases and can provide microscopic information of many different disease processes.

Cytological evaluation is always an adjunct to other diagnostic procedures. A final diagnosis often requires information from the clinical history, physical examination, and evaluation of other laboratory samples obtained from the bird, radiographs, surgical investigations, post-mortem examination and histopathology. Cytology is a technique based on the study of individual cells. In disease situations it can give information about patho-physiologic changes caused by the disease and aetiologic agents that are involved. The technique provides a simple, rapid, inexpensive method of diagnosis. Cytopathology does not give information about size of the lesion or the invasiveness of a malignant lesion. So, in certain cases, especially for internal organs histopathology also should be performed to achieve the final diagnosis.

A cytological classification divides body tissue into four groups including (1) haemic, (2) epithelial-glandular, (3) connective and (4) nervous. Haemic tissue is composed of cells that are found in the peripheral blood, bone marrow and ectopic haemopoietic sites. Blood cells are a common cell type in post-mortem impression smears from tissue. Epithelial cells tend to exfoliate in clumps or sheets. Connective tissue cells tend to exfoliate poorly and provide cytologic specimens with few cells. Nervous tissue cells are rarely seen on cytological specimens, unless the specimens were made from central or peripheral nervous tissue.

Cytology is a rapid and inexpensive technique that can be used in the clinic for the diagnosis of upper respiratory tract diseases of birds with acute and chronic trachitis which caused by virus, bacteria, and fungi. Aspergillosis is the most common avian mycosis seen in the upper and lower respiratory tract of birds, especially in captive waterfowl, wading birds, penguins, raptors, ostriches, pheasants and passerine birds (Bauck 1994, Deem 2003, Kearns 2003, Kunkle 2003, Plumb 2002,Perez 2003, Pollock C, 2003). Among raptors, goshawks (Accipiter gentilis), gyr falcons (Falco rusticolus), immature red-tailed hawks (Buteo jamaicensis), golden eagles (Aquila chrysaetos) and snowy owls (Nyctea scandiaca) are more likely to develop the disease (Redig 2000). Aspergillosis is the most important cause of morbidity and mortality in falcons trained for falconry in the Middle East (Samour 2000, Silvanose 2011). The disease is caused by Aspergillus fumigatus and less commonly by Aspergillus flavus and Aspergillus niger (Silvanose, 2006).

Diagnostic cytology provides a quick answer to the upper and lower alimentary tract diseases. Upper alimentary tract diseases are acute and chronic stomatitis which caused by virus, bacteria, candida, metazoa and protozoa. Trichomonas sp is commonly seen in the oro-pharynx of birds while in water birds Amoeba sp was reported (Silvanose, 1998). Capillaria sp and Serratospiculum sp are the metazoa reported from the upper alimentary tract of birds (Cooper, 2008). Coccidia sp., Cryptosporidium sp., Giardia sp., Trichomonas sp., Histomonas pp., Hexamita sp. and Toxoplasma sp., are the most commonly diagnosed protozoa in the digestive tract of birds (Silvanose, 1999). The presence of protozoa in the digestive tract of birds does not always constitute a disease process and depends on host resistance, immune status, level of parasitism, pathogenicity of protozoa and concurrent bacterial, viral and fungal infections (Silvanose, 2008). One of the most important oro-pharyngeal diseases widely reported in raptors, pigeons and doves is trichomoniasis (Cooper, 2008). Also, trichomoniasis has been documented from the upper digestive tract of bustards from United Arab Emirates (Silvanose, 2006). Enteritis caused by virus, bacteria, protozoa. Coccidia sp., Cryptosporidium sp., Giardia sp., Trichomonas sp., Histomonas pp., Chilomastix sp, Histomonas sp, Hexamita sp. and Toxoplasma sp., are the most commonly diagnosed protozoa in the digestive tract of birds (Samour 2000, Silvanose,

1999). Trichomonas sp is commonly seen in the upper alimentary tract of birds while it does not contribute any diseases in the lower alimentary tract (Silvanose, 1998). Trichomonas gallinae is the common species seen in the upper alimentary tract, while Trichomonas gallinarum is seen in the lower alimentary tract and it is considered as non-pathogenic (Silvanose, 1999). Coccidia species including Cryptosporidia, Caryospora sp., Isospora sp and Eimeria sp are pathogenic protozoa found in the lower alimentary tract of birds (Bailey 2008). Capillaria sp, Trematode, Ascaridia, Acanthocephala, Cestodes and Serratospiculum sp are the metazoa reported from the lower alimentary tract of birds (Bailey, 2008, Samour 2000, Silvanose 1999). The presence of protozoa in the digestive tract of birds does not always constitute a disease process and depends on host resistance, immune status, level of parasitism, pathogenicity of protozoa and concurrent bacterial, viral and fungal infections (Silvanose, 1998). There is very little information is available in the literature concerning diagnostic cytology of birds specific to raptors and bustards. In view of this, it was decided to carry out a comprehensive diagnostic cytology of lower alimentary tract of birds which is mainly focused on bustards and falcons maintained in captivity in the United Arab Emirates.

Haematology is an important tool to diagnose avian diseases. This includes total red blood cell (RBC) count, pack cell volume (PCV), haemoglobin (Hb), total white blood cell (WBC) count, fibrinogen and cytology of the blood smear including differential WBC count, cellular morphology and hematozoa screening. Morphology of blood cells varies from species to species. If the veterinary centers are unable to evaluate avian blood smears should be sent to specialised avian laboratories for evaluation. There are a few laboratories able to perform avian haemotology and these laboratories may be far from the veterinary centers. So whole blood for evaluation is not possible always. In such situations, fresh blood smears can make and sent to the laboratory for cytological evaluation. If any reason fresh blood smears could not able to produce immediately, the blood can store in potassium-EDTA tubes and the smear should be made within few hours. Anticoagulated blood may change the hematozoa morphology and prolonged blood storage will change the leucocyte morphology. 5 - 8μl of blood is enough to prepare a good blood smear for cytological evaluation.

Chapter 2: Background

Cellular responses are categorised as inflammation, hyperplasia, metaplasia and neoplasia. Inflammatory responses are probably the most common type encountered in birds. Inflammation is due to aetiological infectious agents. Metaplasia is due to long-term infections or toxic actions to the cells by infectious agents. Acute inflammation is primarily characterised by heterophils (more than 70% of inflammatory cells). Chronic active inflammation is characterised by a mixture of heterophils (50% of the inflammatory cells) and mononuclear cells including macrophages, lymphocytes and plasma cells. Chronic inflammation is marked by the presence of predominantly mononuclear cells (more than 50% of the inflammatory cells). Tissue hyperplasia is uncommonly seen in birds and represents an increased growth rate of normal cells in response to an insult. Metaplastic lesions can be diagnosed by nuclear cytoplasmic ratios and may show cellular pleomorphism. Neoplastic lesions can be diagnosed by cellular criteria including nuclear cytoplasmic ratios, prominent nucleoli, multiple nuclei, mitotic figures, cellular pleomorphism, cytoplasmic vacuolation and basophilia. The important neoplasias seen in birds include carcinomas, adenocarcinomas, sarcomas and lymphoid neoplasm. Carcinomas are characterised by round to oval cells, arranged in patterns. Adenocarcinomas (ovary) are characterised by giant cell formation and secretory granules. Sarcomas are characterised by spindle shaped cells, poor exfoliation, increased cell size and nuclear cytoplasmic ratio. Lymphoid neoplasm is characterised by discrete cells, round or oval; increase in lymphoblasts; pleomorphism, basophilia, mitotic figures and multiple nucleoli.

2a. Objectives

There is very little information is available in the literature concerning diagnostic cytology of birds specific to raptors and bustards. In view of this, it was decided to carry out a comprehensive diagnostic cytology survey of birds which is mainly focused on bustards and falcons maintained in captivity in the United Arab Emirates. The data collected in this study were used to prepare a manual of Avian Diagnostic Cytology for

the partial fulfillment of the requirements for Doctorate Degree in Laboratory Science from the Atlantic International University.

2b. Cytology Techniques

Samples from exposed lesions, oro-pharynx, crop, choana and cloaca should be collected by rotating a saline moistened sterile cotton swab on the site. Samples can also be collected by scraping with a wooden spatula to increase the cellular exfoliation. Conjunctiva and nasal samples can be collected using thin swab. Fluid samples from the synovial joints, abdomen and pericardium should be collected by aspiration using a 5 or 10 ml syringe and 23 - 25 G needle. Fine-needle aspiration biopsy often provides a good cytological sample for a rapid presumptive diagnosis without radical tissue removal. Samples from organs (post-mortem) and cut tissue surfaces should be collected by imprint (impression) smears by direct contact with glass slides. Samples from the trachea should be collected using nasolacrimal swab or a sterile endotracheal tube and syringe. Biopsy samples from air sac, lung, liver and the peritoneum of anaesthetised live birds should be collected by rotating a sterile swab on the lesion using endoscopy equipment. Granulamatous lesions from the internal organs of live birds should be removed using endoscopy forceps and impression smears can made using two glass slides. A successful cytologic examination is only possible if the representative sample is from a good quality smear with good staining. During a post-mortem examination, impression smears should be routinely made from liver, spleen, kidney and lungs. Figures 2.1 to 2.15 shows various sample collection technique adapted in avian cytology.

Fig.2.1. Collection of abdominal fluid from a saker falcon.

Accumulation of fluid is occasionally encountered in the elbow joints of the wing and the hock joint of the leg. Synovial fluid is poorly cellular but cellular fluids are found in inflammatory conditions. Accumulation of fluid in the abdominal cavity (ascitic fluid) is seen and is occasionally encountered in the pericardium (pericardial fluid). Pericardial fluid and ascetic fluids are obtained by aspiration.

Fig.2.2. Sample collecting from the trachea of a saker falcin using ENT swab.

Fig.2.3. Samples collecting from the oro-pharynx of a houbara bustard using cotton swab.

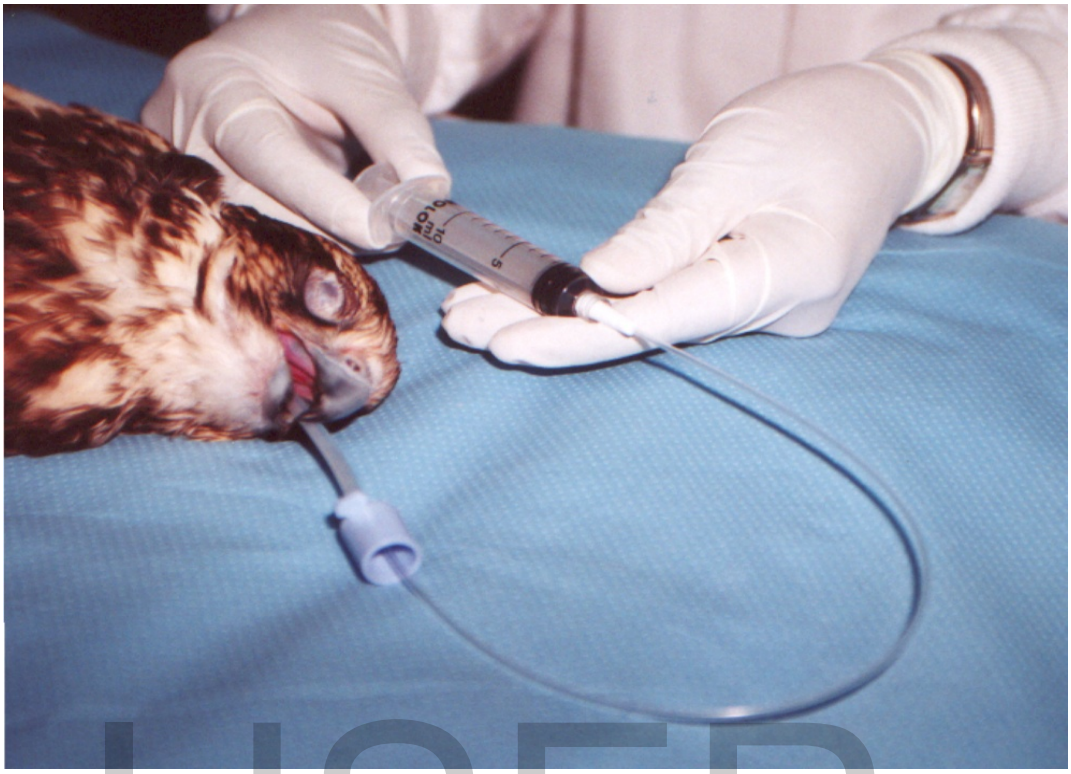

Fig.2.4. Sample collecting from the trachea using endo-tracheal tube.

Fig.2.5. Nasal washing sample collection from a saker falcon.

Fig.2.6. Blood sample collection from a hybrid falcon.

Fig.2.7. Crop aspirate from a hybrid falcon.

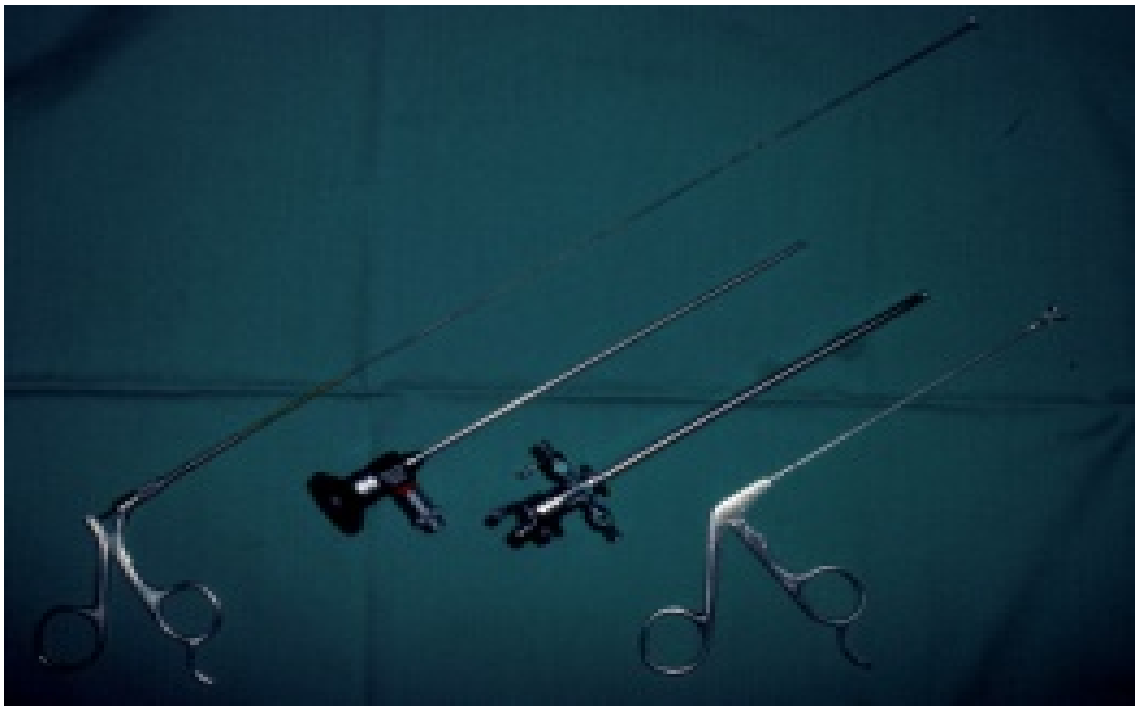

Fig.2.8. Endoscopy forceps used for biopsy sample collection.

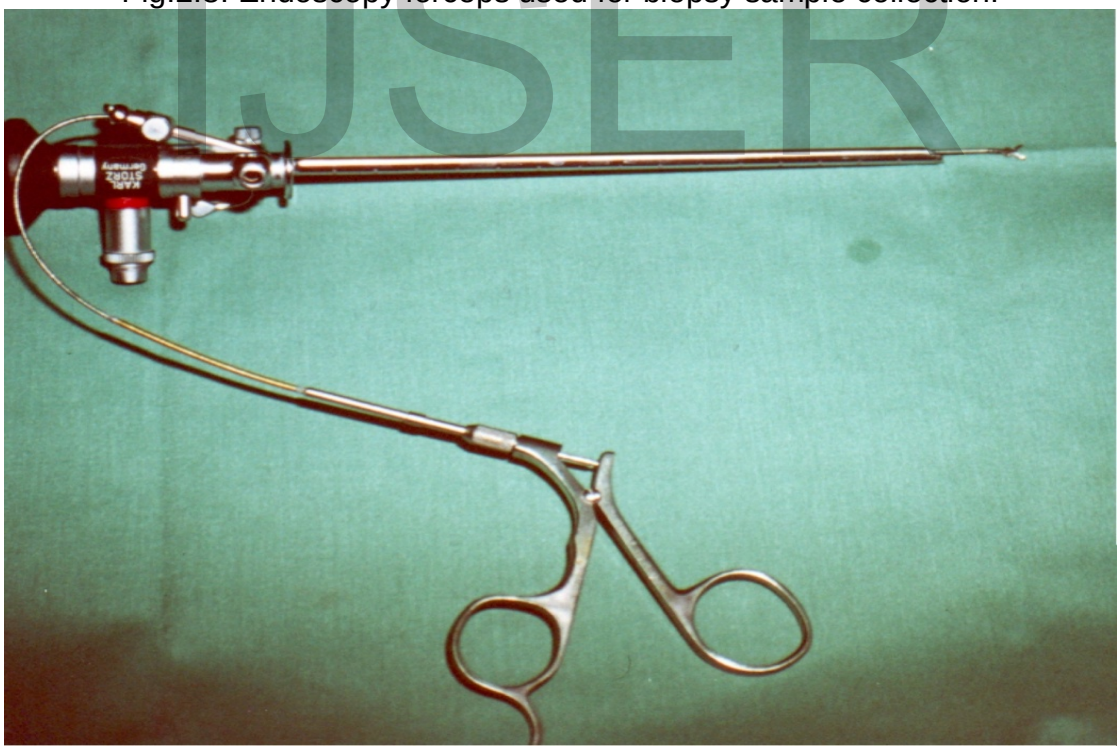

Fig.2.9. Endoscopic viewing lens with biopsy forceps.

Fig.2.10. Biopsy sample collection during endoscopy.

Fig.2.11. A peregrine falcon under anesthesia before biopsy sample collection.

Fig.2.12. Semen sample collection from a houbara bustard.

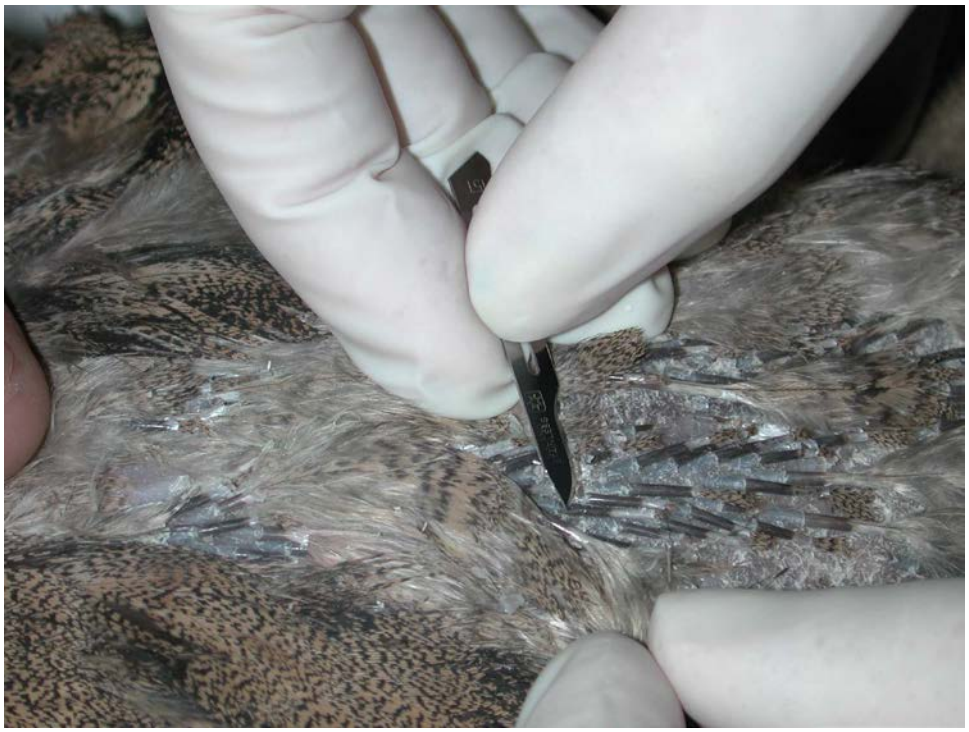

Fig 2.13: Skin scrapping from a houbara bustard using scalpel blade.

Fig 2.14: Sample collection from the leg of a houbara bustard using scalpel blade.

Samples from dry skin lesions caused by fungi or ectoparasites are collected using scalpel blades by scraping. Subcutaneous lesions and swellings are sampled by aspiration. Bumble foot and skin abscess samples are collected by using a thin sterile cotton swab moistened in saline rotating on the surface of lesion. Skin scrapping or infected feather samples are collected for diagnosis fungi and ectoparasite.

Fig 2.15: Sample collection from the nares of a houbara bustard using ENT swab.

2b. Smear preparation

Direct smears should be made from swabs, scrapings, tissue and aspirated fluids with good cellularity. Excessive peripheral blood contamination of a specimen will dilute and mask diagnostic cells; this will make interpretation difficult. Impression smears from the organs should therefore be made after blotting the blood with Whatman filter paper. The thickness of the smear will affect the appearance of the cells and the quality of the smear. Thick areas do not allow the cells to expand on the slide, so they appear smaller

and more dense when compared with the same type on thinner areas of the smear. Therefore, examination of the cells in thick smears should be avoided. Washed samples or poorly cellular fluids require concentration technique to increase the smear cellularity by centrifugation at 600 - 800 g for 5 minutes, discarding the supernatant, and smearing the sediment on to a glass slide or smear made with cytocentrifuge. Figures 2.16 to 2.19 show various smear preparation technique used in avian cytology.

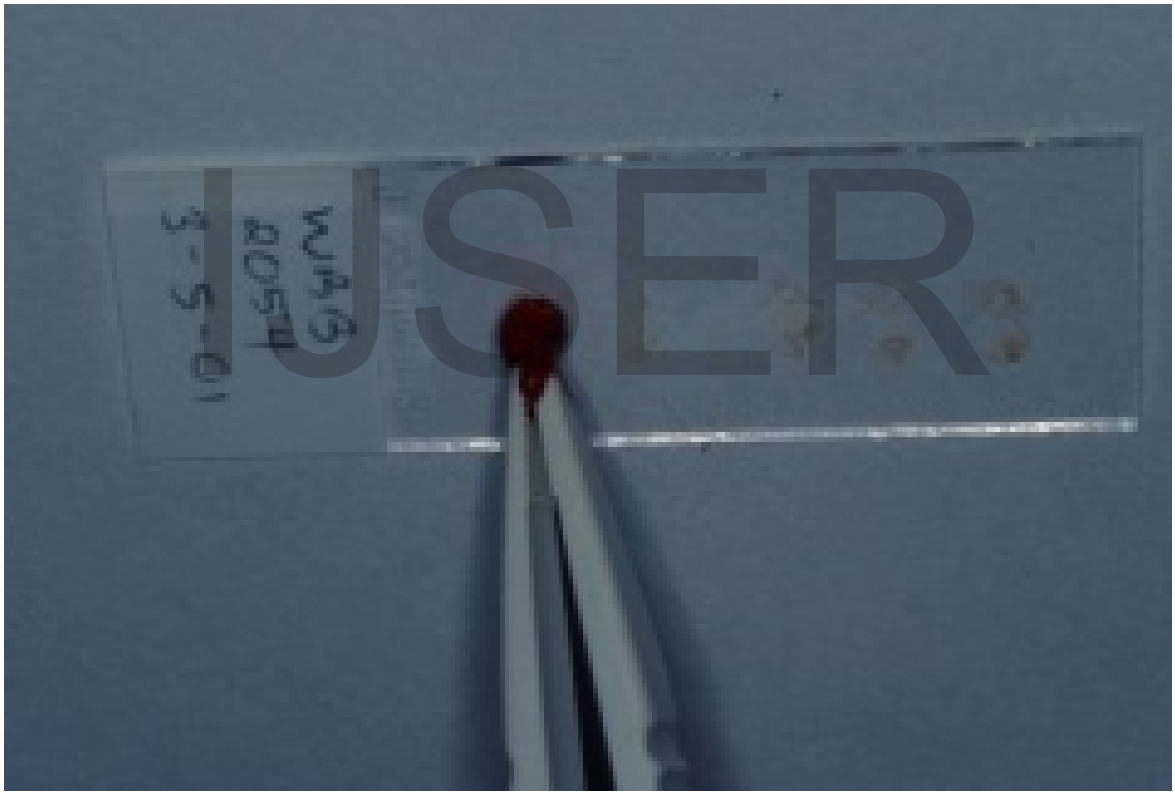

Fig.2.16. Imprint smear preparation from biopsy samples.

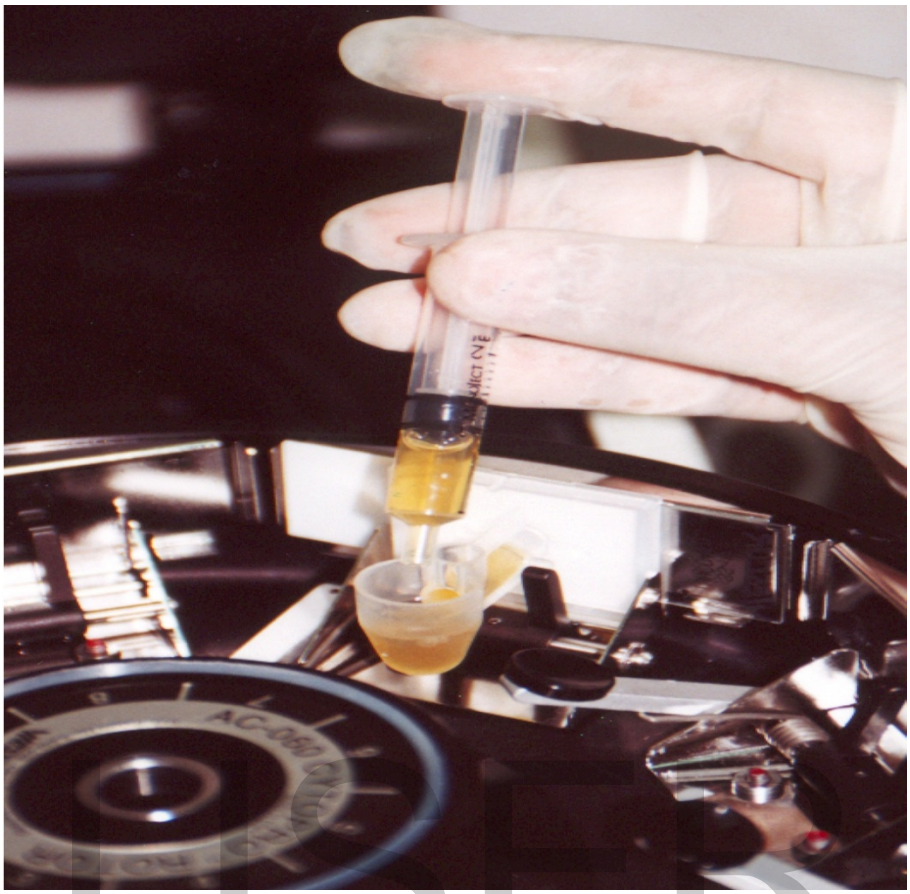

Fig.2.17. Transferring the aspirate into a cyto-centrifuge cuvette for smear preparation.

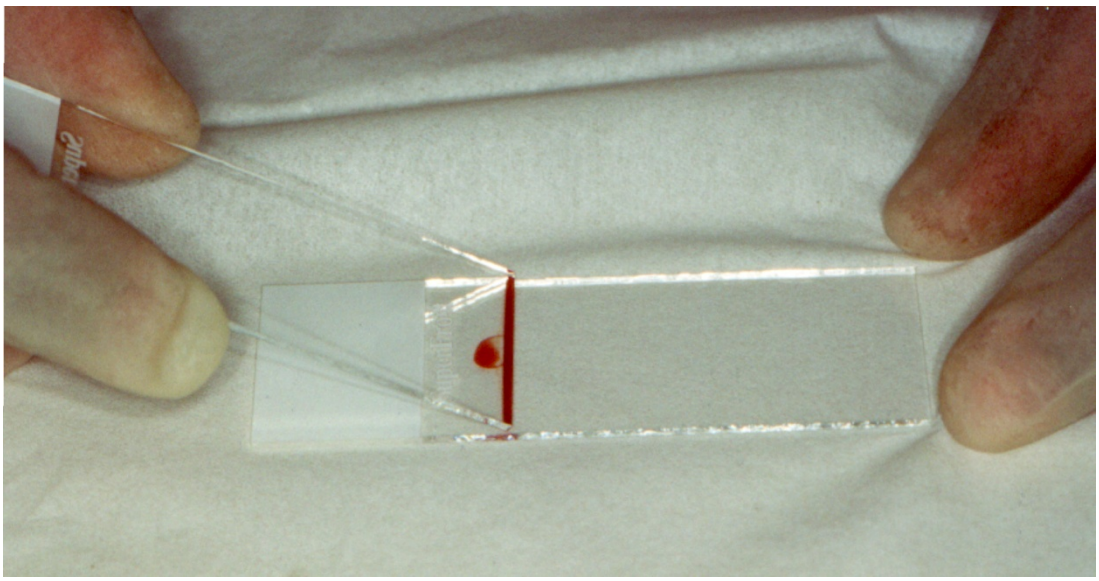

Fig.2.18. Blood smear preparation.

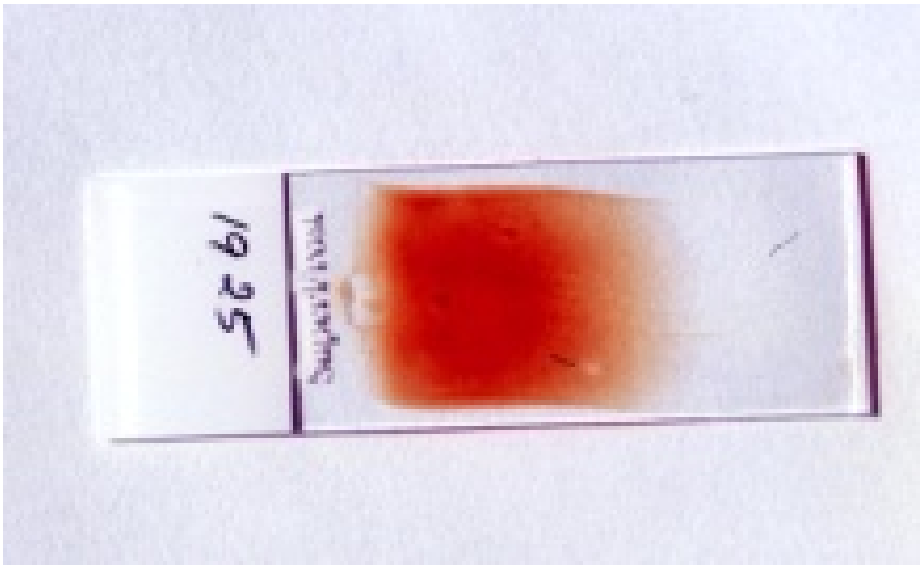

Fig.2.19. Blood smear.

2c. Fixation

Adequate fixation of the smear is essential, prior to the staining so that the material does not wash off during the staining procedure. Methanol is used as a fixative in most of the staining techniques. If smears are to be sent to a diagnostic laboratory, they must air dried, fixed in methanol for 2-5 minutes, well packed and accompanied by a distinct identification and case history.

2d. Staining

Biological stains are generally required to visualise cell structure, inflammatory agents and other biological substances. Stains consist of aqueous and organic preparations of dyes, or groups of dyes, that impart a variety of colours to cells, micro-organisms and to other biologic substances. Microscopic examination of stained smears provides a rapid method for primary diagnosis.

The choice of staining method is a never-ending matter of discussion among cytopathologists. Pathologists are more familiar with wet-fixed smears stained with haematoxylin-eosin (H&E) or Papanicolaou method. Various substitution techniques have been devised in the field of cytopathology. May-Grunwald Giema (MGG) stain is one of these and in a number of situations, particularly when it comes to cytoplasmic detail, it is superior to other stains. In wet fixation staining procedure there is a tendency for cells to shed off the glass slide. MGG illustrations predominate here, as we prefer air-dried cytological preparations before staining. This polychrome staining method provides better results especially in the aspiration biopsy smears.

The commercially available rapid stains of modified May-Grundwald Giemsa (eg: Rapi-Diff, Diff Quick and RAL 555) and Wright's Giemsa (eg: Neat and Hema) are commonly used to stain air-dried smears and the great advantage of the quick stains is a short staining time, which allows rapid examination of the specimen and provide satisfactory staining quality.

May Grunwald-Giemsa stain preparation:

A. May-Grunwald stain

Stock solutions:
Eosin-methylene blue - 0.5 gm and absolute methanol - 100 ml
Working solution:
May - Grunwald - 100 ml and absolute methanol - 50 ml

B. Giemsa stain

Stock solution:

Azure II-Eosin - 0.6 gm, Azure II - 0.16 gm, Glycerine - 50 ml and absolute methanol - 100 ml

Working solution: Giemsa stock - 10 ml and Distilled water - 90 ml

Procedure

- Fix the smear with methanol for 5 minutes.
- Stain the slide with May - Grunwald for 5 minutes and wash in distilled water
- Stain the slide with Giemsa for 15 minutes and wash in distilled water and allow to air dry.

Ziehl-Neelsen stain

Ziehl-Neelsen stain is used to demonstrate the acid-fast reaction of *Mycobacterium* sp. and *Nocardia* sp. Oocysts of *Cryptosporidia* sp and *Sarcocystis* sp. also show acid-fast positive reactions.

Staining solutions**:** Concentrate carbol fuchsin, Methylene blue and 3% Sulphuric acid in 95% ethyl alcohol.

Procedure

- Make a thin smear of the sample in a clean glass slide using a bacterial loop, allow to air dry and fix the smear by passing the slide over the flame of a bunsen burner flame three or four times.
- Place the slide on a staining rack and flood with concentrated carbol fuchsin stain for 5 minutes. Heat two or three time, but not allow to boil.
- Wash with water and decolourise with acid-alcohol (use 1% sulphuric acid for *Nocardia* sp) for 1 to 2 minutes.
- Wash with water and flood with methylene blue stain for 1 minute. Wash with water, allow to dry and examine under oil immersion.

Gram stain

Gram stain is used to differentiate the bacteria into two groups as Gram-positive and Gram-negative. This stain is also useful to the study of size, shape and arrangement of bacteria.

Staining solutions

1. Crystal violet or gention violet - 2 gm%.
2. Gram's iodine (iodine 10 gm% + potassium iodide 20 gm%).
3. Acetone - ethyl alcohol (1:1).
5. Safranin - 0.25%.

Procedure

- Make a thin smear of sample or bacterial suspension of colony on a clean glass slide using a bacterial loop and allow to air dry.
- Fix the smear by passing the slide over a bunsen burner flame three or four times so that the material does not wash off during the staining procedure.
- Place the smear on a staining rack and overlay the surface with crystal violet or gention violet solution for one minute and wash in water.
- Overlay the smear with Gram's iodine solution for one minute.
- Wash in water and decolourise with acetone-alcohol (1:1) for 10 seconds and wash immediately in water.
- Overlay the surface with safranin for one minute and wash in water, air dry and examine under oil immersion.

Chapter 3: Materials and Methods:

3a. Sample collection

Samples from exposed lesions, oro-pharynx and crop were collected by rotating a saline moistened sterile cotton swab on the site. Swabs from bustards including Kori bustard (*Ardeotis kori*), Houbara bustard (*Chlamydotis undulata macqueenii*), Buff-crested bustard (*Eupodotis ruficrista*) and falcons including Gry falcon (*Falco rusticolus*), Peregrine falcon (*Falco peregrinus*), Saker falcon (*Falco cherrug*) and hybrid falcon were collected to prepare the data. Multiple swabs were taken for direct microscopic examination, staining and bacterial culture. Samples from the lower respiratory tract including lungs and airs sacs were collected from anaesthetised birds during endoscopy using a biopsy forceps or swabs. Endoscopic observations such as congestion of lungs, granuloma, airsacculitis and the presence of metazoa should be recorded to support the cytological evaluation. These observations should also be reported during post-mortem examination. In post-mortem cases, lung imprint smears are preferable after blotting in a filter paper to minimise the contamination of haemic cells. Upper and lower respiratory tract are susceptible for fungal diseases and thus swabs were taken for fungal culture. Samples from the synovial swelling were collected by aspiration method using a syringe (2-5ml) and needle (23-25G). Samples from the abdominal cavity and pericardium were collected by aspiration using by 5 to 10 ml syringe with 22 to 25 gauge needle syringe and needle. Conjunctival samples are collected by using a thin sterile cotton swab moistened in saline rotating on the surface of lesion. Samples from dry skin lesions caused by fungi or ectoparasites are collected using scalpel blades by scraping. Subcutaneous lesions and swellings are sampled by aspiration. Bumble foot and skin abscess samples are collected by using a thin sterile cotton swab moistened in saline rotating on the surface of lesion. Skin scrapping or infected feather samples are collected for diagnosis fungi and ectoparasites. Samples from the liver were collected from anaesthetised birds during endoscopy using a biopsy forceps. Endoscopic or post-mortem observations such as enlargement of liver, granuloma and multifocal lesions were recorded to support the cytological evaluation.

Blood samples from 400 bustards including Kori bustard (Ardeotis kori), Houbara bustard (Chlamydotis undulata macqueenii), Buff-crested bustard (Eupodotis ruficrista) and 1500 falcons including Gry falcon (Falco rusticolus), Peregrine falcon (Falco peregrinus), Saker falcon (Falco cherrug) and hybrid falcon were collected to prepare the data. 0.5 ml of blood samples were collected from the metatarsal or brachial vein using 1 ml syringe with 23 gauge needle and transferred in EDTA tubes and mix it in a roller mixture.

Post-mortem samples from 13 bustards including Kori bustard (*Ardeotis kori*), Houbara bustard (*Chlamydotis undulata macqueenii*), Buff-crested bustard (*Eupodotis ruficrista*) and 22 falcons including Gry falcon (*Falco rusticolus*), Peregrine falcon (*Falco peregrinus*), Saker falcon (*Falco cherrug*) and hybrid falcon were done and imprint smears were done from the internal organs such as liver, spleen, heart, kidney, lung and small intestine. Table 1 shows the details of samples collected from various sites.

Table1. Samples collected from various sites of bustards and falcons.

Sample site	Bustards	Falcons
Upper Alimentary Tract	110 (swabs)	900 (swabs)
Lower Alimentary Tract	110 (Swabs) 13 (autopsy)	900 (swabs) 22 (autopsy)
Upper Respiratory Tract	200 (swabs)	1200 (swabs)
Lower Respiratory Tract	80 (biopsy) 13 (autopsy)	600 (biopsy) 22 (autopsy)
Conjunctiva and cornea	80 (swabs)	100 (swabs)
Aspirated fluids	21	16
Internal organs - Liver	47 (biopsy) 13 (autopsy)	36 (biopsy) 22 (autopsy)
Internal organs - Heart	13 (autopsy)	22 (autopsy)
Internal organs - Kidney	13 (autopsy)	22 (autopsy)
Internal organs - Spleen	13 (autopsy)	22 (autopsy)
Skin and subcutis	80	100

Blood	400	1500

3b. Smear preparation:

Swabs were collected, smeared by rotation it on glass slides and dried it at room temperature. Imprint smears from biopsy or autopsy samples from the organs were prepared after blotting in a filter paper to minimise the contamination of haemic cells. A small drop of blood (5 - 8μl) of blood is placed on the end of a clean glass slide and smeared it using a spreader and dried at room temperature. Wet smears are also made with samples collected from skin and alimentary tract to examine parasites.

3c. Fixation and staining:

Adequate fixation of the smear is essential, prior to the staining so that the material does not wash off during the staining procedure. Ethanol is used as a fixative.

Staining was done using modified May-Grunwald Giema (MGG) stain and it is one of the best cytoplasmic stains in a number of situations, particularly when it comes to cytoplasmic detail (Cooper 1994). In wet fixation staining procedure there is a tendency for cells to shed off the glass slide. MGG illustrations predominate here, as we prefer air-dried cytological preparations before staining. This polychrome staining method provides better results. The commercially available rapid stains (Diff Quick and Neat) are commonly used to stain air-dried smears and the staining qualities of the smears are similar to those of MGG stain. The great advantages of the quick stains are short staining time, which allows rapid examination of the specimen and provide satisfactory staining quality.

3d. Microscopic Examination:

Microscopic examination of wet smear was done at 100X and 400 X, but the stained smears were examined at 1000X magnification using Olympus BX51 microscope and photography were done using DP70 microscopic camera.

Chapter 4: Results

4a. Tables

Table 1 and 2 show the results obtained from the upper respiratory tract and table 3 and 4 show the results obtained from the lower respiratory tract of falcons and bustards.

Table 1shows the bacteria, fungi and parasitic findings from the upper respiratory tract in bustards and falcons.

Causative agent		Bustards (n= 200)*	Falcons (n=1200)*
Bacteria	Pasterulla sp Chlamydia psittaci Streptococcus bovis Staphylococcus aureus Mycoplasm sp Pseudomonas aeruginosa Bordetella sp	16	52
Fungi	Aspergillus sp	5	27
Protozoa	Cryptosporidia sp	-	2
Metazoa	Syngamus sp	-	6
	Serratospiculum sp	-	23

*n = Number of birds

Table 2 shows the microscopic findings of upper respiratory tract associated with various pathological conditions.

Diagnostic cytology	Microscopic findings
Normal cytology	Few numbers of lining columnar squamous cells, small amount of mixed normal flora bacteria.
Acute bacterial trachitis	Predominate of single type bacterial colonization, > 70% heterophils, < 30% macrophages.
Chronic bacterial trachitis	Predominate of single type bacterial colonization, > 70% macrophages, <30% heterophils.
Chronic active bacterial stomatitis	Predominate of single type bacterial colonization, Mixed population of heterophils and macrophages approximately 1:1 ratio
Aspergillosis	Fungal hyphae, spores, giant cells, goblet cells and mixed inflammatory cells.
Cryptosporidiasis	Cryptosporidia oocyst, mucous, inflammatory cells and cellular debrics
Syngamus sp	Syngamus eggs, excessive exfoliation of superficial, intermediate and basal cells and inflammatory cells.
Serratospiculum sp	Serratospiculum sp eggs and superficial squamous cells.

Table 3 shows the bacteria, fungi and parasitic findings from the lower respiratory tract in bustards and falcons.

Causative agent		Bustards (n= 93)*	Falcons (n=622)*
Bacteria	Pasterulla sp Chlamydia psittaci Streptococcus bovis Staphylococcus aureus Mycoplasm sp Pseudomonas aeruginosa Bordetella sp	12	18
Fungi	Aspergillus sp	6	37
Protozoa	Cryptosporidia sp	-	2
Metazoa	Serratospiculum sp		21

*n = Number of birds

Table 4 shows the microscopic findings from the lower respiratory tract of bustards and falcons associated with various pathological conditions.

Diagnostic cytology	Microscopic findings
Normal cytology	RBC seen in lung imprints. Airsac lining squamous cells seen in airsac samples
Acute bacterial airsacculitis	Predominate of single type bacterial colonization, > 70% heterophils, < 30% macrophages.
Chronic bacterial airsacculitis	Predominate of single type bacterial colonization, > 70% macrophages, <30% heterophils.
Pneumonia	Mixed population of heterophils and macrophages with bacterial colonisation
Aspergillosis	Fungal hyphae, spores, giant cells and mixed inflammatory cells.
Cryptosporidiasis	Cryptosporidia oocyst, mucous, inflammatory cells and cellular debrics
Serratospiculum sp	Serratospiculum sp eggs.

Table 5 and 6 show the results obtained from the upper alimentary tract and table 7 and 8 show the result obtained from the lower alimentary tract of bustards and falcons

Table 5 shows the upper alimentary tract inflammatory response caused in bustards and falcons.

Causative agent		Bustards (n= 110)	Falcons (n=900)
Bacteria	Pasterulla sp Pseudomonas sp Staphylococcus sp Streptococcus sp Salmonella sp Compylobacter sp Mycobacterium sp	21	109
Virus	Pox	7	-
Protozoa	Trichomonas sp	16	111
	Entamoeba sp	3	-
Metazoa	Capillaria sp	-	19
	Serratospiculum sp	-	36

Table 6 shows the microscopic findings from the upper alimentary tract associated with various pathological conditions.

Diagnostic cytology	Microscopic findings
Normal cytology	Superficial squamous epithelial cells, small amount of mixed normal flora bacteria.
Acute bacterial stomatitis	Predominate of single type bacterial colonization, > 70% heterophils, < 30% macrophages.
Chronic bacterial stomatitis-	Predominate of single type bacterial colonization, > 70% macrophages, <30% heterophils.
Chronic active bacterial stomatitis	Predominate of single type bacterial colonization, Mixed population of heterophils and macrophages approximately 1:1 ratio
Pox virus	Intermediate and basal squamous cells with Bollinger bodies and Borrel bodies
Trichomonas sp	Trichomonas sp, mucous, inflammatory cells
Entamoeba sp	Entamoeba sp and inflammatory cells.
Capillaria sp	Capillaria eggs, excessive exfoliation of superficial, intermediate and basal cells and inflammatory cells.
Serratospiculum sp	Serratospiculum sp eggs and superficial squamous cells.
Hypovitaminosis A	Excessive exfoliation of huperkeratinised superficial squamous cells.

Table 7shows the lower alimentary tract bacterial enteritis and parasitic findings in bustards and falcons.

Causative agent		Bustards (n= 128)*	Falcons (n=912)*
Bacteria	Salmonella sp Vibrio cholera Chlamydia psittaci Compylobacter sp Clostridium botulinum Clostidium perfringens Streptococcus bovis Enterococcus avium Toxic E. coli Mycobacterium sp	37	52
Protozoa	Trichomonas sp	16	-
	Coccidia sp	3	310
Metazoa	Capillaria sp	-	17
	Serratospiculum sp	-	36
	Trematode	4	41
	Acanthocephala sp	44	2
	Ascaridia sp	7	9

*n = Number of birds

Table 8 shows the microscopic findings from the lower alimentary tract associated with various pathological conditions.

Diagnostic cytology	Microscopic findings
Normal cytology	Squamous epithelial cells, mixed normal flora bacteria.
Bacterial stomatitis	Exfoliation of columnar squamous cells with predominant of single type bacteria
Coccidia sp	Heavy infection showed Coccidia sp, Columnar cells, mucous, RBC, inflammatory cells
Metazoa sp	No changes, Heavy infection of cestode and trematode cause blood in feces

Table 9 and 10 show the microbial and cytological findings from the conjunctiva of bustard and falcons.

Table 9 shows the bacteria, virus and parasitic findings from the conjunctiva of bustards and falcons.

Diseases	Causative agent	Bustards (n= 80)*	Falcons (n=100)*
Conjunctivitis	Aeromonas sp Pasterulla sp Chlamydia psittaci Streptococcus bovis Staphylococcus aureus Mycoplasm sp Pseudomonas aeruginosa Bordetella sp	12	13
Pox	Pox virus	3	2
Cryptosporidiasis	Cryptosporidia sp	-	2
Trematode infection	Eye flukes	2	-

*n = Number of birds

Table 10 shows the microscopic findings associated with various pathological conditions of conjunctiva and cornea.

Diagnostic cytology	Microscopic findings
Normal cytology	Conjunctiva columnar cells or squamous cells
Conjuctivitis	Predominate of single type bacteria and inflammatory cells.
Pox	Squamous cells with Bollinger bodies
Cryptosporidiasis	Squamous cells, inflammatory cells and Cryptosporidia oocysts
Trematode infection	Eye flukes

Table 11 and 12 show the microbial and cytological findings from the aspirated fluids of bustard and falcons.

Table 11 shows the bacteria, virus and parasitic findings in the aspirated fluids of bustards and falcons.

Diseases	Causative agent	Bustards (n= 21)*	Falcons (n=16)*
	Streptococcus bovis Staphylococcus aureus Mycoplasm sp	7	5
Pox	Pox virus	2	-
Serratospiculosis	Serratospiculum	-	1
Aspergillosis	Aspergillus sp	-	1

*n = Number of birds

Table 12 shows the microscopic findings of aspirated fluids associated with various pathological conditions.

Diagnostic cytology	Microscopic findings
Normal cytology	Mesothelial cells, RBC
Inflammation	Reactive mesothelial cells, bacteria and inflammatory cells.
Pox	Synovial cells with Bollinger bodies
Serratospiculosis	Serratospiculum eggs

Table 13 and 14 show the microbial and cytological findings from the post-mortem samples collected from the heart of bustard and falcons.

Table 13 shows the cardiac diseases and causative agents in bustards and falcons.

Diseases	Causative agent	Bustards (n= 13)*	Falcons (n=22)*
Pericarditis	Streptococcus bovis Clostridium perfringens	2	3
Tuberculosis	Mycobacterium	-	1
Gout	Urate crystal deposition	2	4
Filaria	Endo parasite	1	-

*n = Number of birds

Table 14 shows the microscopic findings associated with various pathological conditions of heart.

Diagnostic cytology	Microscopic findings
Normal cytology	RBC
Pericarditis	Inflammatory cells including heterophils and macrophages, bacteria
Tuberculosis	Macrophages, AFB rods in Z-N stain
Gout	Urate crystals
Filaria	Microfilaria

Table 15 and 16 show the microbial and cytological findings from the post-mortem samples collected from the kidney of bustard and falcons.

Table 15 shows the renal diseases and causative agents in bustards and falcons.

Diseases	Causative agent	Bustards (n= 13)*	Falcons (n=22)*
Septicemia	Streptococcus bovis Clostridium perfringens	2	2
Avian leucosis	Leucosis virus	1	-
Septicemic pox	Pox virus	1	-
Gout	Urate crystals	2	4

*n = Number of birds

Table 16 shows the microscopic findings associated with various pathological conditions of kidney.

Diagnostic cytology	Microscopic findings
Normal cytology	Renal squamous cells
Septicemia	Reactive renal cells, inflammatory cells and bacteria
Avian leucosis	Plasma cells, reactive renal cells.
Septicemic pox	Plasma cells, reactive renal cells.
Gout	Urate crystals

Table 17 and 18 show the microbial and cytological findings from the biopsy and post-mortem samples collected from the liver of bustard and falcons.

Table 17 shows the findings associated with liver diseases in bustards and falcons.

Diseases	Causative agent	Bustards (n= 47)*	Falcons (n=36)*
Bacterial hepatitis	Chlamydia psittaci Streptococcus bovis Salmonella sp Clostridium botulinum Clostridium perfringens	7	5
Herpes	Viral disease	1	6
Avian leucosis	Viral disease	1	-
Fatty liver	Fat deposition	5	2
Amyloidosis	Amyloid protein deposition	2	7
Mycobacterium tuberculosis	Mycobacterium tuberculosis	-	3

*n = Number of birds

Table 18 shows the microscopic findings in liver cytology associated with various pathological conditions.

Diagnostic cytology	Microscopic findings
Normal cytology	Hepatocytes and RBC.
Hepatitis	Heterophils, macrophages, kupffer cells.
Fatty liver	Hepatocytes with fat vacuolation
Amyloid	Hepatocytes and amyloid
Herpes	Hepatocytes with perinuclear inclusions
Avian leucosis	Hepatoytes with reactive changes, lymphoid cells.
Tuberculosis	Hepatocytes with reactive changes, Numerous kupffer cells. Z-N stain shows acid fast bacilli.

Table 19 and 20 show the microbial and cytological findings from the post-mortem samples collected from the spleen of bustard and falcons.

Table 19 shows the splenic diseases and causative agents in bustards and falcons.

Diseases	Causative agent	Bustards (n= 13)*	Falcons (n=22)*
Septicemia	Streptococcus bovis Clostridium perfringens	2	2
Chlamydiosis	Chlamydia	2	2
Herpes	Herpes virus	2	4
Avian leucosis	Leucosis virus	1	-
Tuberculosis	Mycobacterium tuberculosis	-	1
Septicemic pox	Pox virus	1	-

*n = Number of birds

Table 20 shows the microscopic findings associated with various pathological conditions of spleen.

Diagnostic cytology	Microscopic findings
Normal cytology	Lymphoid cells
Septicemia	Plasma cells,reactive lymphoid cells and bacteria
Chlamydiosis	Plasma cells, reactive lymphoid cells and chlamydia inclusions
Herpes	Plasma cells, reactive lymphoid cells with perinuclear inclusions
Avian leucosis	Plasma cells, reactive lymphoid cells.
Tuberculosis	Plasma cells, reactive lymphoid cells. AFB positive rods in Z-N stain.
Septicemic pox	Plasma cells, reactive lymphoid cells and with karyorrhexis.

Septicemia normally affects all internal organs and thus spleen also shows enlargement and reactive changes. Streptococcus sp, Mycobacterium sp, Clostridium sp. are the pathogens isolated from such cases. Viral disease includes hepes, avian leucosis and septicemic pox.

Table 21 and 22 show the microbial and cytological findings from the skin and subcutis of bustard and falcons.

Table 21shows the bacteria, virus fungi and parasitic findings from the skin of bustards and falcons.

Diseases	Causative agent	Bustards (n= 80)*	Falcons (n=100)*
Ectoparasites	*Degeeriella rufa* (louse)	-	14
	Colpocephalum zerafae (louse)	-	10
	Otidoecus houbarae (louse)	3	-
	Laemobothrion tinnunculi (louse)	-	3
	Hyalomma marginatum (tick)	1	2
	Pseudo lynchia canariensis (louse-fly)		1
	Ornithonyssus sp (mite)	-	2
	Amblyoma sp (tick)	1	-
Pox	Pox virus	3	2
Fungi	*Trichophyton* sp	1	1
Bacterial abscess or bumble foot	*Staphylococcus aureus* *Streptococcus pyogens* *Enterococcus* sp	2	9

*n = Number of birds

Table 22 shows the microscopic findings associated with various pathological conditions of skin.

Diagnostic cytology	Microscopic findings
Normal cytology	Keratinised squamous cells
Abscess or Bumble foot	Bacteria and inflammatory cells.
Pox	Squamous cells with Bollinger bodies
Ectoparasite	Louse, mite and nymph of ticks
Dermatitis	Fungal filaments and keratinized squamous cells.

4b. Gross Pathology

Oro-pharyngeal lesions are seen in association with bacterial stomatitis, candidiasis, capillariasis and trichomoniasis. Images 4.1 to 4.4 show the appearance of oro-pharyngeal lesions and 4.5 and 4.6 show the gross pathology of lower alimentary tract in bustards and falcons. Aspergillosis and bacterial airsacculitis are the common fungal diseases seen in respiratory tract of birds (images 4. 7 to 4.10). Synovial arthritis (image 4.11) and conjunctivitis (images 4.12 to 4.13) are common diseases in bustards and falcons.

Hepatomegaly seen in falcons is mainly caused by fatty liver, amyloid accumulation, bacterial hepatitis caused by tuberculosis, chlamydiosis, and salmonellosis. Viral hepatitis caused by herpes and adeno virus infections. While in bustards, hepatomegaly is associated with fatty liver, hepatitis, chlamydiosis, salmonellosis and leucosis. Adeno virus infection was seen in a falcon that showed massive hepatic necrosis and pale yellow (discoloration) of liver. Images 4.24 to 4. 33 show the gross pathology of liver associated with various viral and bacterial diseases.

Systemic infections may be confirmed by the presence of the aetiologic agent in spleenic samples. Salmonella sp., Chlamydia sp. and herpes virus infections cause splenomegaly in falcons. Images 4.34 to 4. 39 show the normal and abnormal spleen of bustards and falcons.

Viseral gout is a common post-mortem finding in bustards. Articular gout has only occasionally been observed in bustards. Gout should not be considered as a disease entity, but as a clinical manifestation of any severe renal dysfunction that result in a persistent hyperuricaemia and very often gout can be attributed to renal diseases. If uric acid crystals crystals precipitate in the kidney, an acute obstructive uropathy occurs and the secretion of uric acid is severely compromised. Images 4.21 to 4.23 show the gross pathology of kidney in response to viral, bacterial and gout depositions. Skin diseases are caused by fungi, ectoparasites and pox (Images 4. 40 to 4.42).

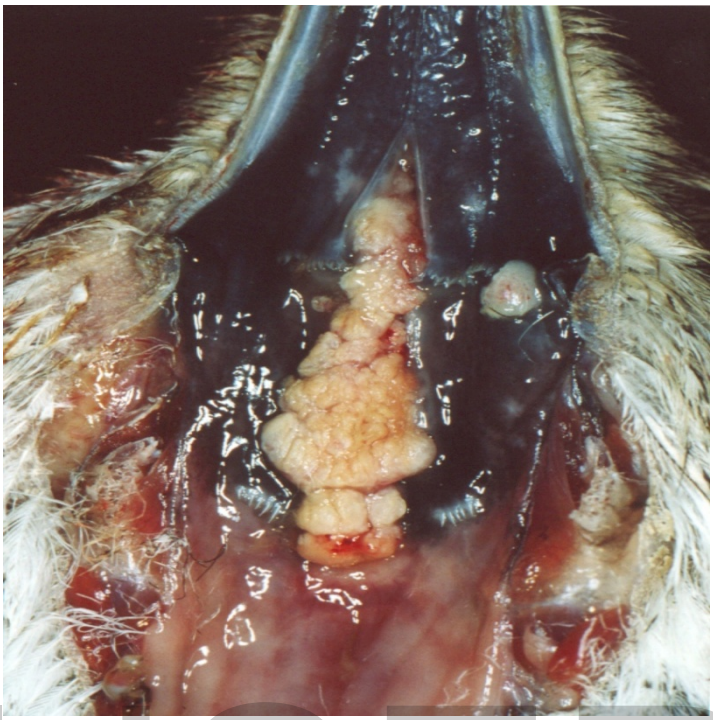

Fig.4.1 Oral cavity of a Houbara bustard (*Chlamydotis undulata macqueenii*) with pox lesion.

Fig.4.2. Oral cavity of a Gry falcon (*Falco rusticolus*) with Trichomonas lesion.

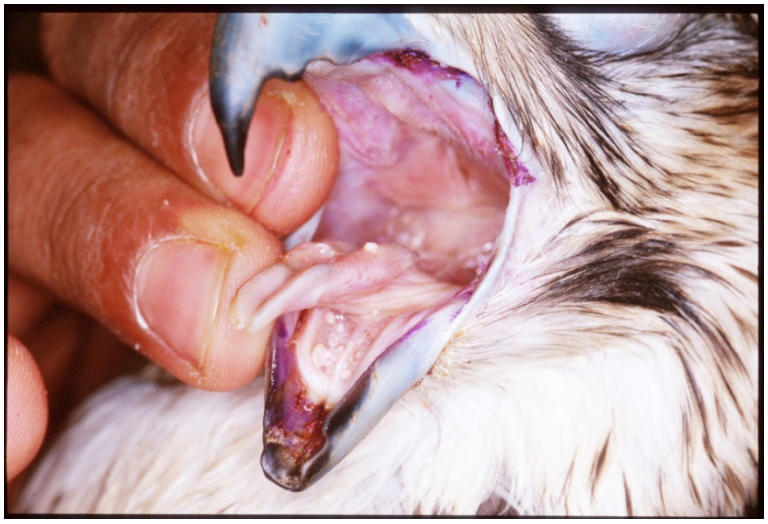

Fig.4.3. Oral cavity of a Gry falcon (*Falco rusticolus*) with Capillaria lesion.

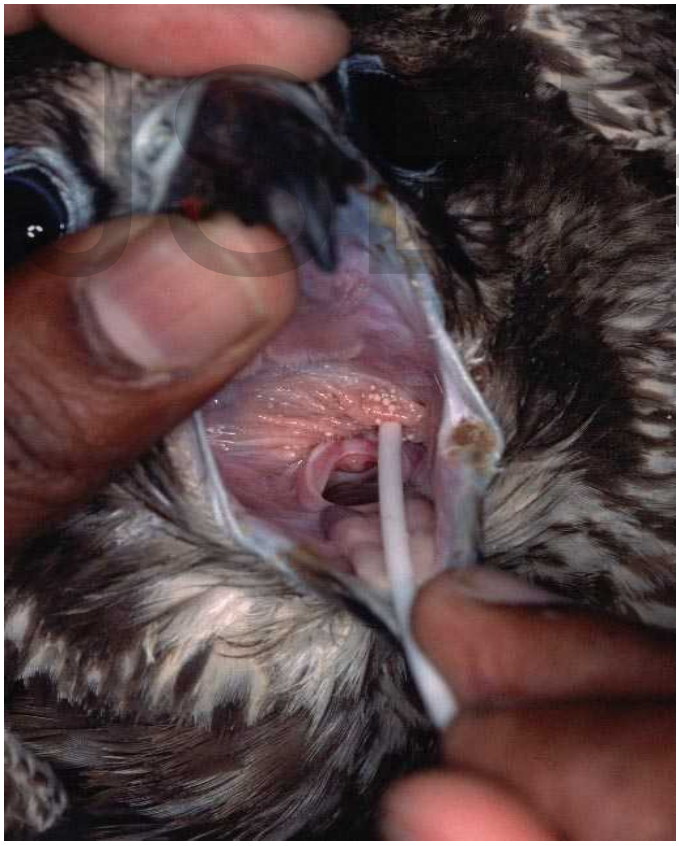

Fig.4.4. Oral cavity of a hybrid falcon with Candida lesion.

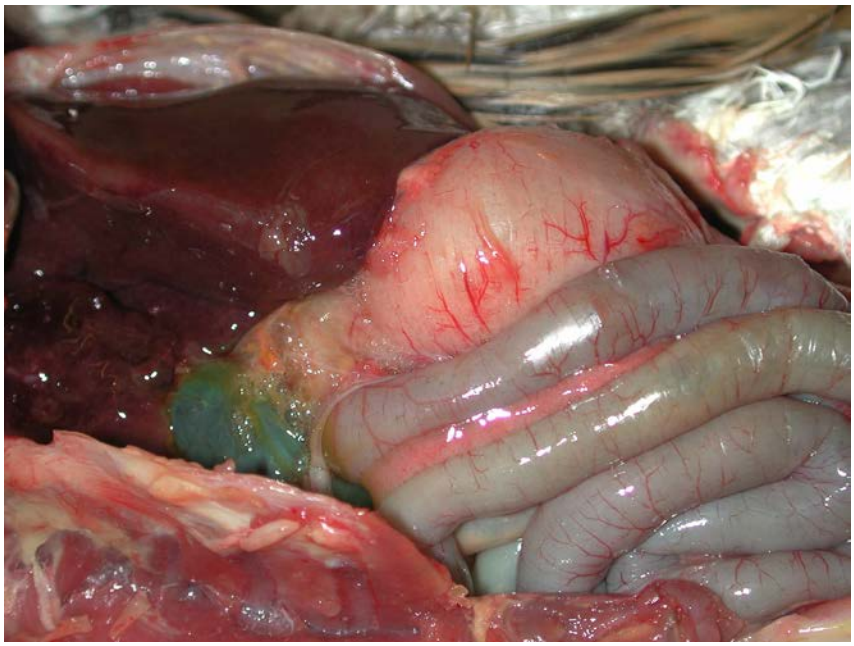

Fig.4.5. Post mortem view of a Gyr falcon (*Falco rusticolus*) showing gas gangrene due to Salmonella enteritis.

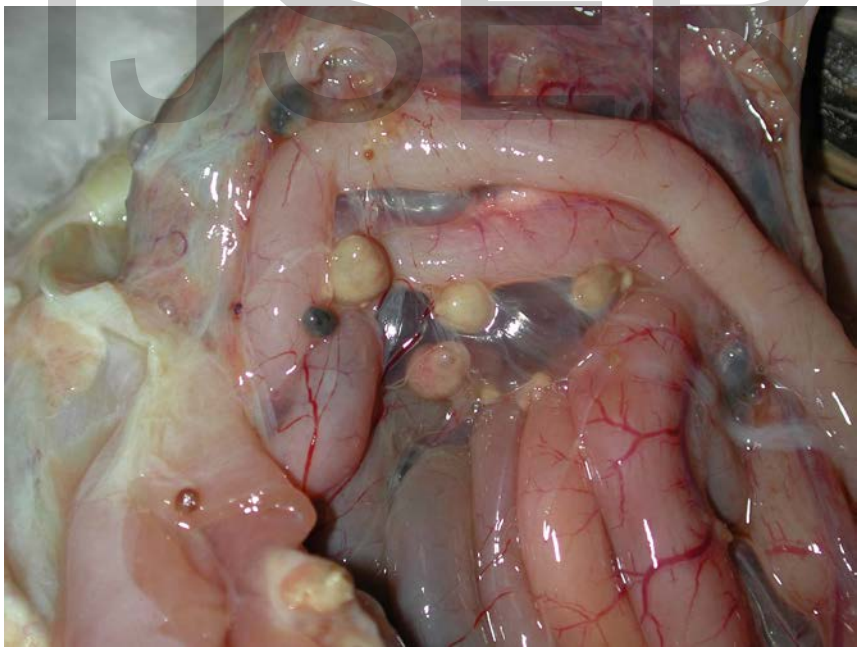

Fig.4.6. Post mortem view of a Gyr falcon (Falco rusticolus) showing Mycobacteriaum tuberculosis lesion attached to the small intestins.

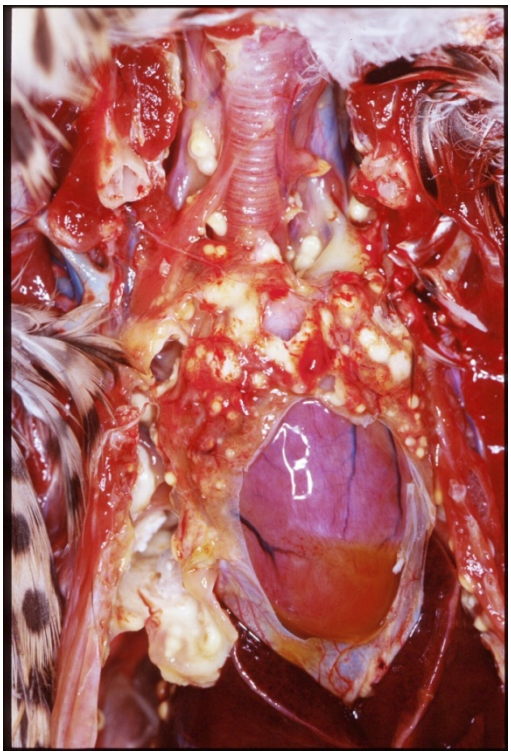

Fig 4.7. Post-mortem view of a gyr falcon showing Aspergillus granulomas in the airsac.

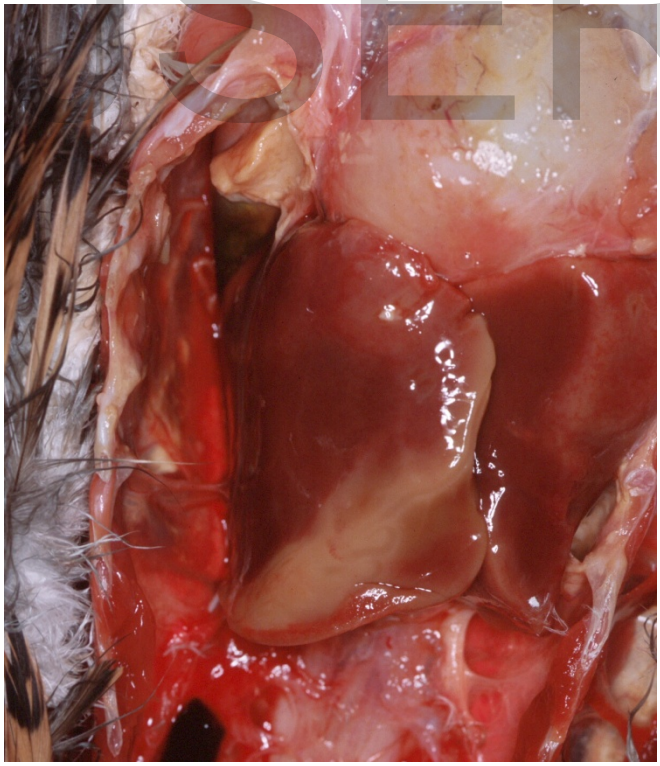

Fig 4.8. Post-mortem view of a gyr-peregrine hybrid falcon showing bacterial air sacculitis.

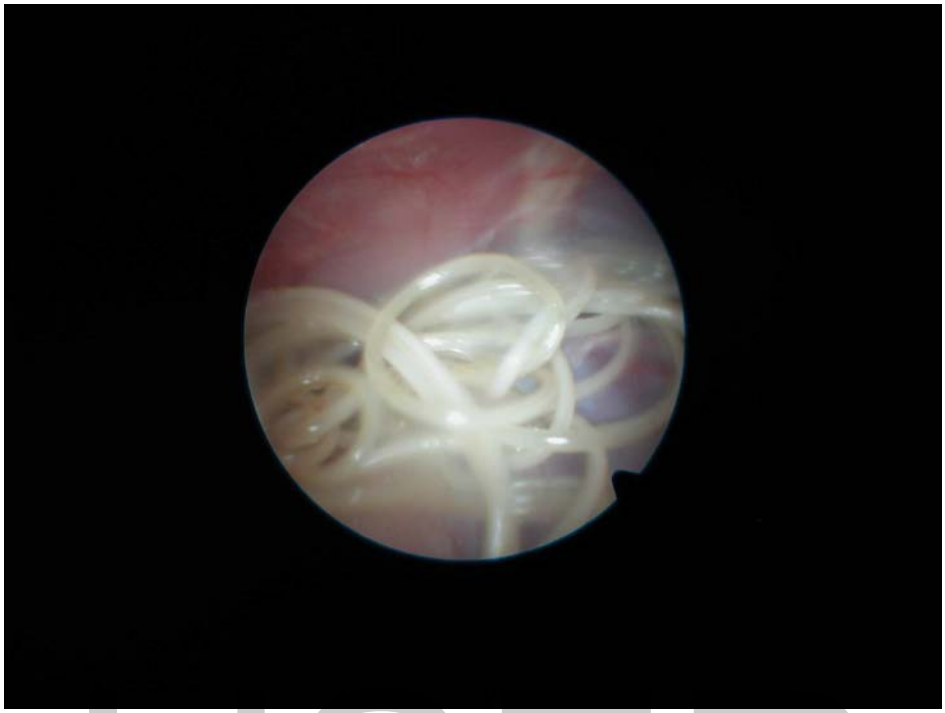

Fig 4.9. Endoscopic view of a saker falcon showing Serratospiculum worms in the airsac.

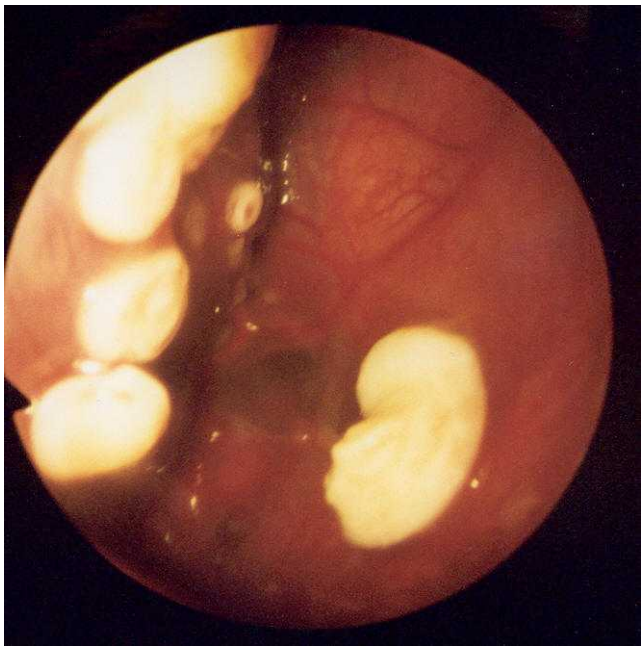

Fig 4.10. Endoscopic view of a gyr falcon showing aspergillosis in the airsac.

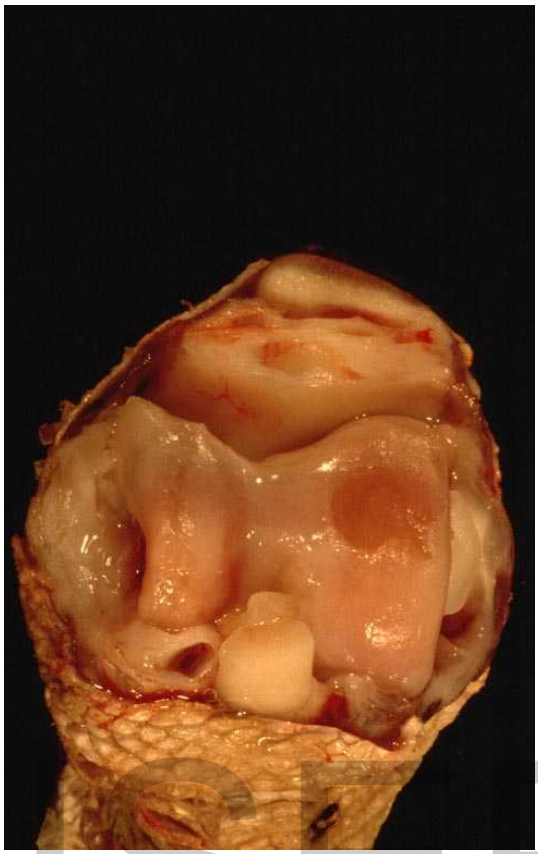

Fig.4.11. Synovial joint of a houbara bustard with arthritis.

Fig.4.12. A white bellied bustard showing eye flukes infection.

Fig.4.13. A saker falcon showing bacterial sinusitis.

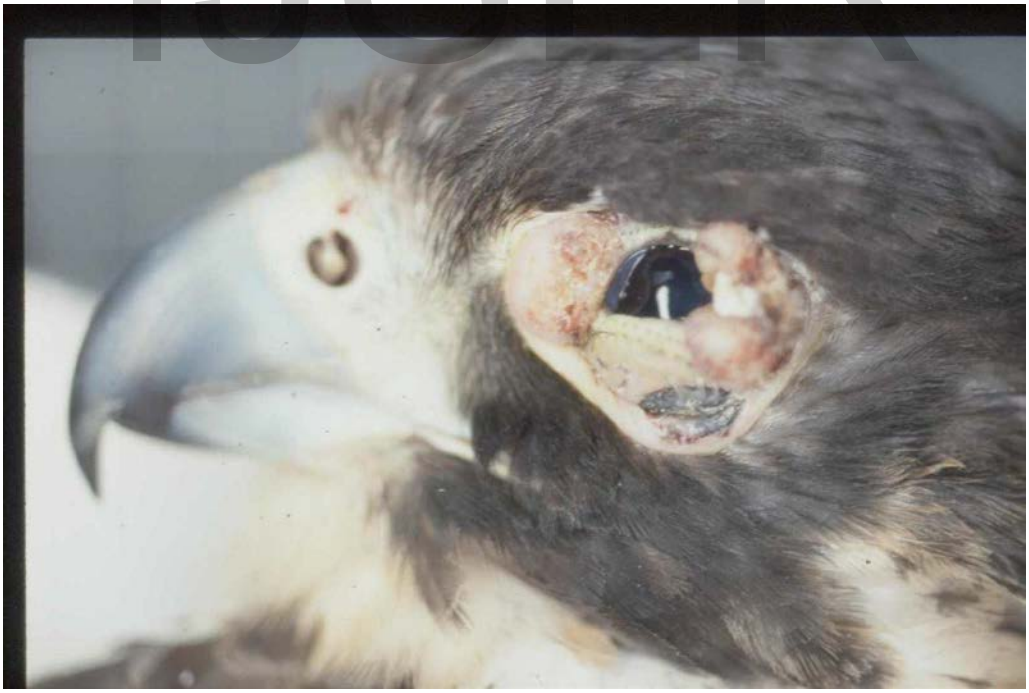

Fig.4.14. A peregrine falcon showing falcon pox in the eyelid.

Fig.4.15 A hybrid falcon showing cataract.

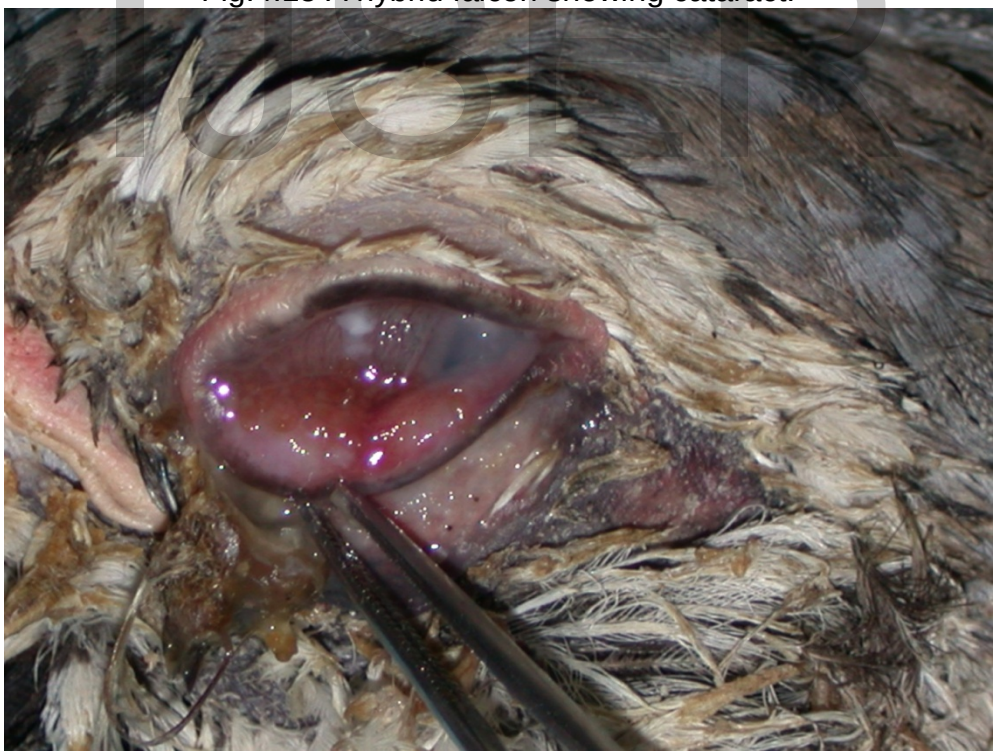

Fig.4.16. A houbara bustard showing bacterial conjunctivitis.

Fig.4.17. A houbara bustard showing pox in the third eye lid.

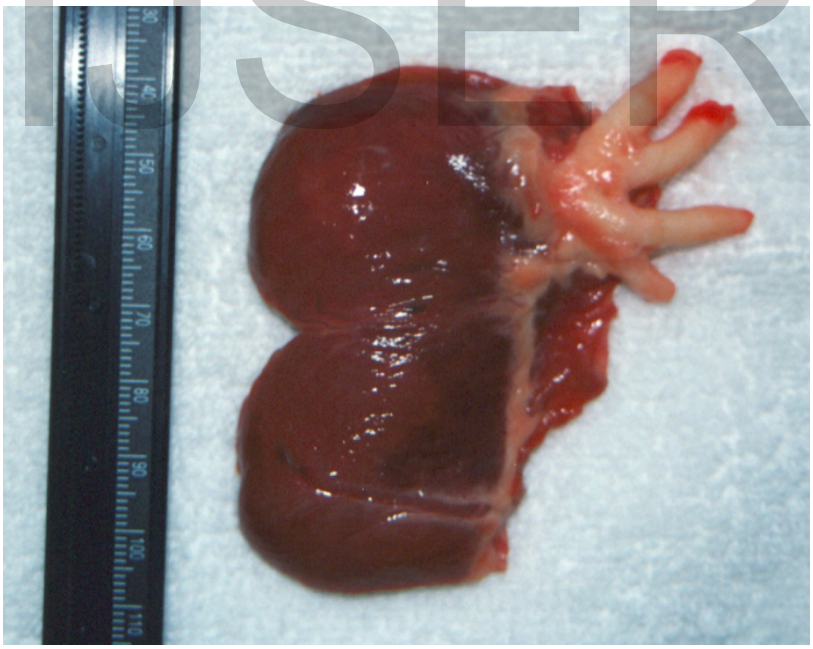

Fig.4.18. Heart of a healthy houbara bustard.

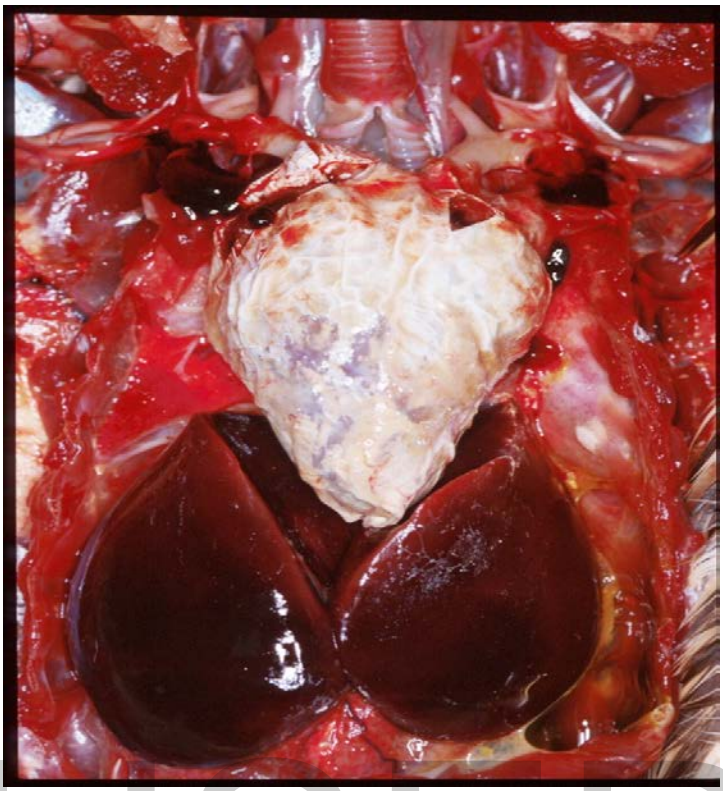

Fig. 4.19. Post-mortem view of a Gyr falcon showing pericardial gout.

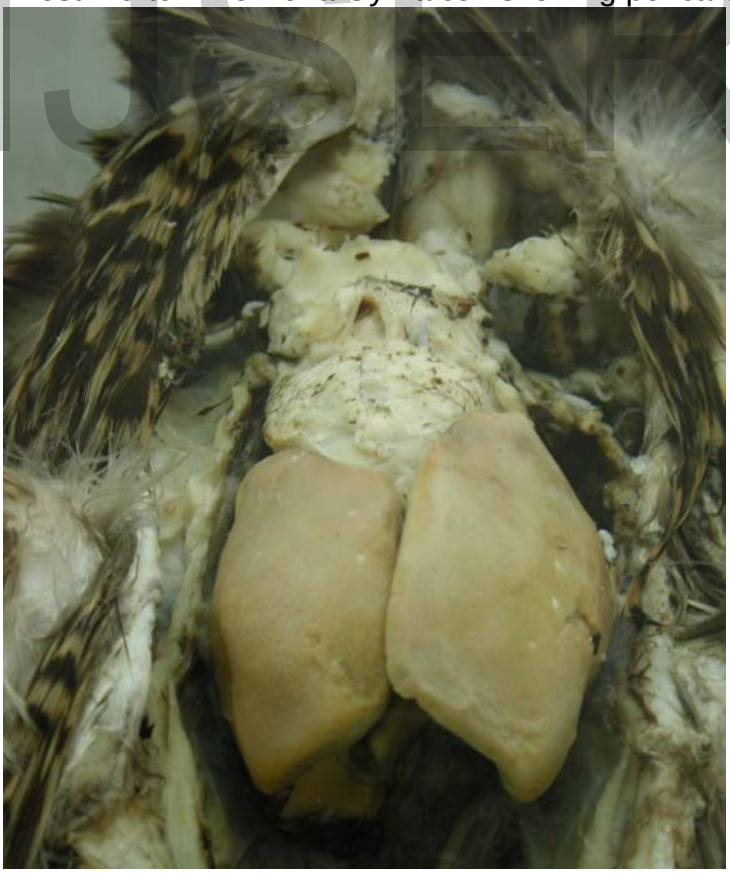

Fig.4.20. Post-mortem view of a peregrine falcon showing pericardial tuberculosis.

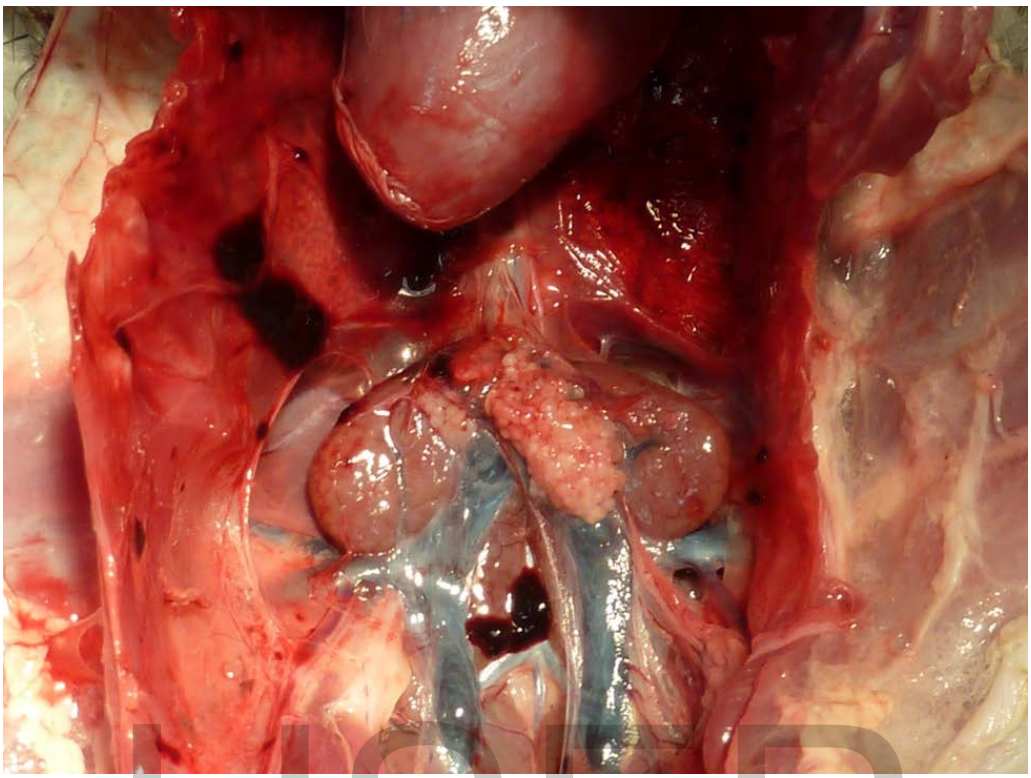

Fig.4.21. Post-mortem view of a gyr falcon showing normal kidney.

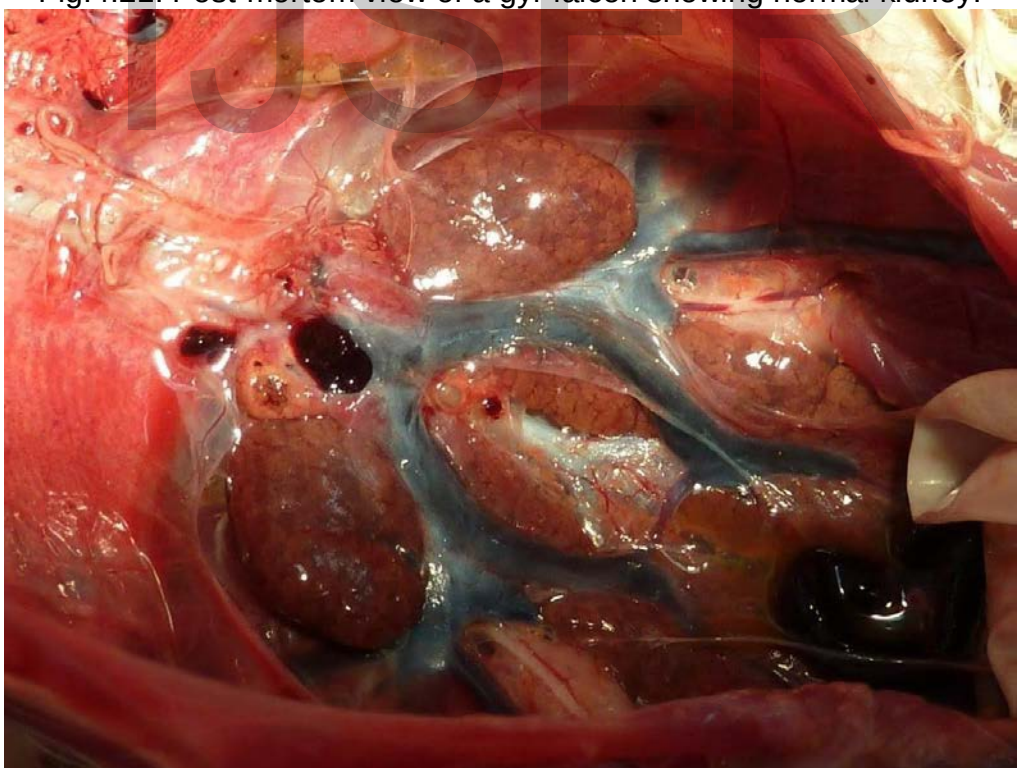

Fig.4.22. Post-mortem view of a houbara bustard showing mottled kidney due to septicemia.

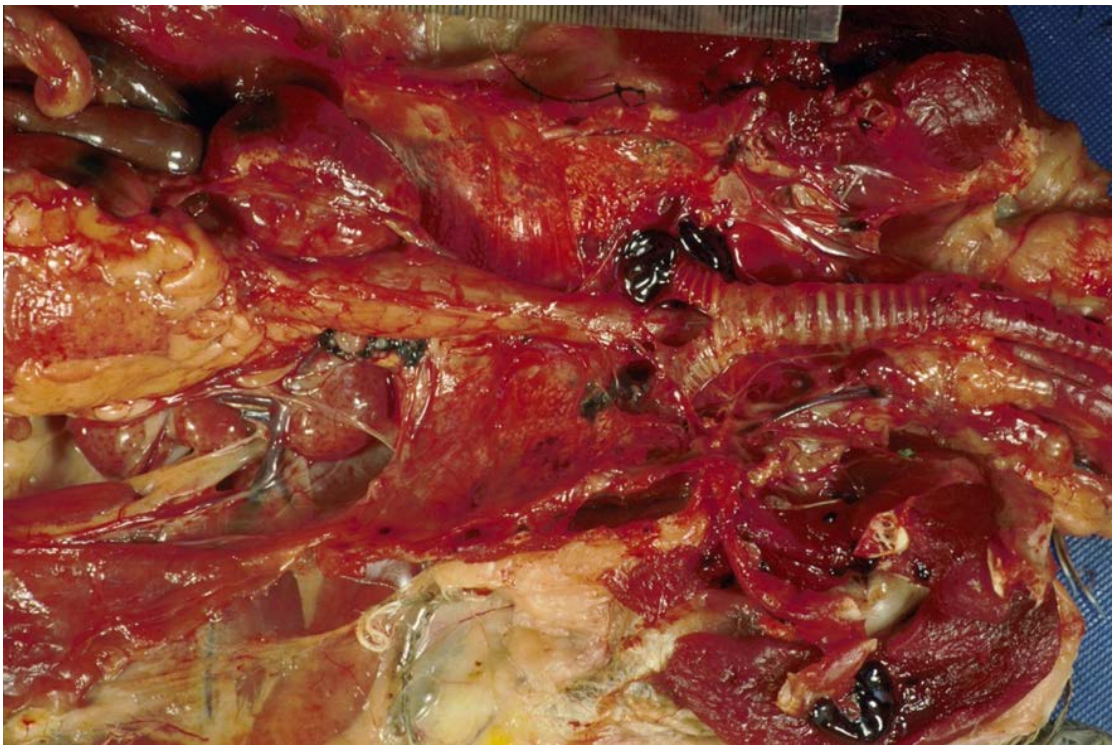

Fig.4.23. Enlarged kidney of a houbara bustard with avian leucosis.

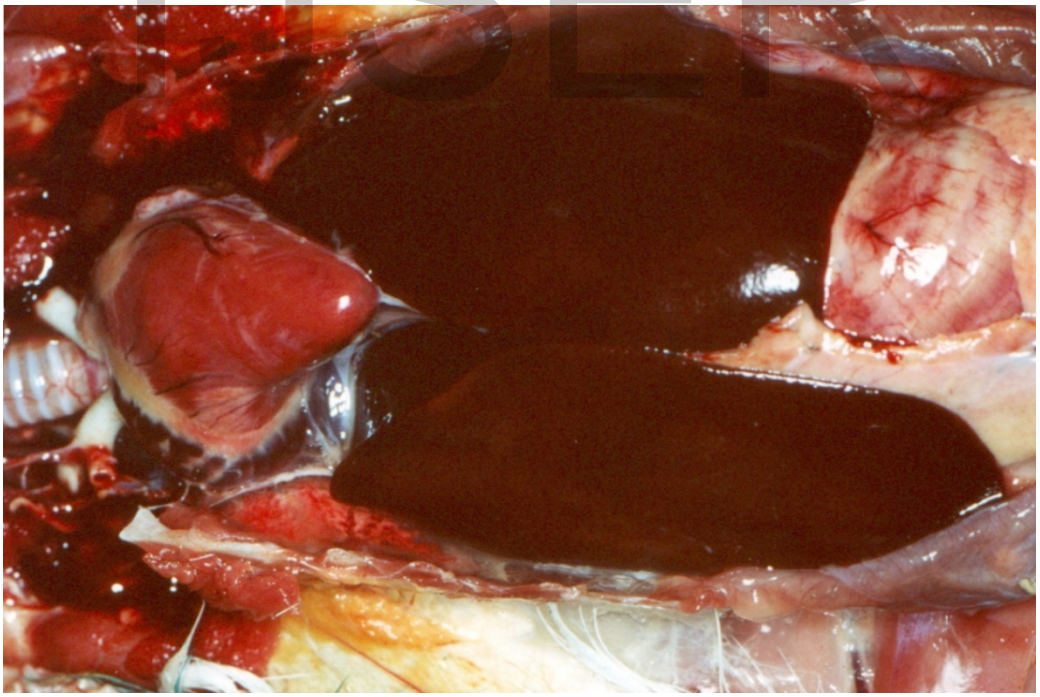

Fig.4. 24Post-mortem view of a Gyr falcon showing enlarged liver (hepatomegaly) due to hepatitis.

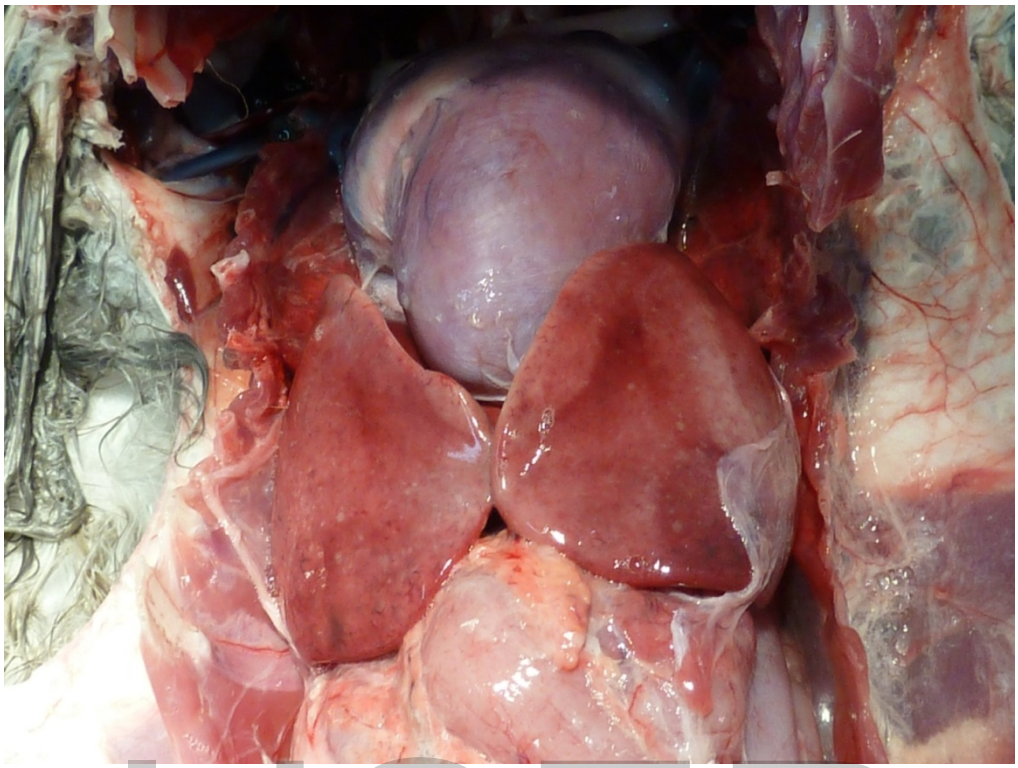

Fig.4.25. Post-mortem view of a houbara bustard showing pale liver due to bacterial hepatitis.

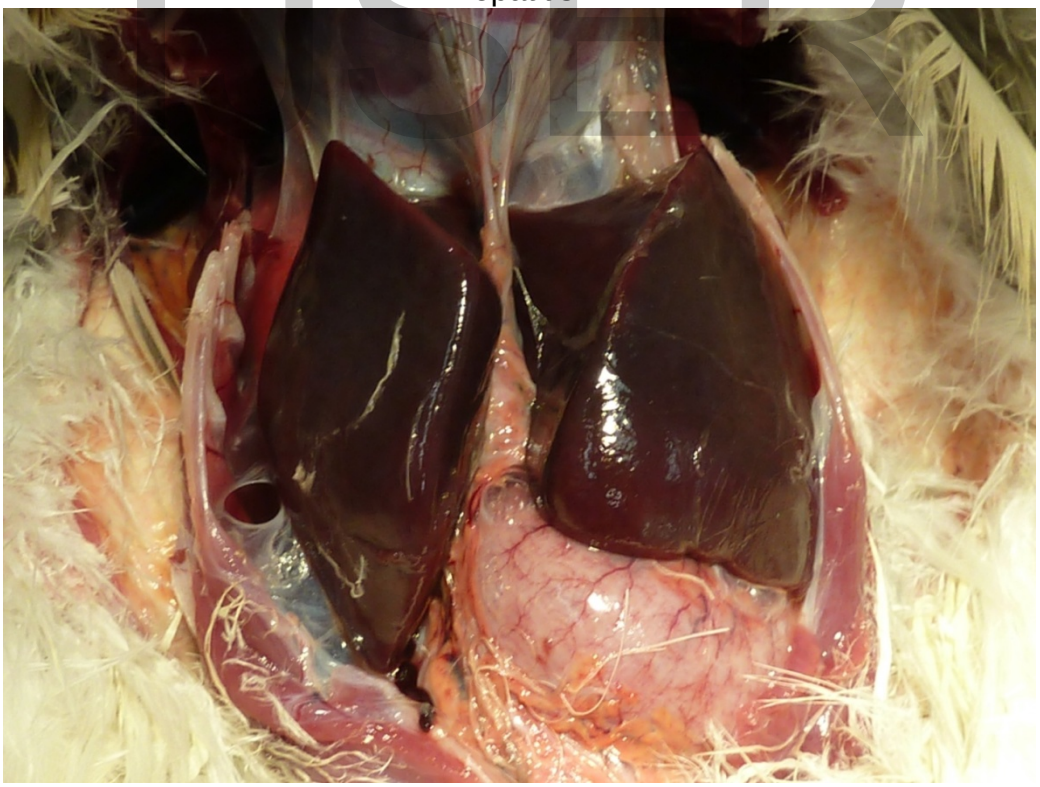

Fig.4.26. Post-mortem view of a houbara bustard showing dark liver due to bacterial septicemia.

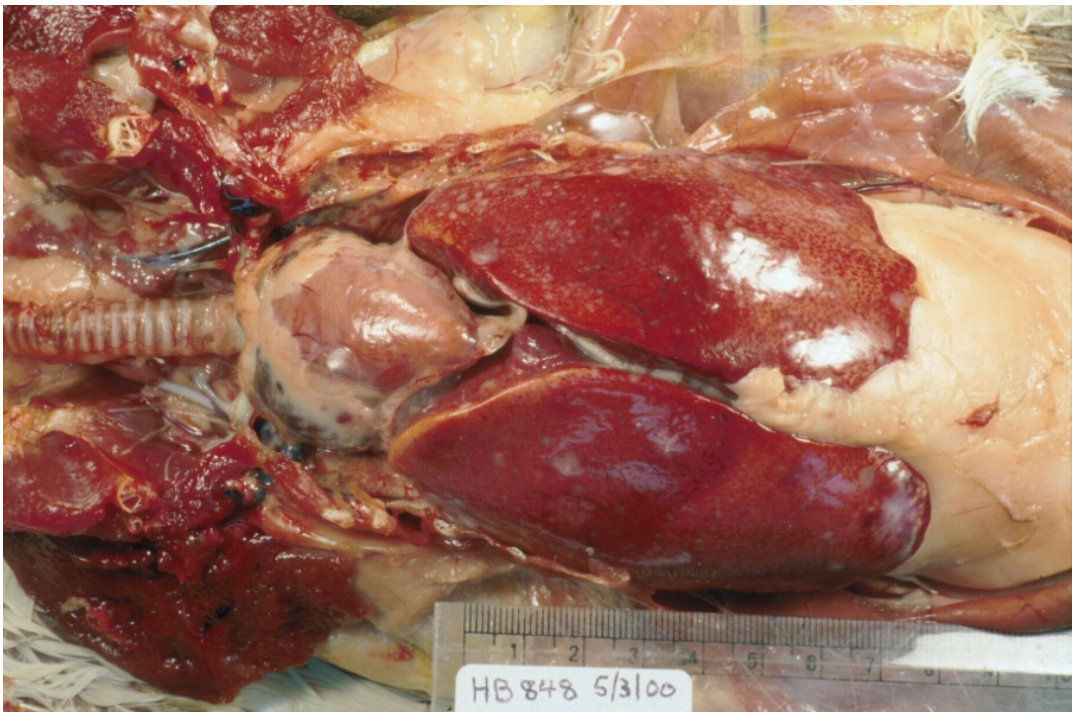

Fig.4.27. Post-mortem view of a houbara bustard showing hepatomegaly due to avian leucosis.

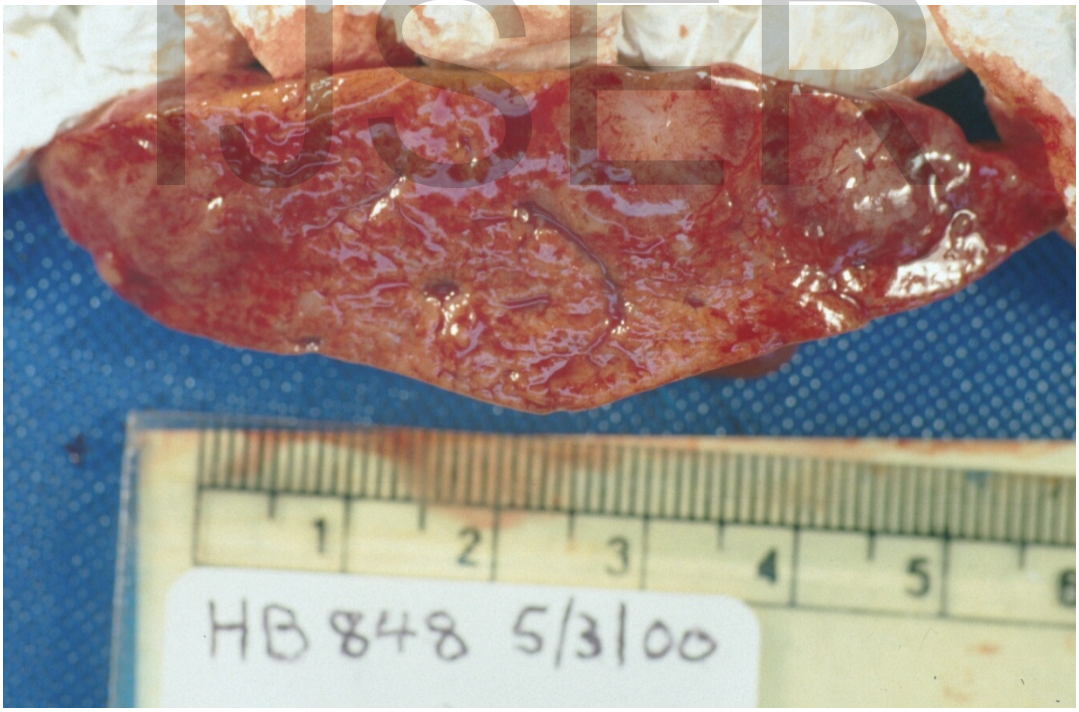

Fig.4.28. Cut surface of liver of a houbara bustard showing avian leucosis.

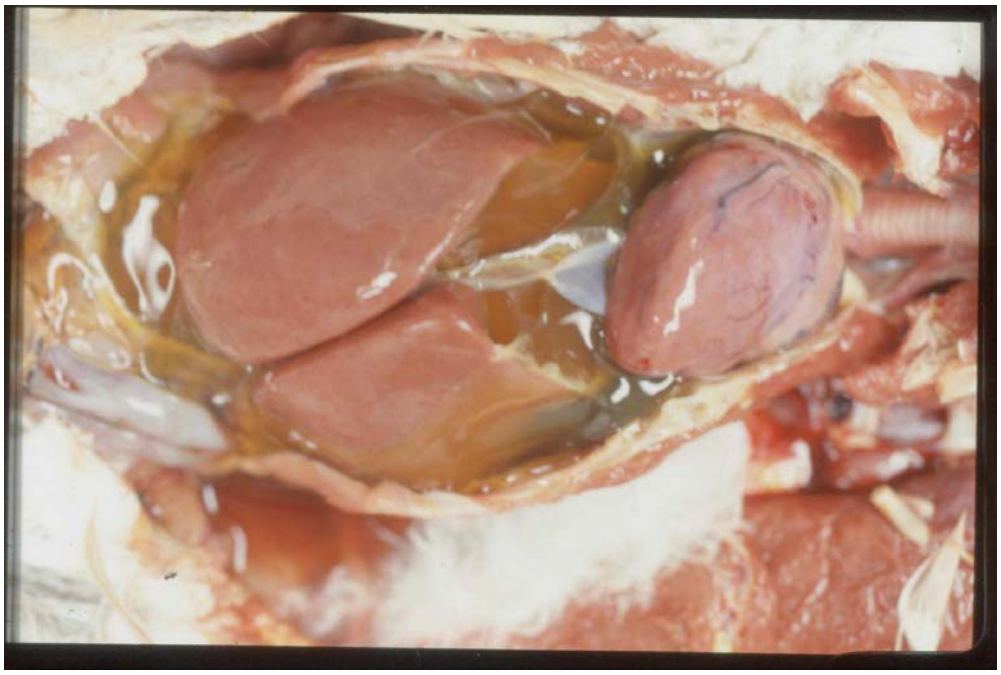

Fig.4.29. Post-mortem view of a houbara bustard showing pale liver due to amyloidosis.

Fig.4.30. Cut surface of liver of a falcon showing amyloidosis.

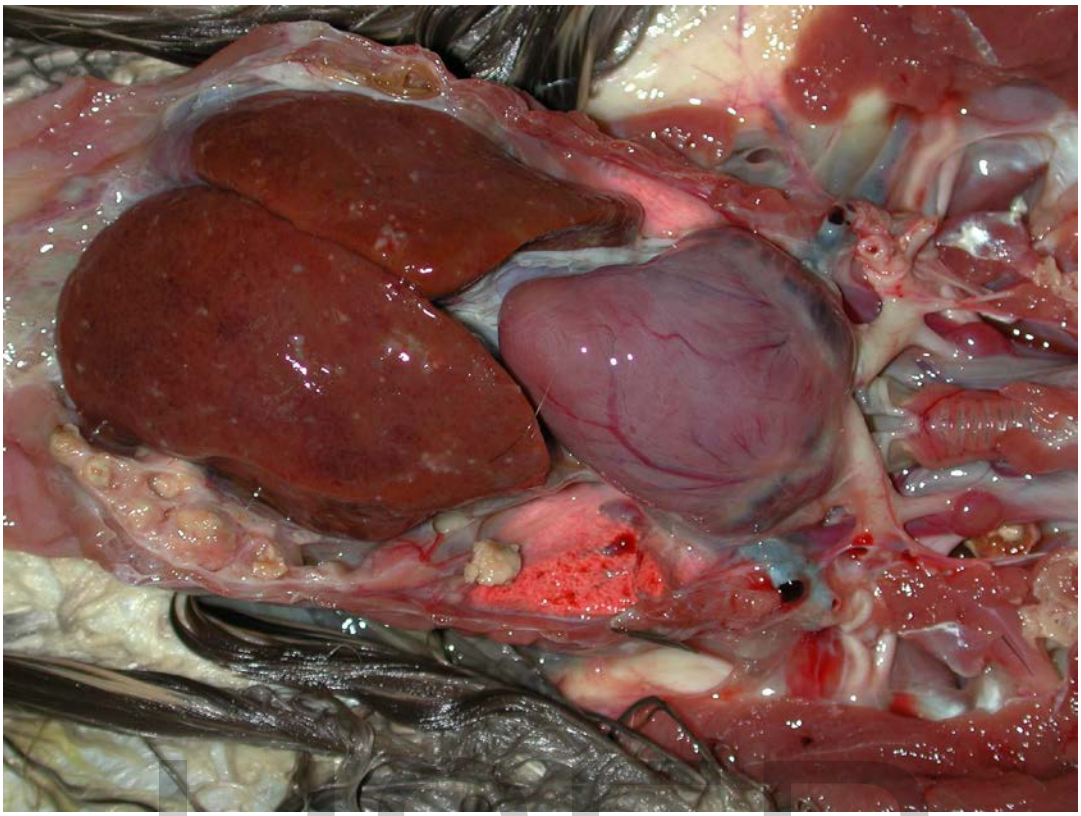

Fig.4.31. Post-mortem view of a peregrine falcon showing tuberculosis lesions in the liver.

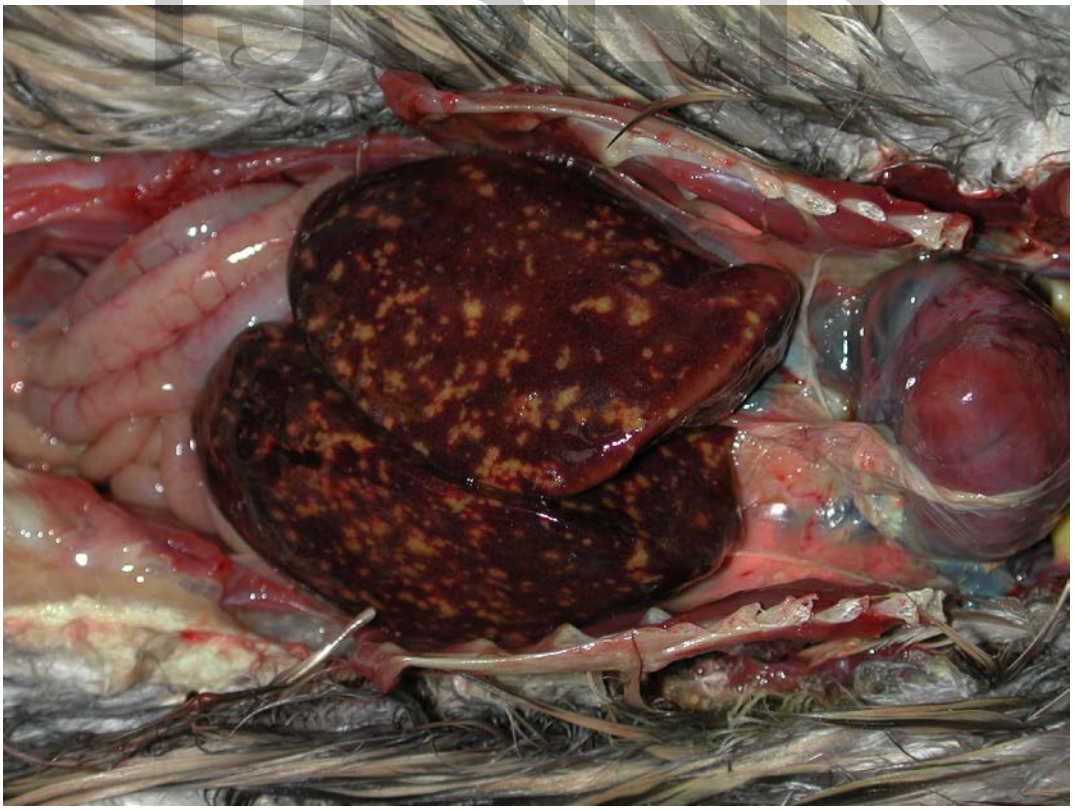

Fig.4.32. Post-mortem view of a falcon showing multifocal herpes lesions in the liver.

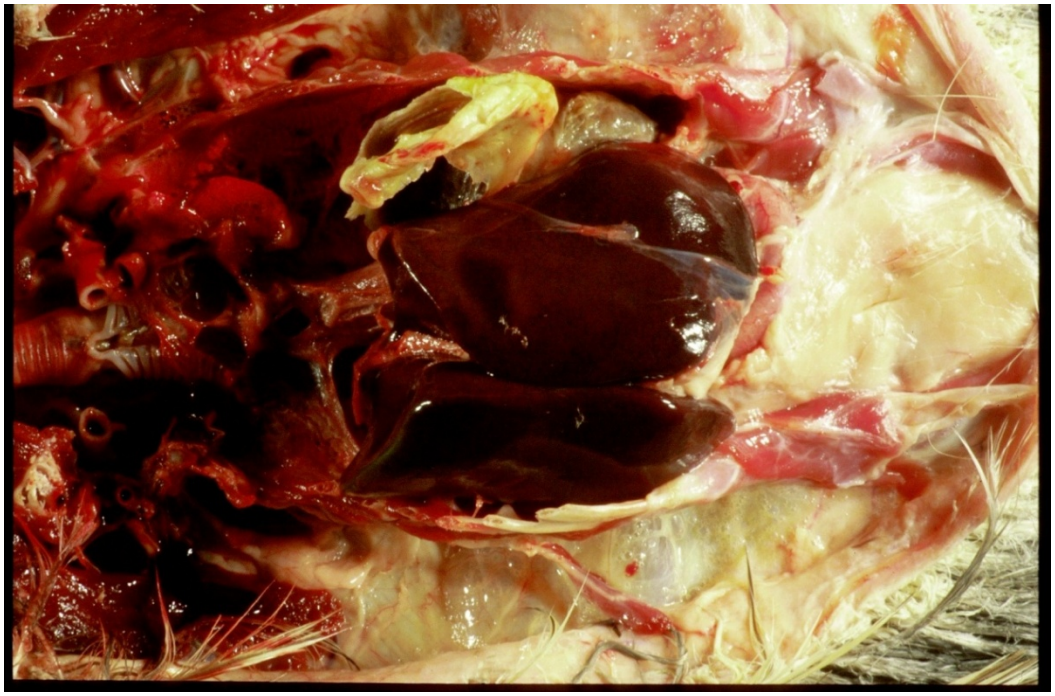

Fig.4.33. Post-mortem view of a falcon showing aspergilloma lesions near to the the liver.

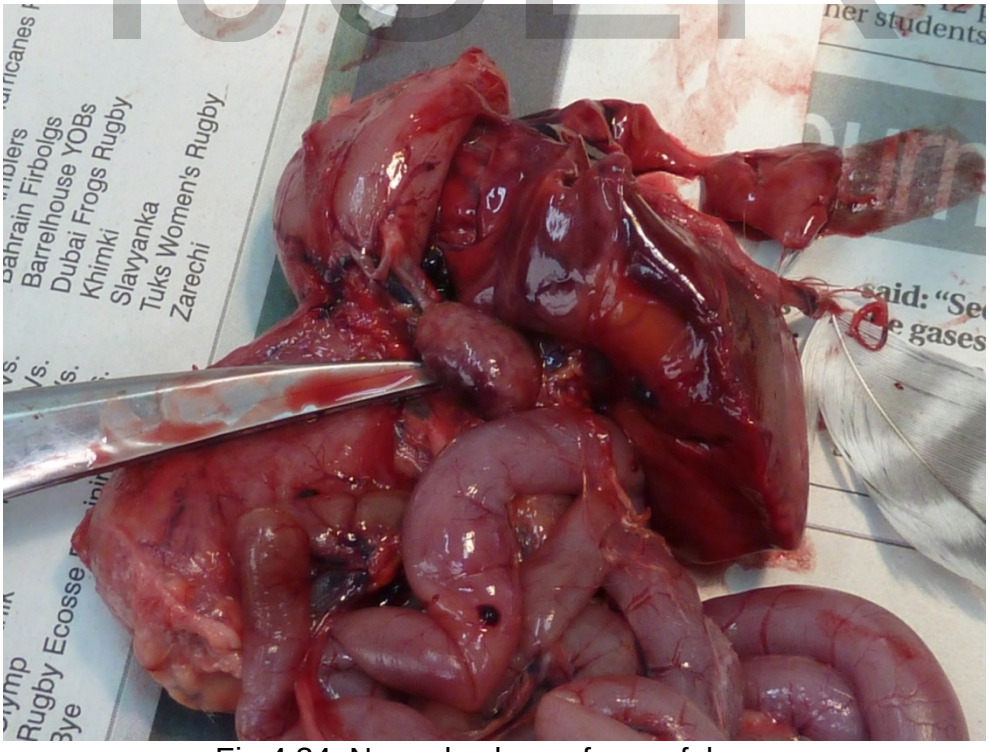

Fig.4.34. Normal spleen of a gyr falcon.

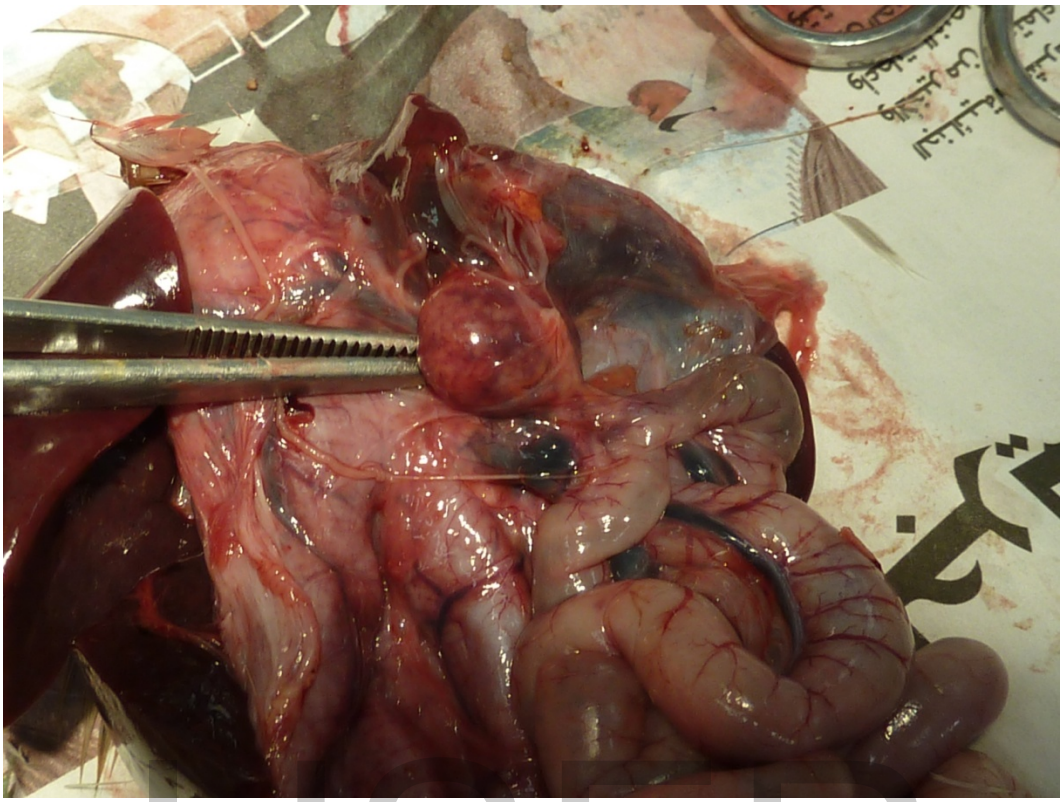

Fig 4.35. Mottled and enlarged spleen of gyr falcon due to septicemia.

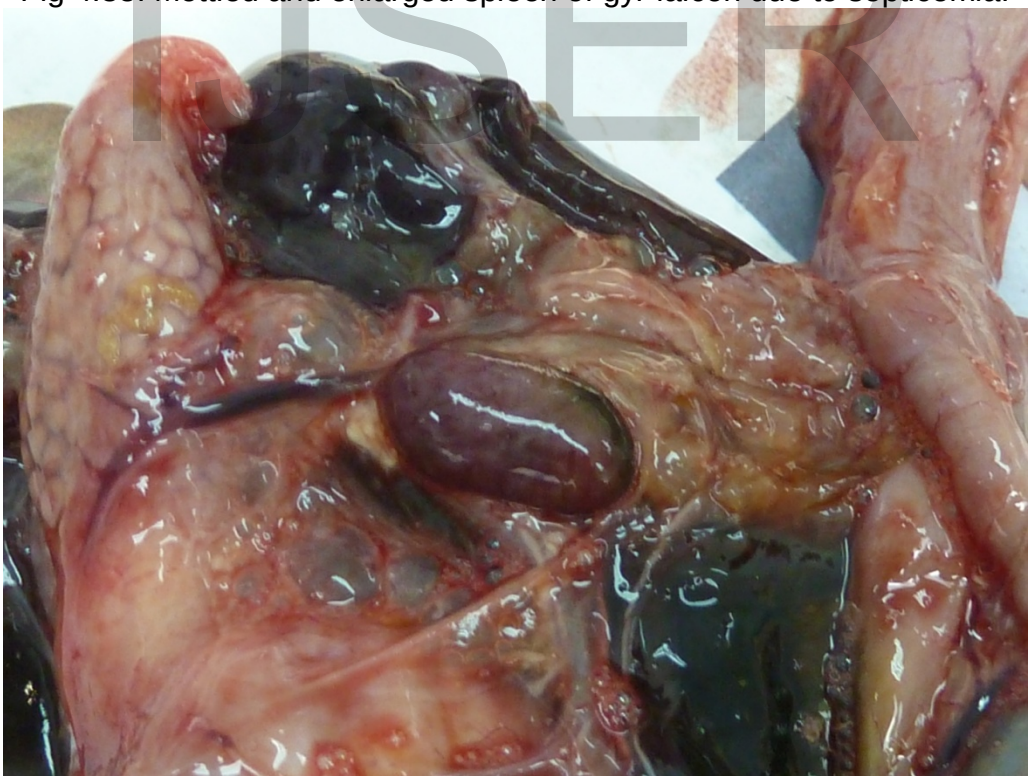

Fig.4.36. Enlarged spleen (Spleenomegaly) of a houbara bustard.

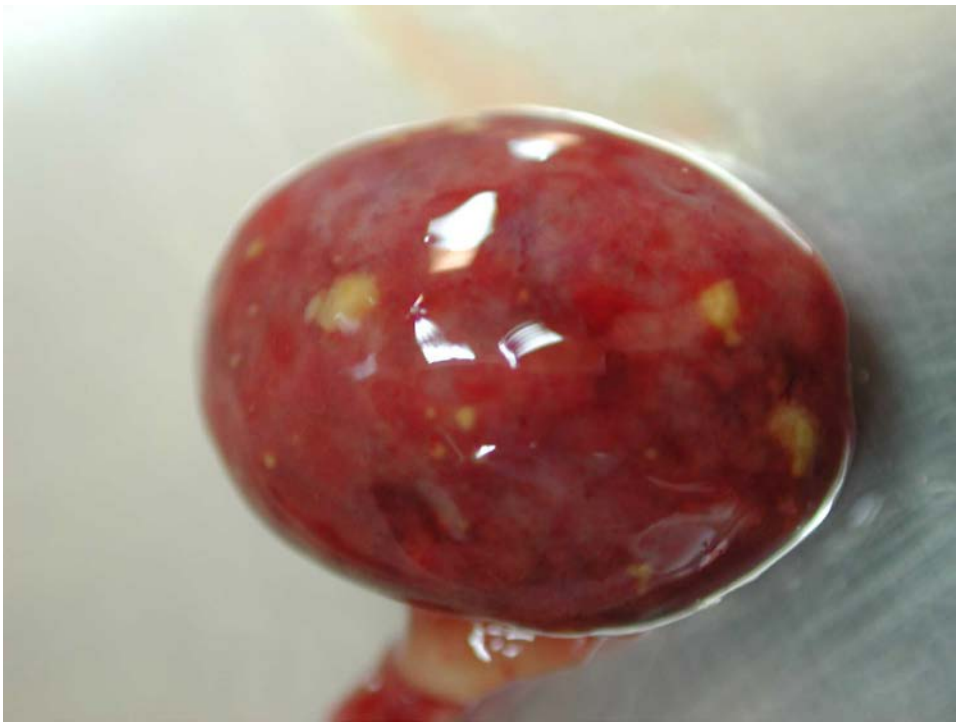

Fig.4.37. Enlarged spleen of a gyr falcon with multifocal herpes lesion.

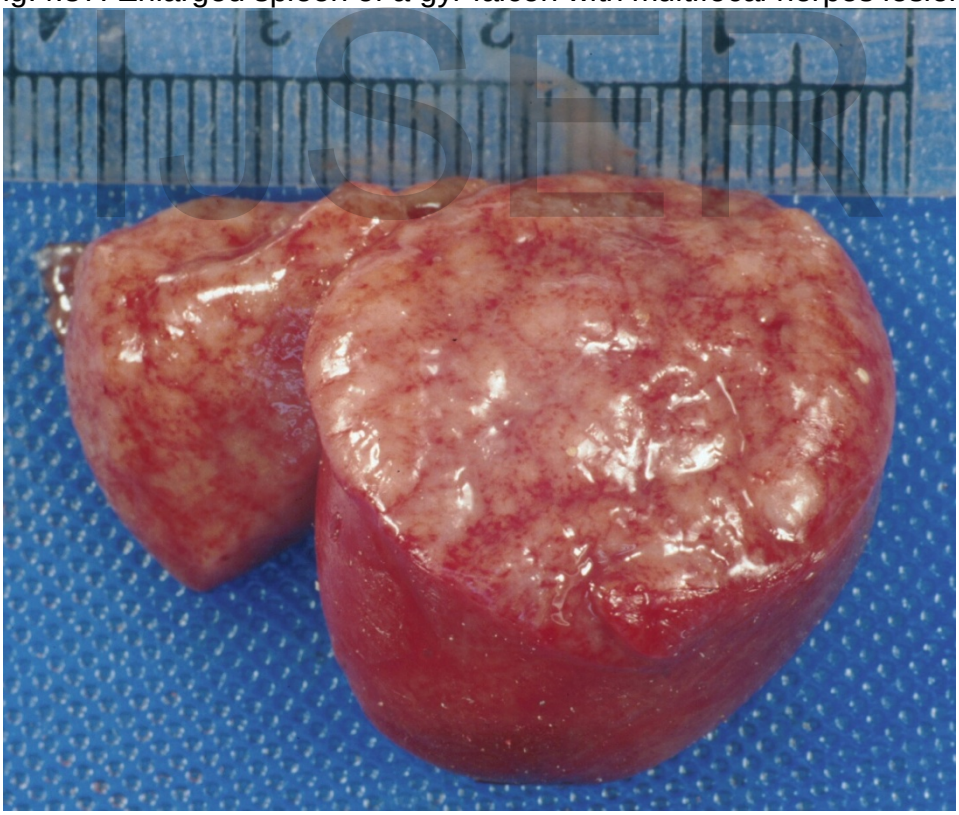

Fig.4.38. Cut spleen surface of a houbara with avian leucosis.

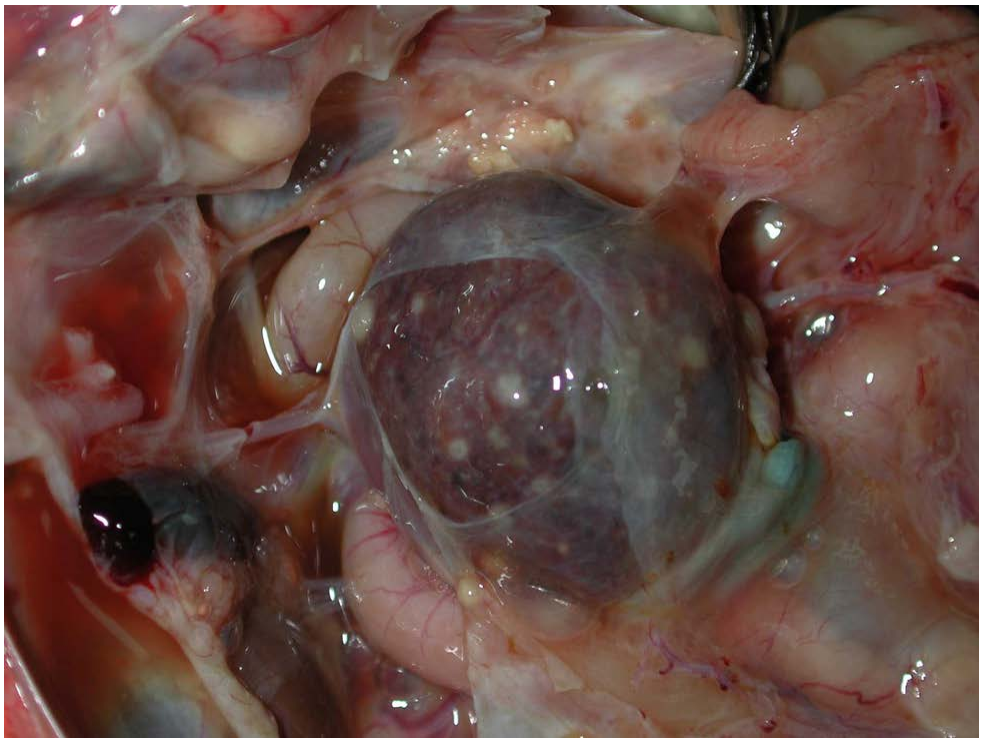

Fig.4.39. Enlarged spleen of a gyr falcon with multifocal tuberculosis lesions.

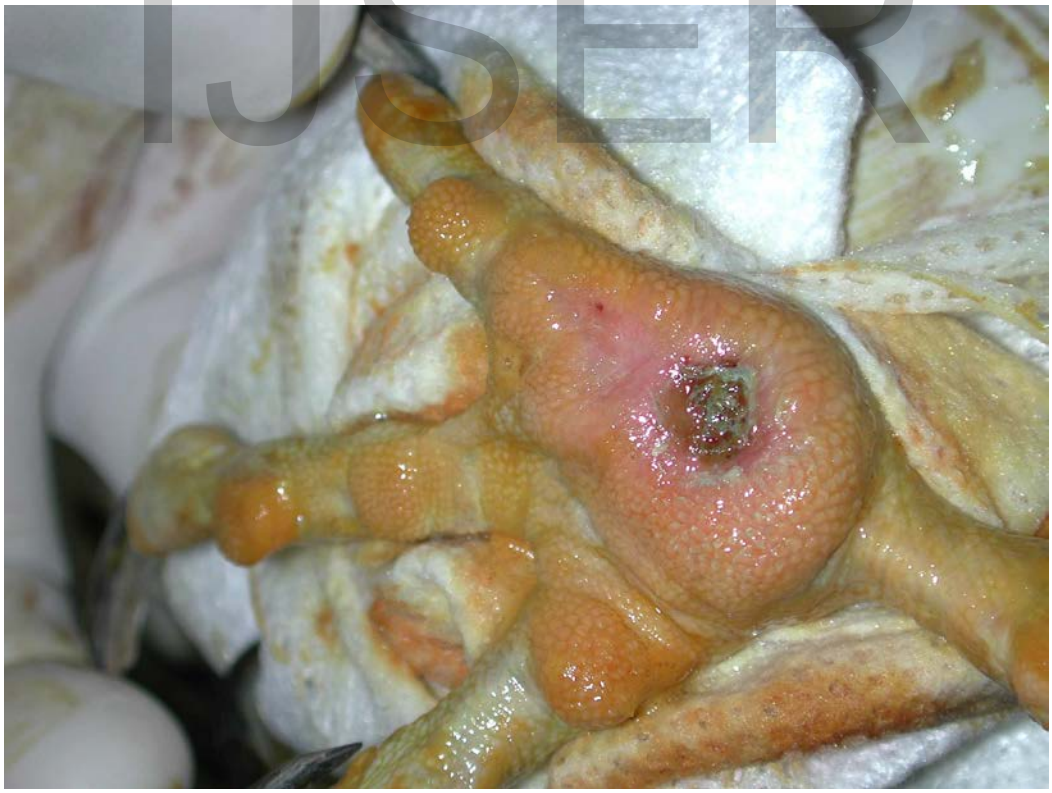

Fig.4.40. Bumble foot diseases in a falcon.

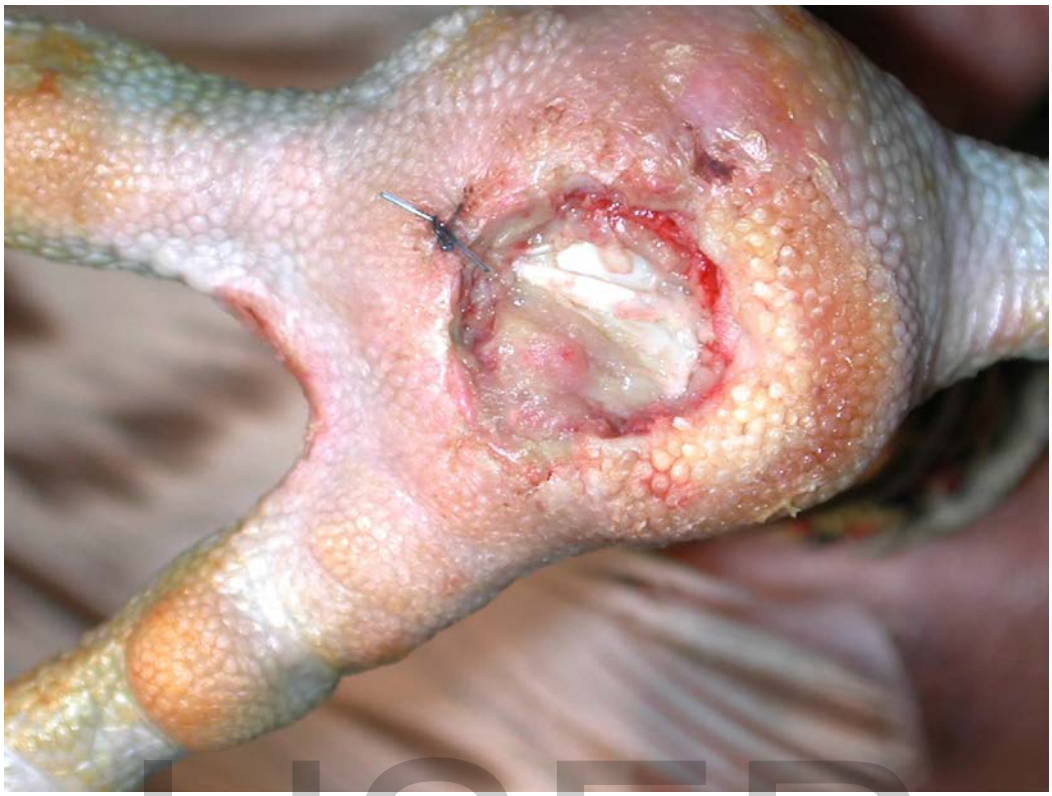

Fig.4.41. Infected bumble foot in a falcon.

Fig.4.42. A saker falcon with pox lesion.

(4c) Cytology of Alimentary Tract

The oral cavity of bustards and falcons is lined with cornified squamous epithelium. The outer layer of the epithelium is lined by anucleated squamous cells. In healthy birds, the normal exfoliation of the superficial cells of the epithelium that lines the upper alimentary tract reveals normal, mature squamous epithelial cells. Superficial cells usually exfoliate singly, and the cells are polygonal in shape, show varying degrees of cornification, often possessing angular or folded cytoplasmic margins, and with condensed pyknotic nucleus. Inflammatory changes caused by viruses, bacteria, candida and trichomonas have been detected in the upper alimentary tract of bustards and falcons, while in falcons, metazoa eggs are also commonly detected. In certain cases, inflammatory lesions are noticed in the upper alimentary tract (stomatitis, oesophagitis) with a mucous discharge or white - yellow lesions. Figures 4c.1 to 4c.7 show the normal and abnormal cytology of upper alimentary tract including oral cavity and crop.

The intestine and cloaca of bustards and falcons are lined with columnar epithelial cells. The vent (cloacal opening) is lined by cornified, stratified squamous epithelial cells. The normal cloacal cytology is poorly cellular with a predominance of squamous epithelial cells. Many basal cells and erythrocytes are seen in smears after traumatic injury. Inflammatory lesions in the intestine and cloaca show a predominance of inflammatory cells, erythrocytes and elevated exfoliation of columnar cells. Figures 4c.8 to 4c.17 show the normal and abnormal cytology of lower alimentary tract including small intestine and cloaca.

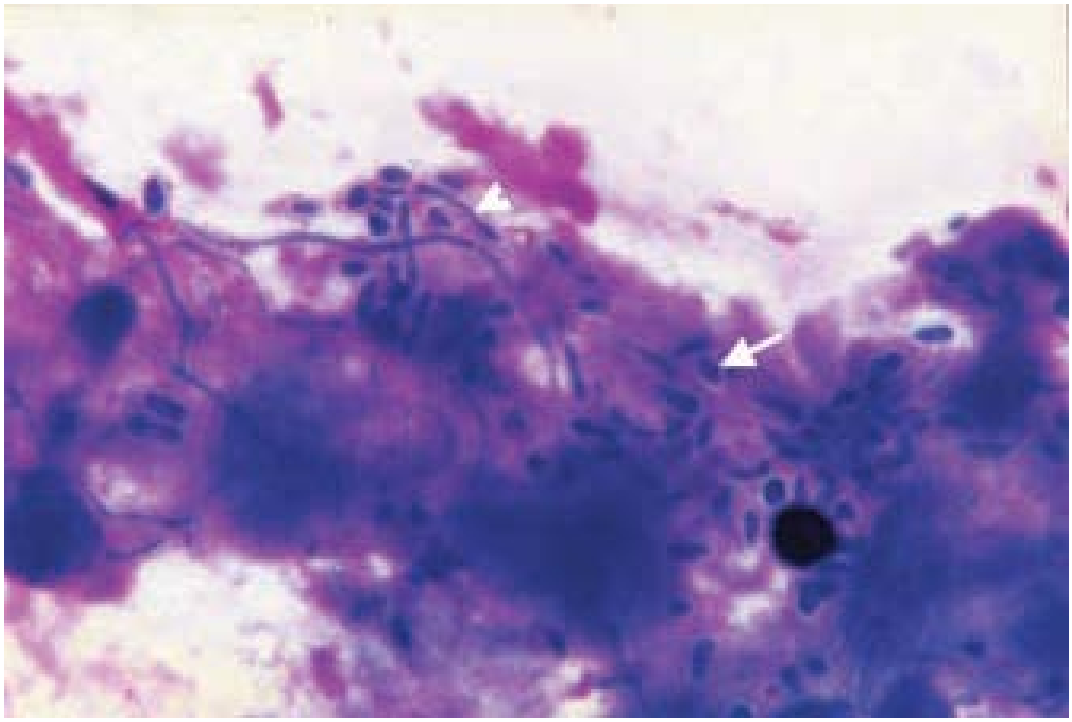

Fig. 4c.1 Smear from the oral cavity of a Heuglin's bustard (Neotis heuglinii) showing Candida sp. with budding cells and hyphae formation (May–Grünwald–Giemsa stain, 1000 X).

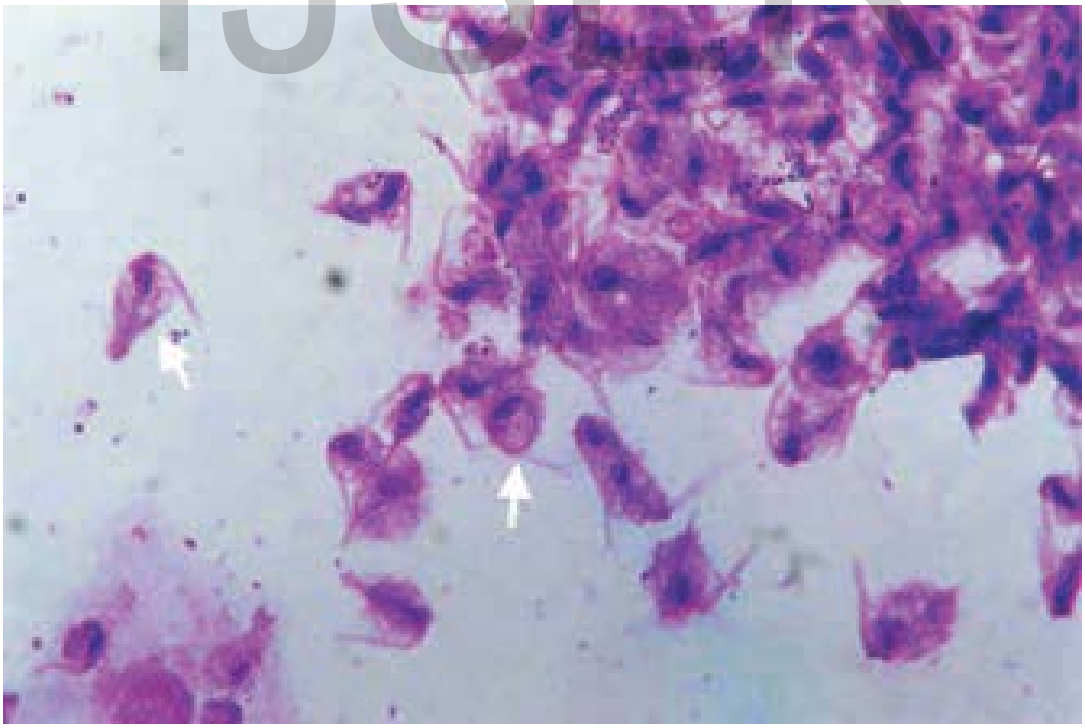

Fig. 4c.2. Trichomonas gallinae in a smear from the oral cavity of a kori bustard (Ardeotis kori). (May–Grünwald–Giemsa stain, 1000X)

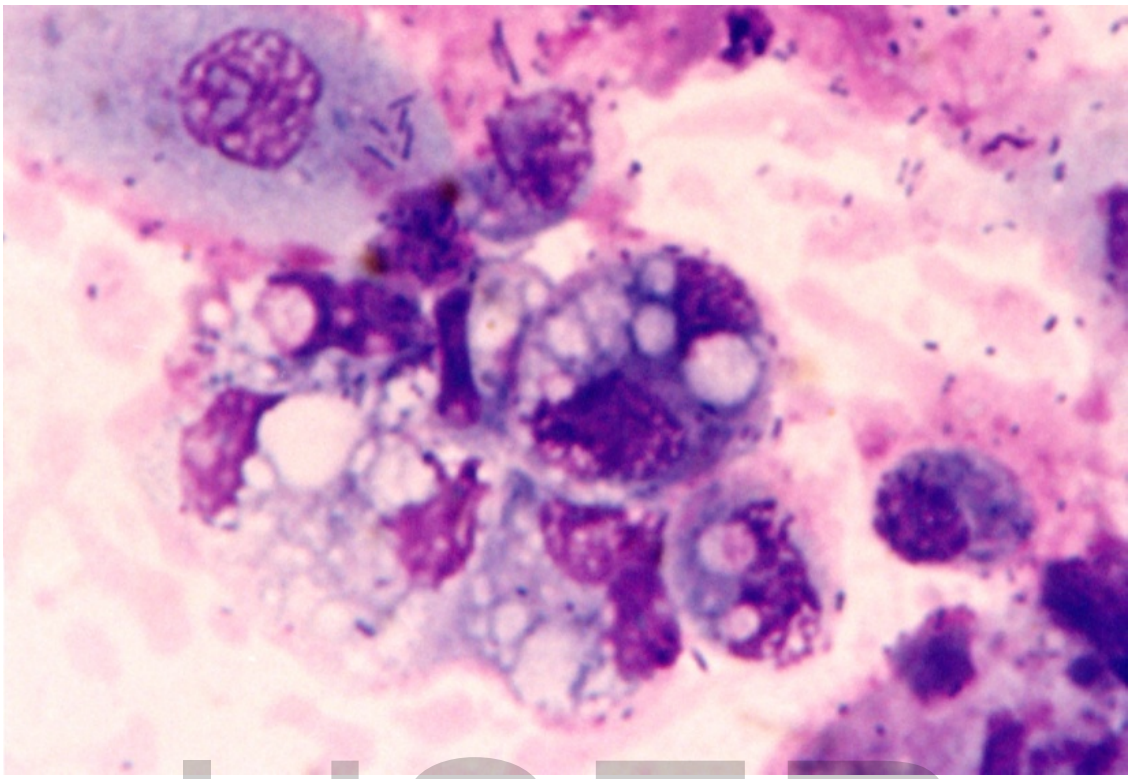

Fig. 4c.3. Chronic bacterial stomatitis in a smear from the oral cavity of a kori bustard (Ardeotis kori). (May–Grünwald–Giemsa stain, 1000X)

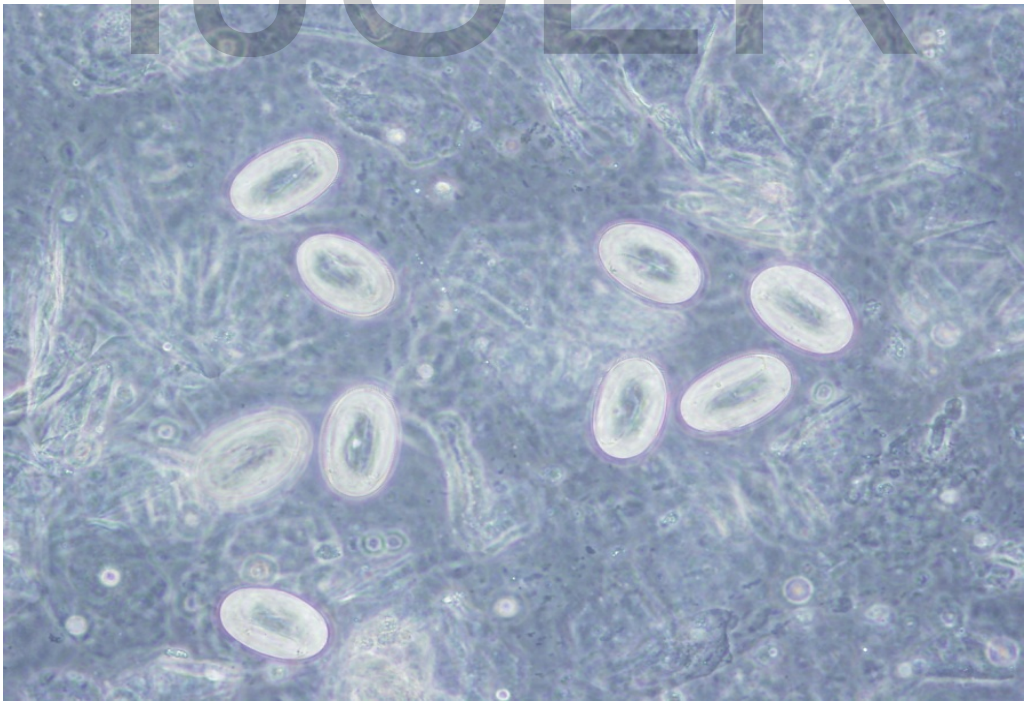

Fig. 4c.4. Serratospiculum eggs in a smear from the oral cavity of a Saker falcon (Falco cherrug). (Direct smear, 400X)

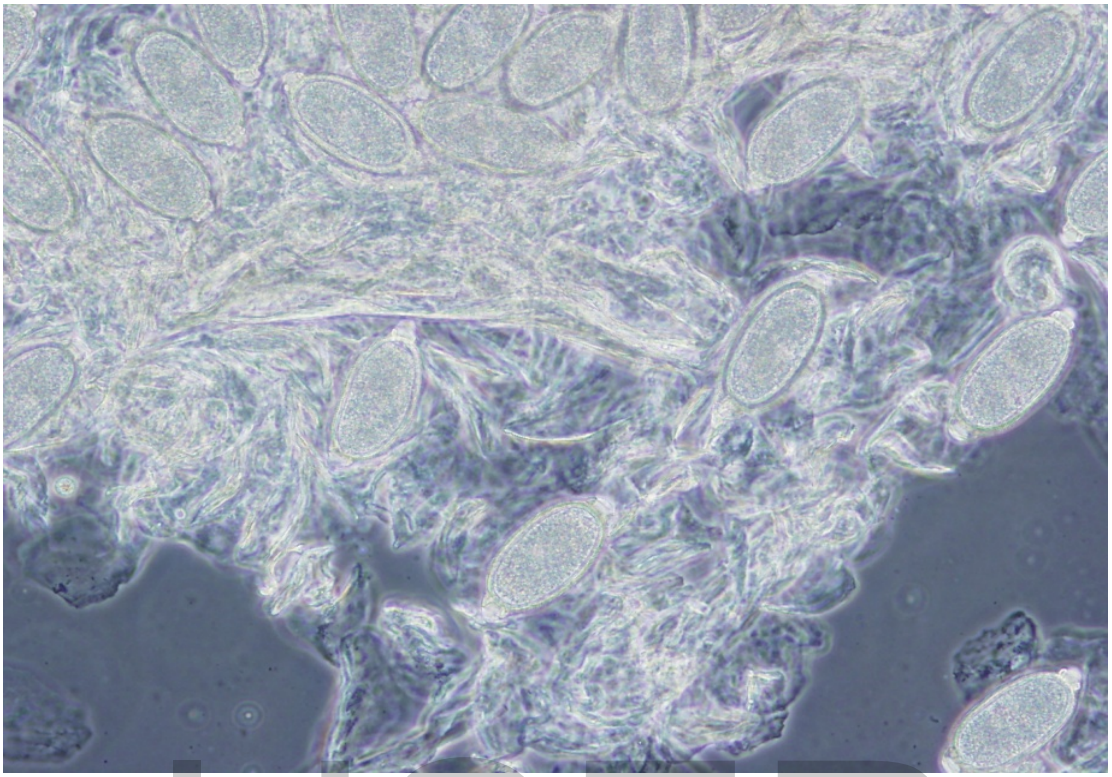

Fig. 4c.5. Capillaria eggs in a smear from the oral cavity of a Perigrine falcon (Falco peregrinus). (Direct smear, 400X)

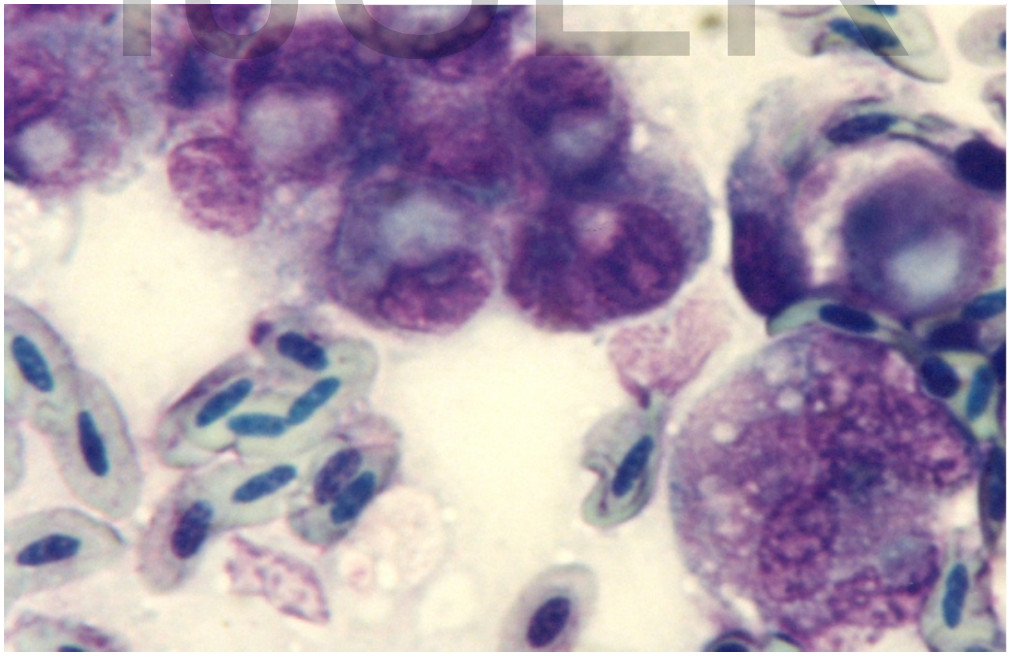

Fig.4c.6. Smear from the oral cavity of a Houbara bustard (*Chlamydotis undulata macqueenii*) showing intermediate and basal squamous cells with Bollinger bodies and Borrel bodies (May–Grünwald–Giemsa stain, 1000 X).

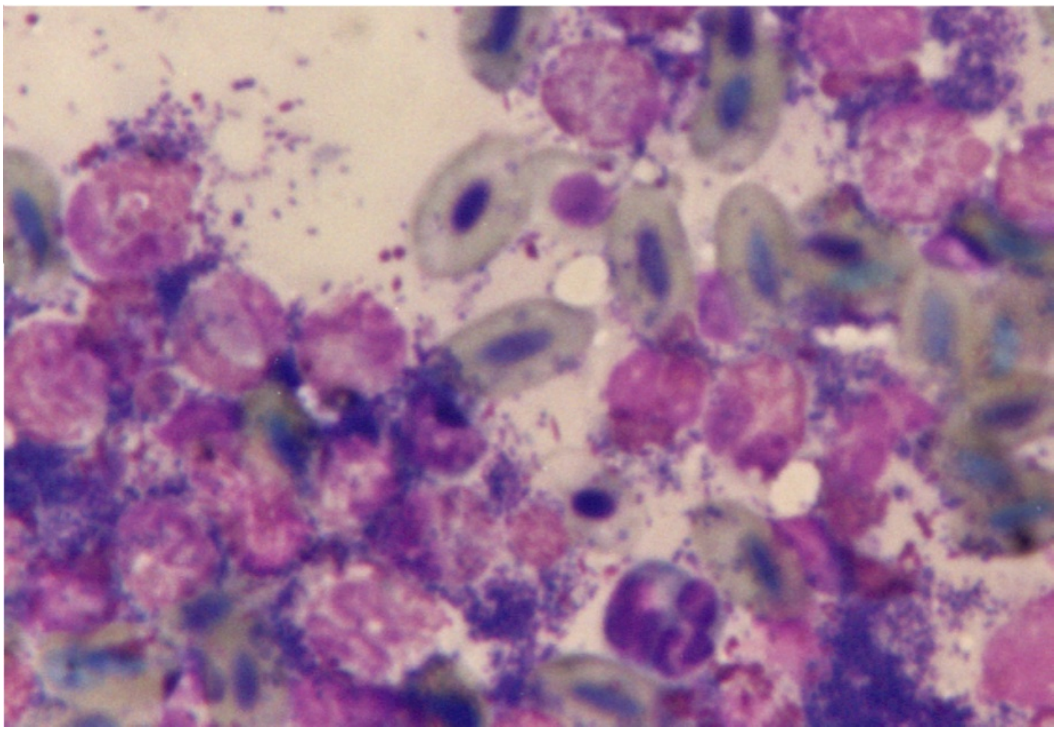

Fig.4c.7.Smear from the oral cavity of a Houbara bustard (*Chlamydotis undulata macqueenii*) showing acute bacterial stomatitis (May–Grünwald–Giemsa stain,1000 X).

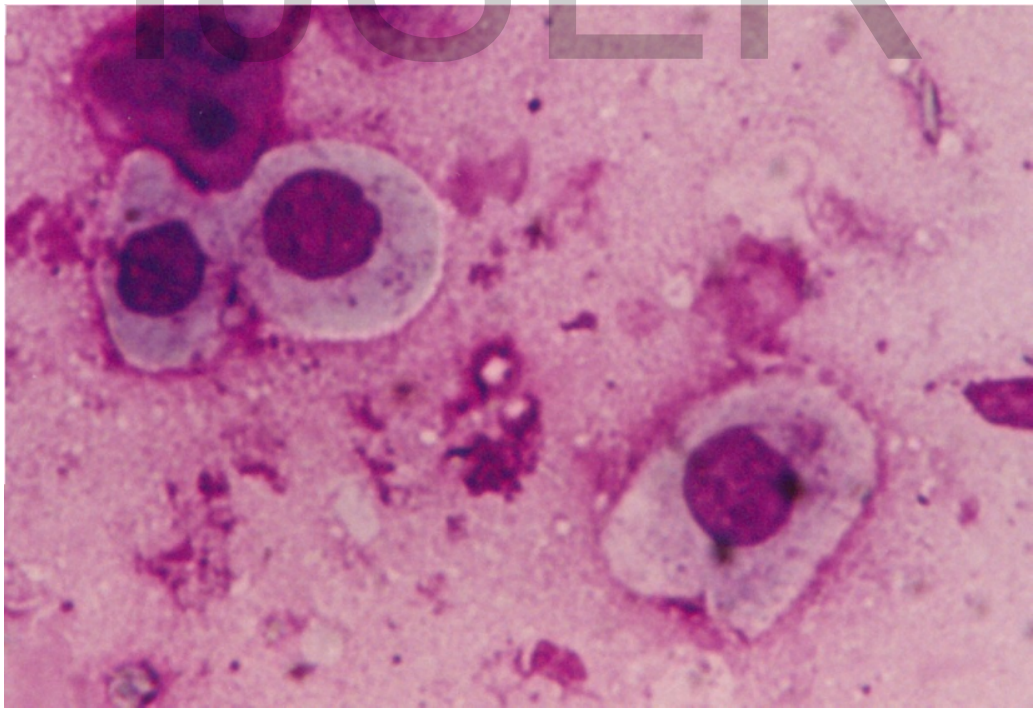

Fig.4c.8. Squamous cells from the cloaca of a Houbara bustard (*Chlamydotis undulata macqueenii*) 1000 X.

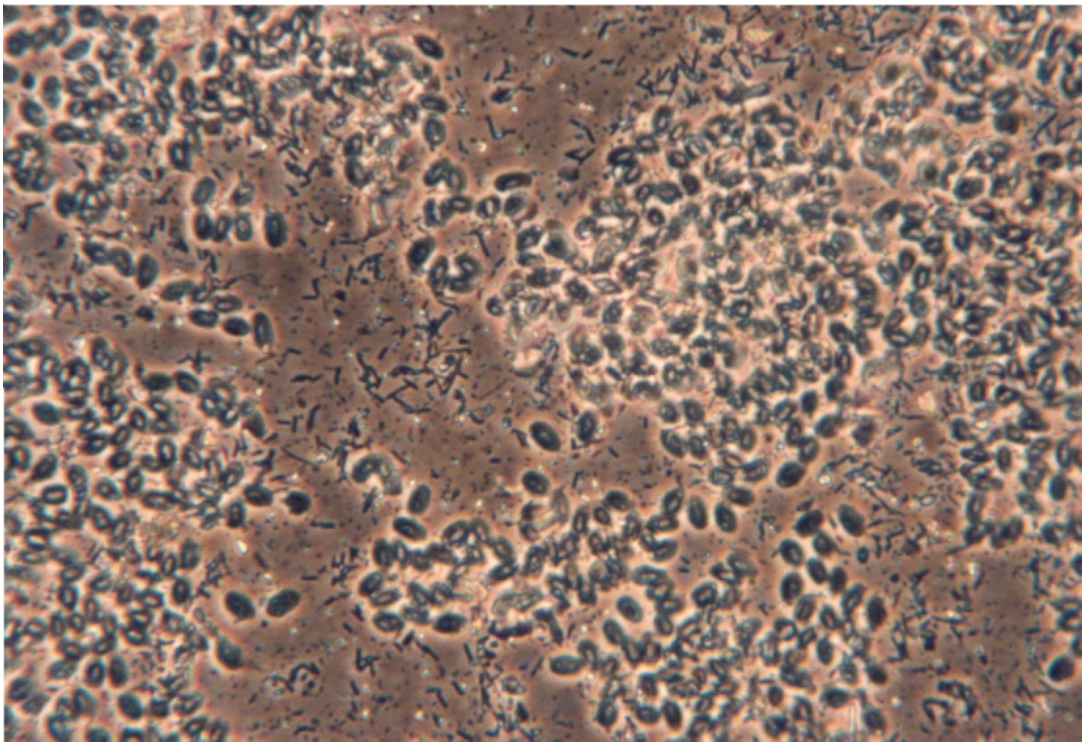

Fig.4c.9. A fecal sample from a Gry falcon (*Falco rusticolus*) under microscopy shows RBC (400X).

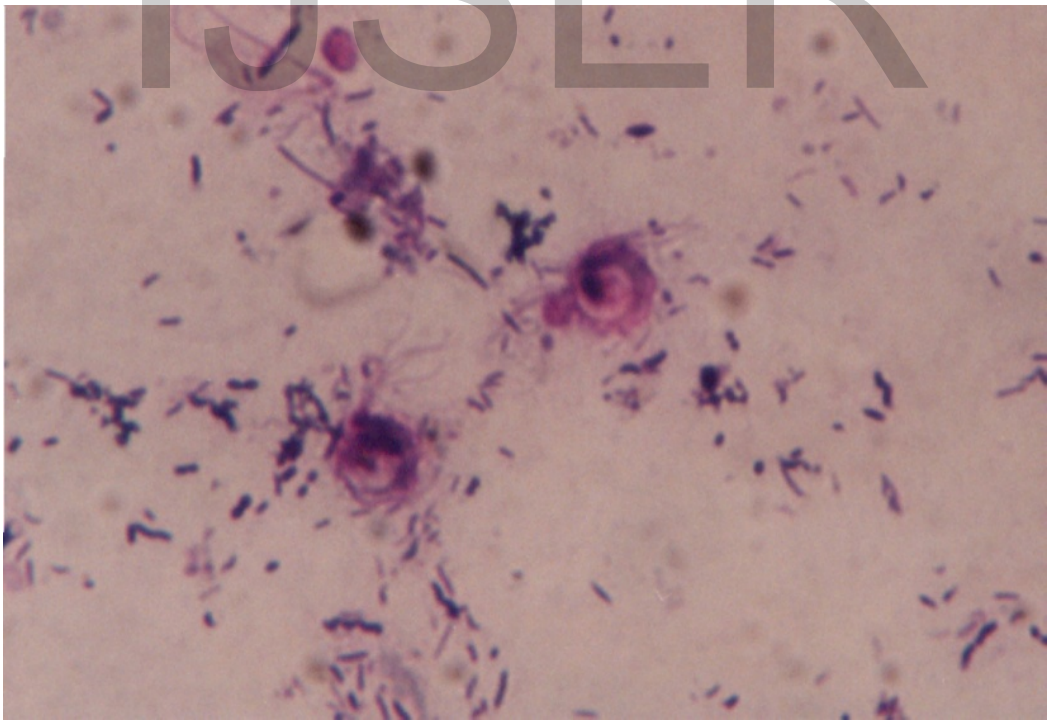

Fig.4c.10. Stained fecal smear from a Houbara bustard (*Chlamydotis undulata macqueenii*) shows Trichomonas gallinarum under 1000X

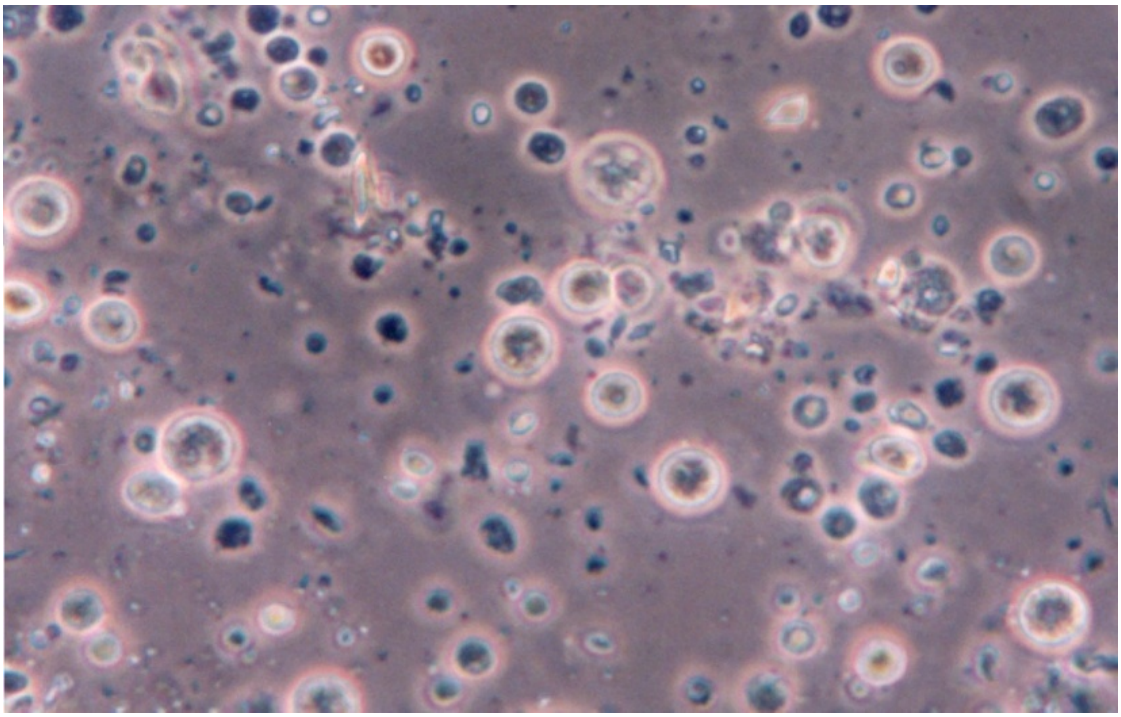

Fig.4c.11. Feces wet smear from a Gry falcon (Falco rusticolus) shows Cryptococcus under 400X

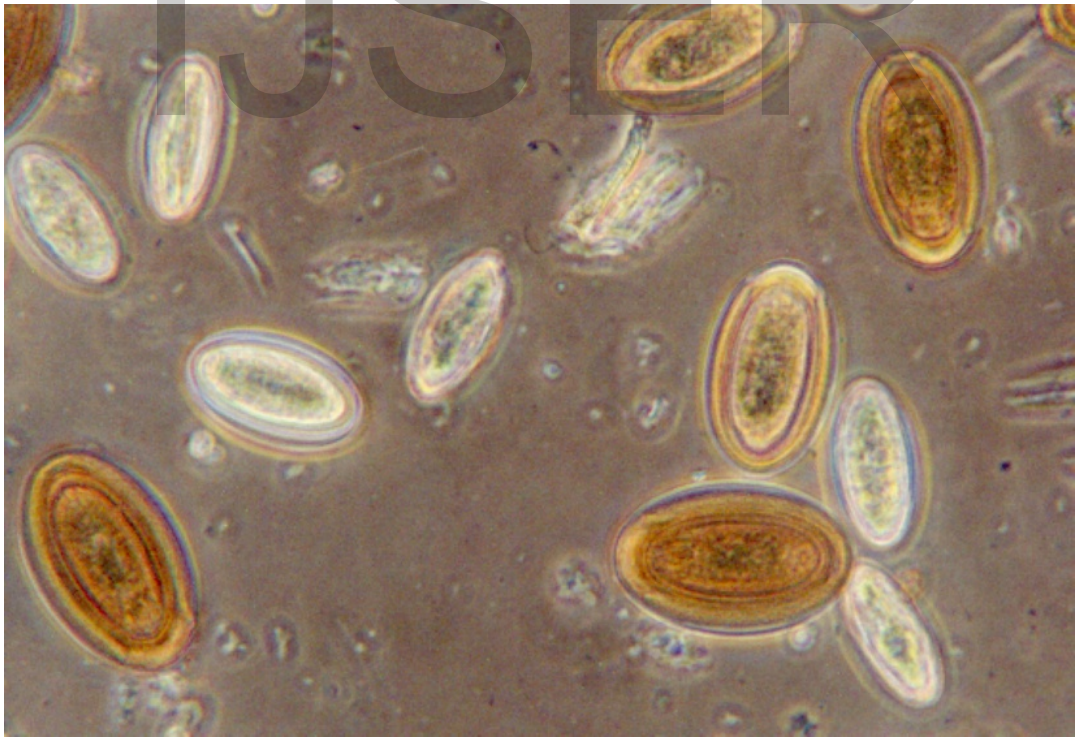

Fig. 4c.12. Smear from the feces of a Heuglin's bustard (Neotis heuglinii) showing Acanthocephala eggs at 100 X).

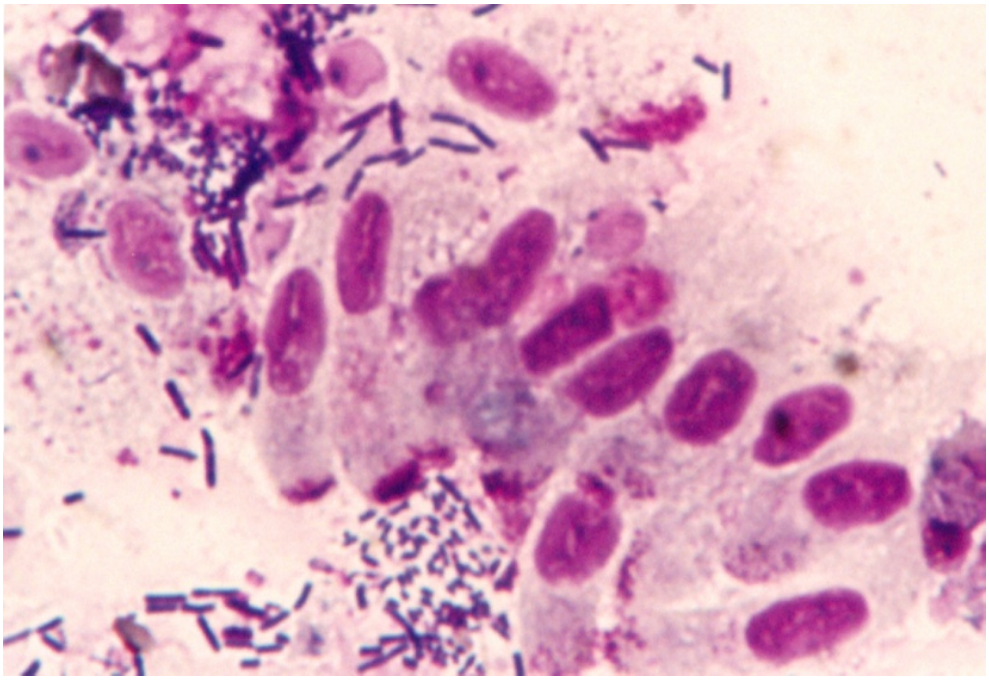

Fig. 4c.13. Exfoliation of columnar cells in a smear from the intestine of a kori bustard (Ardeotis kori) which has died due to clostridiosis. (May–Grünwald–Giemsa stain, 1000X)

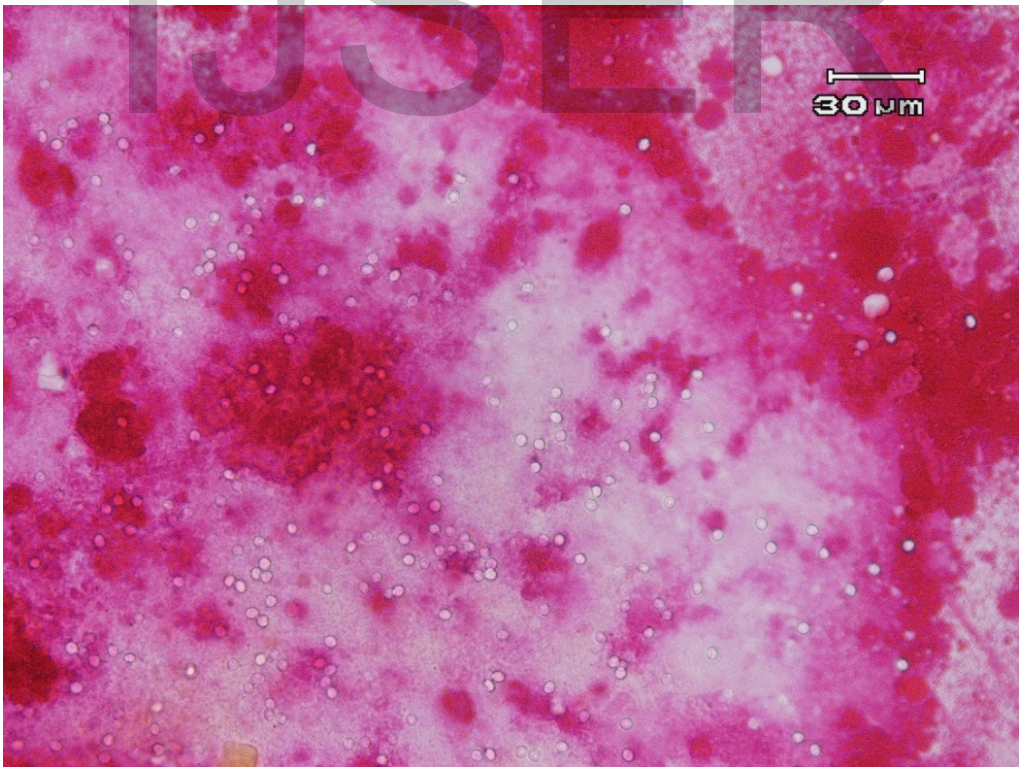

Fig.4c.14. Cryptosporidiasis sp in feceal smear of a Saker falcon (Falco cherrug) wit Z-N stain at 400X.

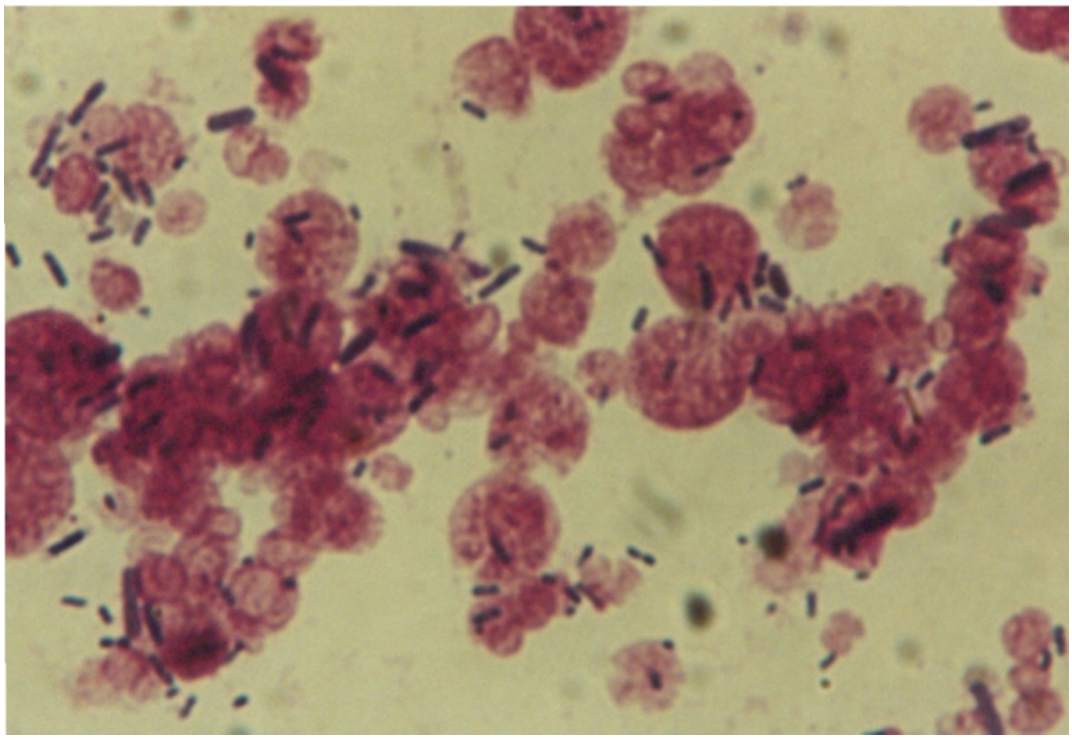

Fig.4c.15. Urate crystals and bacteria in a normal fecal smear of a Perigrine falcon (Falco peregrinus). (Direct smear, 1000X)

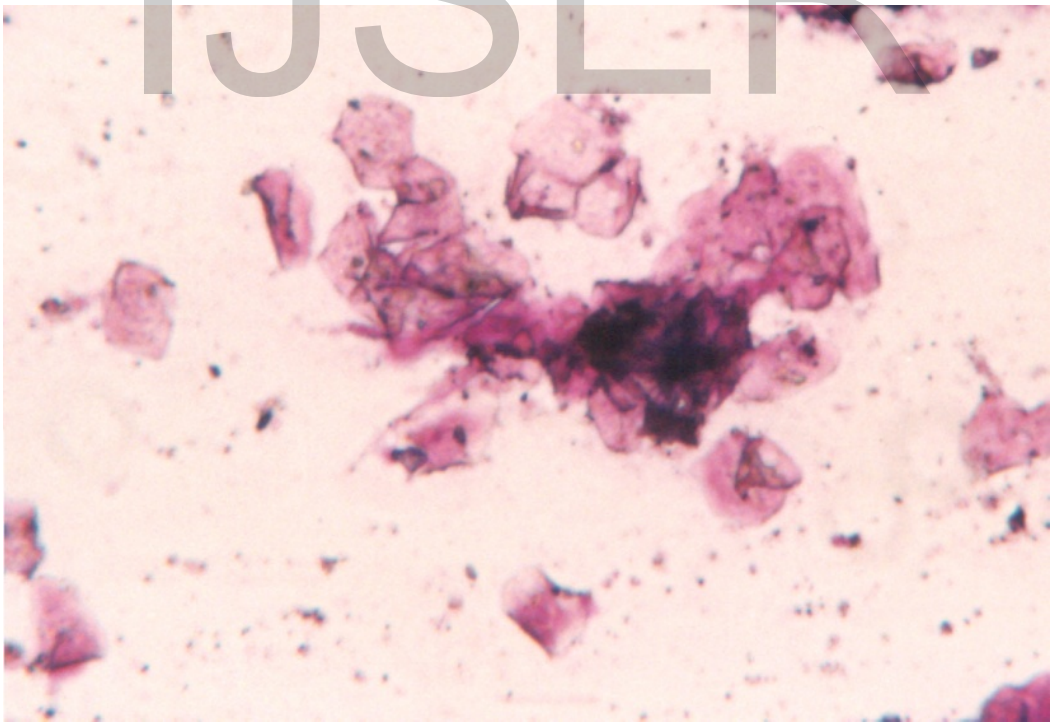

Fig.4c.16.Smear from the cloaca of a Houbara bustard (*Chlamydotis undulata macqueenii*) showing normal squamous cells (May–Grünwald–Giemsa stain, 1000 X).

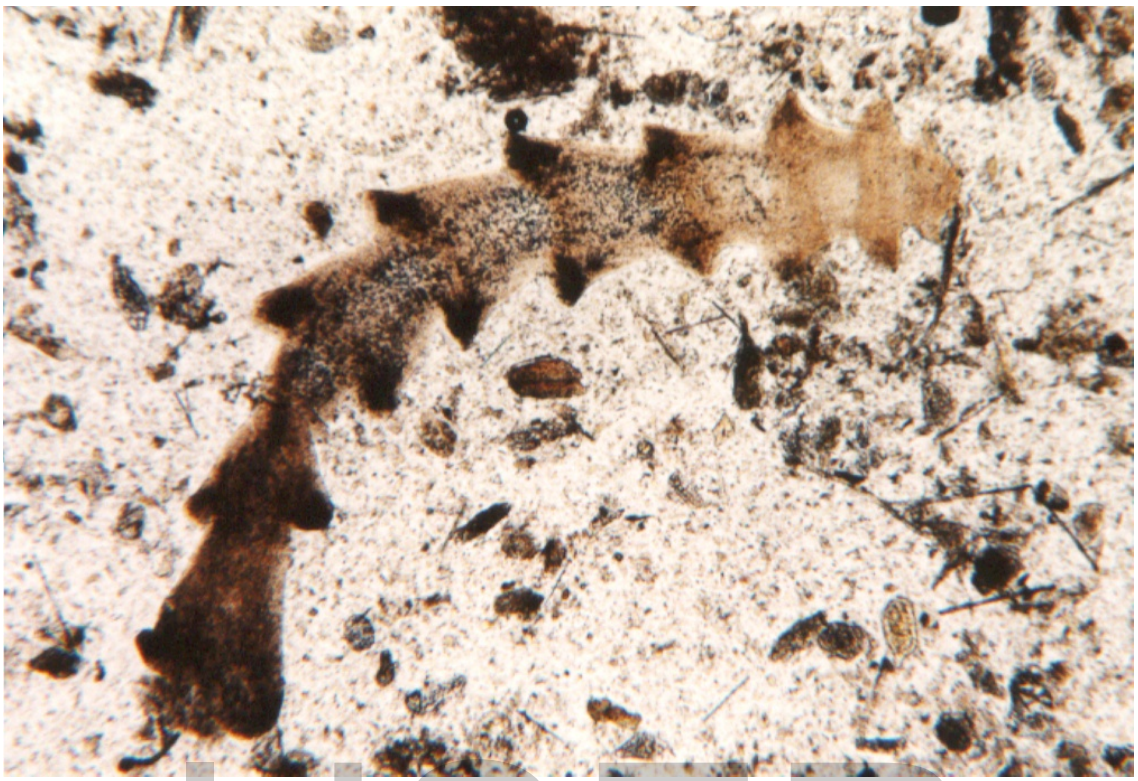

Fig.4c.17.Wet smear from the feces of a Houbara bustard (Chlamydotis undulata macqueenii) showing cestode proglottids at 40 X.

(4d) Cytology of Respiratory Tract

The nares and choana of bustards and falcons are lined by stratified squamous epithelium. The trachea is lined with pseudostratified ciliated columnar epithelium with goblet cells. Abnormal cytology of the upper respiratory tract (sinusitis, tracheobronchitis) shows inflammatory cells and infectious agents. Squamous cells from the choana may show nuclear changes associated with inflammation. Many plasma cells, macrophages and lymphocytes are seen in smears of chronic inflammation. Macrophages often have a highly vacuolated cytoplasm, indicating increased phagocytic activity, and they often contain phagocytised material. An increase in heterophils is seen in acute inflammation. Figures 4d.1 to 4d.12 show the normal and abnormal cytology of upper respiratory tract including nares, choana and trachea.

The lung air capillaries and air sacs are lined by simple squamous epithelium. The normal lung imprint cytology reveals a marked amount of peripheral blood contamination and an alveolar-like pattern. Normal air sac cytology is poorly cellular with occasional non-cornified epithelial cells seen in a clear background. Abnormal cytology of the lungs and air sacs (pneumonia and air sacculitis) are characterised by numerous inflammatory cells and the causative agents. Figures 4d.13 to 4d.22 show the normal and abnormal cytology of lower respiratory tract including lungs and airsacs.

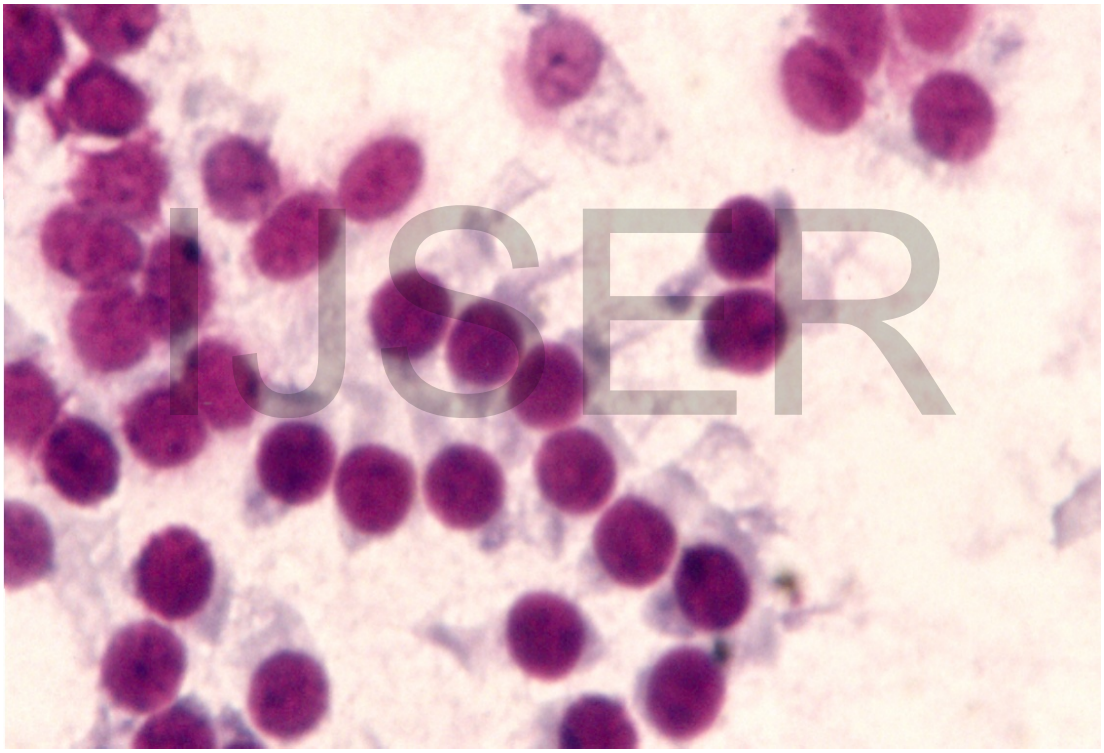

Fig.4d.1. Smear from the trachea of a houbara bustard showing ciliated columnar cells at 1000x.

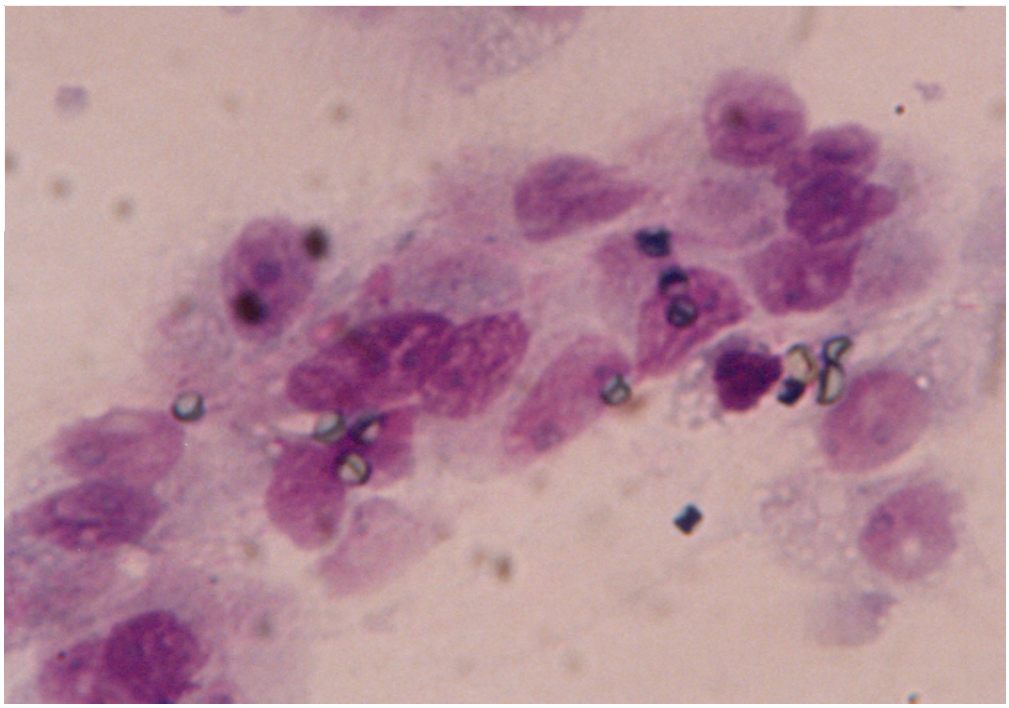

Fig.4d.2. Smear from the trachea of a Gyr falcon showing macrophages and fungal spores at 1000 X.

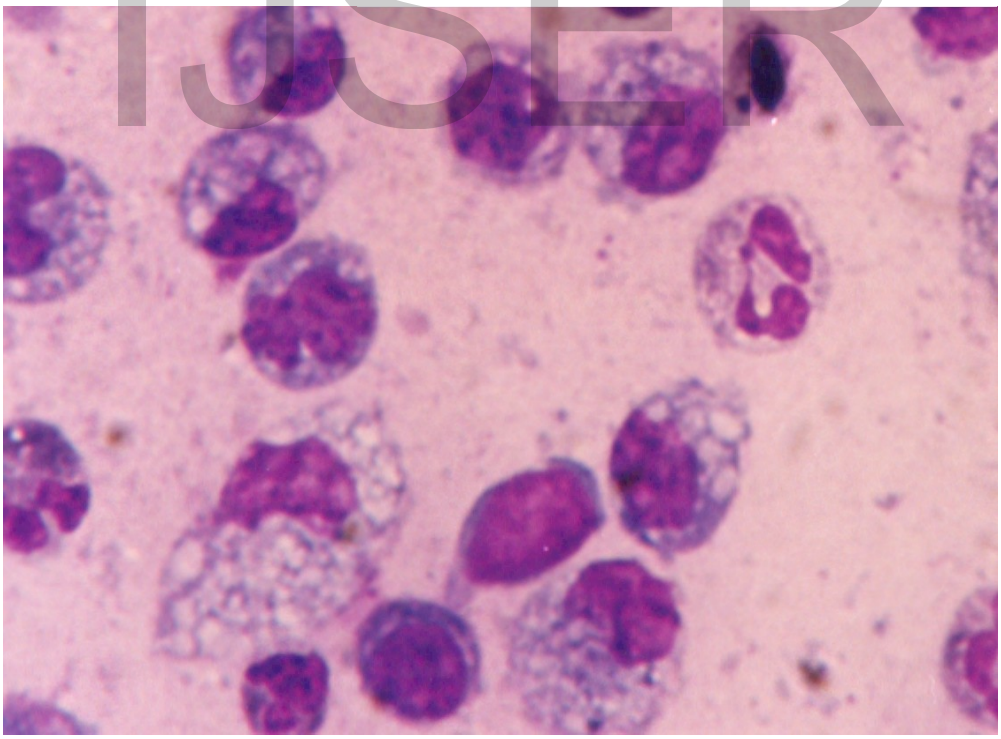

Fig.4d.3. Smear from the trachea f a Peregrine falcon showing macrophages at 1000X indicate chronic inflammatory response.

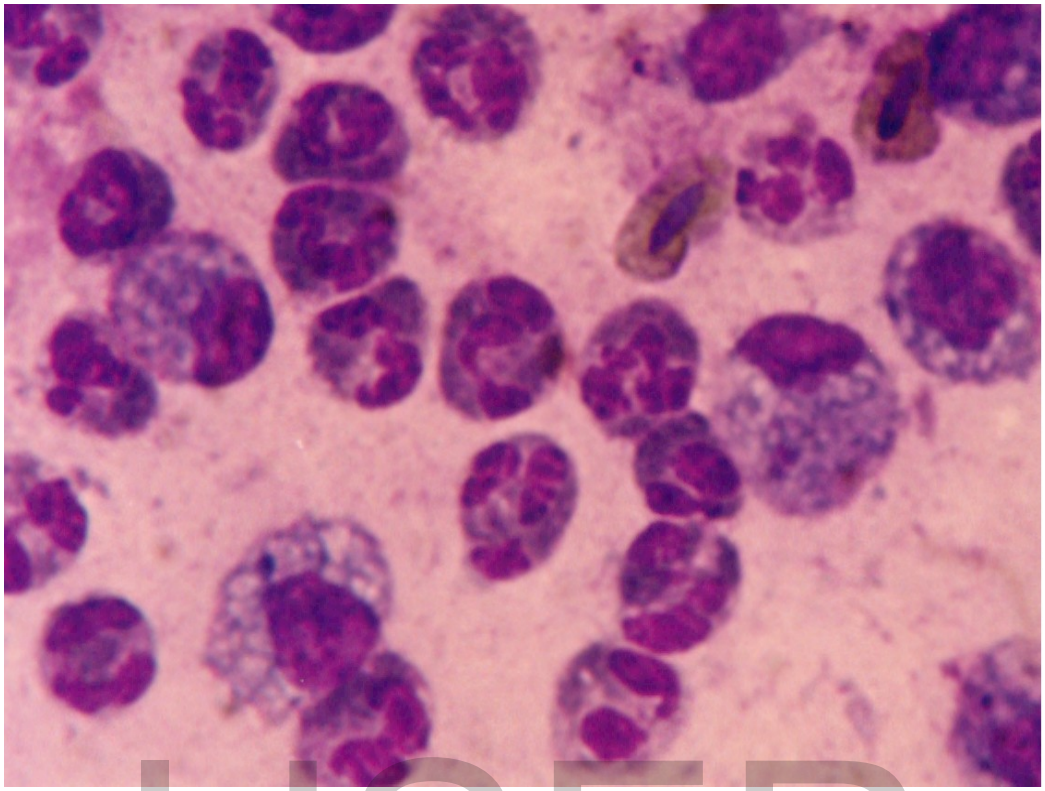

Fig.4d.4. Smear from the trachea f a Peregrine falcon showing heterophils at 1000X indicate acute inflammatory response.

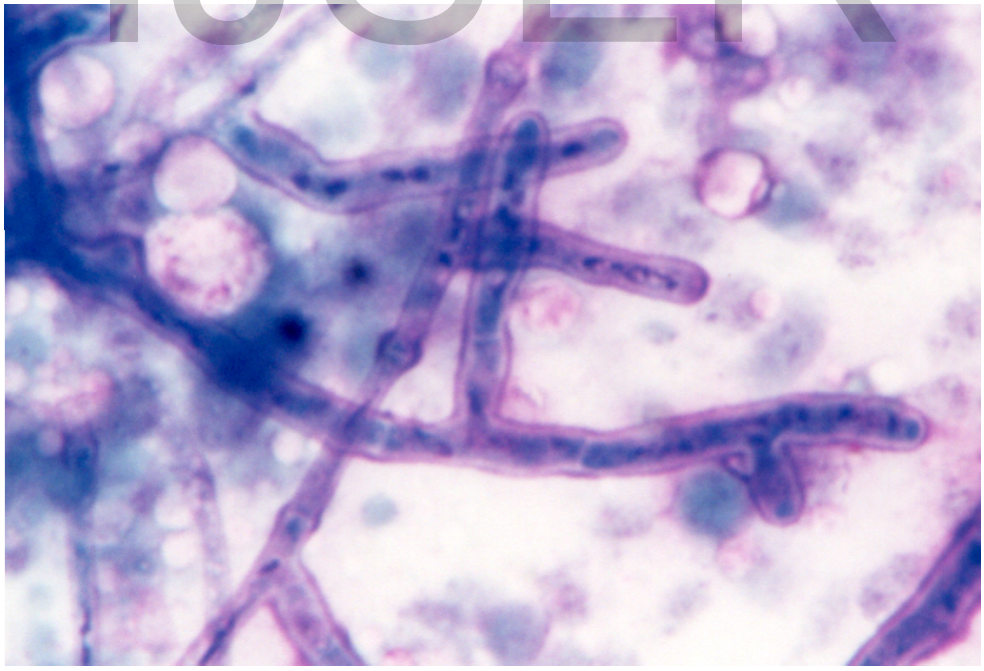

Fig.4d.5. Smear from the trachea of a Gyr falcon showing fungal hyphae. *Aspergillus fumigatus* was isolated in mycology culture.

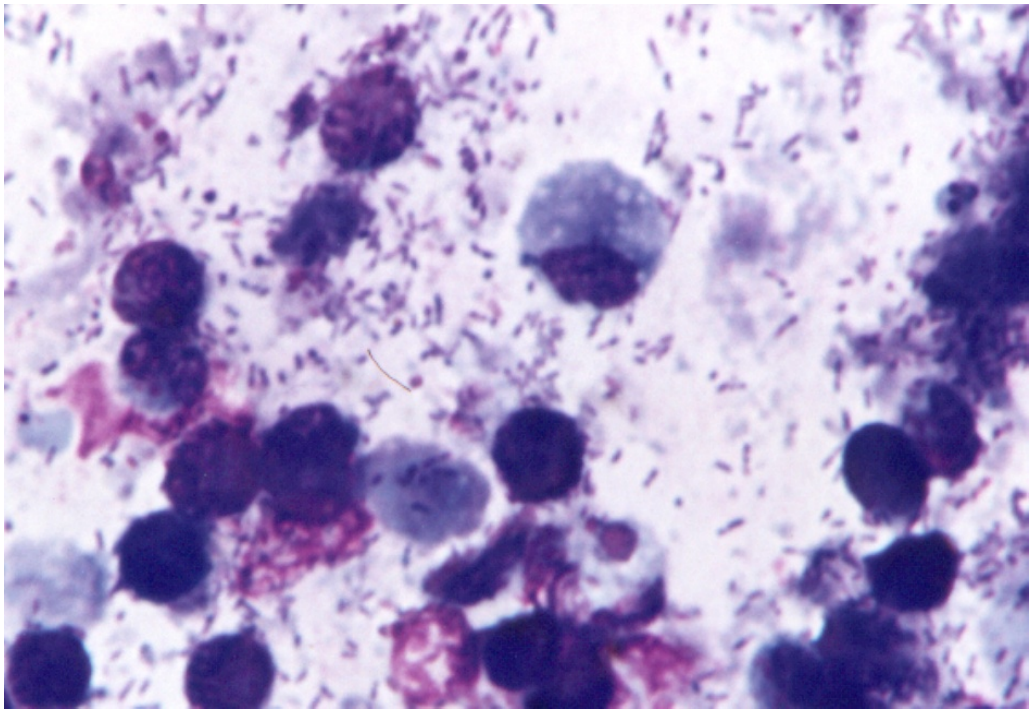

Fig.4d.6. Smear from the nares of a Heuglin's bustard (Neotis heuglinii) showing chronic active inflammatory response due to bacterial sinusitis (1000 X).

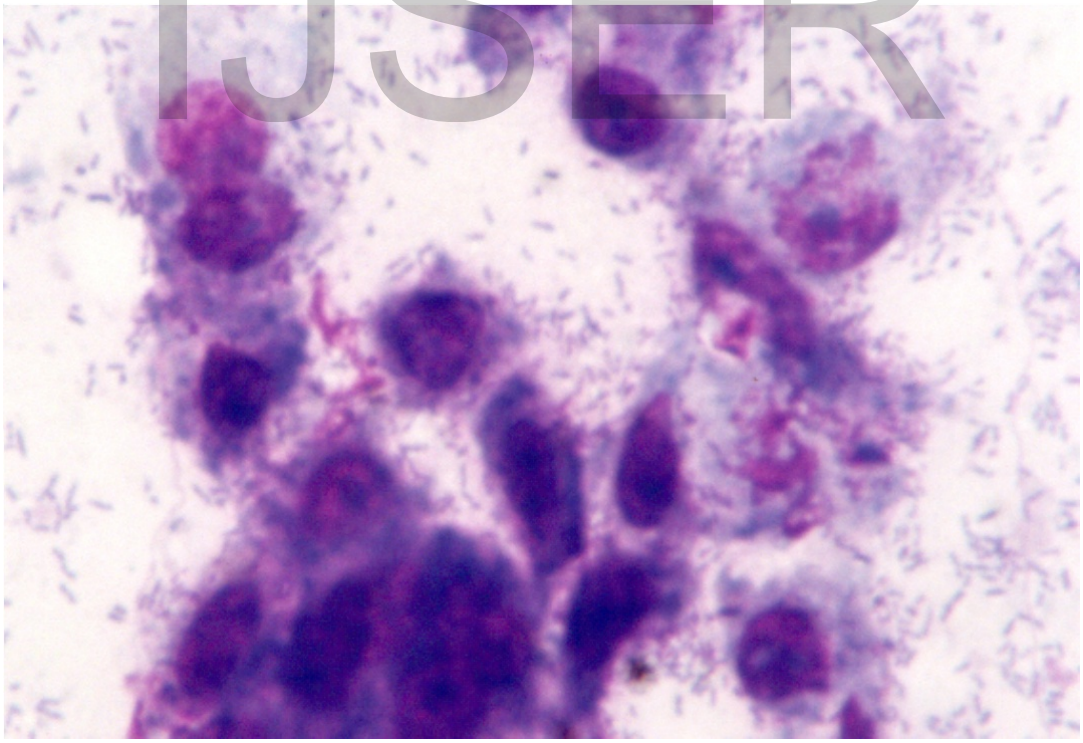

Fig.4d.7. Smear from the trachea of a Peregrine falcon showing chronic bacterial trachitis (1000X)

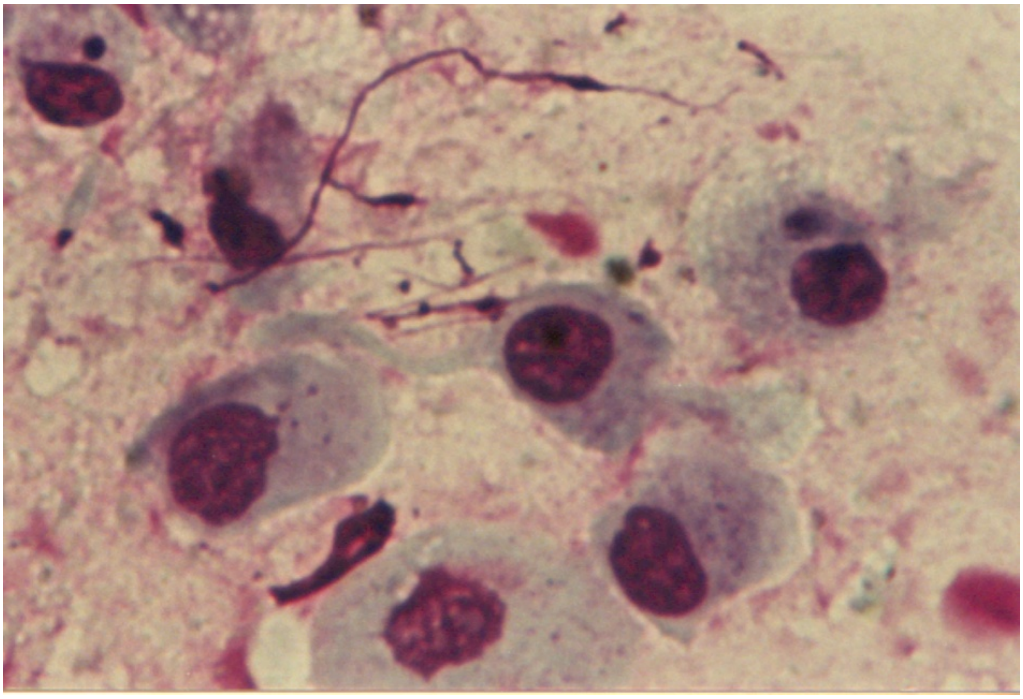

Fig.4d.8. Smear from the choana of a Houbara bustard showing normal choanal lining squamous cells at 1000X.

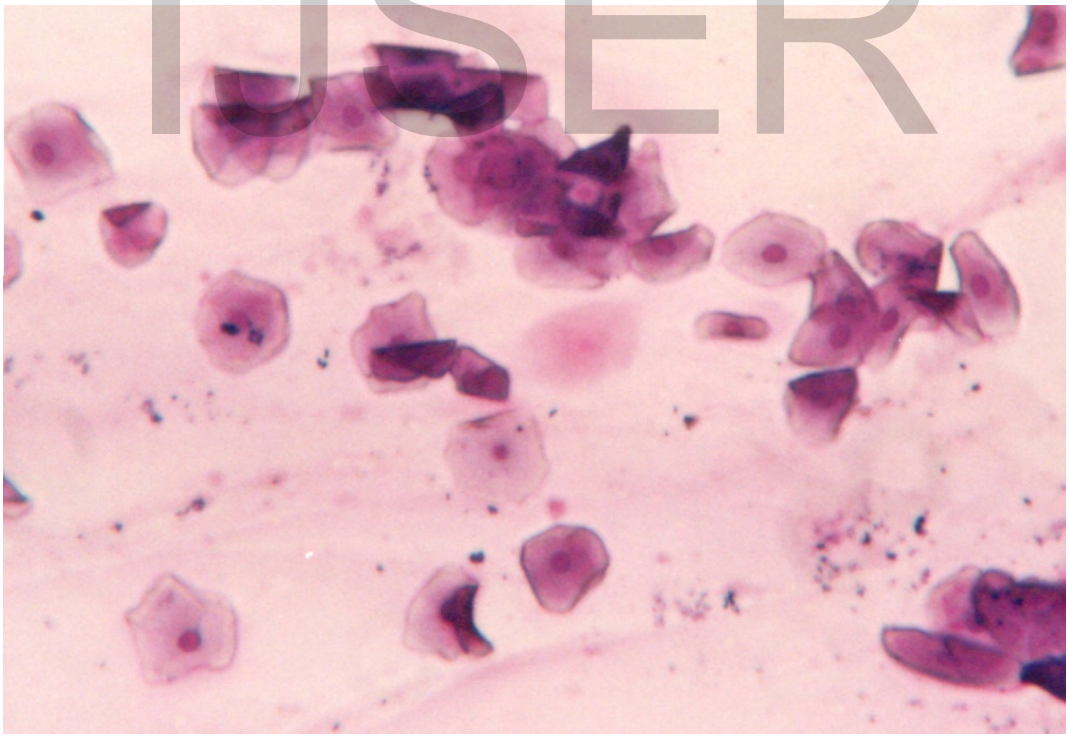

Fig.4d.9. Smear from the nares of a Houbara bustard showing normal nasal lining squamous cells at 1000X.

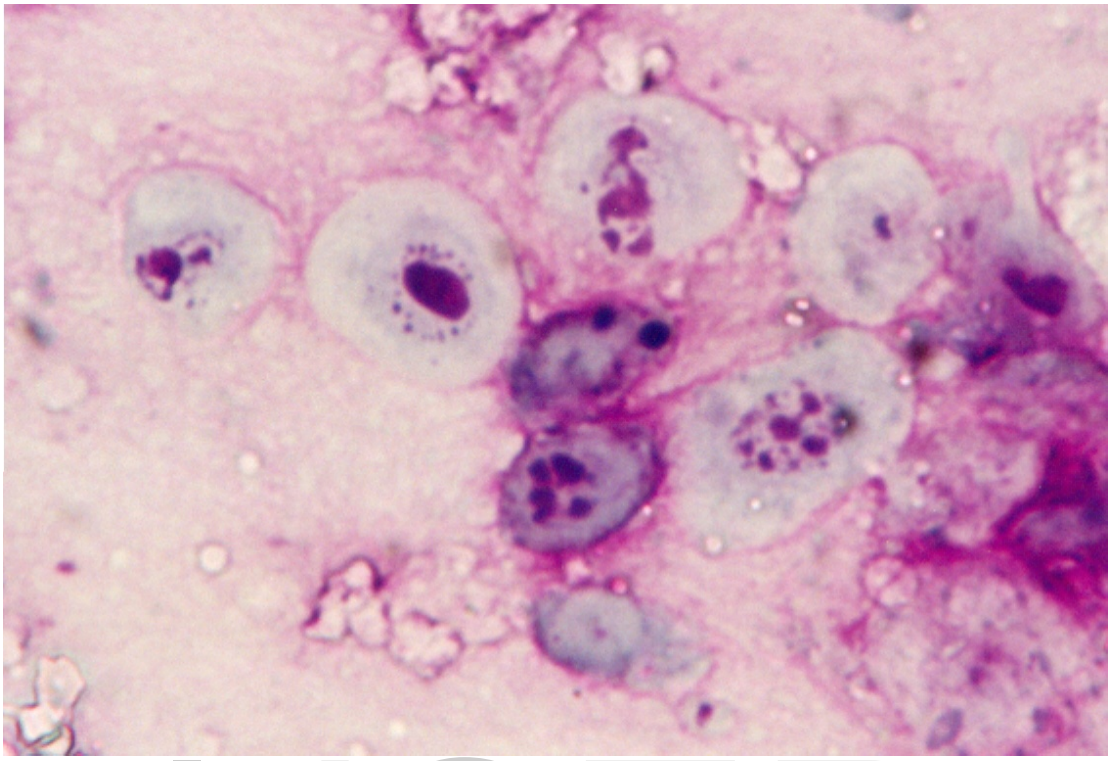

Fig.4d.10. Smear from the choana of a Houbara bustard showing choanal lining squamous cells with karyorrhexis at 1000X.

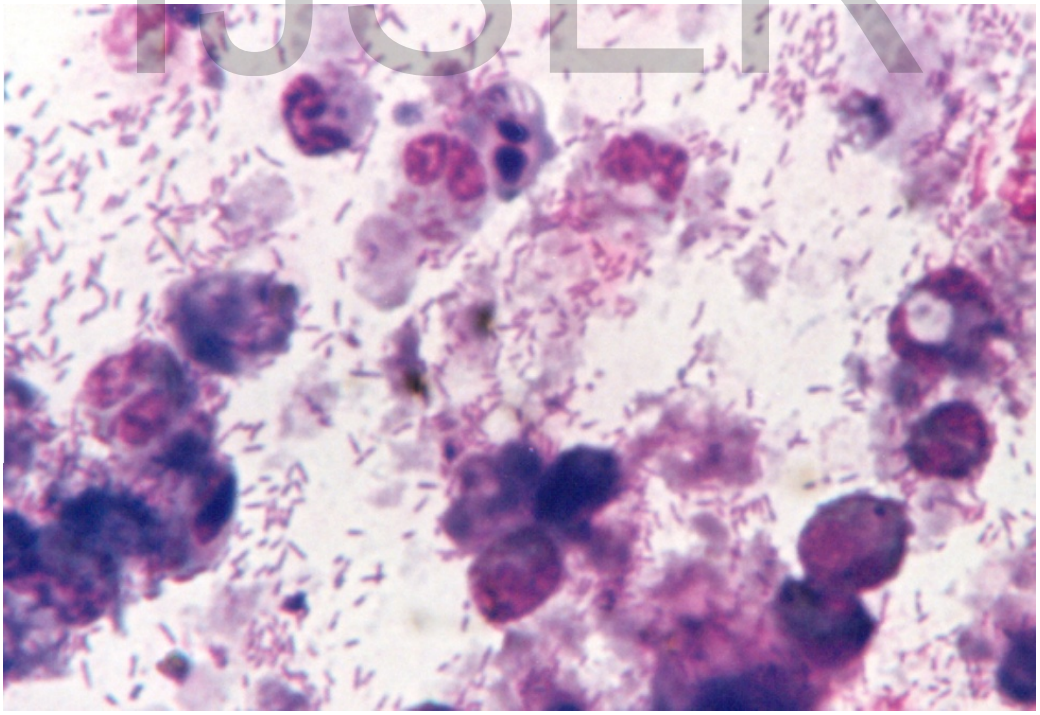

Fig. 4d.11.Smear from the trachea of a Saker falcon showing bacterial trachitis at 1000X.

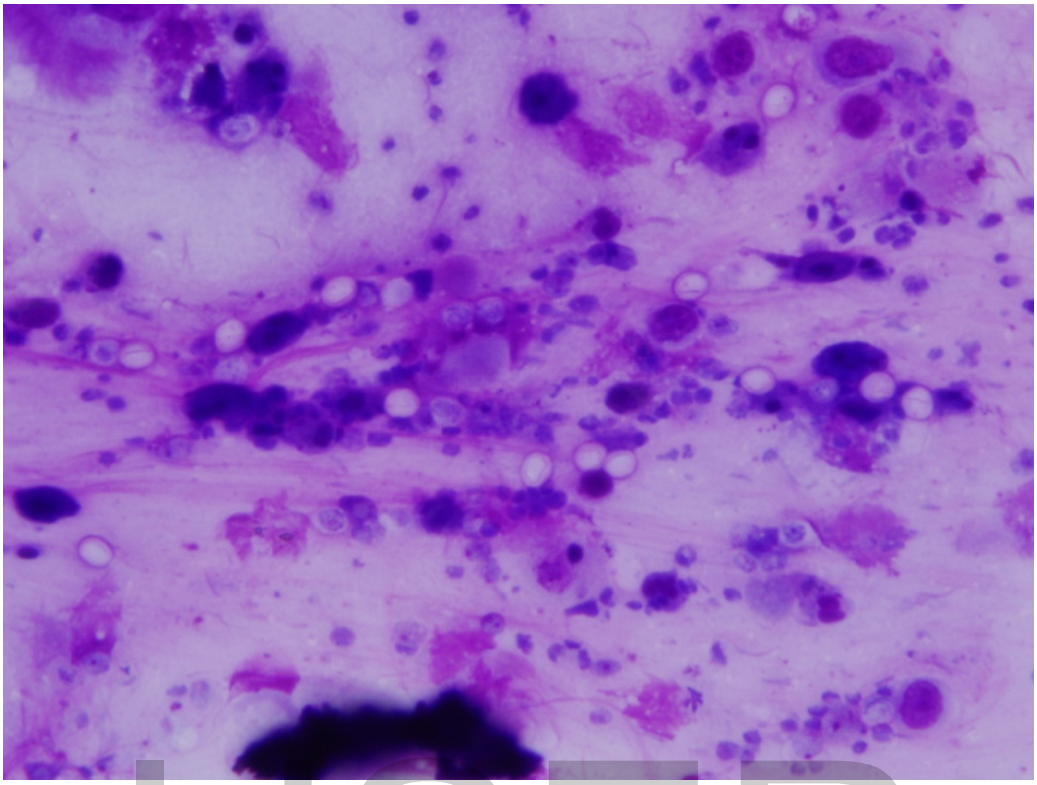

Fig. 4d.12.Smear from the trachea of a Peregrine falcon showing oocyst of Cryptosporida at 1000X.

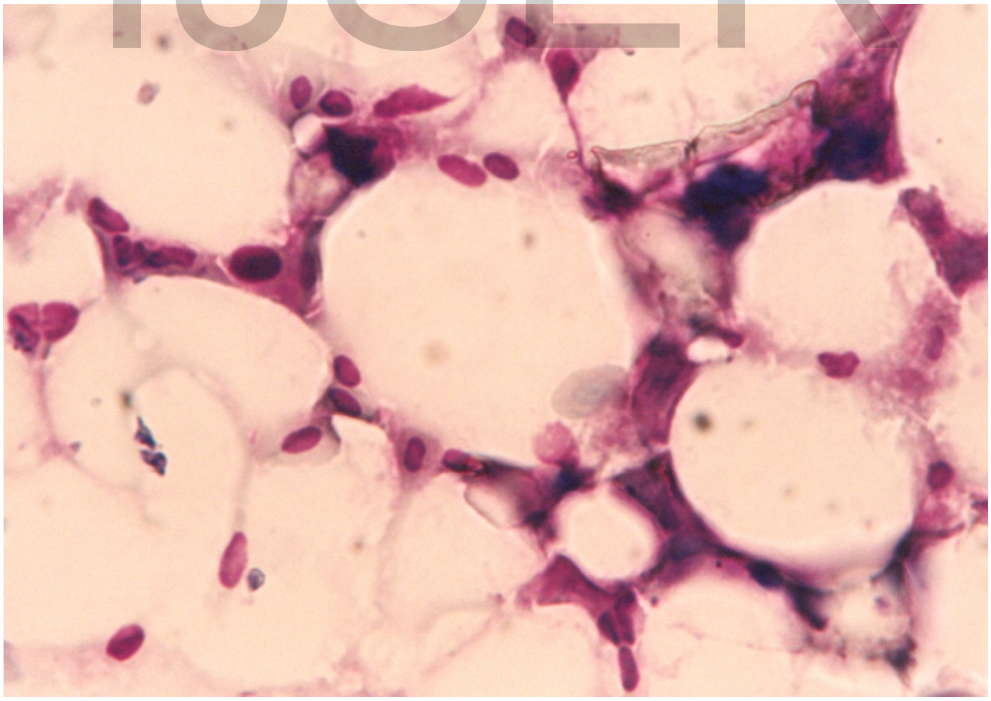

Fig.4d.13. Normal imprint smear from the lung of a falcon showing red blood cells at 1000x.

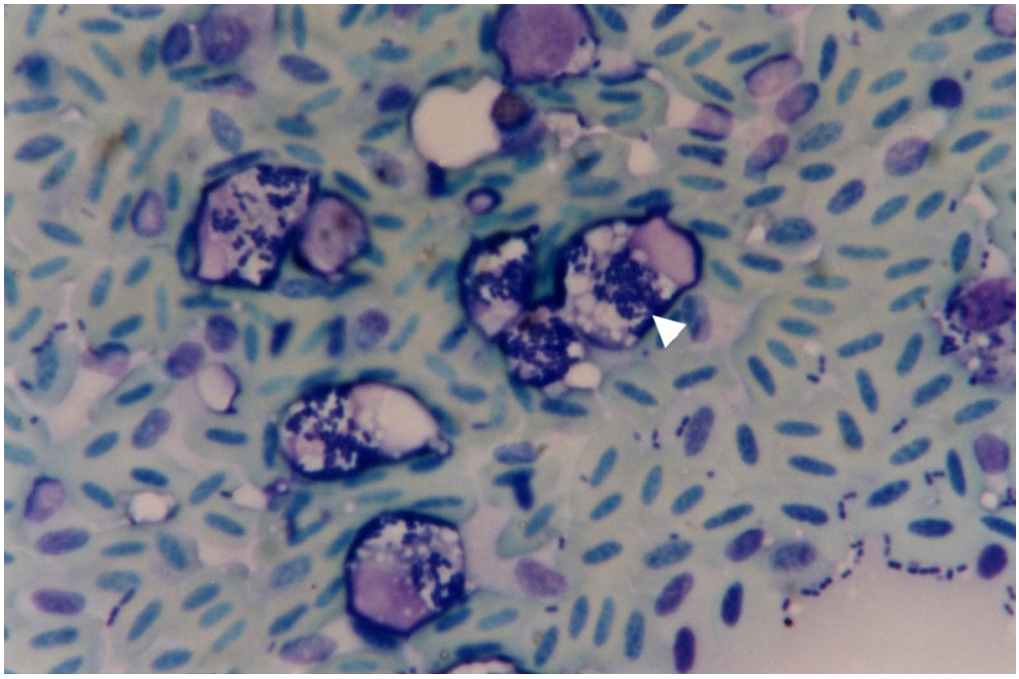

Fig.4d.14. Smear from the lung of a Gyr falcon showing macrophages engulfed with bacteria (pneumonia) at 1000 X.

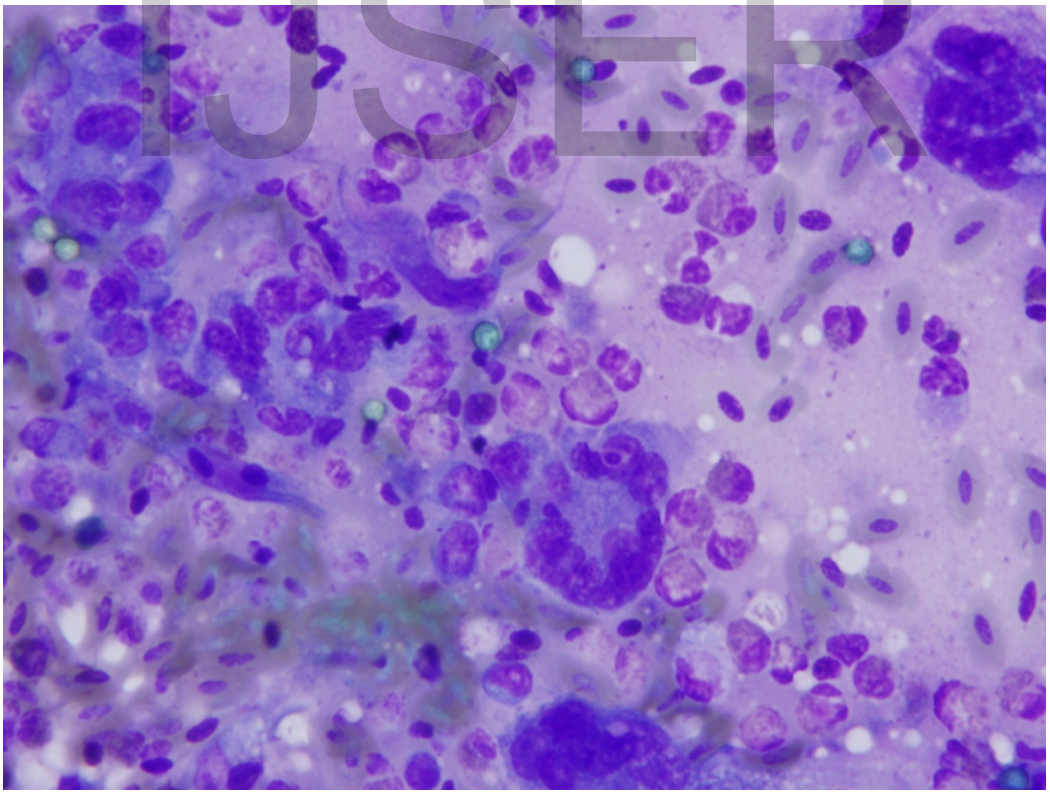

Fig.4d.15. Smear from the airsac of a Peregrine falcon showing mixed inflammatory cells, fungal spores and giant cells at 1000X. It is a typical cytology view of aspergillosis.

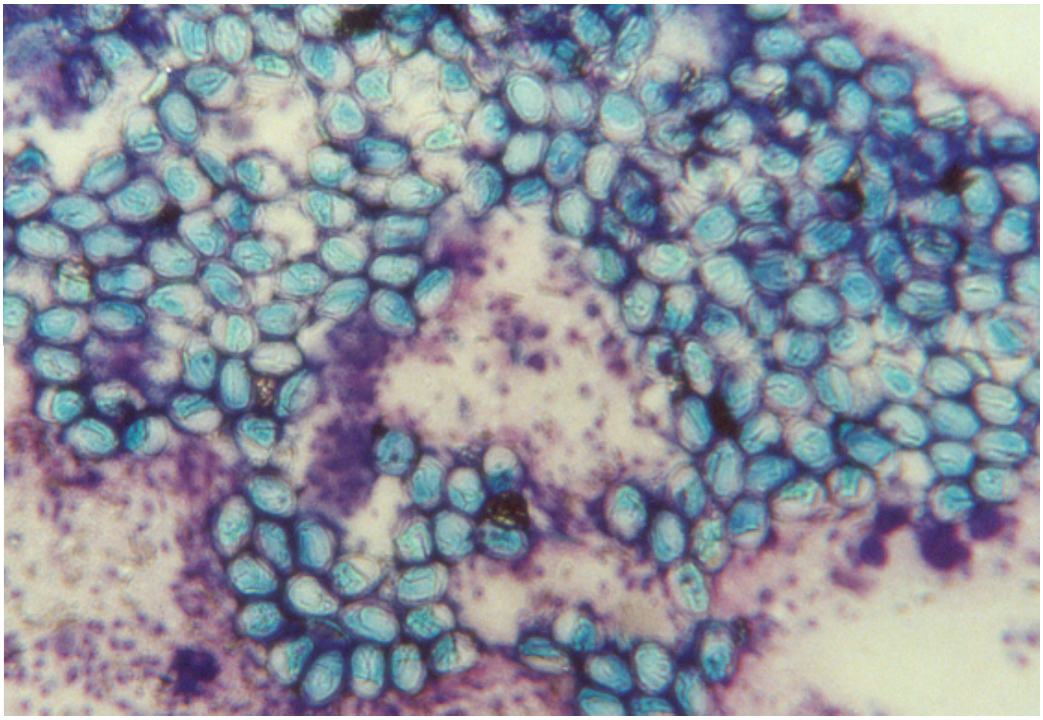

Fig.4d.16. Smear from the airsac of a Saker falcon showing Serratospiculum eggs at 100X.

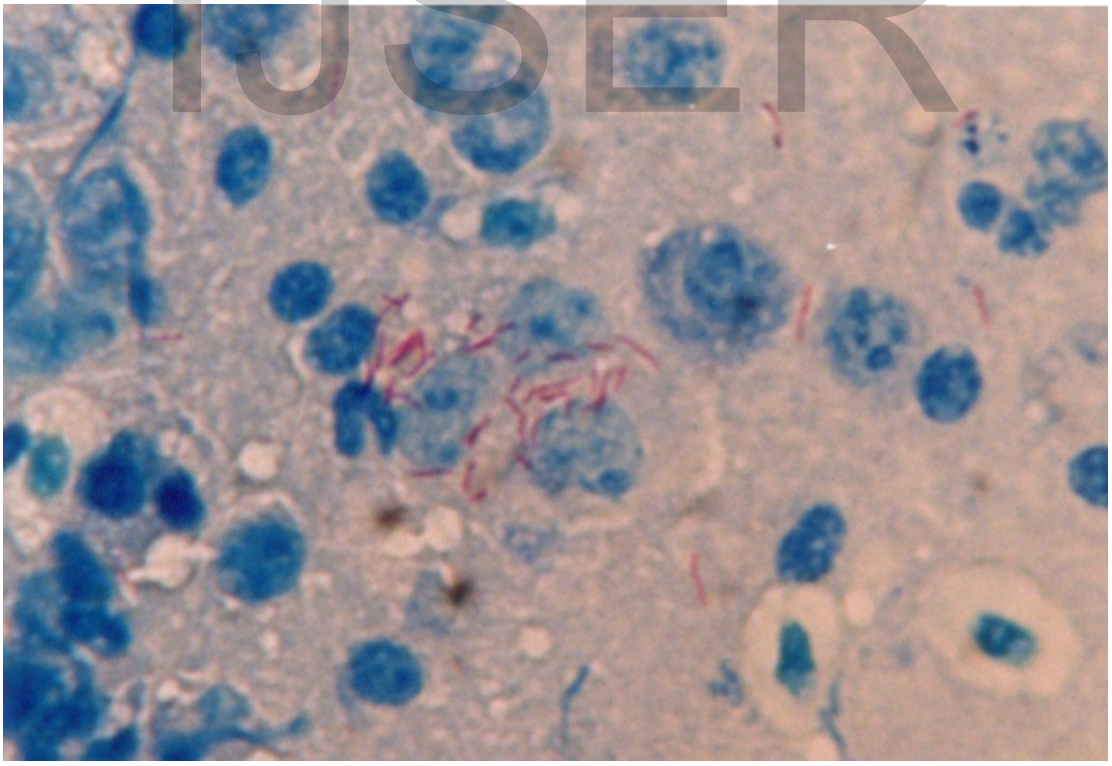

Fig.4d.17. Smear from the airsac of a Peregrine falcon showing AFB positive rods in Z-N stain and it is confirmed as Mycobacterium tuberculosis.

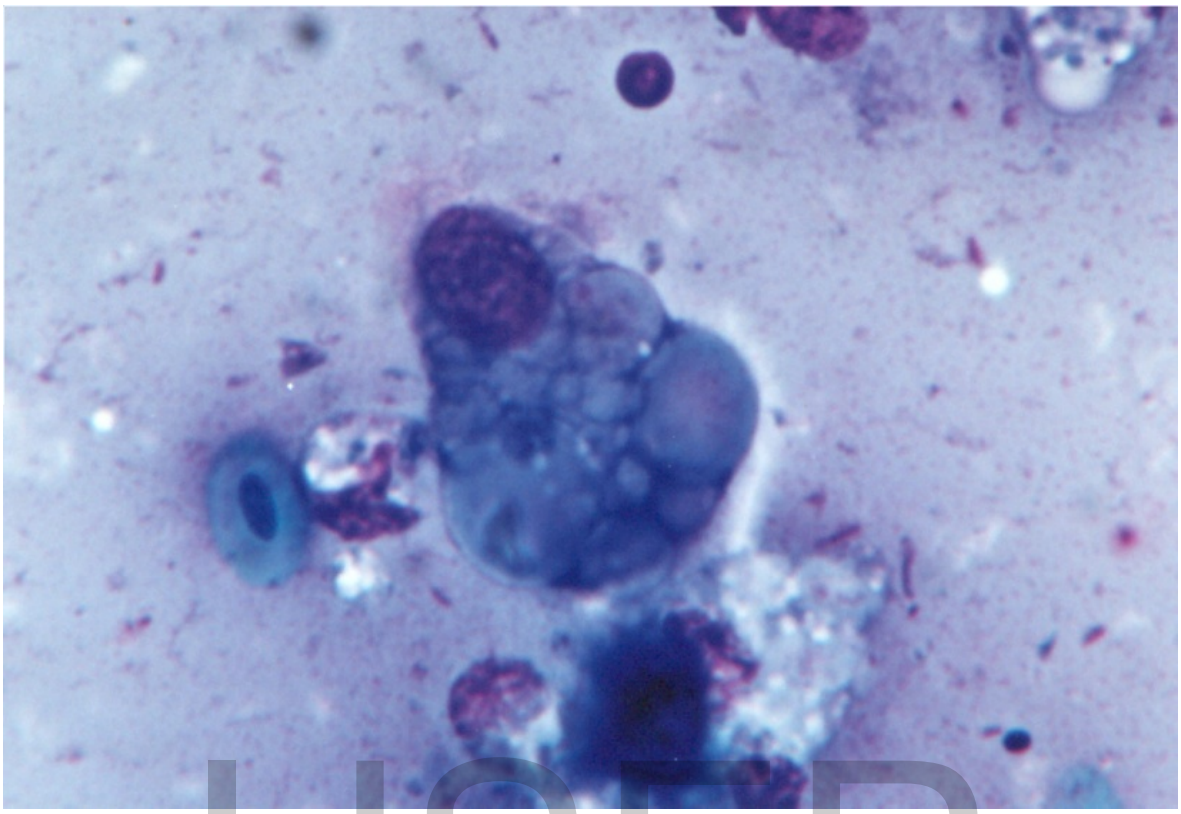

Fig.4d.18. Smear from the lungs of a Kori bustard showing foreign body pneumonia (1000 X).

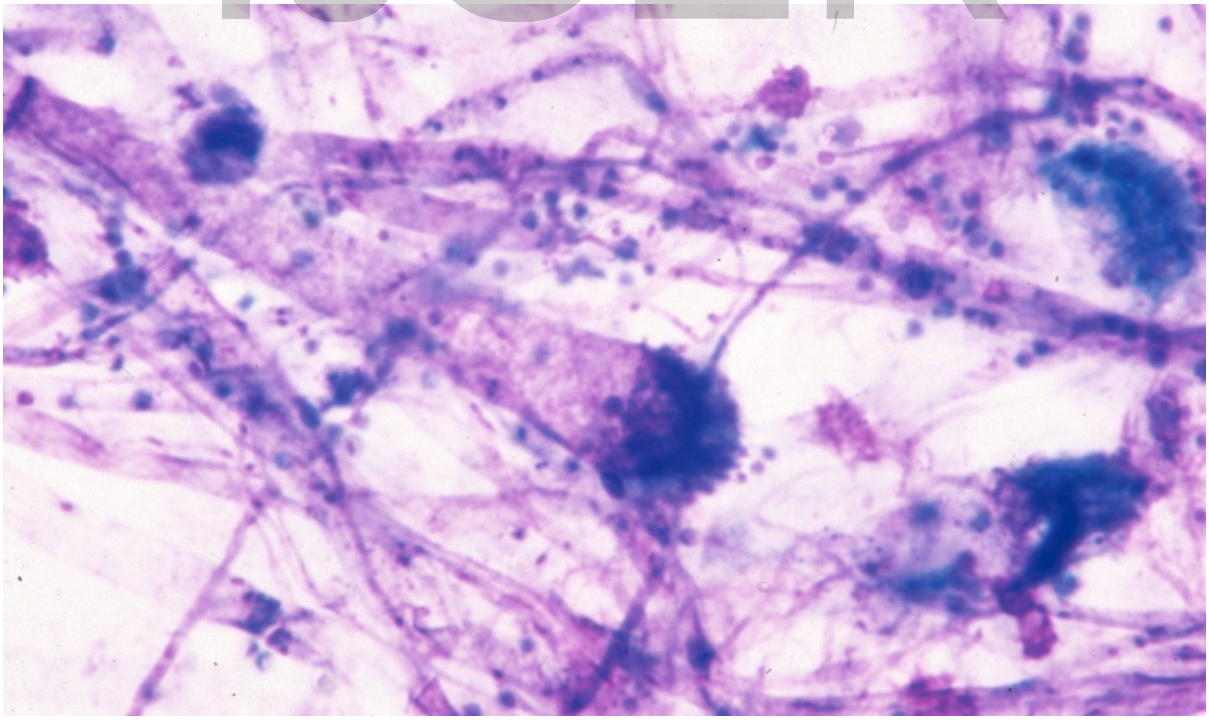

Fig.4d.19. Smear from the lungs of a Houbara bustard showing aspergillosis (1000X)

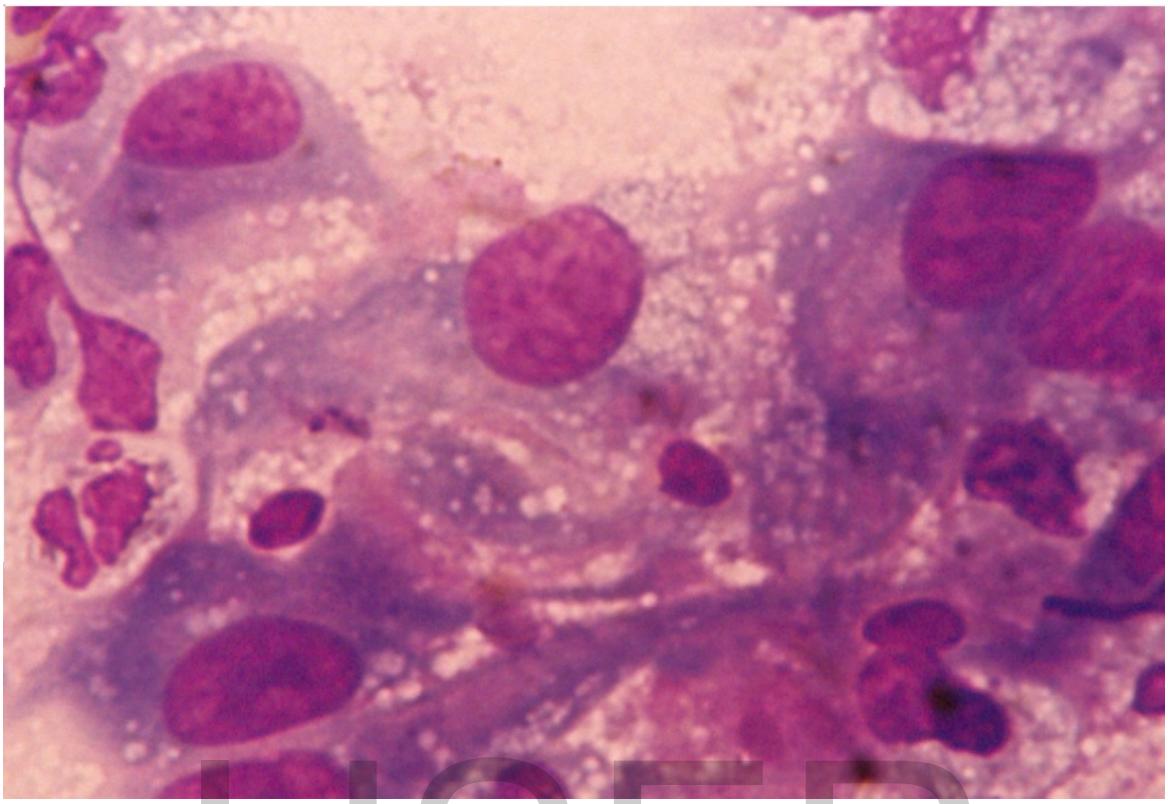

Fig.4d.20. Smear from the airsac of a gyr falcon showing metaplasia changes in the lining squamous cells at 1000X.

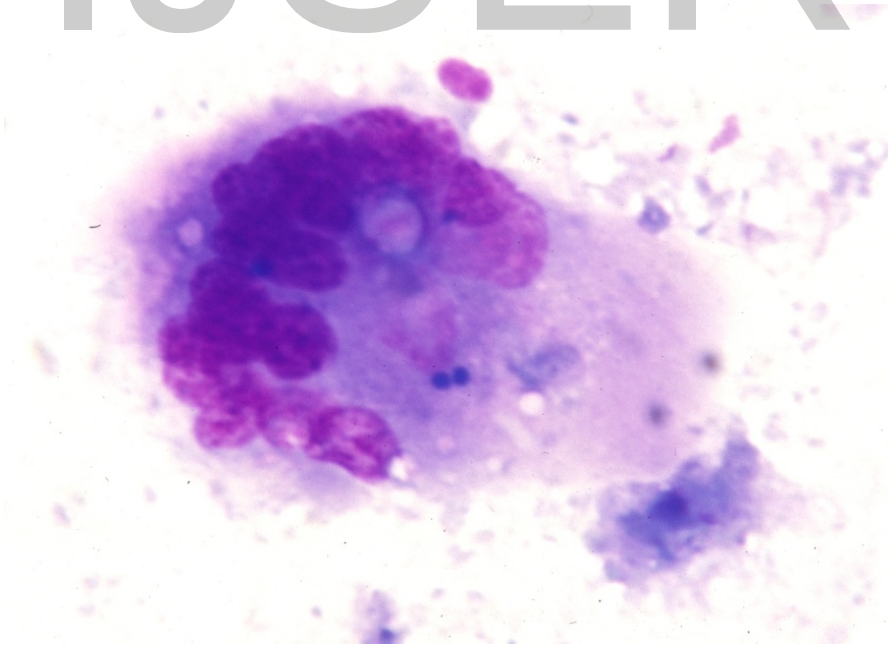

Fig.4d.21. Smear from the air sac of a gyr falcon showing giant cells, which is an indication of fungal response or tuberculosis.

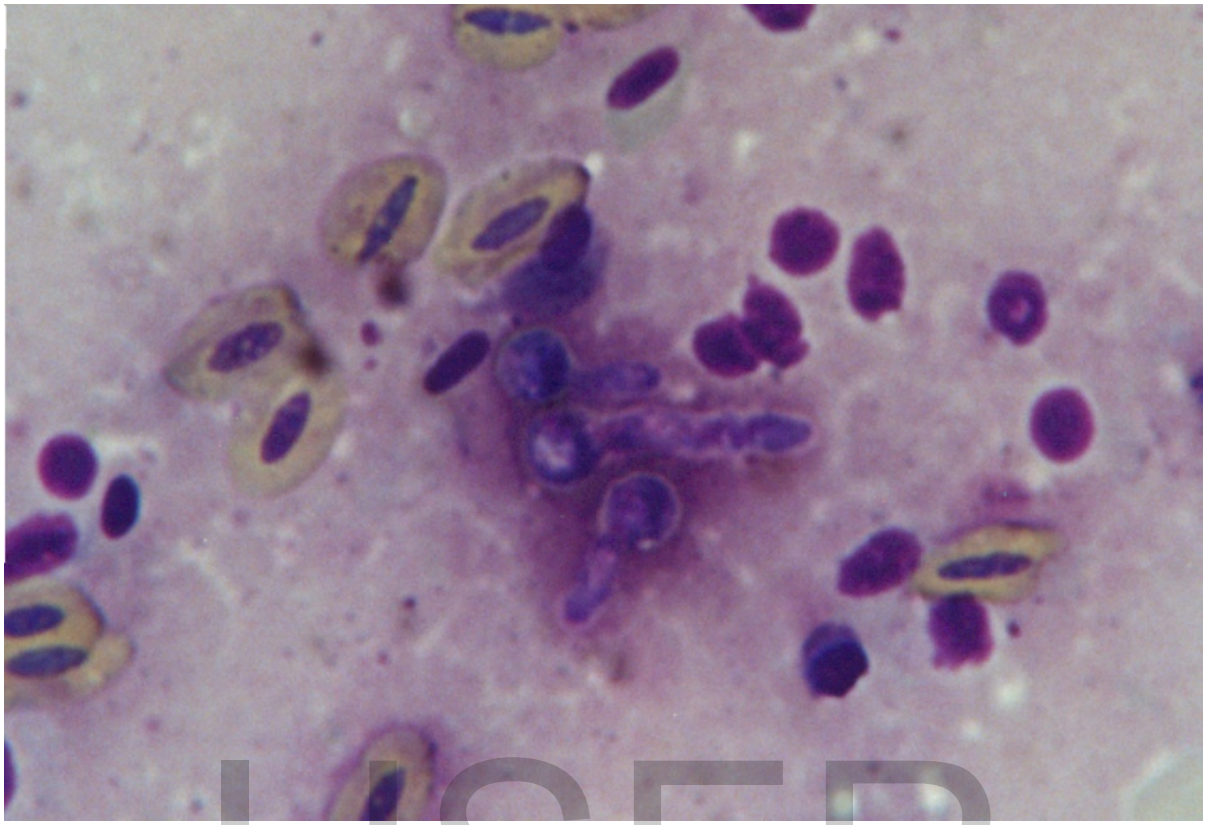

Fig.4d.22. Smear from the airsac of a gyr falcon showing germinating fungal spores at 1000X.

(4e) Cytology of Conjunctiva and Cornea

The conjunctival mucosa is composed of stratified columnar epithelium. Normal smears made from the conjunctiva are usually poorly cellular with a few epithelial cells present in a clear background. The conjunctival cells have an eccentric, round to oval nucleus with coarse nuclear chromatin and abundant weakly basophilic cytoplasm with distinct margins. The cornea is composed of stratified squamous epithelium. Normal smears from the cornea are poorly cellular with noncornified squamous epithelial cells. Abnormal cytology of the conjunctiva, (conjunctivitis and keratoconjunctivitis) caused by bacteria reveal numerous inflammatory cells and the inflammatory cells may show phagocytosis of bacteria and degenerative epithelial cells. The degenerative changes

include cytoplasmic vacuolation, karyolysis or karyorrhexis. Acute inflammatory lesions are represented by an increase in heterophils. Chronic inflammatory lesions are represented by an increase in mononuclear leucocytes. Conjunctival goblet cells may also be present in chronic cases. Figures 4e.1 to 4e.7 show the normal and abnormal cytology of conjunctiva and cornea.

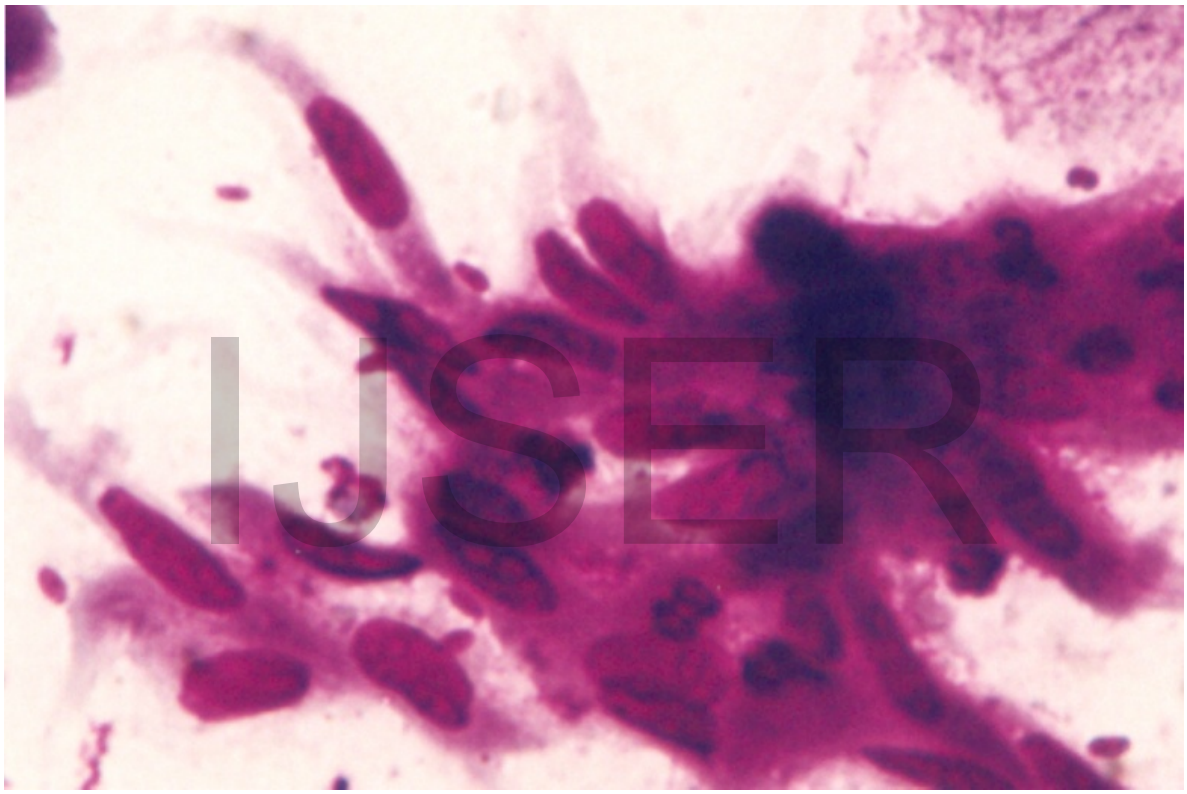

Fig.4e.1. Conjunctiva smear from a houbara bustard showing columnar cells.

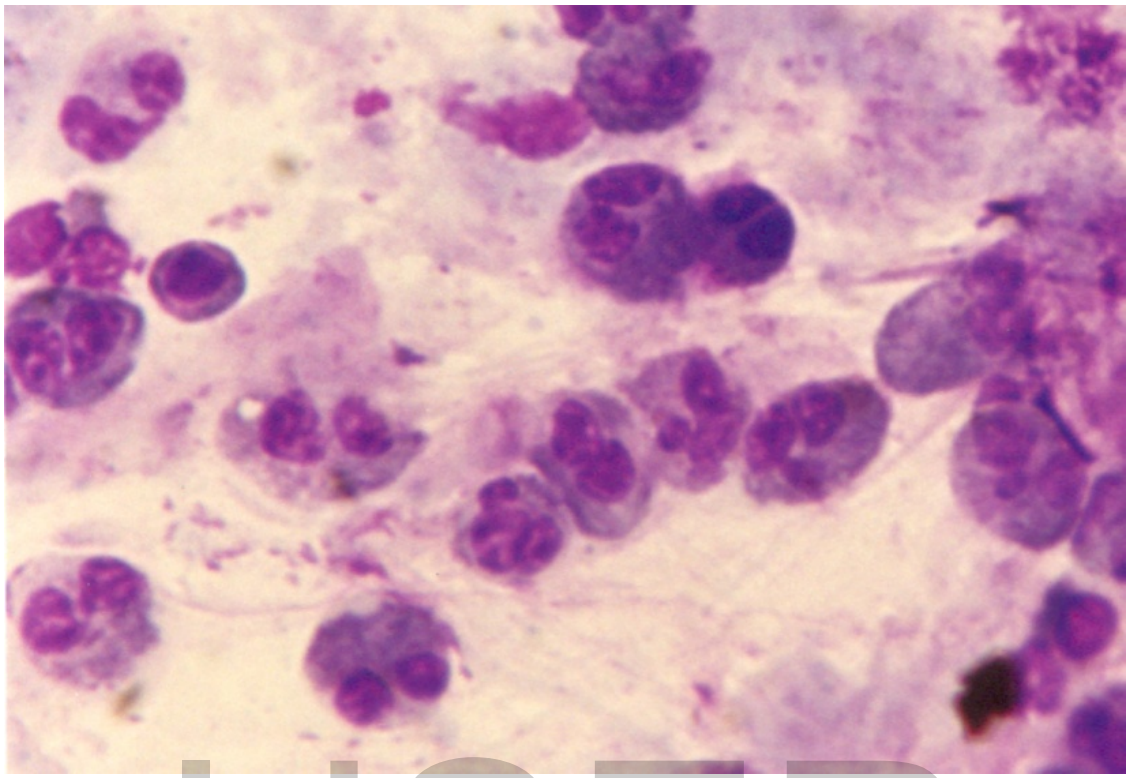

Fig.4e.2. Conjunctiva smear from a gyr falcon showing heterophils which is an indication of bacterial conjunctivitis.

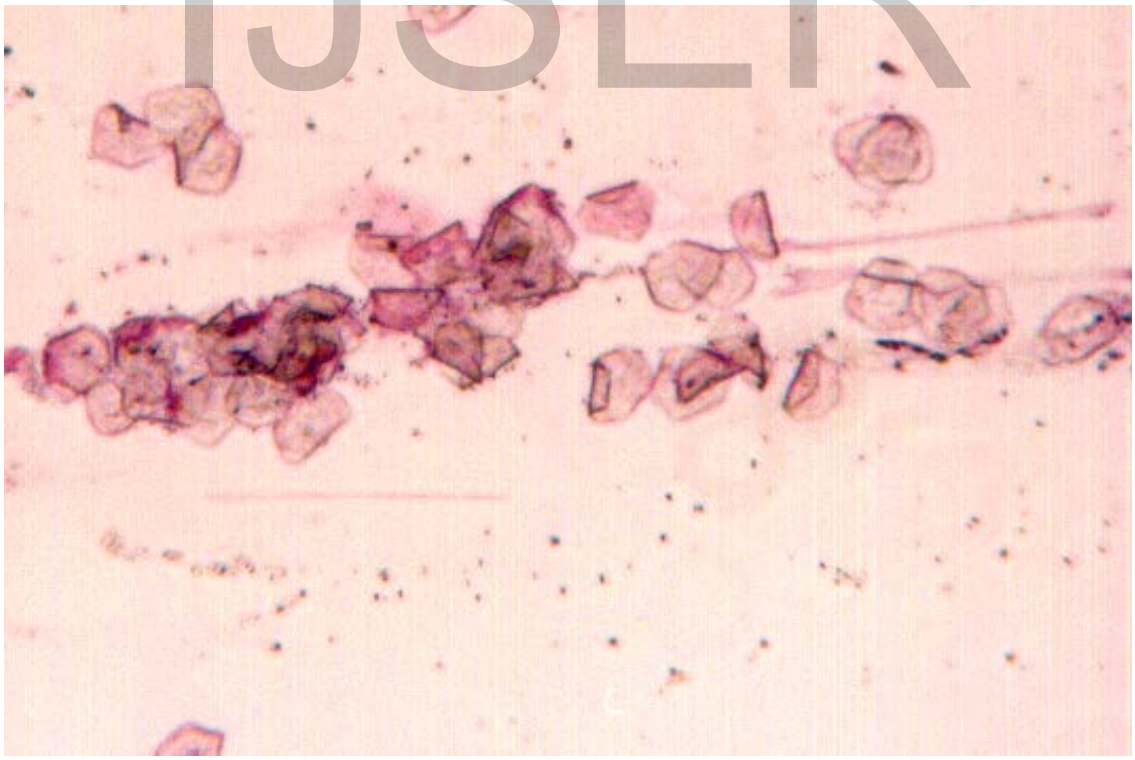

Fig.4e.3. A conjunctiva smear shows squamous cells which is contaminated from the surrounding area.

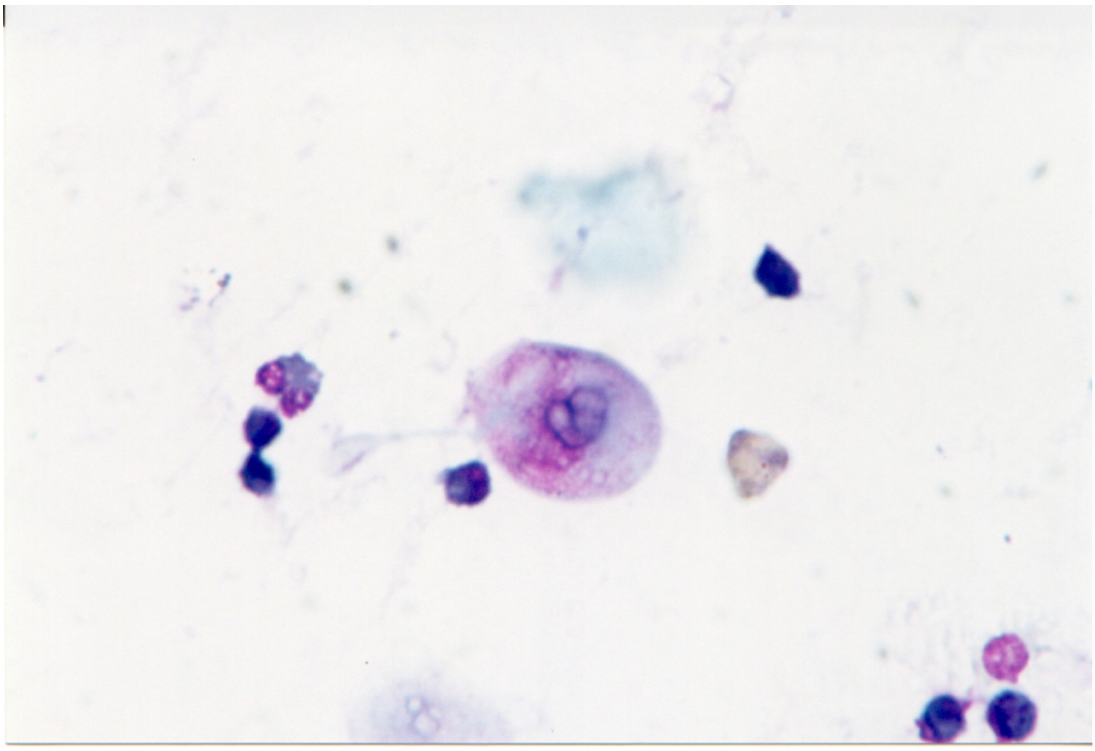

Fig.4e.4. Conjunctiva cell with pox response.

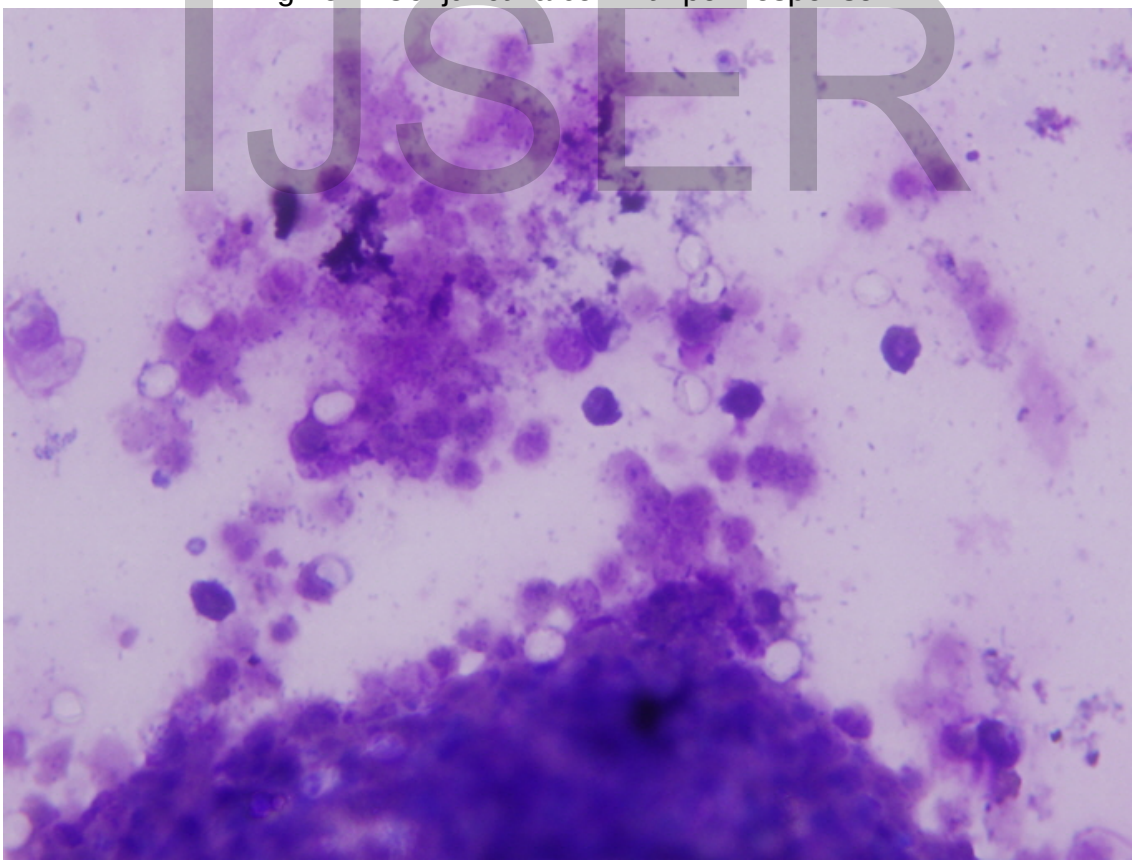

Fig.4e.5. Conjunctiva smear from a peregrine falcon showing Cryptosporidium oocysts.

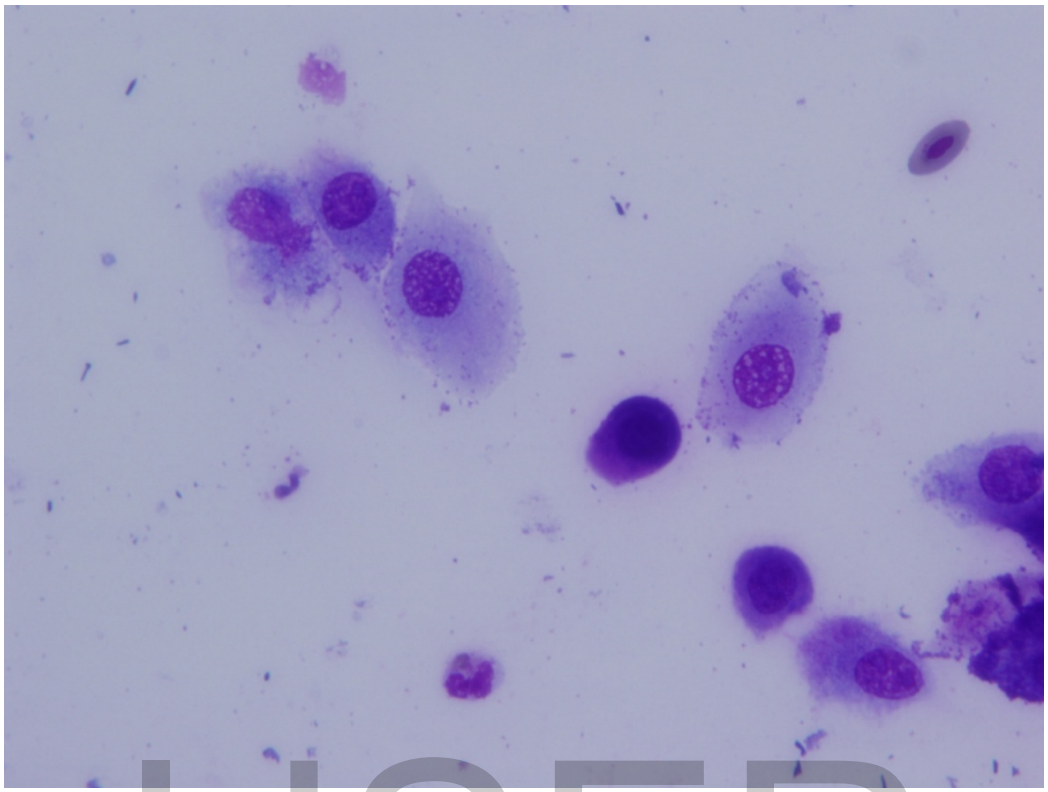

Fig.4e.6. Conjunctiva smear from a gyr falcon showing chlamydia inclusions.

Fig.4e.7. Anterior portion of an eye flukes from a white bellied bustard.

(4f) Cytology of Aspirated Fluids

Synovial fluid with increased numbers of leucocytes and alternation in colour, turbidity and viscosity is considered abnormal. An increase of heterophils is seen in smears of inflammatory joint diseases. Non-inflammatory joint disorders show a normal cytology picture. The amount of granular back- ground particles indicates the amount of mucin present in the fluid. The presence of erythrocytes usually indicates contamination from the peripheral blood). Articular gout can often be diagnosed by presence of needle shaped monosodium urate crystals.

The peritoneum produces a small amount of lubricating fluid to facilitate organ movements, but little or no fluid can be collected from the abdomen of normal birds. The cytology of abdominal fluid from a normal bird shows the occasional mesothelial cell and a few macrophages. Mesothelial cells are round or oval and variable in size, and have a homogenous basophilic cytoplasm and a centrally positioned round nucleus. An abdominal effusion can be classified as transudate and exudate according to the cellularity. Transudates are characterised by low cellularity and exudates are characterised by high cellularity.

Exudate and transudate effusions in the abdominal cavity (ascites, peritonitis and haemoperitoneum) are a result of inflammatory processes within the peritoneal cavity. Acute inflammatory effusions are characterised by a predominance of heterophils. Septic exudates are characterised by macrophages with bacterial phagocytosis. Malignant exudates have a haemorrhagic character and contain malignant cells. It is difficult to conclude the origin of undifferentiated malignant cells in peritoneal effusion. Bacteria or degenerated heterophils may be present in purulent exudates. Reactive

mesothelial cells may show cytoplasmic vacuolisation. Plasma cells frequently occur in chronic inflammatory exudates. Figures 4f.1 to 4f.8 show the normal and abnormal cytology of aspirated fluids.

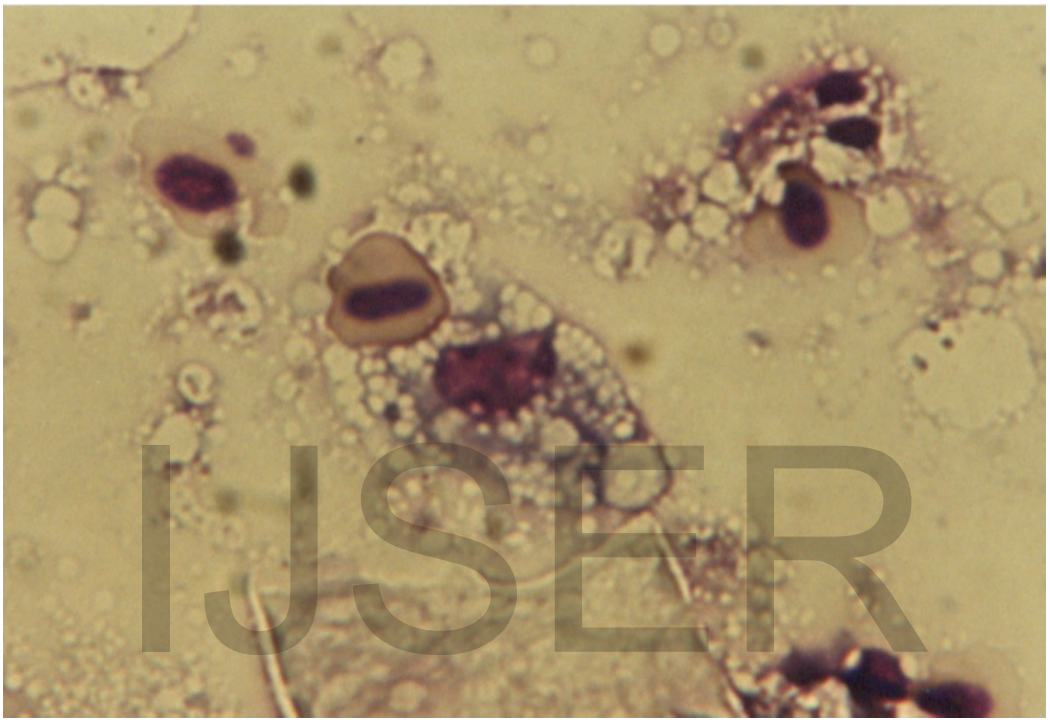

Fig.4f.1. Normal synovial smear from a gyr falcon.

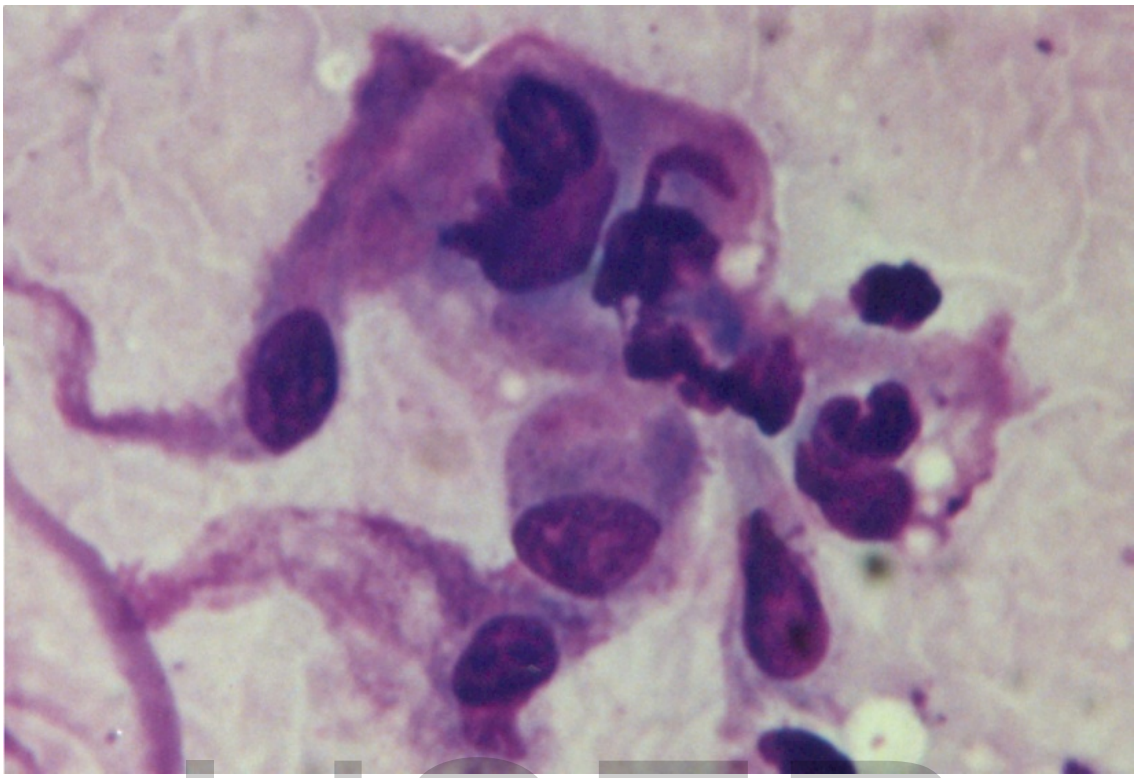

Fig.4f.2. Mesothelial cells and fibrin strands in an abdominal aspirate from a gyr falcon.

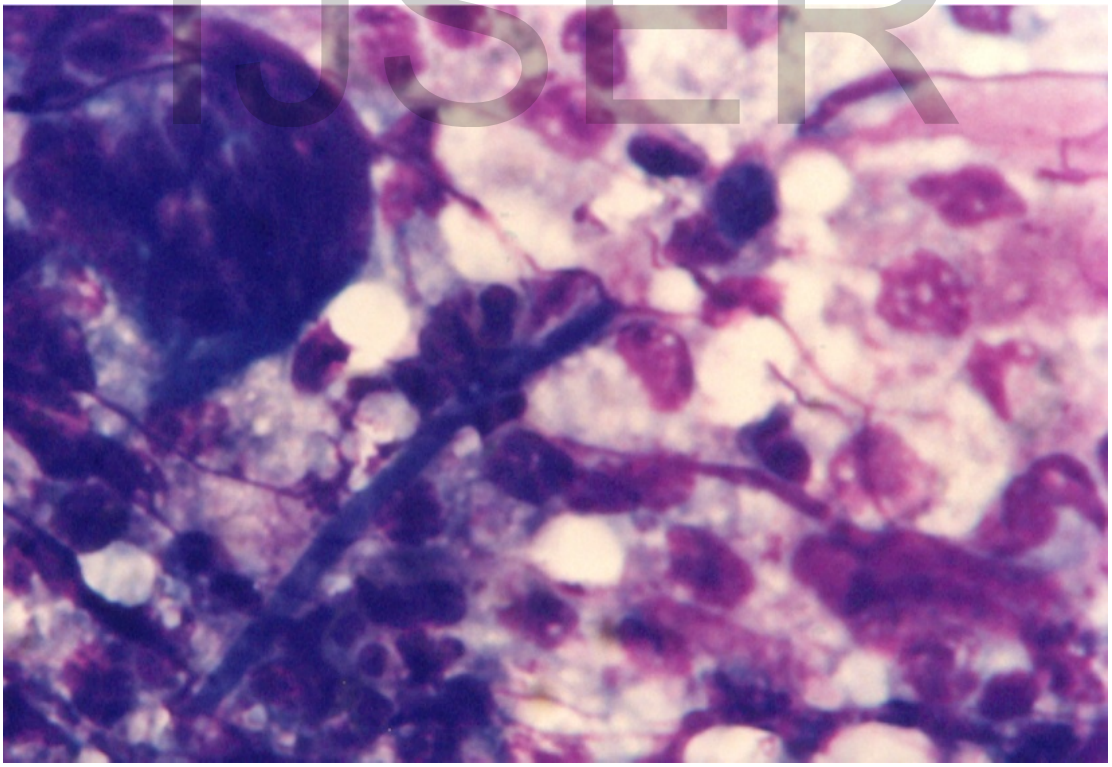

Fig.4f.3. A smear from the abdominal aspirate of a gyr falcon showing Aspergillus hyphae and macrophages.

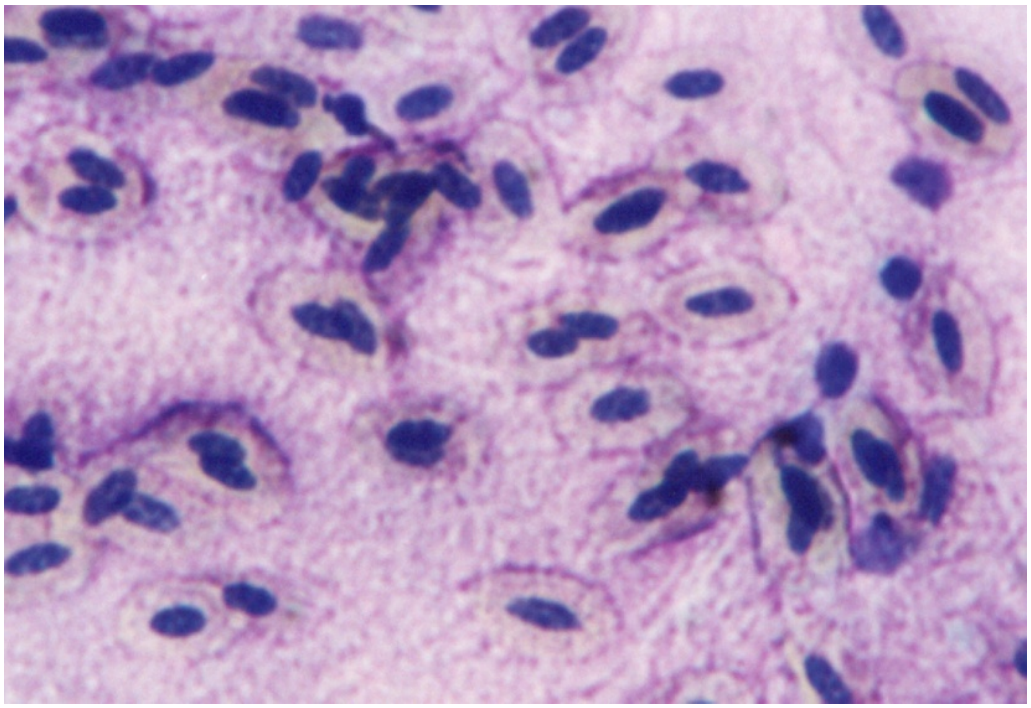

Fig.4f.4. Normal smear from the synovial aspirate of a houbara bustard showing erythrocytes in a granular protein background.

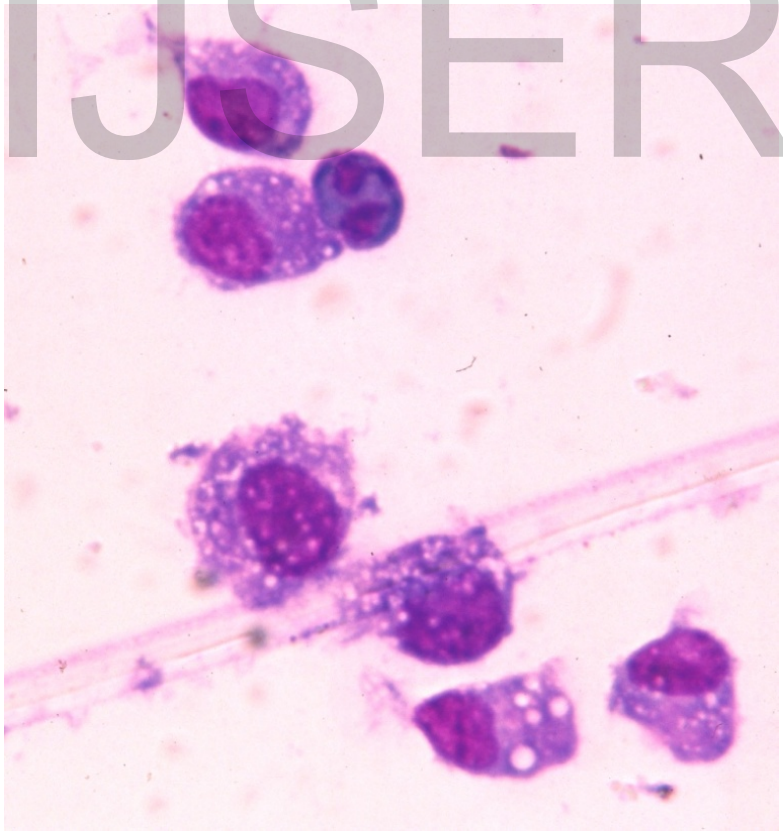

Fig.4f.5. A smear from the abdominal aspirate of saker falcon showing reactive mesothelial cells.

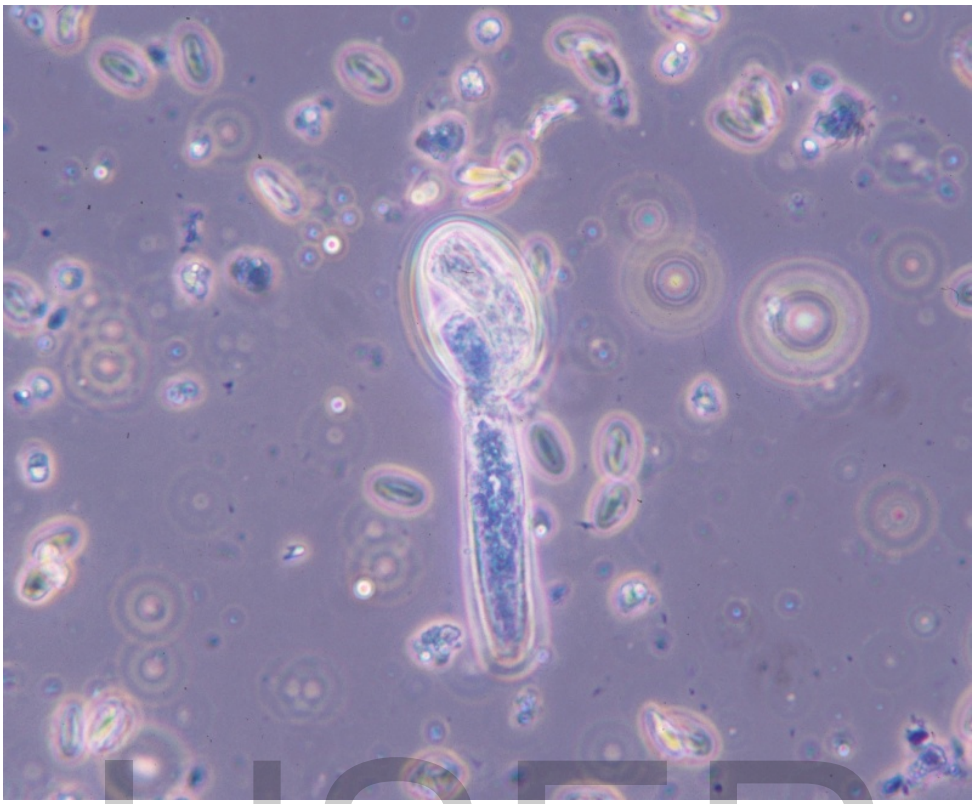

Fig.4f.6. A smear from the abdominal aspirate of saker falcon showing an emerging microfilaria from its egg.

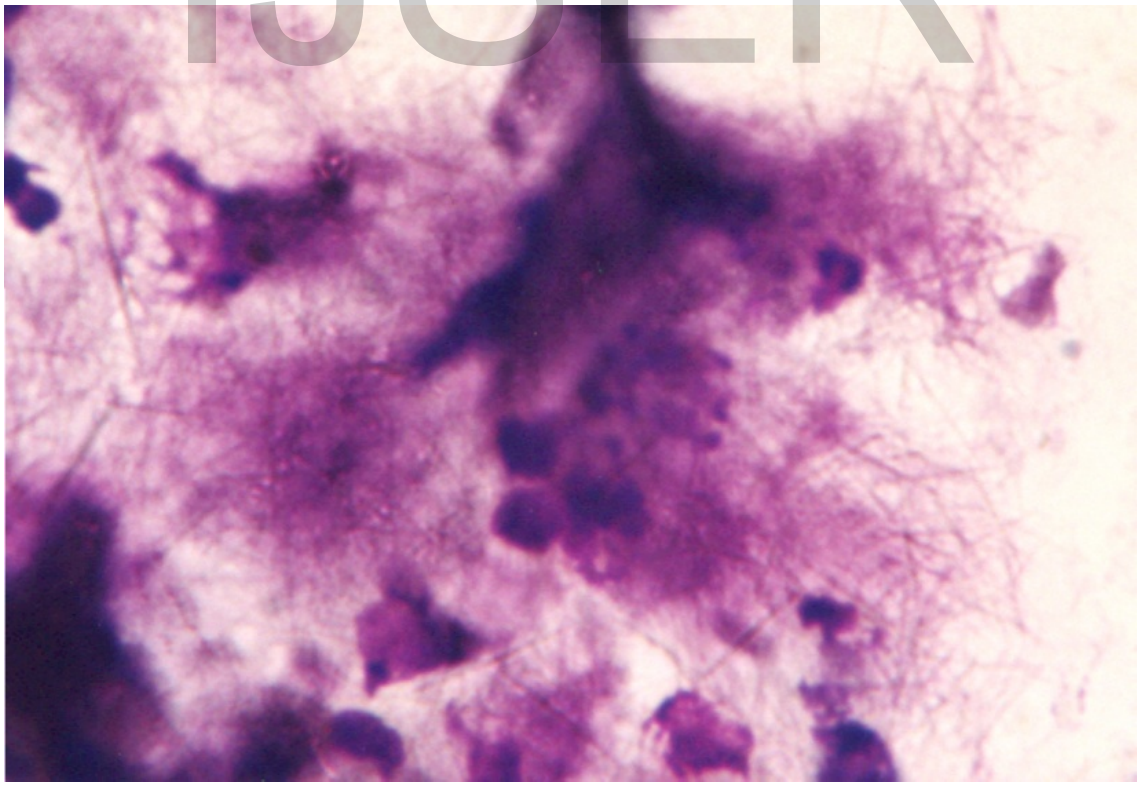

Fig.4f.7. Synovial joint of a houbara bustard showing urate crystals (gout).

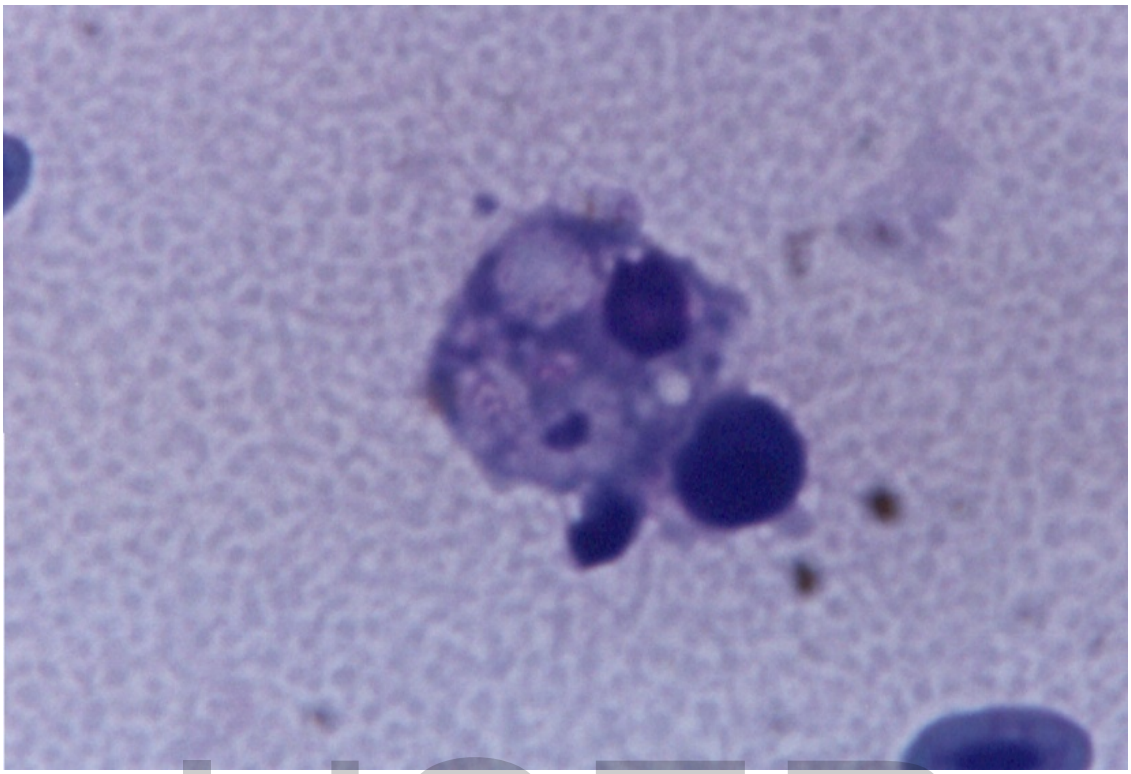

Fig.4f.8. A smear from synovial joint of a houbara bustard with pox response.

(4g) Cytology of Internal Organs – Liver

Imprint cytology smears from the cut surface of the liver show primarily hepatocytes and a variable numbers of blood cells. Hepatocytes are large cells with round or oval nuclei and an abundant cytoplasm. Hepatocytes occur singly, in sheets or in clusters. These cells are easily damaged, resulting in free nuclei. Lymphocytes, macrophages and spindle-shaped cells are occasionally seen in normal smears. Abnormal cytology of liver includes non-infectious diseases (fatty liver, amyloidosis, hepatoma and gout) and infectious diseases. Fatty liver syndrome is often seen in captive bustards and is characterised by fat deposition in hepatocytes. Cytology smears are characterised by vacuolated hepatocytes. Figures 4g.1 to 4g.16 show the normal and abnormal cytology of liver.

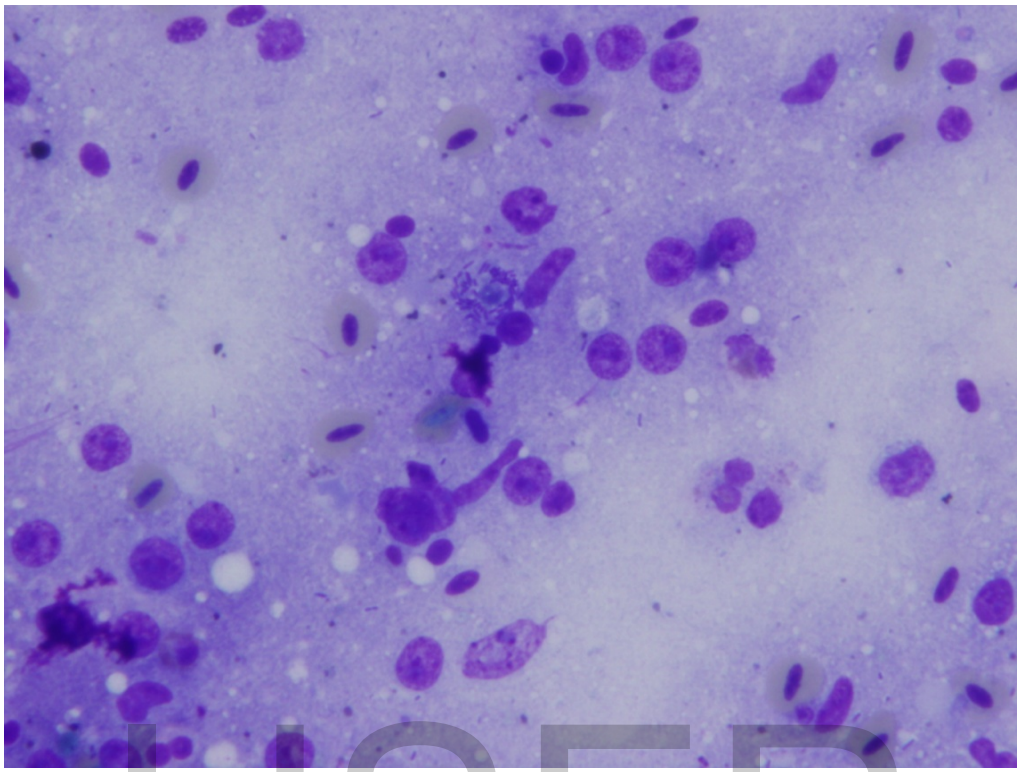

Fig.4g.1. Liver imprint smear from a gyr falcon shows bacterial rods and it died due to septicemia.

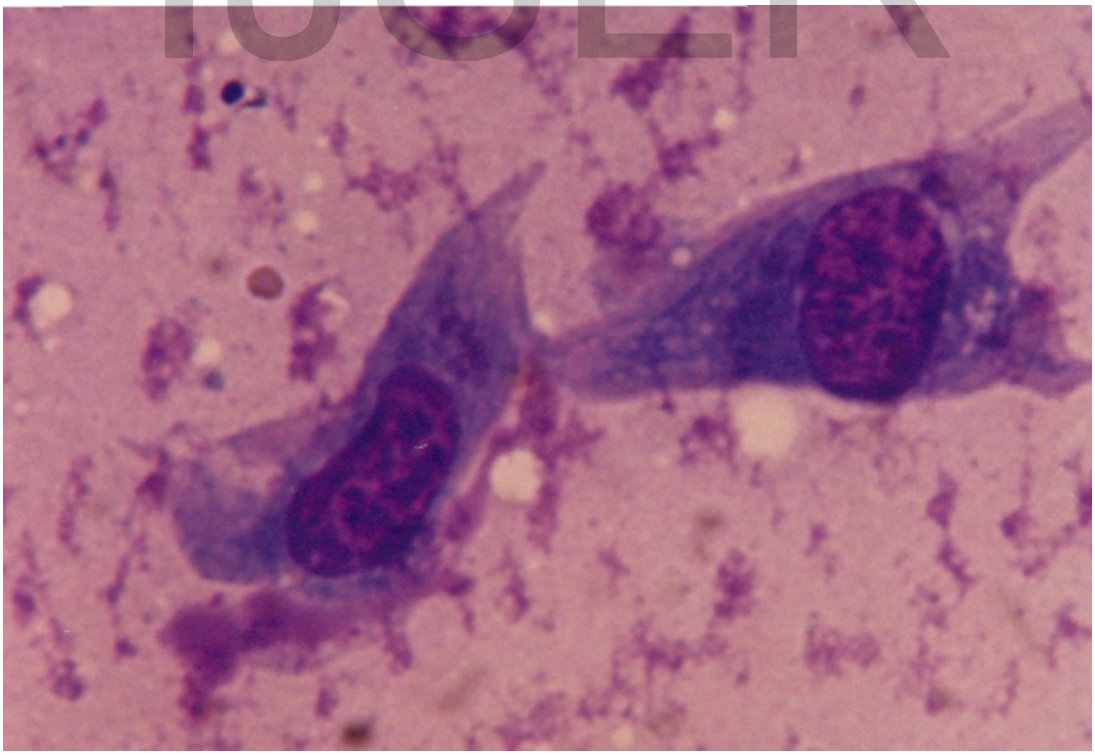

Fig.4g.2. Liver imprint smear from a peregrine falcon showing changes in the hepatocytes due to adeno virus hepatitis.

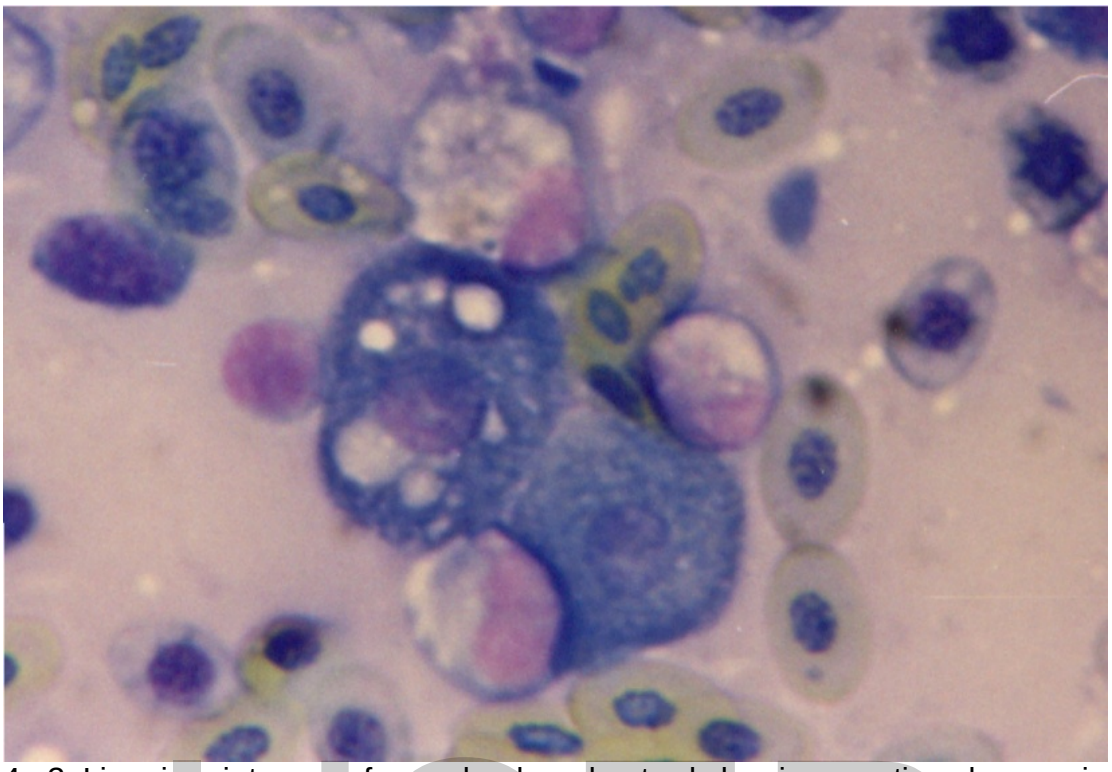

Fig.4g.3. Liver imprint smear from a houbara bustard showing reactive changes in the hepatocytes due to avian leucosis.

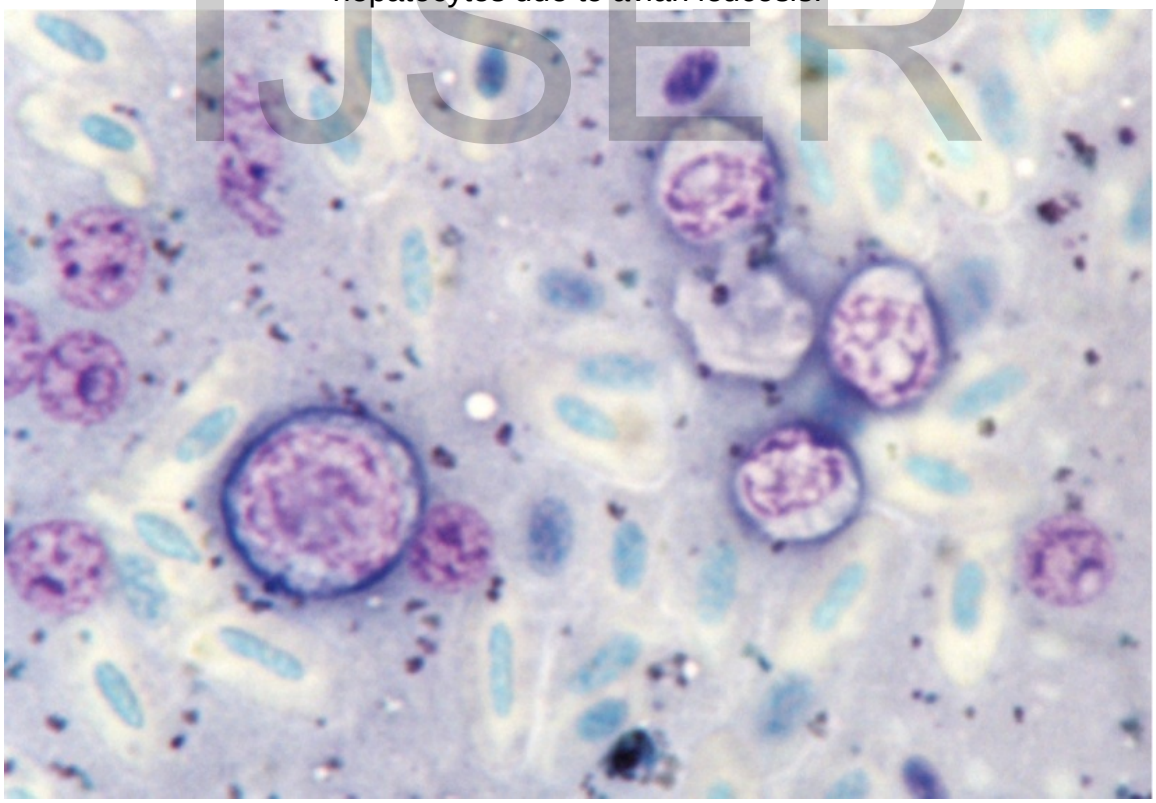

Fig.4g.4. Liver imprint smear from a kori bustard showing bile pigments.

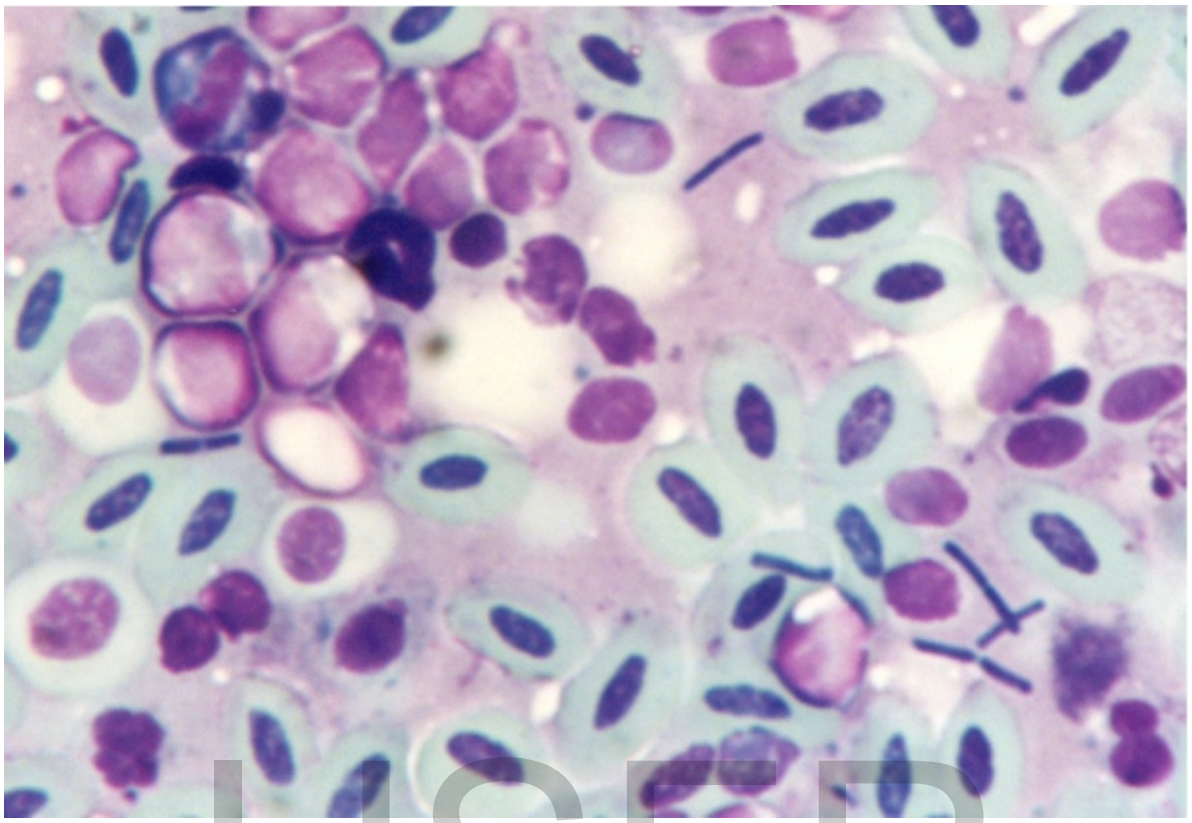

Fig.4g.5. Liver imprint smear from a gyr falcon showing bacterial rods which died due to clostridiosis.

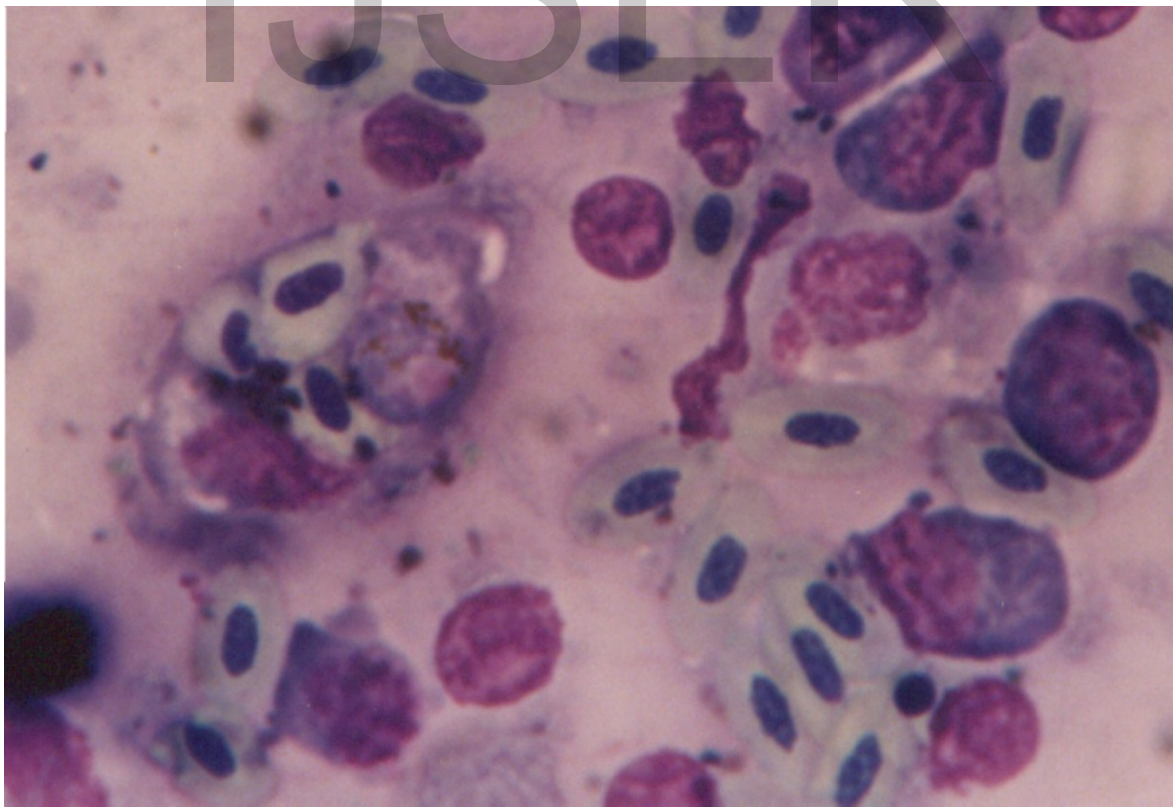

Fig.4g.6. Liver imprint smear from a houbara bustard showing hemoproteus.

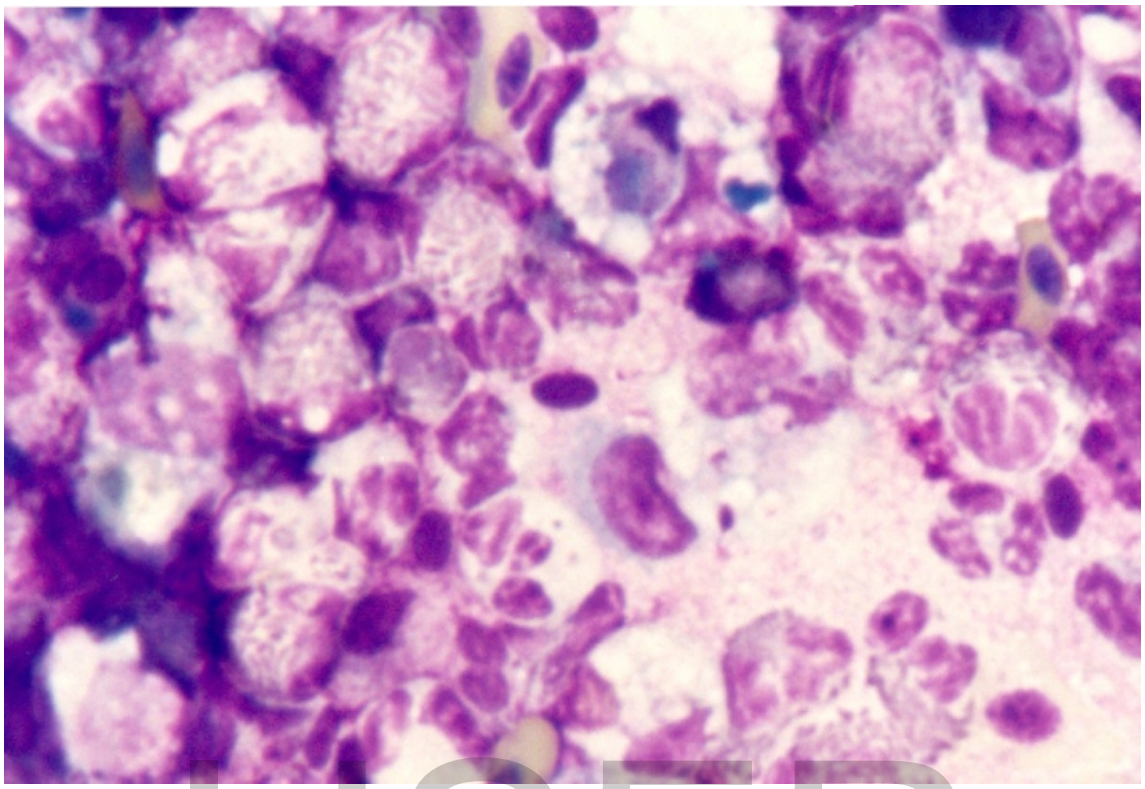

Fig.4g.7. Liver imprint smear from a gyr falcon showing hepatitis.

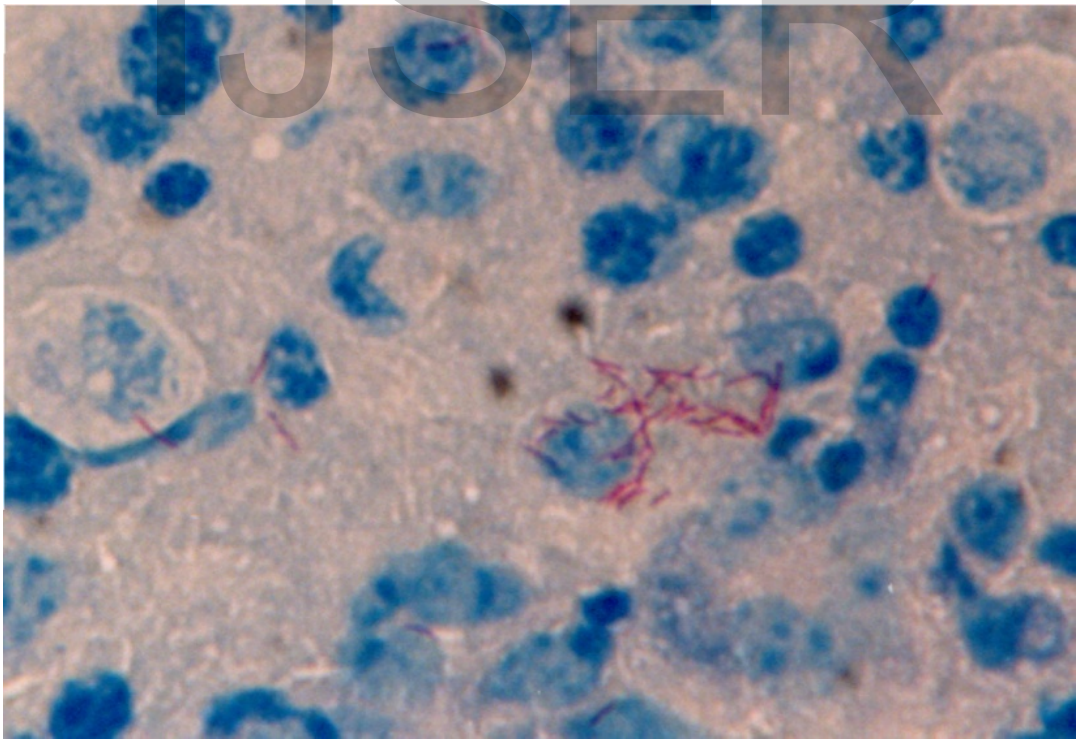

Fig.4g.8. Liver imprint smear from a peregrine falcon showing AFB rods in Z-N stain.

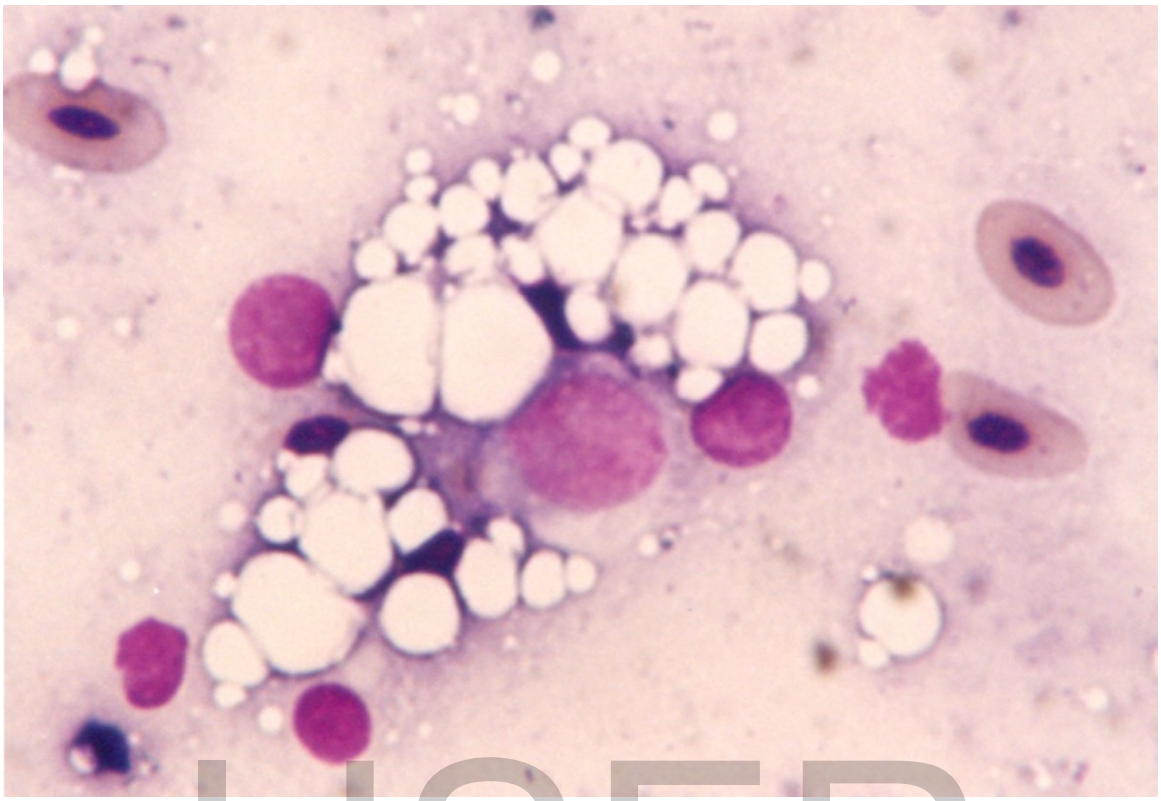

Fig.4g.9. Liver imprint smear from a gyr falcon showing hepatocytes with fatty vacuole.

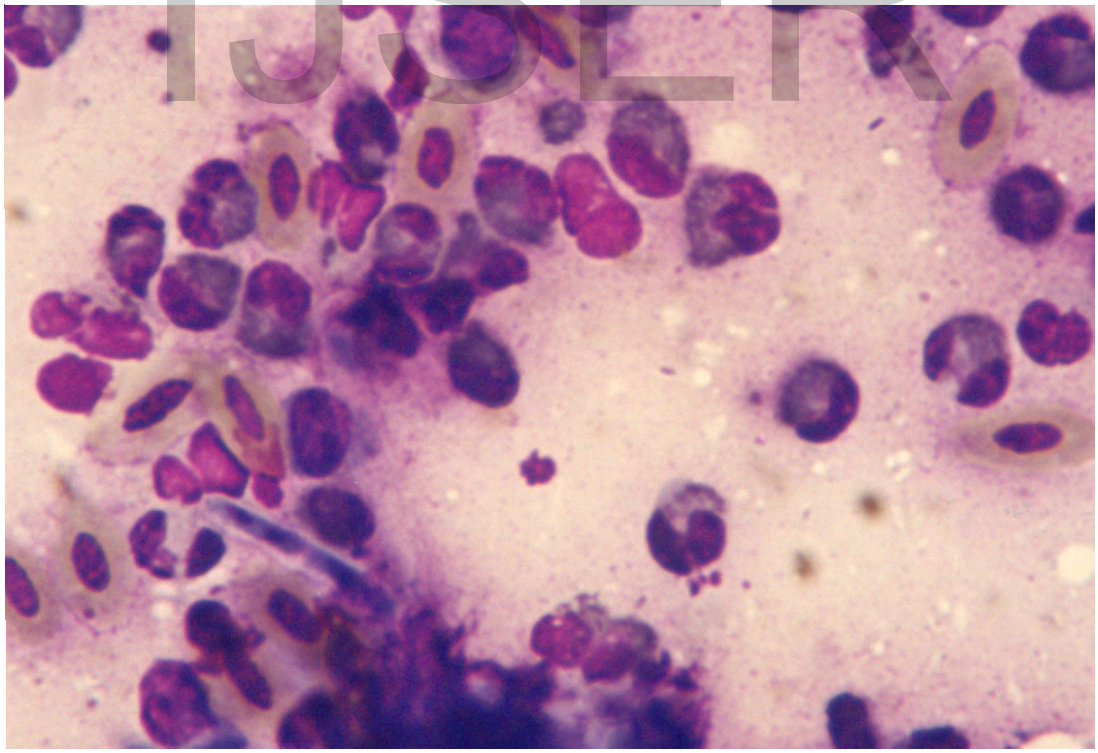

Fig.4g.10. Liver imprint smear from a gyr falcon showing aspergillus hyphae and inflammatory cells.

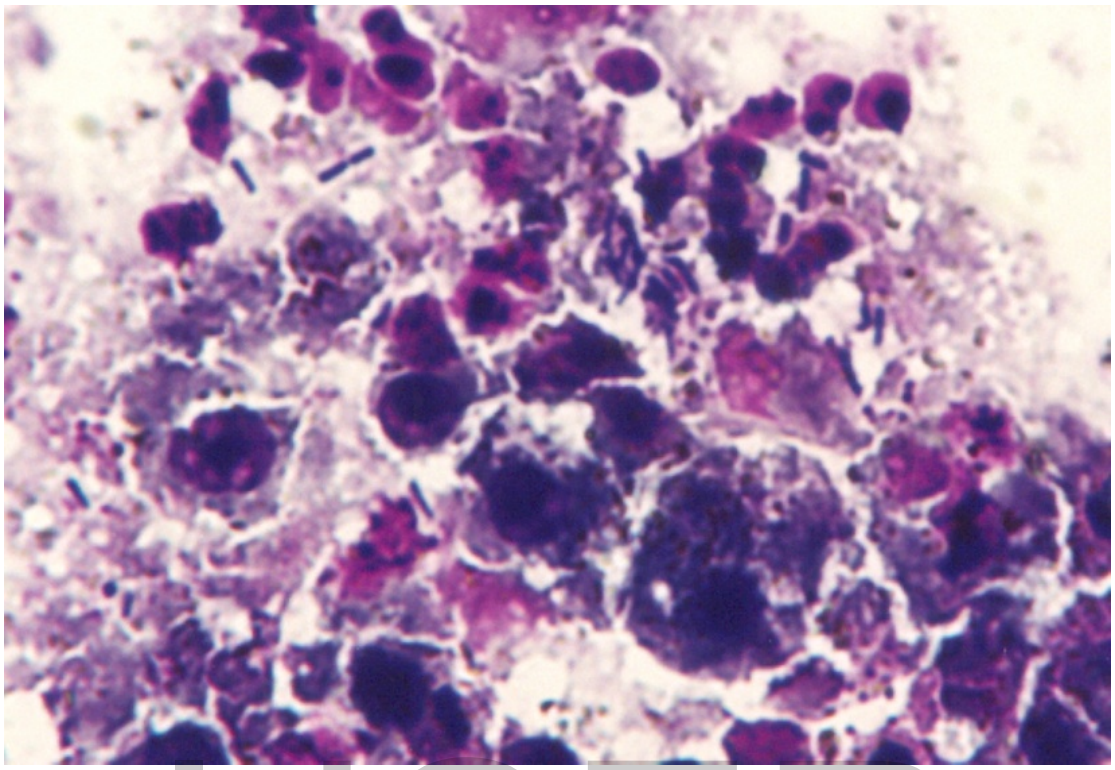

Fig.4g.11. Liver imprint smear from a gyr falcon showing necrotic changes and cadaver bacteria.

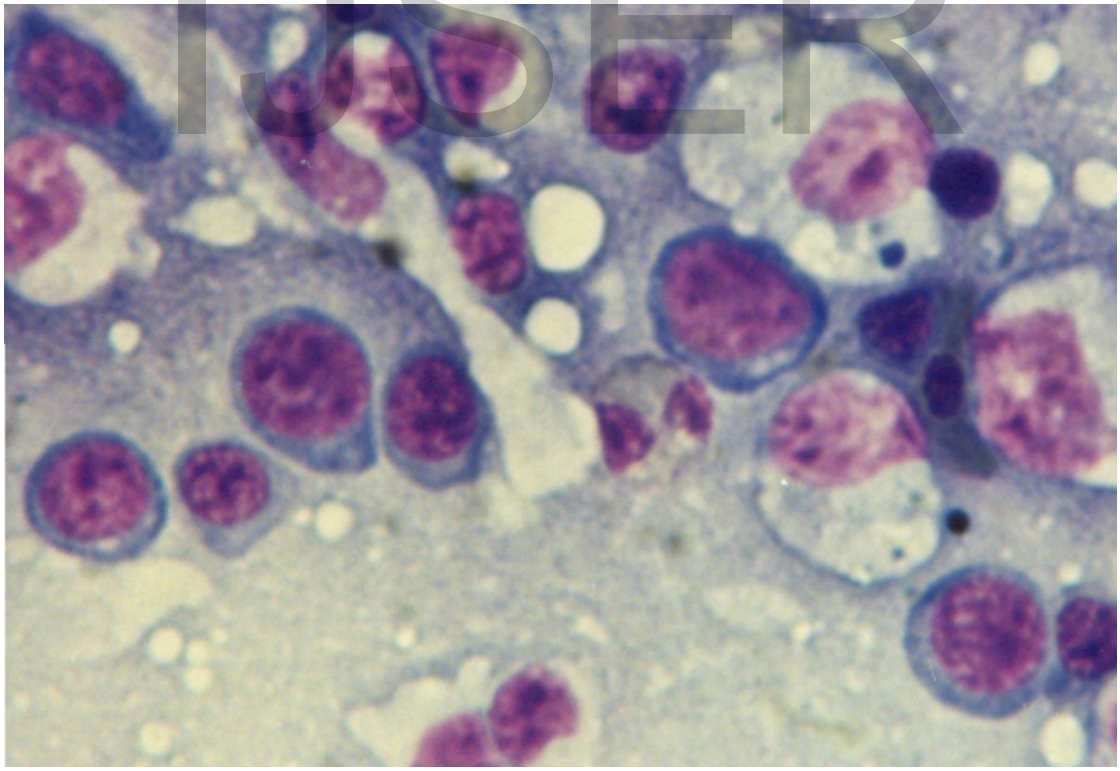

Fig.4g.12. Liver imprint smear from a gyr falcon showing many kupffer cells due to mycobacterium tuberculosis.

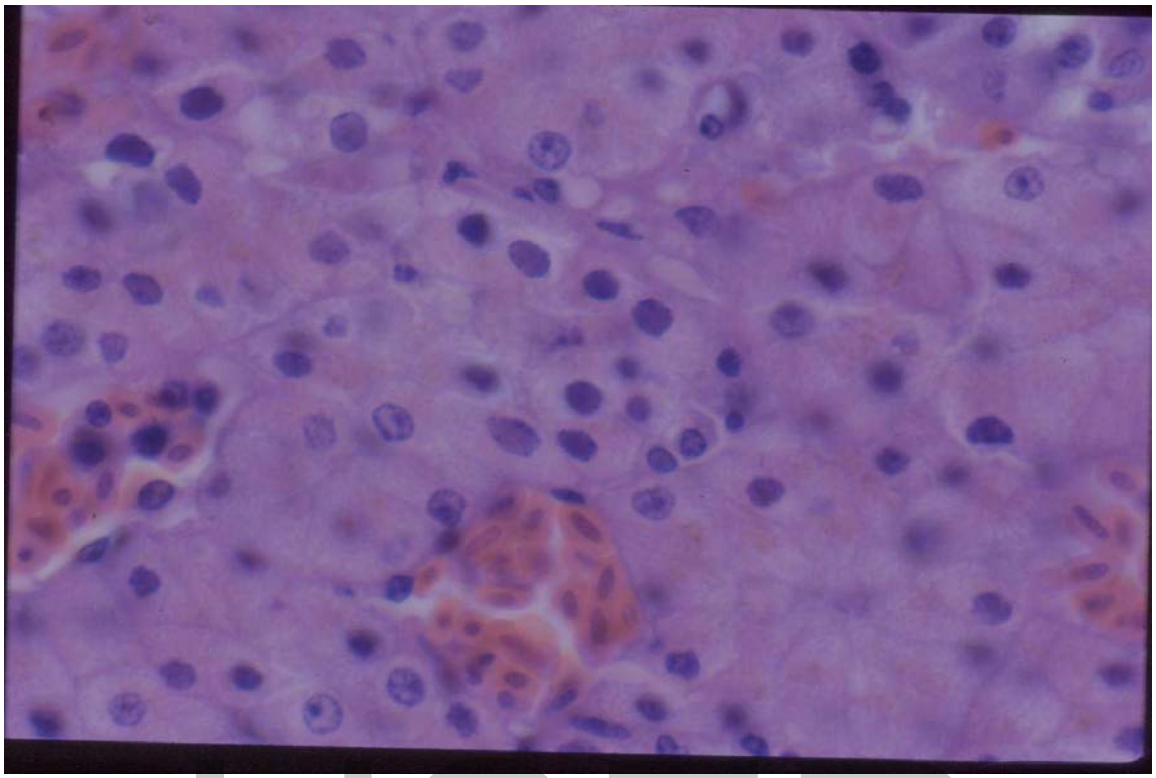

Fig.4g.13. Liver histology (H & E stain) from a gyr falcon showing perinuclear inclusions due to herpes infection.

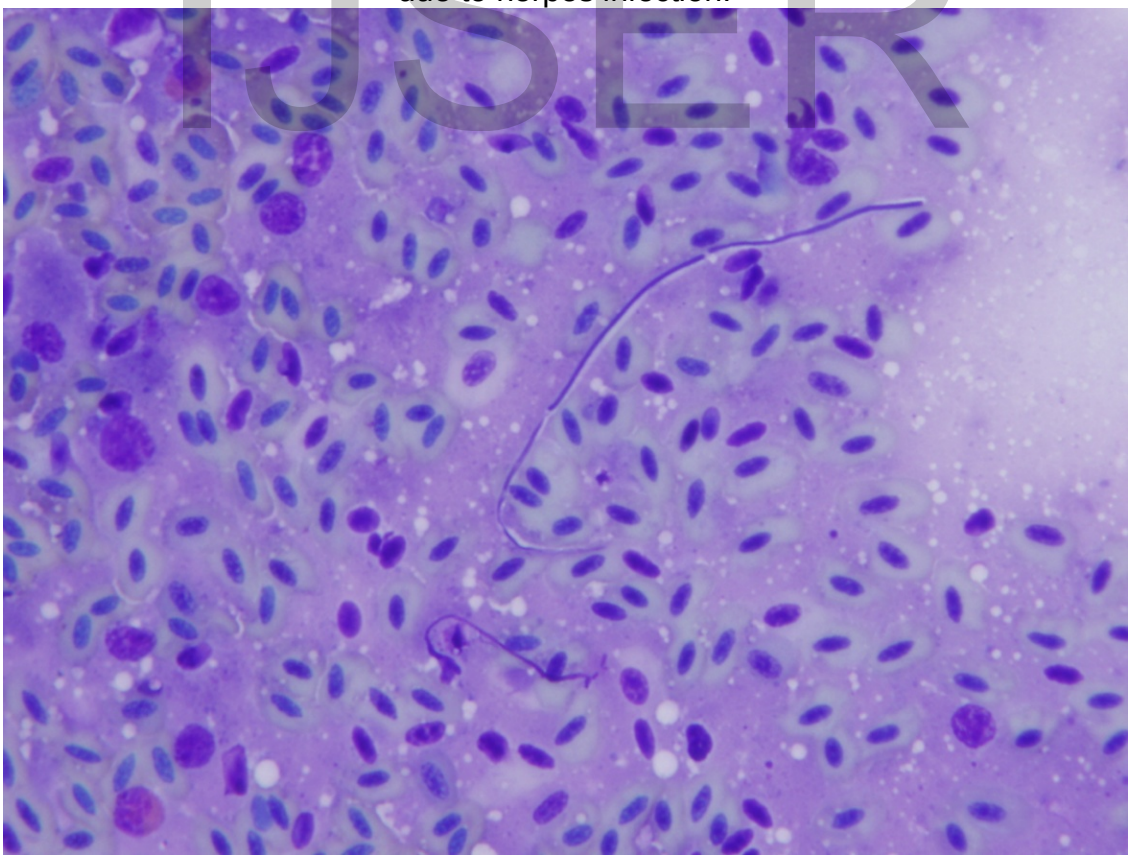

Fig.4g.14. Liver imprint smear from a gyr falcon showing bacterial rods (clostidiosis).

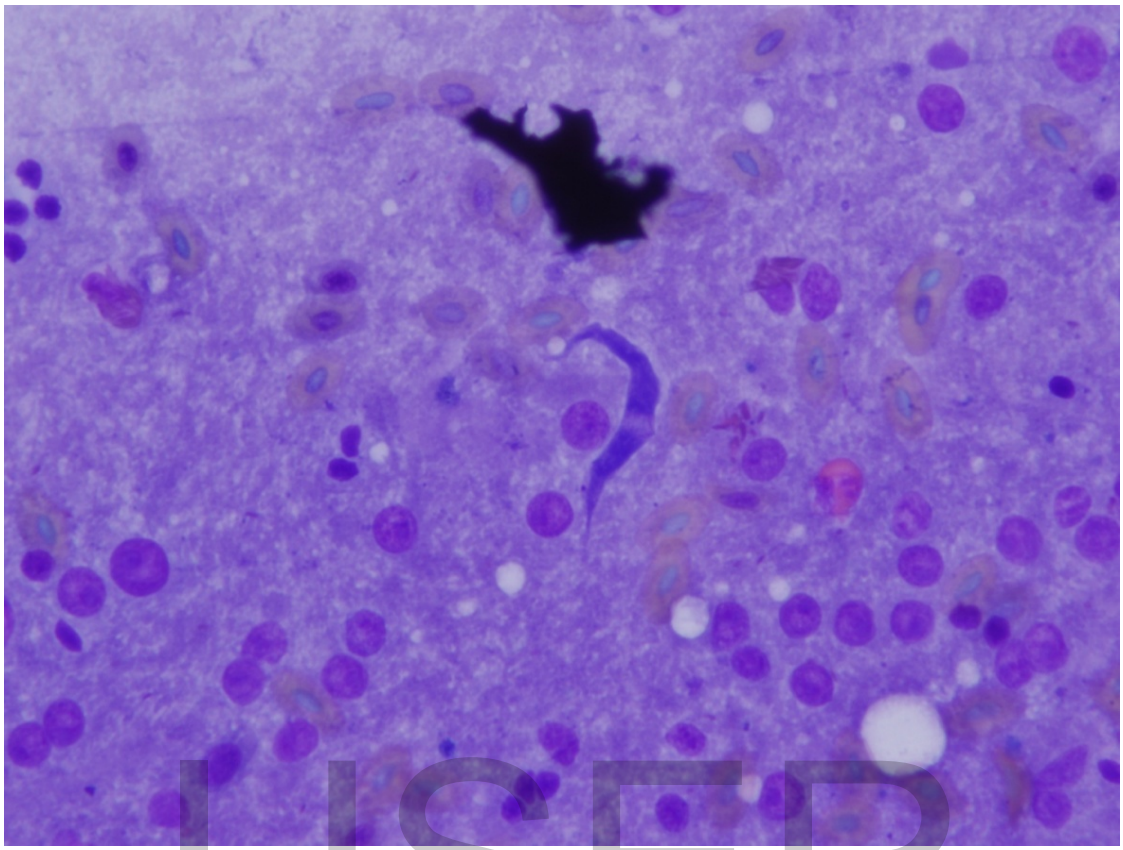

Fig.4g.15. Liver imprint smear from a gyr falcon showing Trypanosoama.

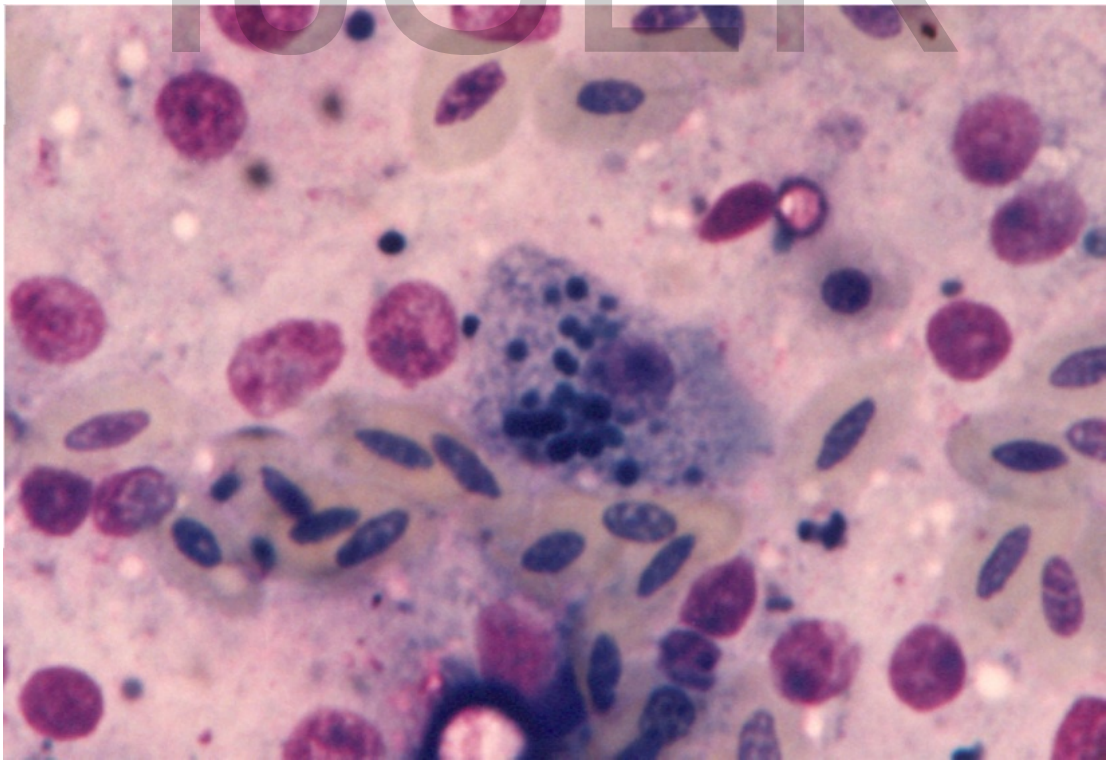

Fig.4g.16. Liver imprint smear from a gyr falcon showing hemosiderin pigments.

(4h) Cytology of Internal Organs – Spleen

The spleen is a blood-forming and blood-destroying organ. Therefore cytological imprints of normal spleens reveal a marked number of haemic cells. Many lymphocytes (predominantly small mature lymphocytes) are present since the spleen contains lymphoid tissue. Other cells including a few prolymphocytes, lymphoblasts and plasma cells may be seen in normal smears with less than 10% of the cell population of normal lymphoid tissue. The evaluation of the lymphoid elements in the spleen provides an indication of the overall status of the bird's immune system and lymphoid elements. Excessive iron storage within macrophages and erythrophagocytosis may be associated with haemolytic anemias. Systemic infections may be confirmed by the presence of the aetiologic agent in spleenic samples. Salmonella sp., Chlamydia sp. and herpes virus infections cause splenomegaly in falcons. A reactive spleen is characterised by a marked increase of plasma cells and mild to moderate increase in immature lymphocytes. Figures 4h.1 to 4h.7 show the normal and abnormal cytology of spleen.

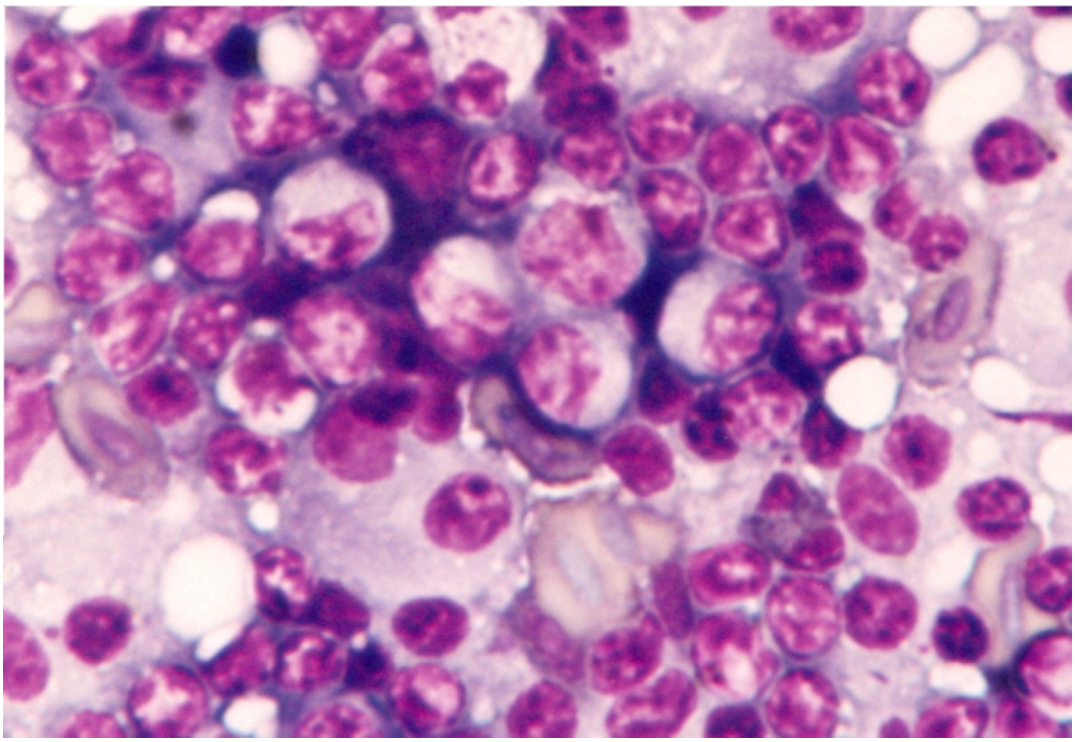

Fig.4h.1. Imprint cytology of spleen showing lymphoid cells.

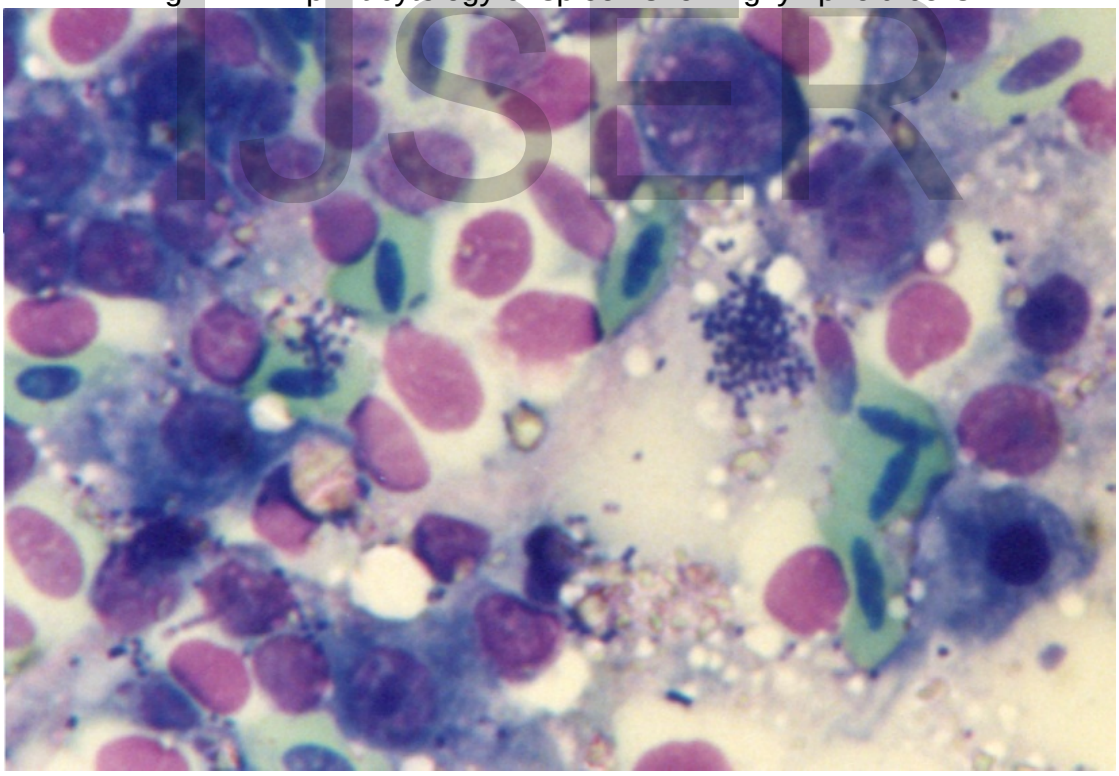

Fig.4h.2. Spleen imprint cytology of a gyr falcon showing bacterial septicemia.

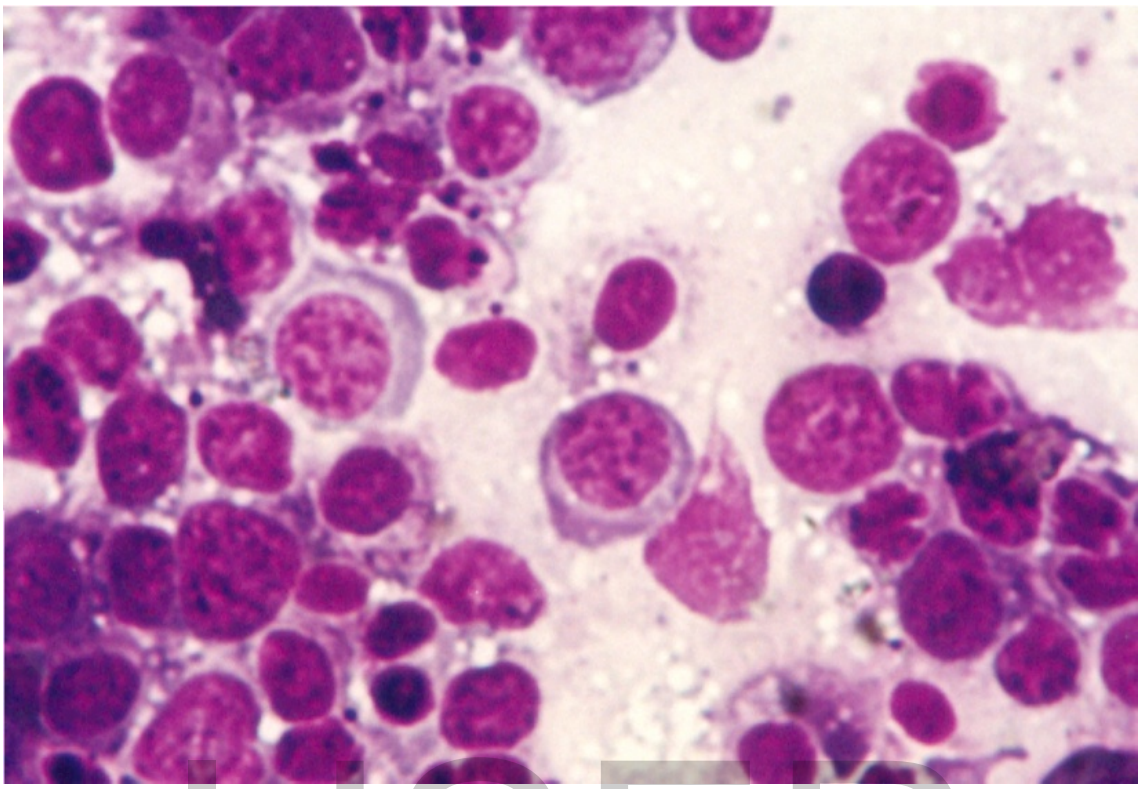

Fig.4h.3. Normal spleen imprints cytology of a houbara showing lymphoblasts.

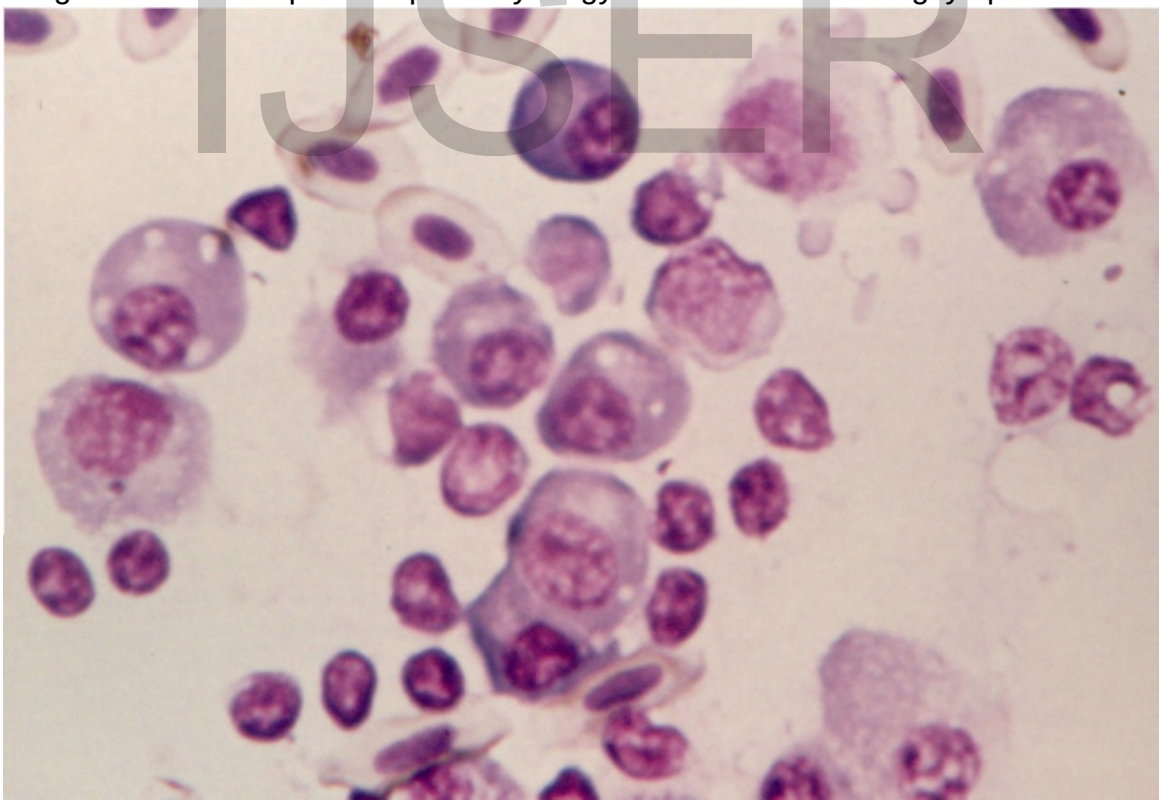

Fig.4h.4. Imprint spleen cytology of a of houbara bustard with many plasma cells showing reactive spleen.

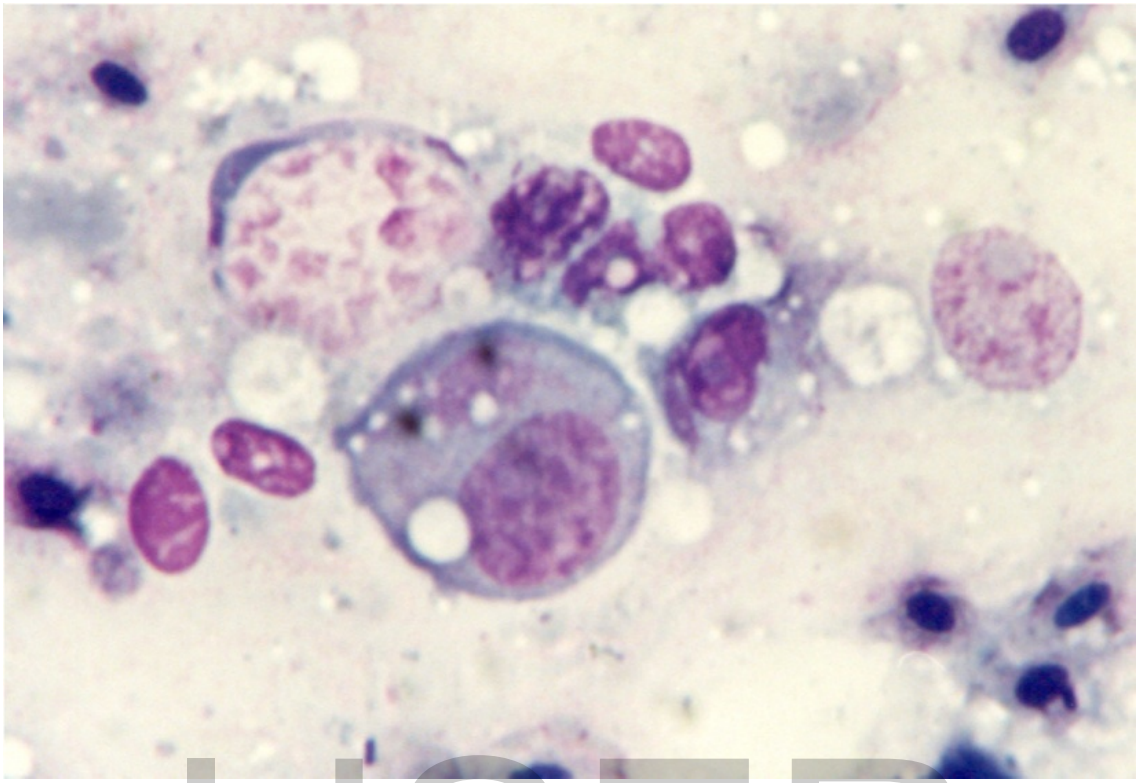

Fig.4h.5. Imprint spleen cytology of a of houbara bustard with septicemic pox showing plasma cells and karyorrhexis.

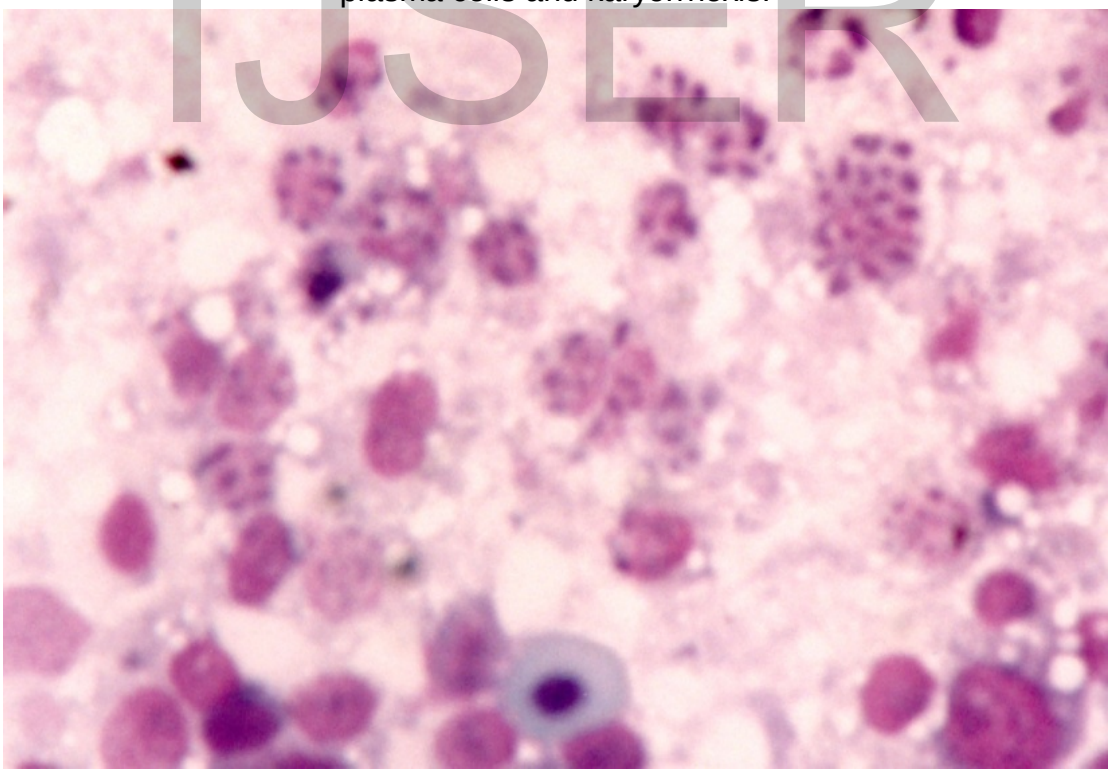

Fig.4h.6. Imprint spleen cytology of a of houbara bustard showing post-mortem changes.

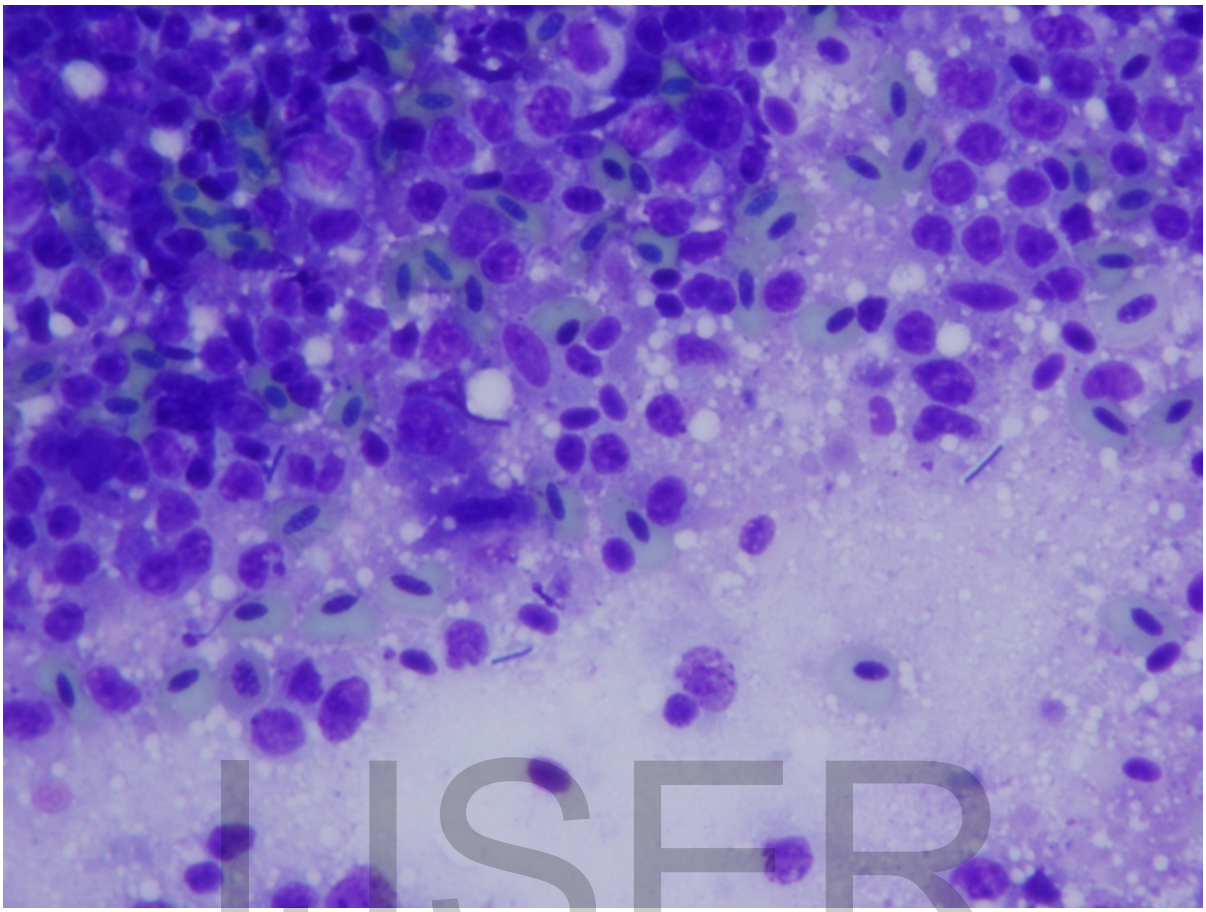

Fig.4h.7. Imprint spleen cytology of a of houbara bustard with with clostrdial septicemia.

(4i) Cytology of Internal Organs – Kidney

Normal cytology imprint smears from normal kidneys show primarily tubular epithelial cells. These cells have an abundant cytoplasm and round to oval slightly eccentric nucleus. Irregular swollen kidneys with white foci are seen in bustards and falcons; and are due to urate crystal deposition (renal gout). Cytology smears from such cases reveal urate crystals. In certain cases, iron pigments (haemosiderin) are seen in cells, indicating haemoglobin catabolism. Chlamydia sp., Clostridium perfringens, Streptococcus bovis and Salmonella sp. are the pathogenic bacteria isolated from the kidney of bustards. Irregular swollen kidneys with multifocal abscessation may be due to bacterial infection. Cytological smears showing bacteria should be differentiated from contaminants. Cytology smears from septicemic pox cases are characterised by vacuolated renal cells. Plasma cells, multinucleated giant cells are also often seen. Figures 4i.1 to 4i.7 show the normal and abnormal cytology of kidney.

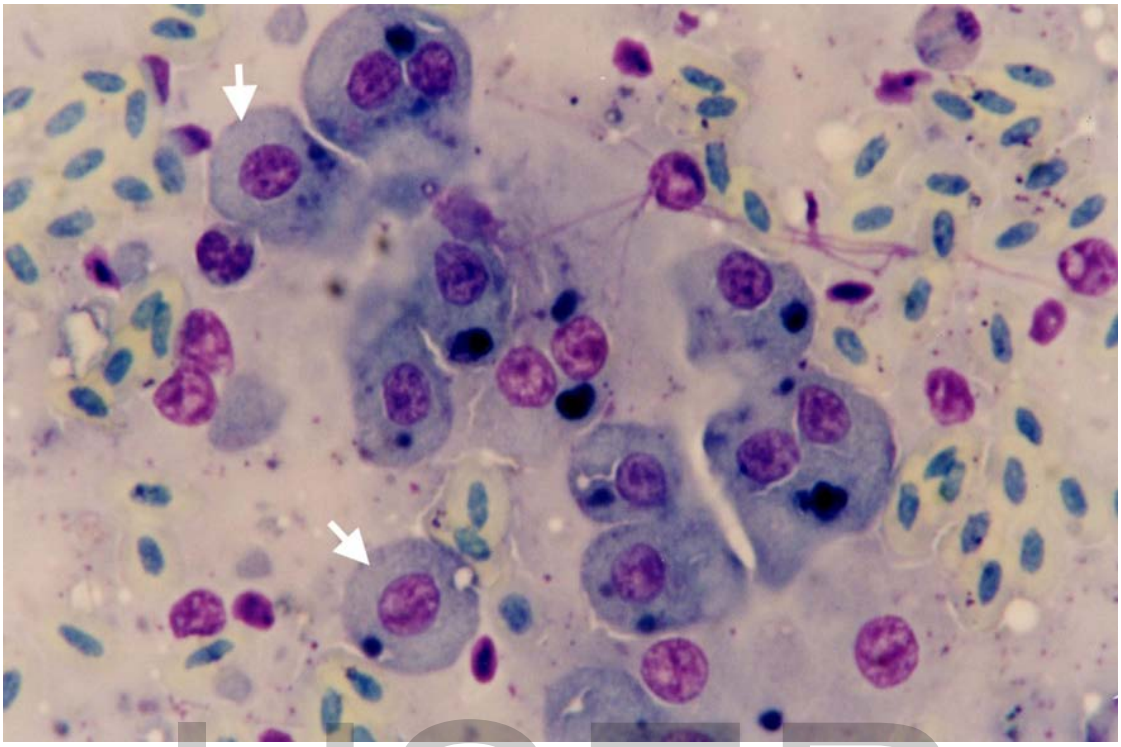

Fig.4i.1. Imprint cytology of kidney showing renal squamous cells.

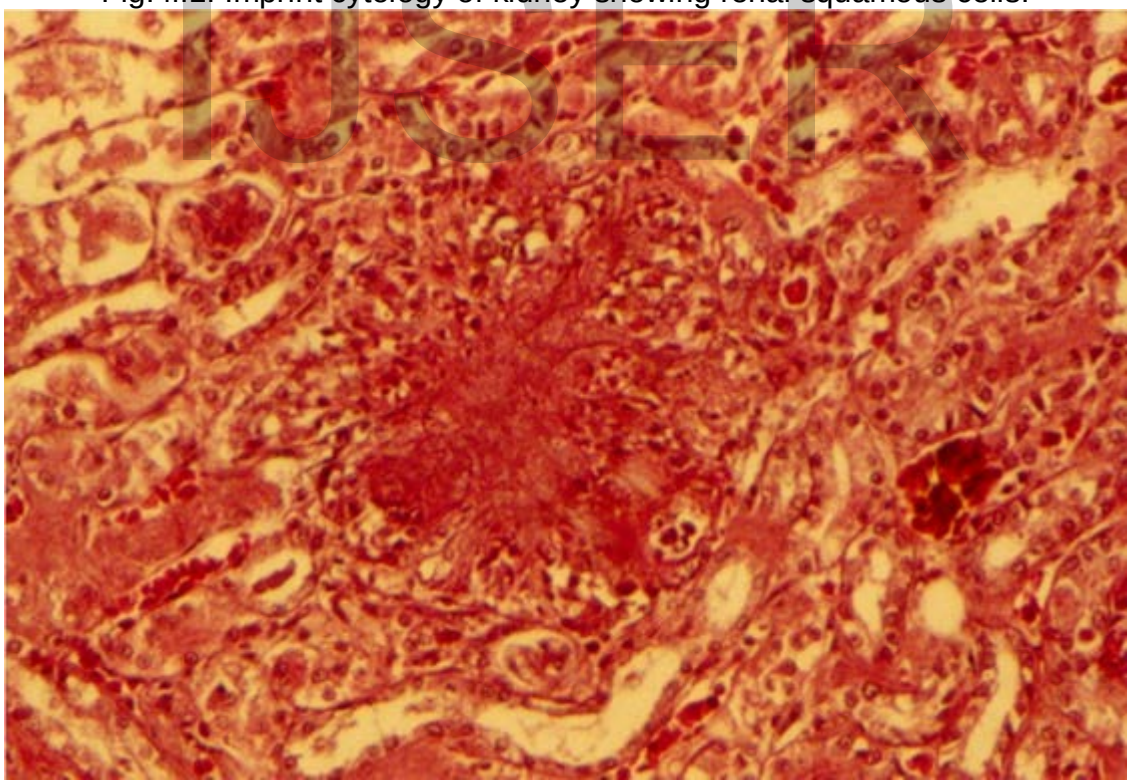

Fig.4i.2. Kidney imprints cytology of a houbara showing urate crystals.

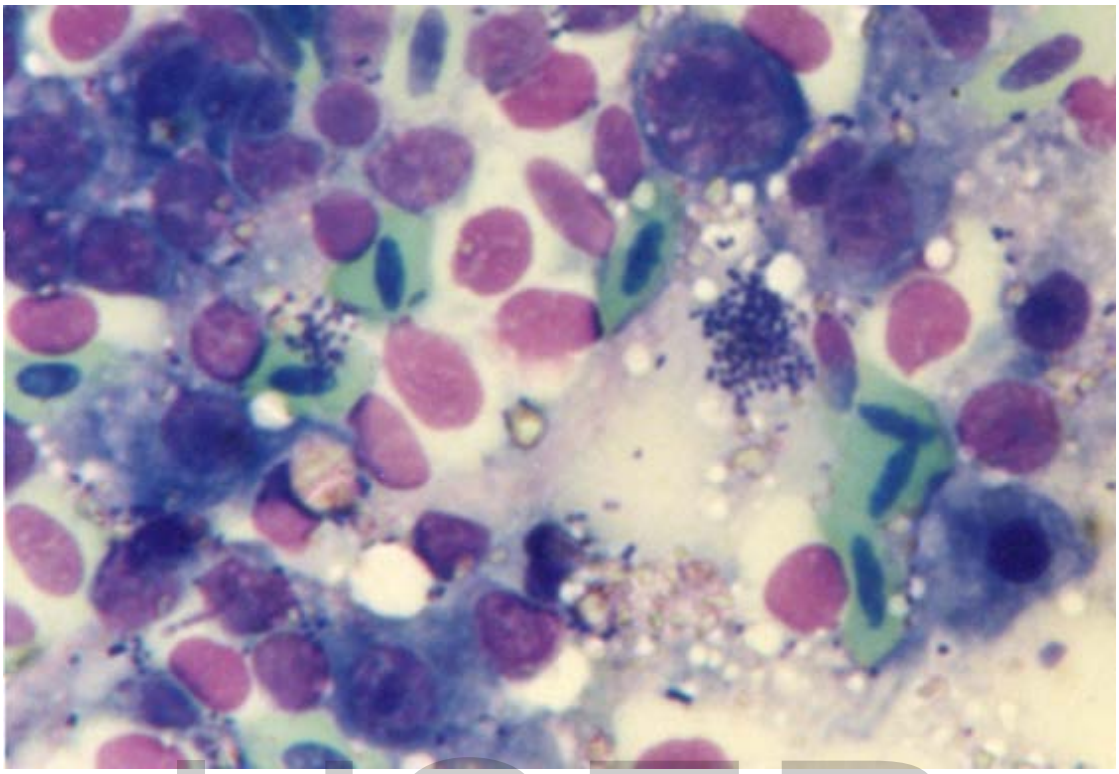

Fig.4i.3. Kidney Imprint cytology of a of houbara bustard with bacterial septicemia.

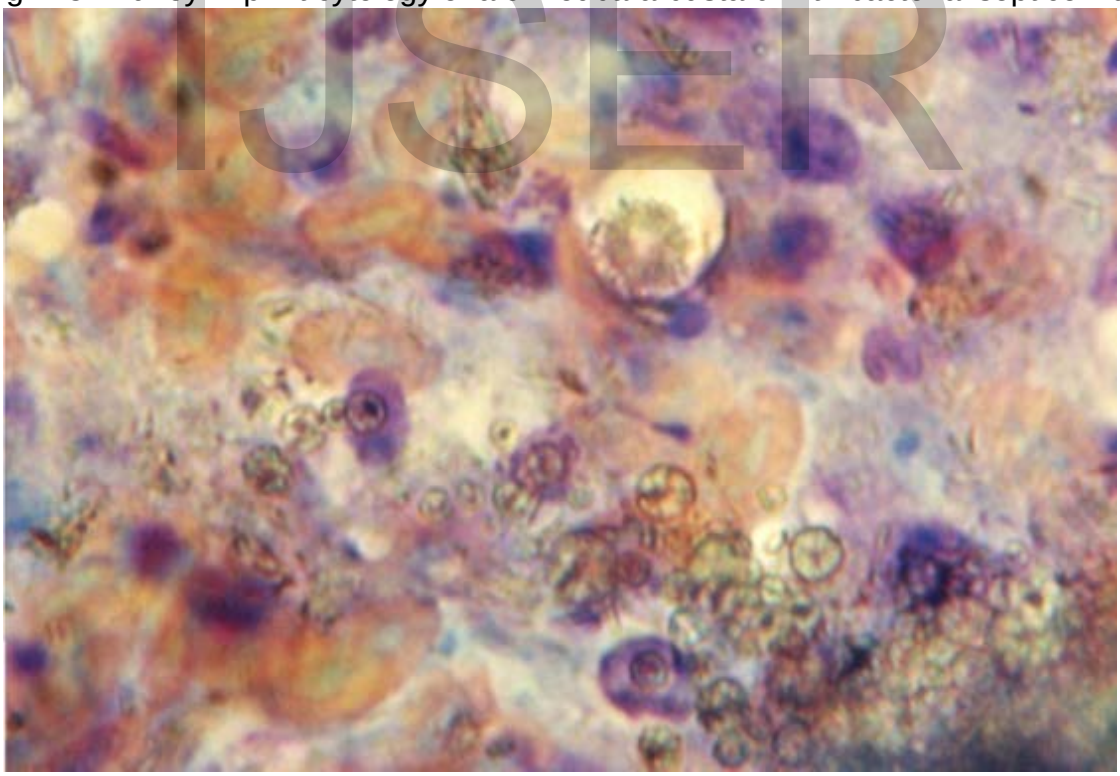

Fig.4i.4. Kidney Imprint cytology of a peregrine falcon showing urate crystals.

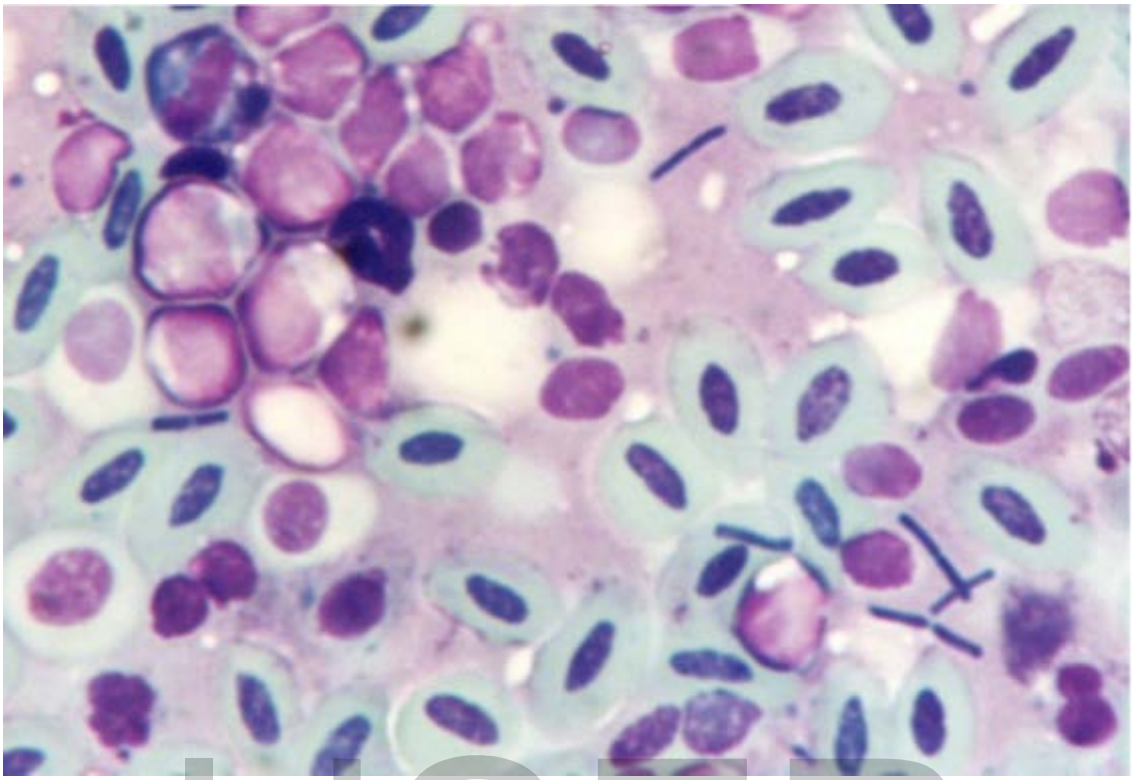

Fig.4i.5. Kidney imprint cytology of a of houbara bustard with with clostrdial septicemia.

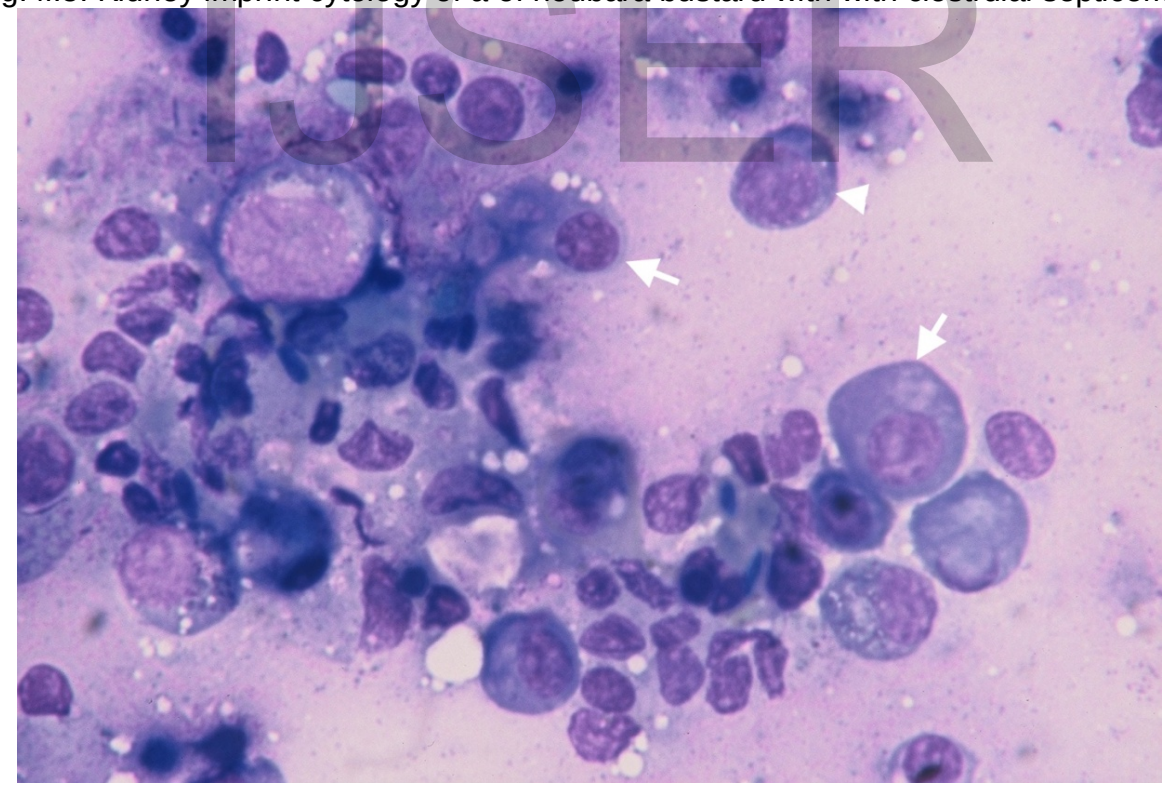

Fig.4i.6. Kidney imprint cytology of a of houbara bustard with avian leucosis.

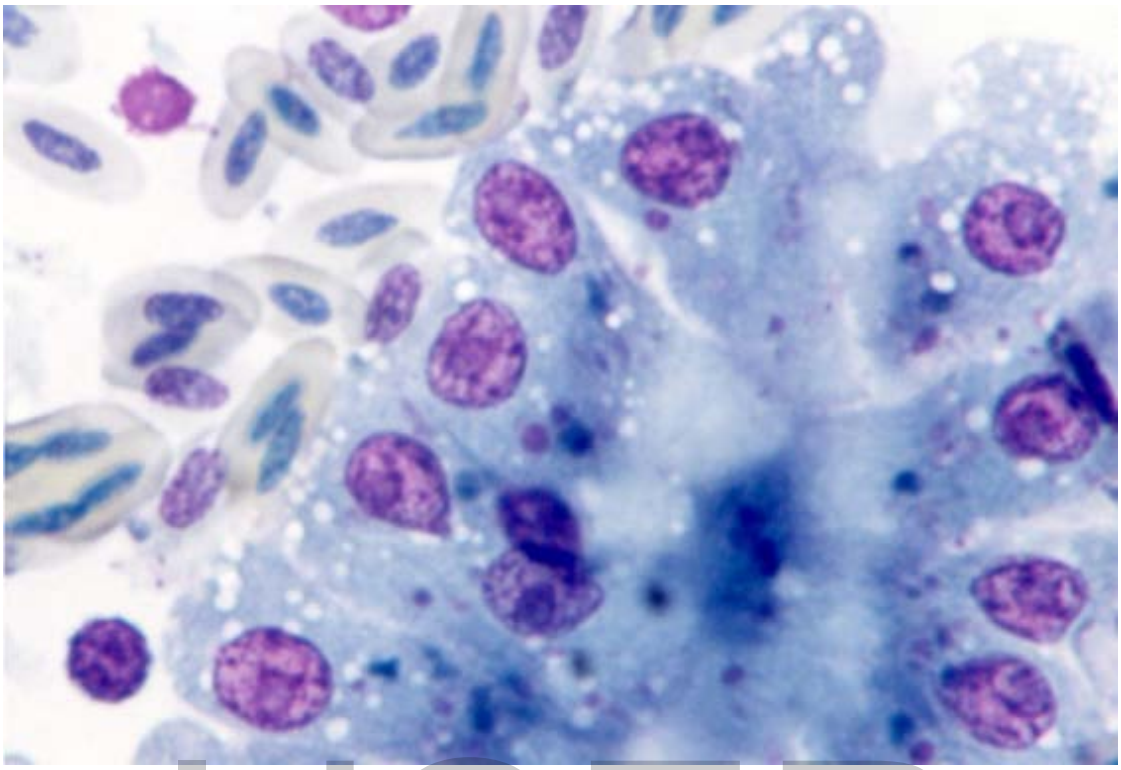

Fig.4.i.7. Kidney imprint cytology of a of houbara bustard with septicemic pox.

(4j) Cytology of Internal Organs – Heart

Normal impression smear from the heart shows primarily blood cells and it is valuable in dead birds to screen samples for blood parasites and to assess the level of parasitemia. Accumulation of pericardial exudates (pericarditis) is due to cardiac inflammatory response and systemic disease that could be due to various infectious agents. *Clostridium perfringens* and *Streptococcus bovis* have been isolated from the pericardial fluid. Non-infectious pericarditis is seen due to the deposition of urates called gout Cytology smears from such lesions are characterised by urate crystals. Transudates can occur with heart failure and hypoproteinemia. Inflammatory cells are indicative of pericarditis. *Paronchocerca filariae* have occasionally been seen in the cardiac arteries of bustards. Figures 4j.1 to 4j.5 show the normal and abnormal cytology of heart.

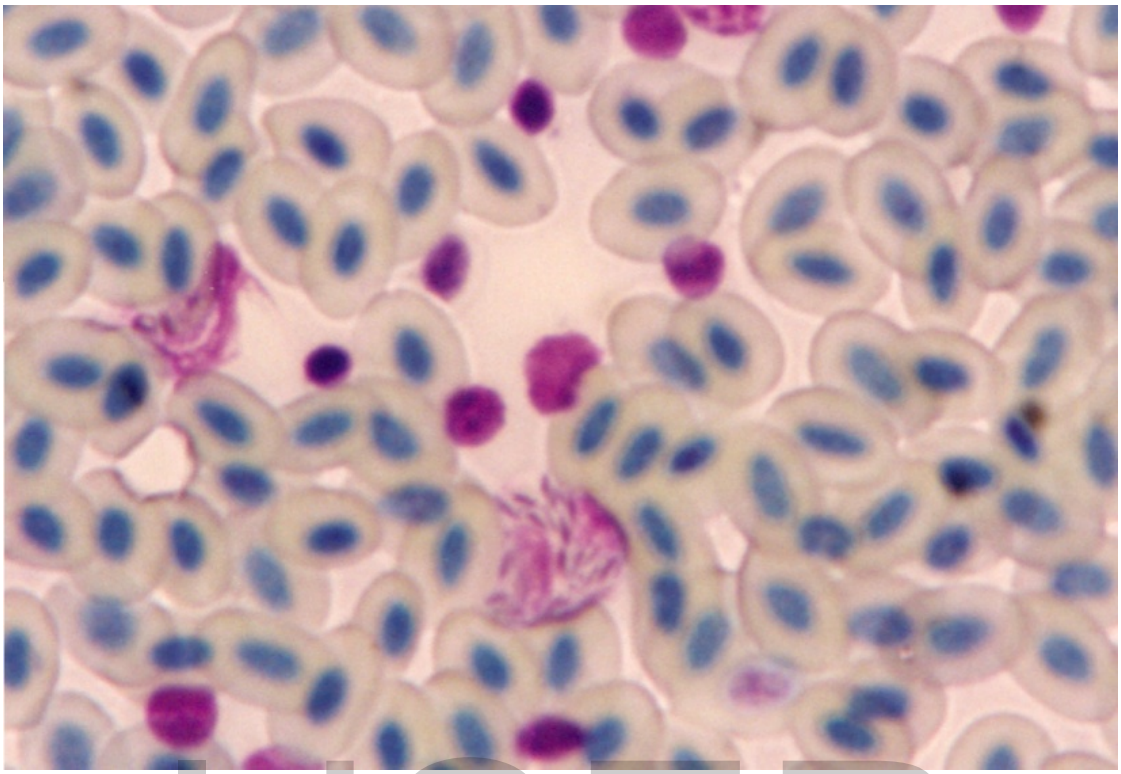

Fig.4j.1. Normal heart imprint cytology of a facon showing blood cells.

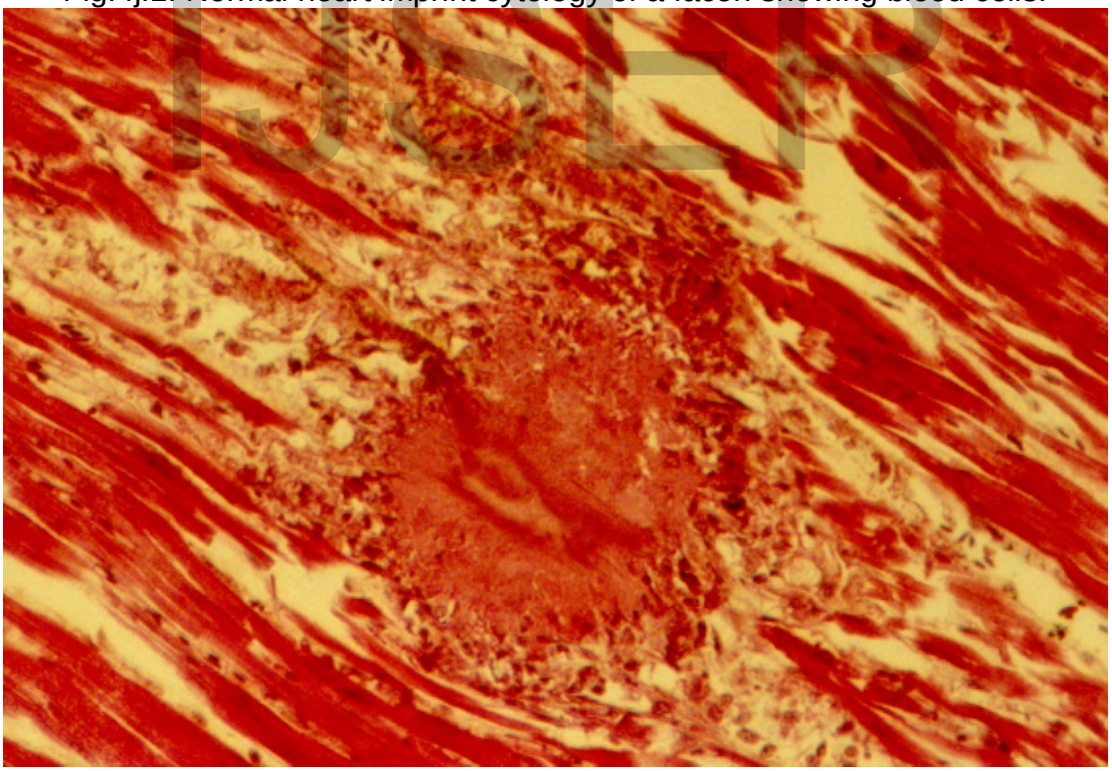

Fig.4j.2. Histology of heart showing gout crystals.

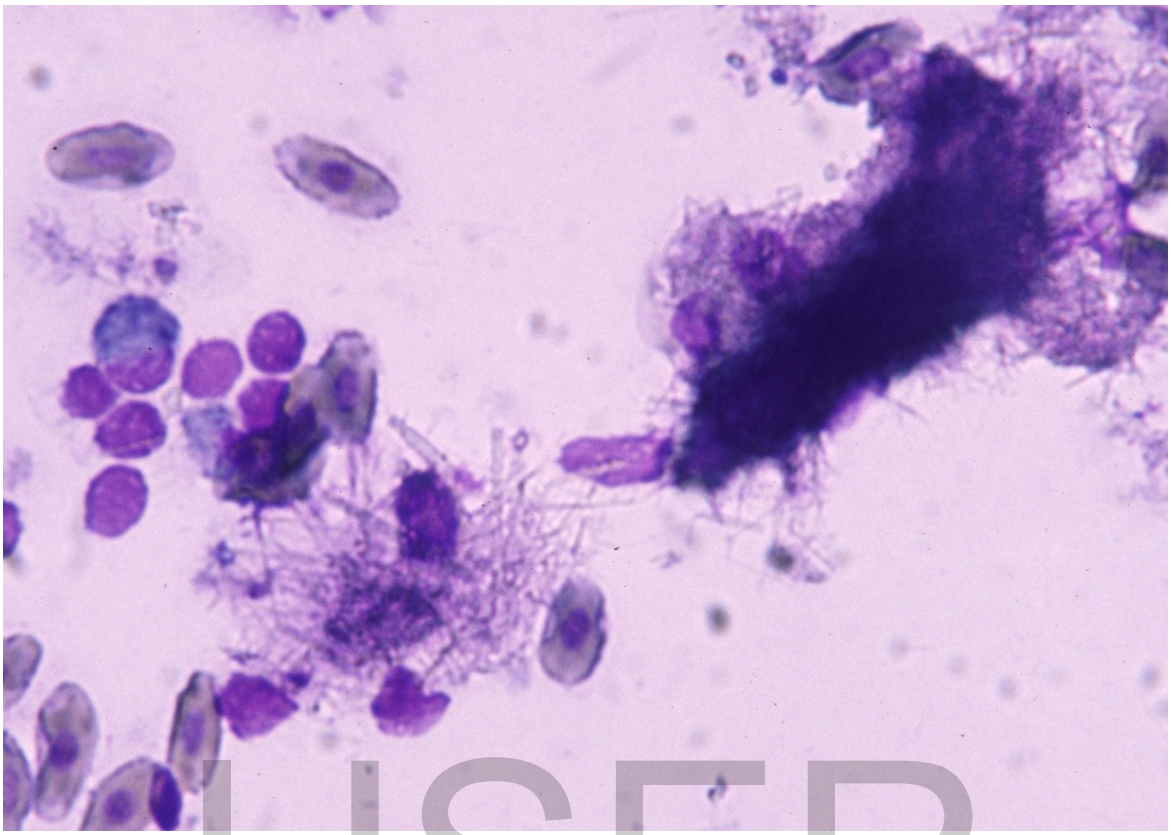

Fig.4j.3. Imprint cytology of pericardium showing urate crystals.

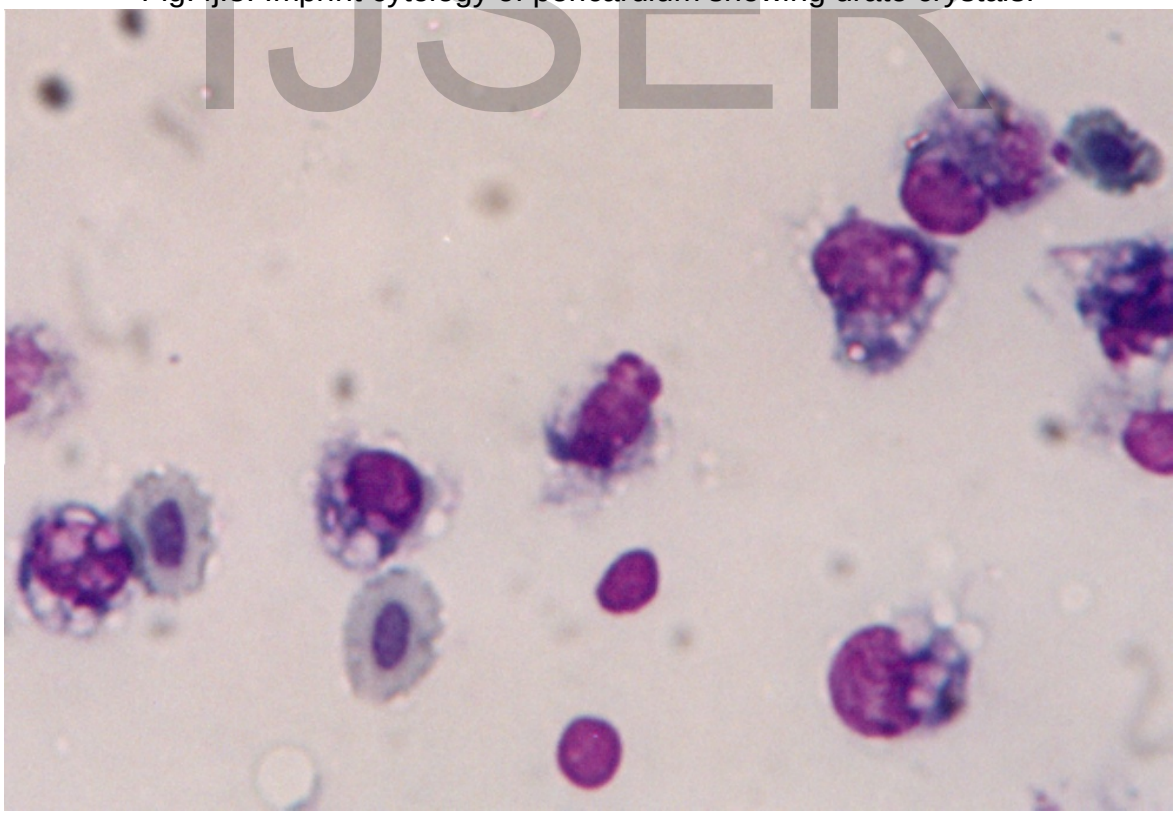

Fig.4j.4. Imprint cytology of pericardium showing macrophages due to pericariditis.

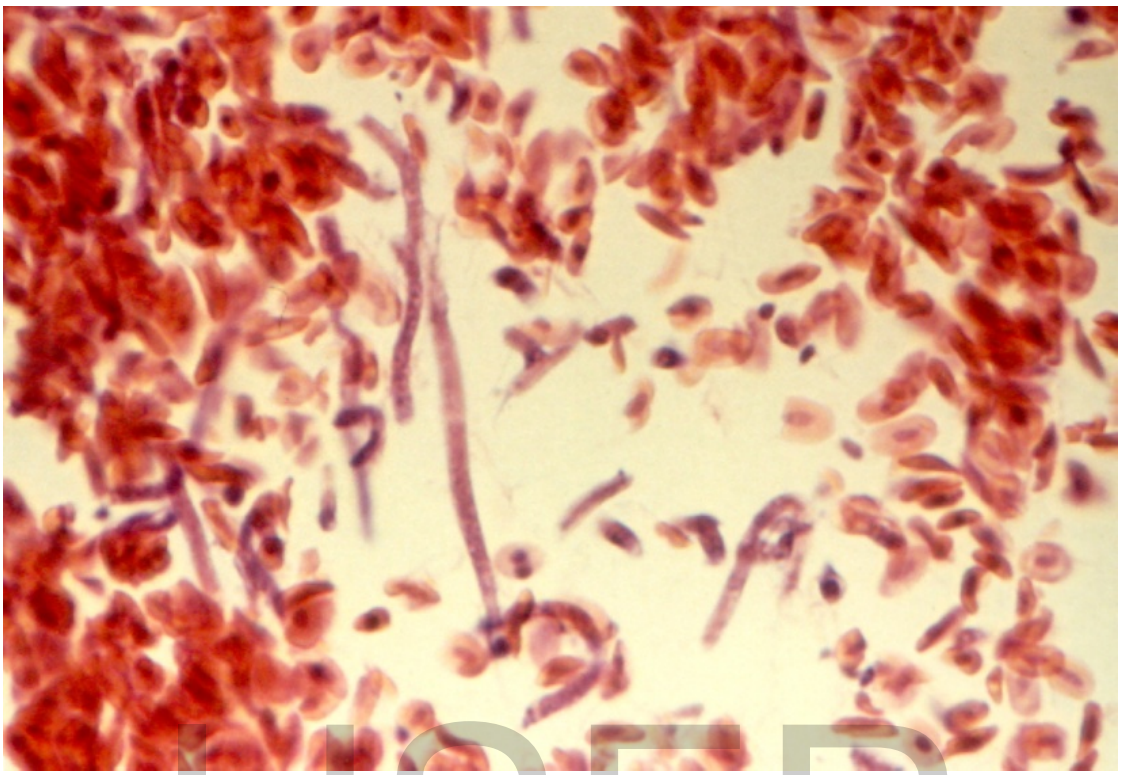

Fig.4j.5. Histology of houbara bustard showing filarial worms.

(4k) Cytology of Skin and Subcutis

The skin of bird is composed of keratinized, stratified squamous epithelium. The normal cytology findings from skin include anucleated, cornified squamous epithelial cells, nucleated cornified squamous cells and variable amount of debris. Lesions caused by bacterial infections are represented by large number of inflammatory cells with bacterial phagocytosis and cellular debris. Non- pathogenic bacteria are often associated with the surface of squamous epithelial cells. Bumblefoot lesions are very common in falcons and Gram stain helps to provide more information about bacterial morphology. Avipox viruses causes cutaneous forms of lesions that appear as scab-like lesions around the eyes, beak, nares, hock joint, tarsometatarsus and feet. Cytology findings of pox lesions show swollen squamous epithelial cells with one or more large cytoplasmic vacuoles that push the cell nucleus to the margin of the cell. The large cytoplasmic vacuoles are

called Bollinger bodies and contain tiny, round, pale eosinophilic inclusions that are called Borrel bodies. Skin scraping smears may contain whole ectoparasites and eggs of lice, mites and ticks. Figures 4k.1 to 4k.12 show the normal and abnormal cytology of skin and ectoparasites.

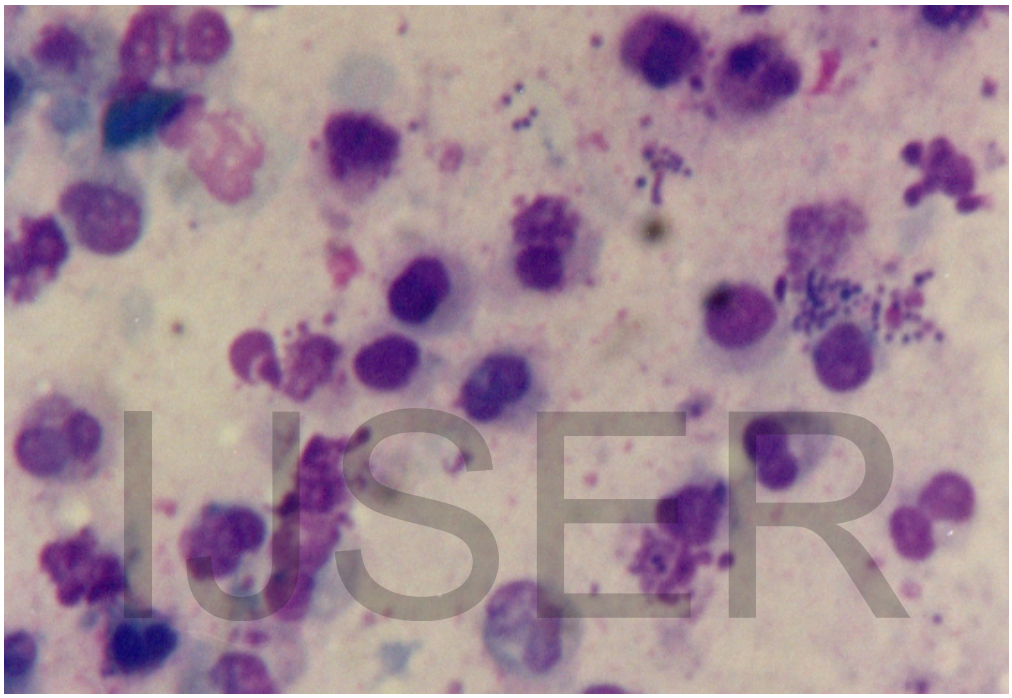

Fig.4k.1. Cytology from the abscess of a houbara bustard showing bacteria and inflammatory cells.

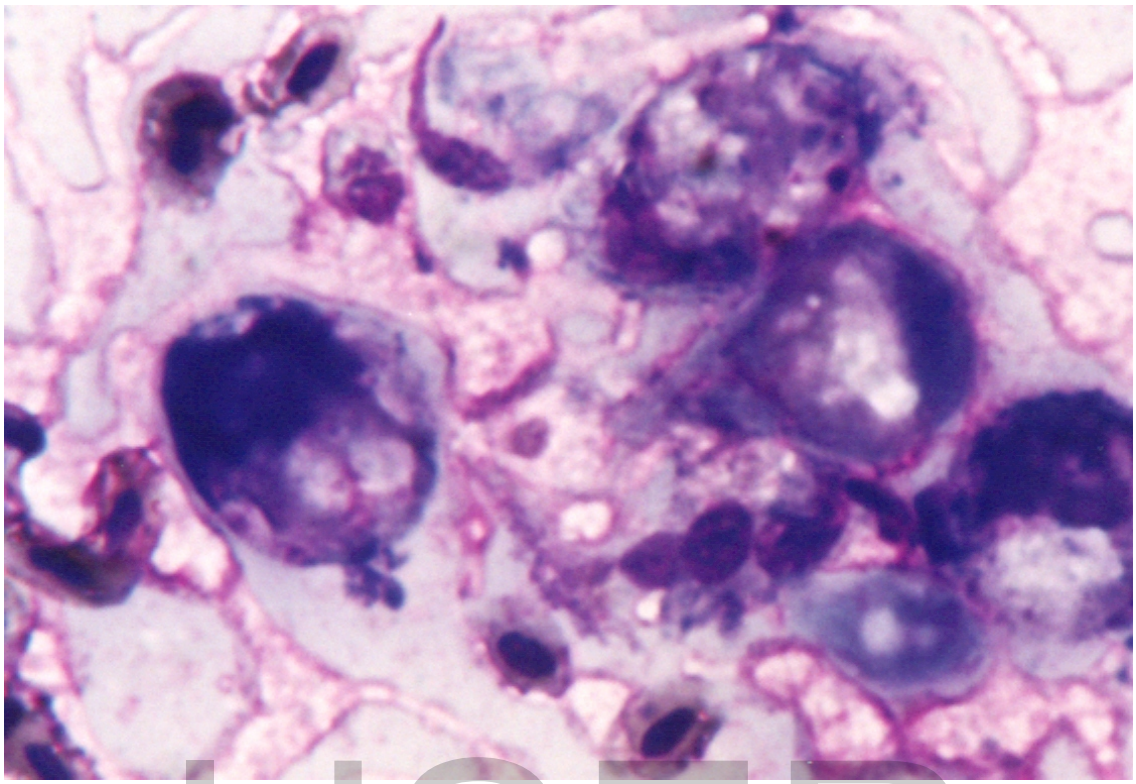

Fig.4k.2. Cytology from the pox lesion of a houbara bustard showing changes in the squamous cells.

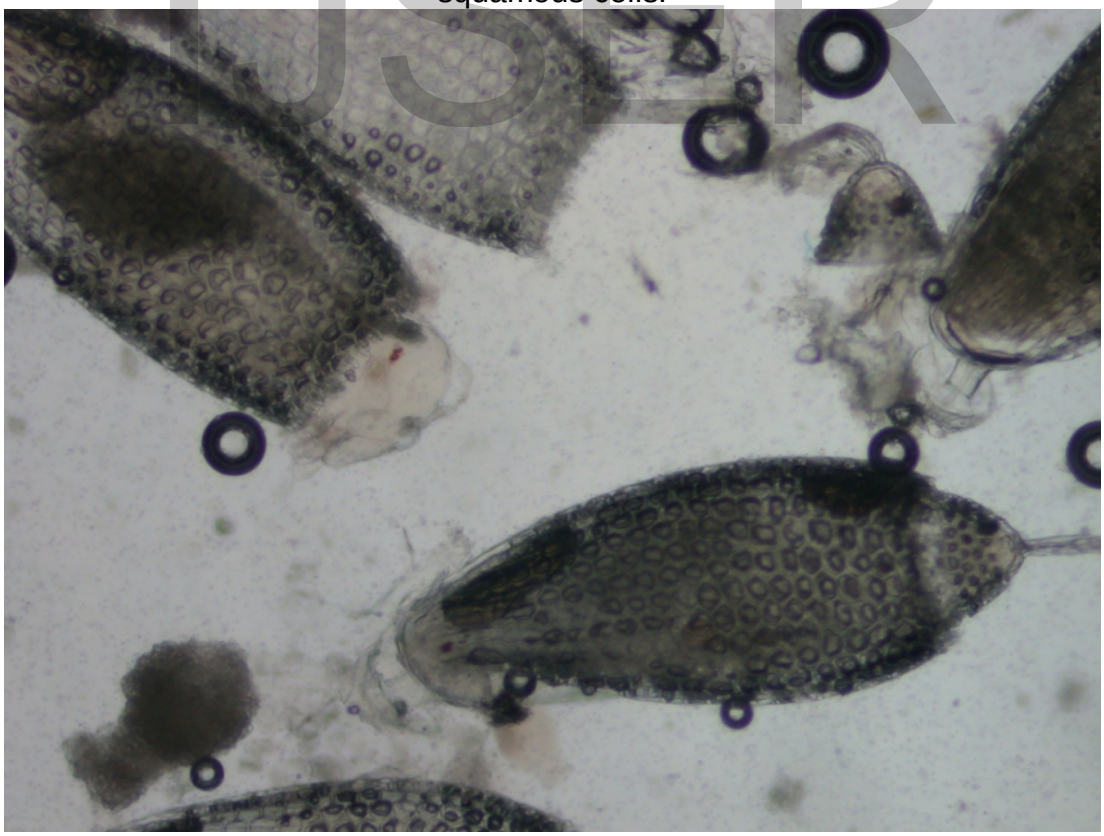

Fig.4k.3. Skin scrapping from a falcon showing hatching mite eggs.

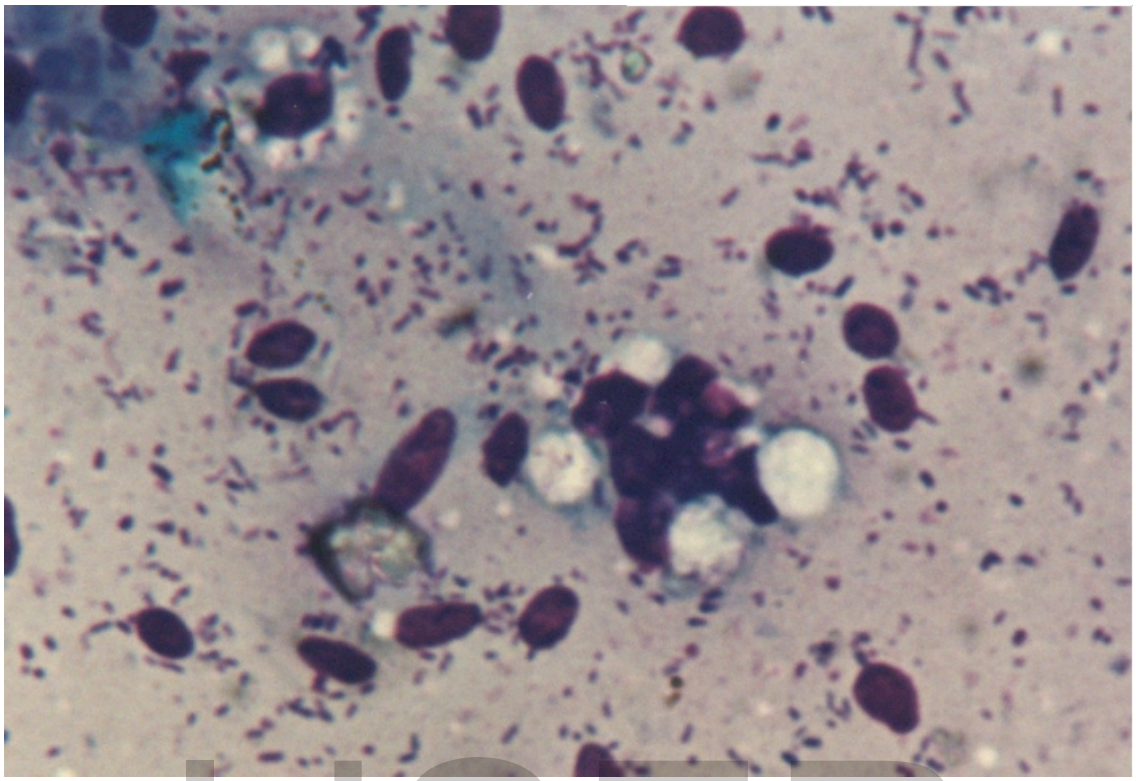

Fig.4k.4. Pox lesion from a houbara bustard showing secondary bacterial infection.

Fig.4k.5. *Degeeriella rufa* (louse) from a saker falcon.

Fig.4k.6. *Colpocephalum zerafae* (louse) from a saker falcon.

Fig.4k.7. *Otidoecus houbarae* (louse) from a houbara bustard.

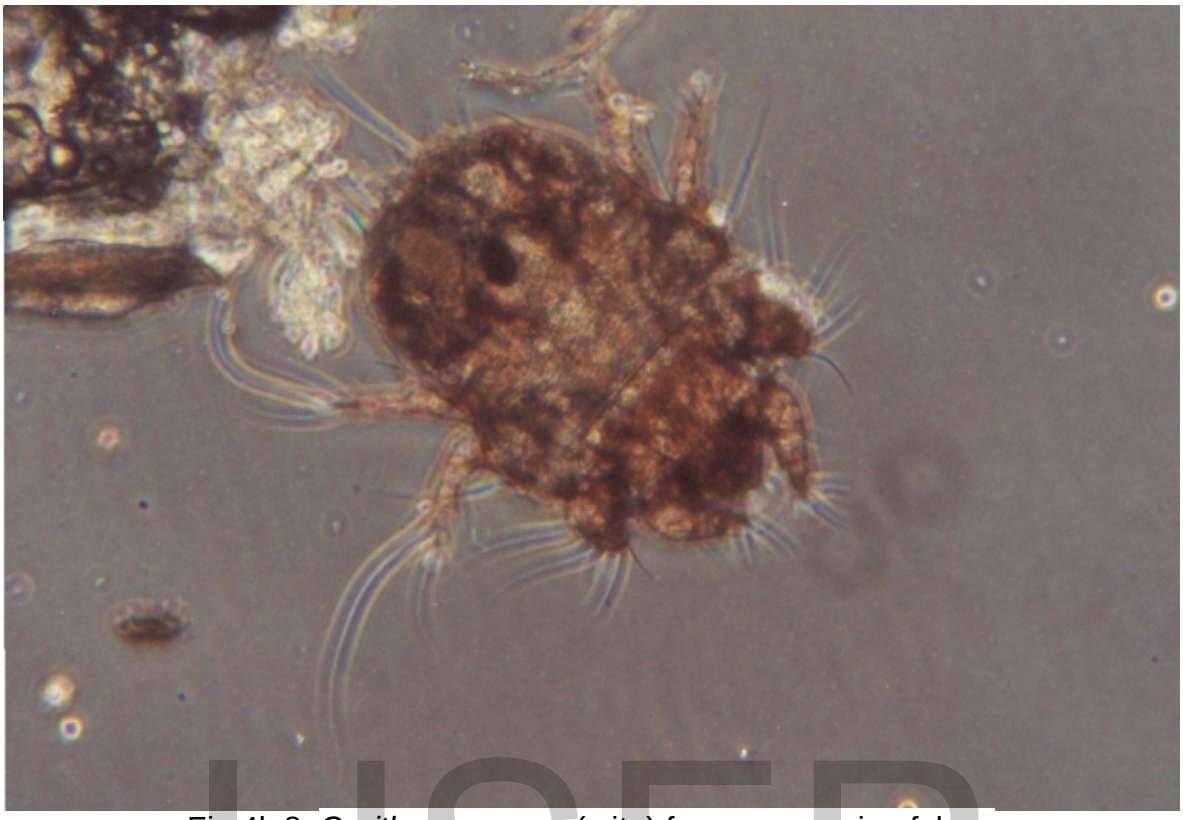

Fig.4k.8. *Ornithonyssus* sp (mite) from a peregrine falcon

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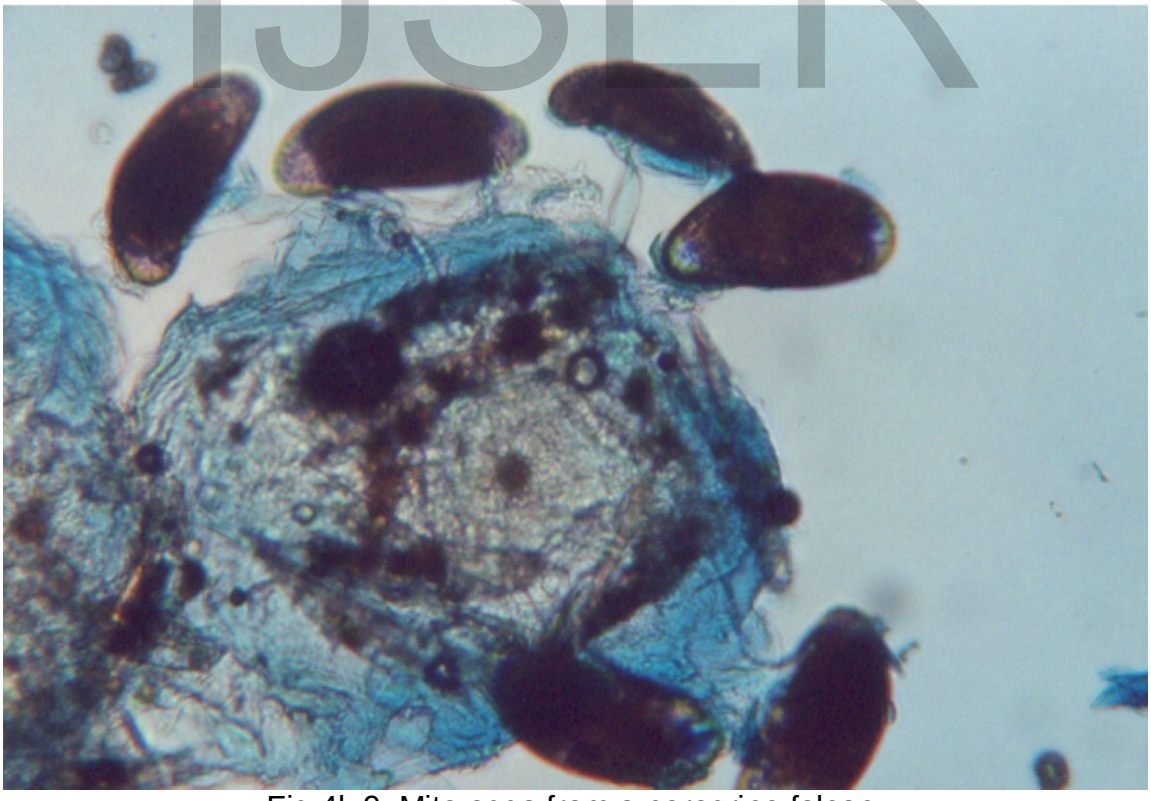


Fig.4k.9. Mite eggs from a peregrine falcon.

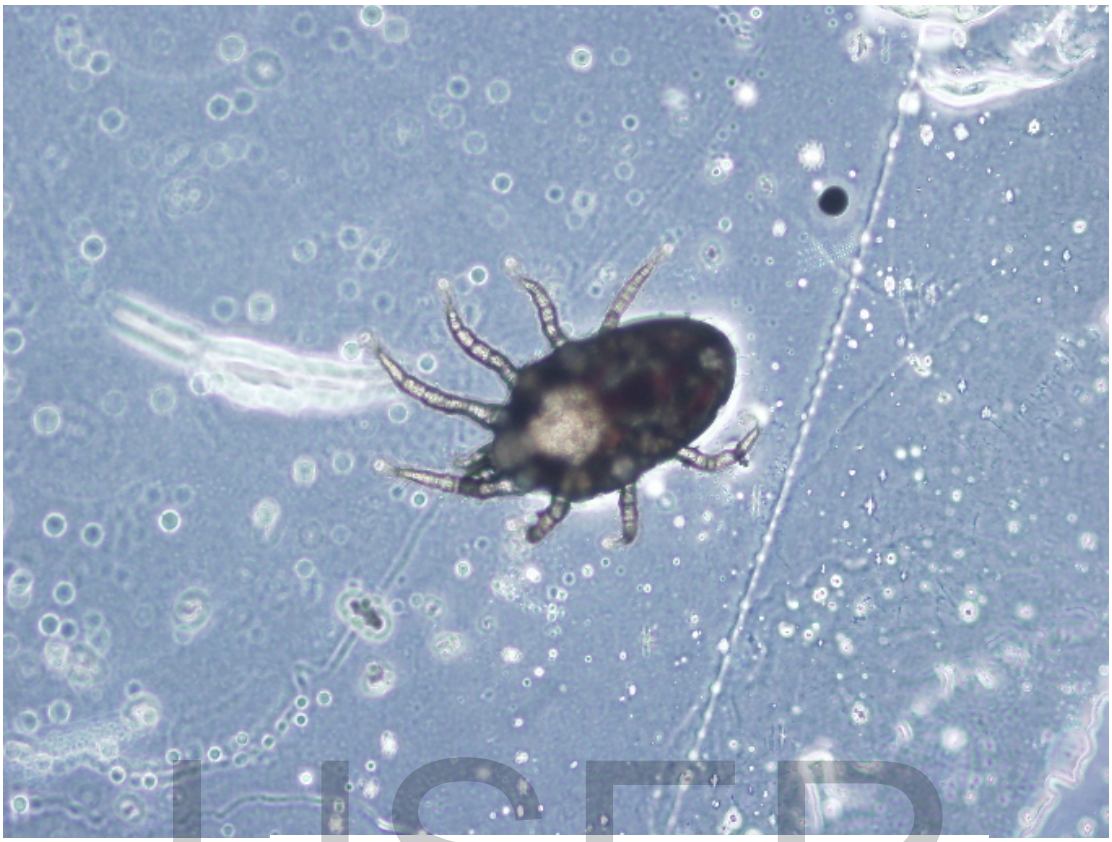


Fig 4k.10: *Amblyoma* sp (tick) nymph from a houbara bustard

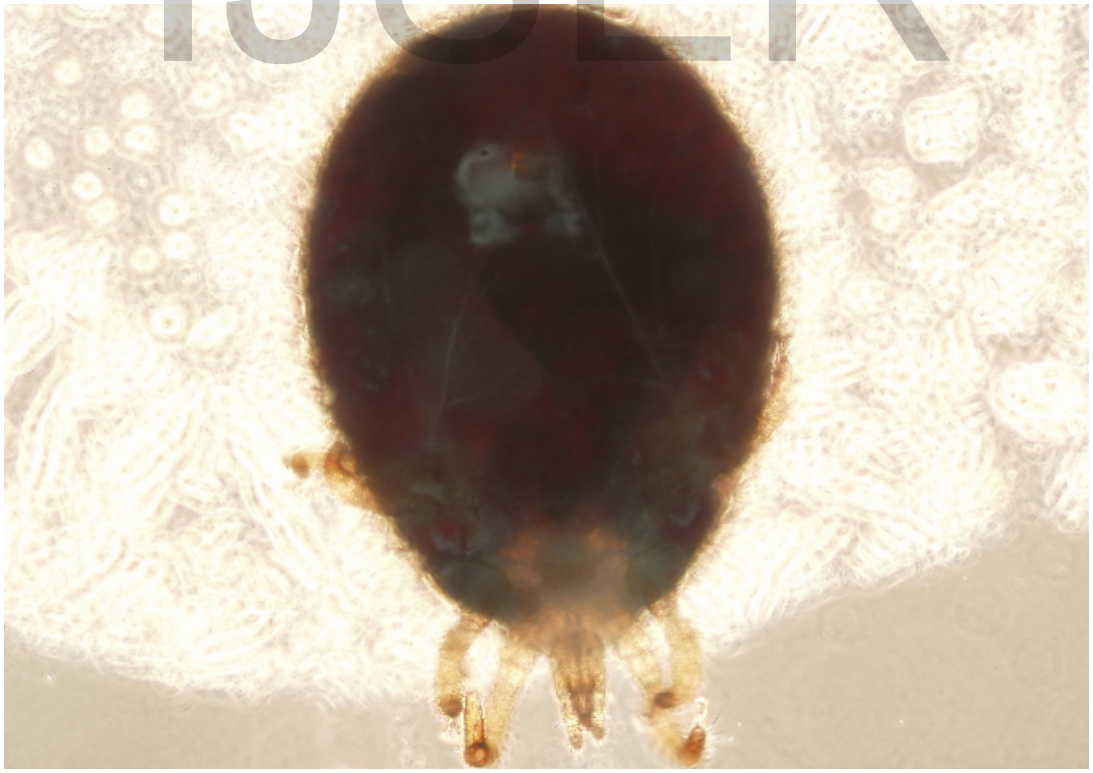


Fig 4k.11: *Hyalomma marginatum* (tick) nymph from a Gyr falcon

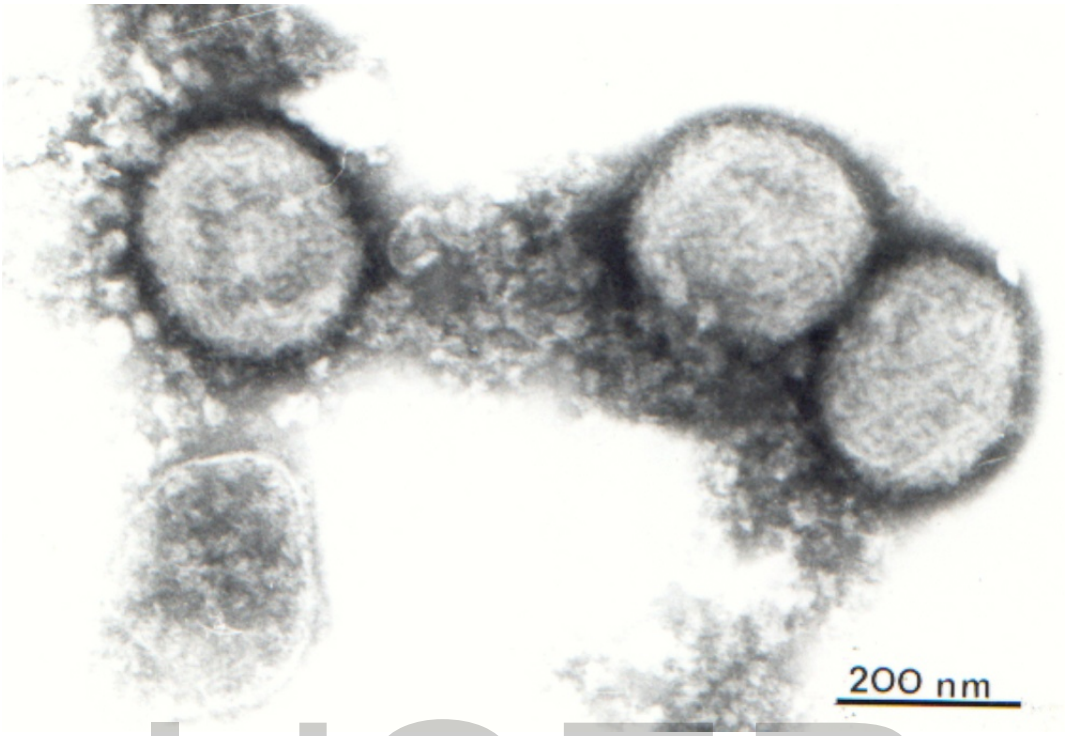


Fig 4k.12: Avian pox virus under electron microscope.

(4l) Hematology of birds

The cellular components of avian blood include erythrocytes (red blood cells), leucocytes (white blood cells) and thrombocytes. Avian erythrocytes are oval and nucleated. Leucocytes include agranulocytes and granulocytes. Agranulocytes include lymphocytes and monocytes which do not have cytoplasmic granules. Granulocytes include heterophils, basophils and eosinophils that contain cytoplasmic granules. Normal stained blood smears from birds show normocytic and normochromic mature erythrocytes.

Erythrocytes: Normocytic erythrocytes from bustards and falcons are oval in shape with relatively large central nuclei, which contains tightly packed coarse chromatin. Normochromic erythrocytes have yellowish-pink cytoplasm in Romonowsky stained smears. Polychromatic erythrocytes are frequently seen in blood smears of falcons and bustards. The cytoplasm of polychromatic erythrocytes is more basophilic than that of

mature erythrocytes. Normal blood smears from healthy birds often show less than 5% polychromatic erythrocytes. Increased numbers of polychromatic erythrocytes are due to erythrocyte regeneration. Anucleated erythrocytes are called erythroplastids, are occasionaly seen normal peripheral blood smears.

Heterophils: Normal heterophils of bustards and falcons are relatively large round cells with a colourless cytoplasm with slightly eosinophilic (greyish- pink), rod (spindle) shaped cytoplasmic granules and deep purplish bi-lobed nucleus (Fig 2). Band form and non-lobulated heterophils are seen occasionally in normal blood smears. The presence of more than 10% non-lobulated and band form heterophils in a blood smear is considered abnormal.

Eosinophils: Normal eosinophils of bustards and falcons are large round cells, containing a bi-lobed nucleus and a slightly basophilic cytoplasm with round, eosinophilic granules. Cytoplasmic granules of eosinophils have different morphology in each species. The saker falcon contains eosinophils with a bi-lobed nucleus and basophilic cytoplasm containing eosinophilic small, round cytoplasmic granules. Kori bustard eosinophils are 9 - 15μm in size with bi-lobed nucleus and basophilic cytoplasm containing distinct bright-red granules.

Lymphocytes: Normal lymphocytes of bustards and falcons are the smallest of the white cells. They are round cells with a small portion of pale blue cytoplasm containing a very large nucleus. Small lymphocytes (6 - 9 μm) and large lymphocytes (9 – 13 μm) are distinct in kori bustards.

Monocytes: The monocytes of bustards and falcons are the largest white cells, slightly rounded, but very often irregular in shape with a clear lace pattern like structure in the cytoplasm containing a large irregular kidney shaped nucleus. The monocytes of kori bustards are larger than those described for houbara bustards.

Basophils: Normal basophils are round cells with purplish cytoplasmic granules that usually overlap the nucleus. Thrombocytes: Normal thrombocytes are small cells with an irregular clear cytoplasm and purple-red nucleus with dense chromatin clumps. To perform a differential leucocyte count, one hundred white cells are classified as heterophils, eosinophils, basophils, lymphocytes and monocytes on a thin blood film stained with MGG stain. Table 4l.1 shows the normal proportion of leucocytes in peripheral blood and Table 4l.2 shows abnormal hematology associated with various pathological conditions. Figures 4l.1 to 4l.14 show normal and abnormal hematology of birds.

Table 4l.1. Normal proportions of leucocytes in the peripheral blood of adult bustards and falcons.

Species	Heterophils	Lymphocytes	Monocytes	Eosinophils	Basophils
Saker falcon	52 – 70%	16 – 34%	1 – 7%	0 – 3 %	0 – 3%
Peregrine falcon	46 – 66%	20 – 38 %	1 – 5%	1 - 4%	0 – 3%
Gyr falcon	48 – 68 %	18 – 40%	0 – 4%	0 – 3%	0 – 2%
Houbara bustard	53 – 73%	22 – 41%	0 – 9%	0 – 5%	0 – 4%
Kori bustard	44 – 68%	18 – 42%	0 - 10%	0 – 8%	0 – 6%
Rufous-crested bustard	40 – 60%	22 – 35%	1 – 3%	0 – 6%	0 – 8%
White-bellied bustard	40 – 60 %	25 – 40%	1 – 9%	1 – 4%	0 – 7%

Table 4l.2 Clinical values of hematological evaluation.

Diagnostic cytology	Microscopic findings**	Bustards (n= 400)*	Falcons (n=1500)*
Normal	Normal series of RBC, WBC and thrombocytes	279	1218
Regenerative anemia	Low hemoglobin with immature RBC	24	33
Non-regenerative anemia	Low hemoglobin with normal RBC	37	121
Acute inflammatory response	Elevated WBC count with heterophilia	36	118
Chronic inflammatory response	Elevated WBC count with macrophages	25	86
Viral response	Lymphocytosis with reactive lymphocytes and megathrombocytes	21	34
Toxemia	Toxic heterophils	6	17
Hematozoa	Blood parasites	30	19

*n = Number of birds

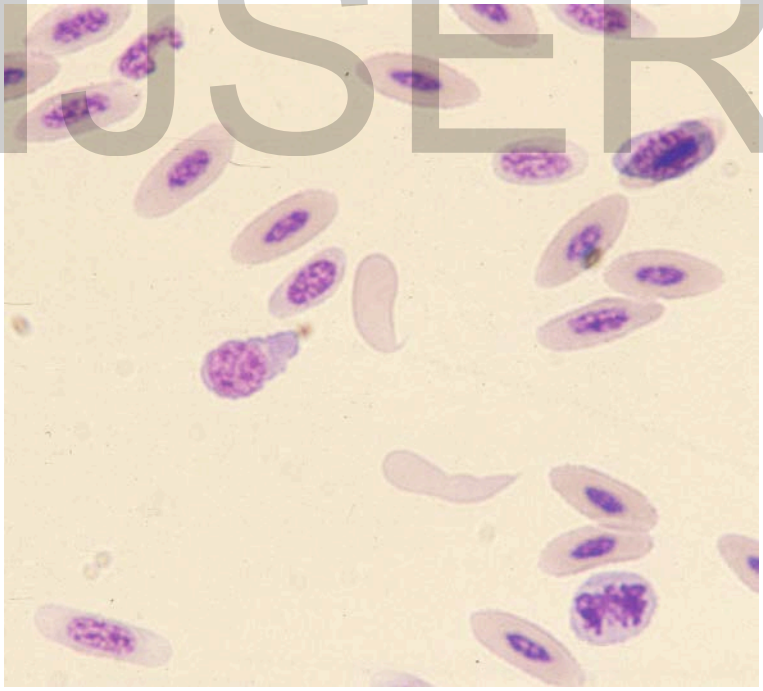


Fig.4l.1. Normal erythrocytes and an erythroplastid (anucleated red cell) from a healthy houbara bustard.

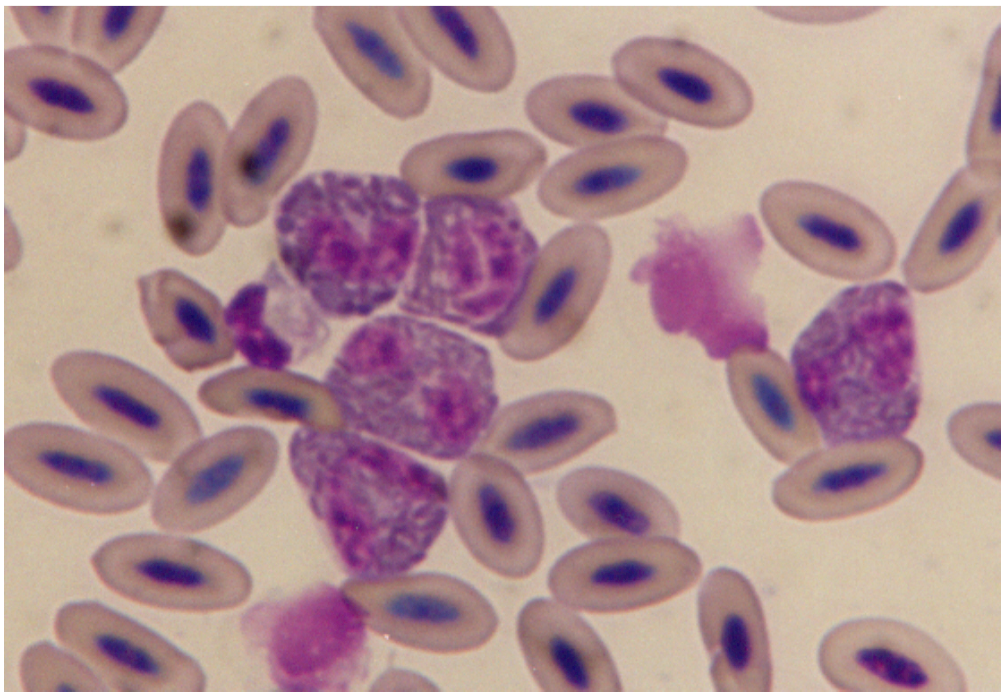


Fig.4l.2. Normal heterophils from a saker falcon. The cytoplasmic granules are cigar-shaped and the nucleus is bilobed.

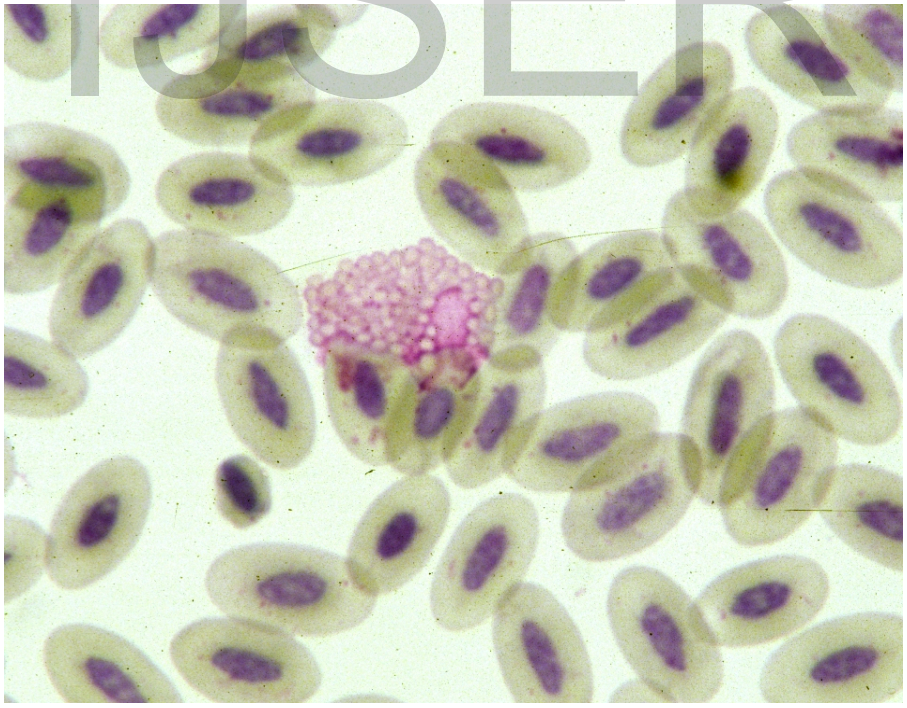


Fig.4l.3. A normal eosinophil with distinct red-brick cytoplasmic granules from a kori bustard.

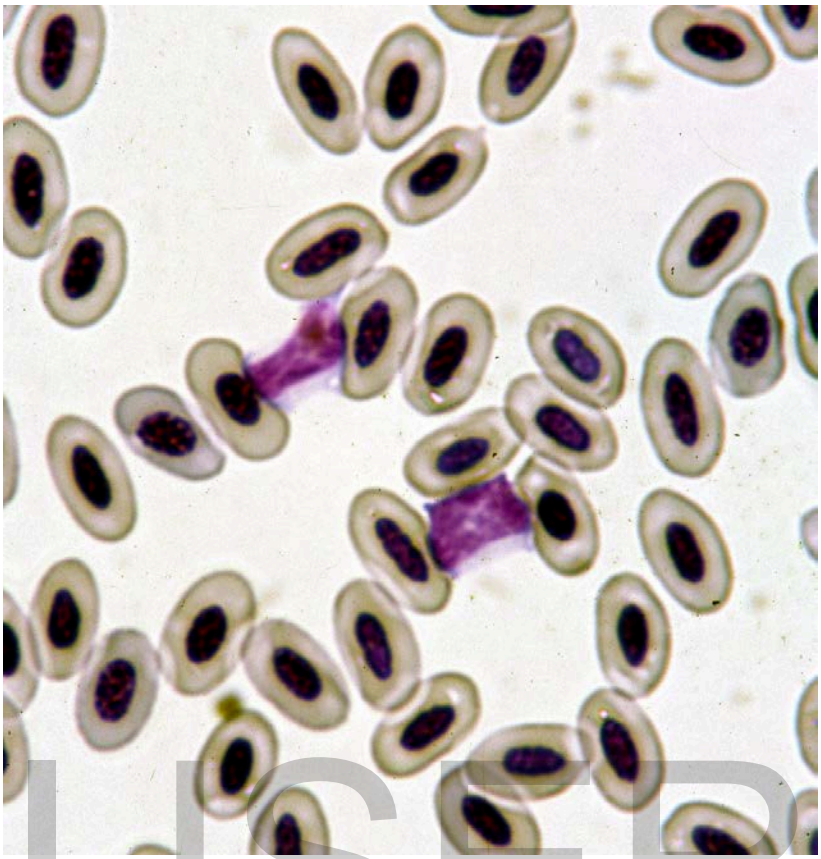


Fig.4l. 4. Small lymphocytes from a kori bustard.

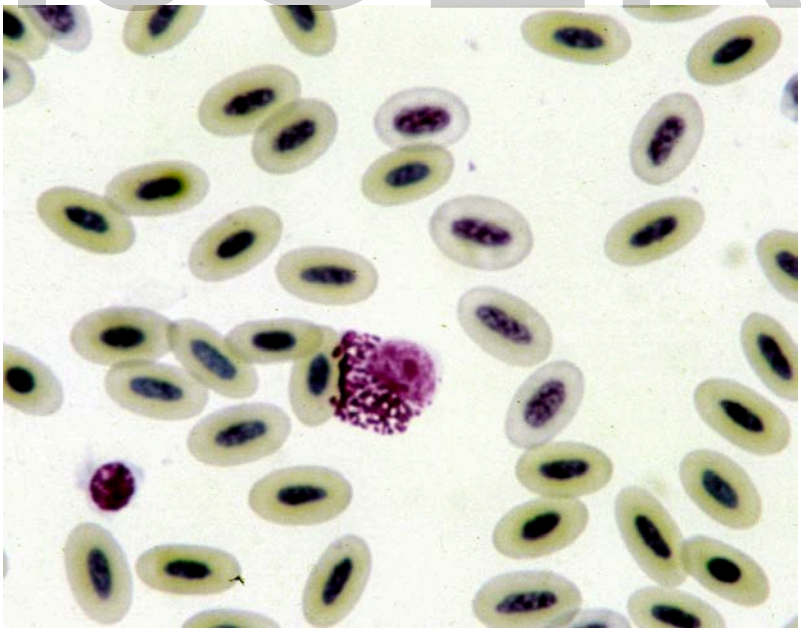


Fig. 4l.5. A normal basophil from a houbara bustard.

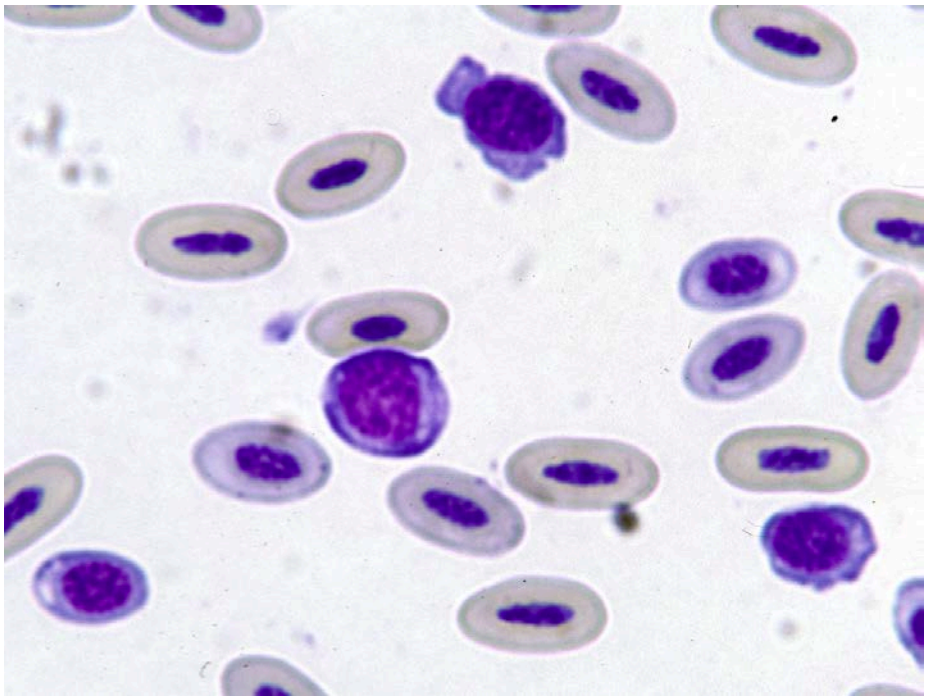


Fig.4l.6. A blood smear field from a houbara bustard showing rubrioblasts and rubriocytes that reveals severe erythropoiesis.

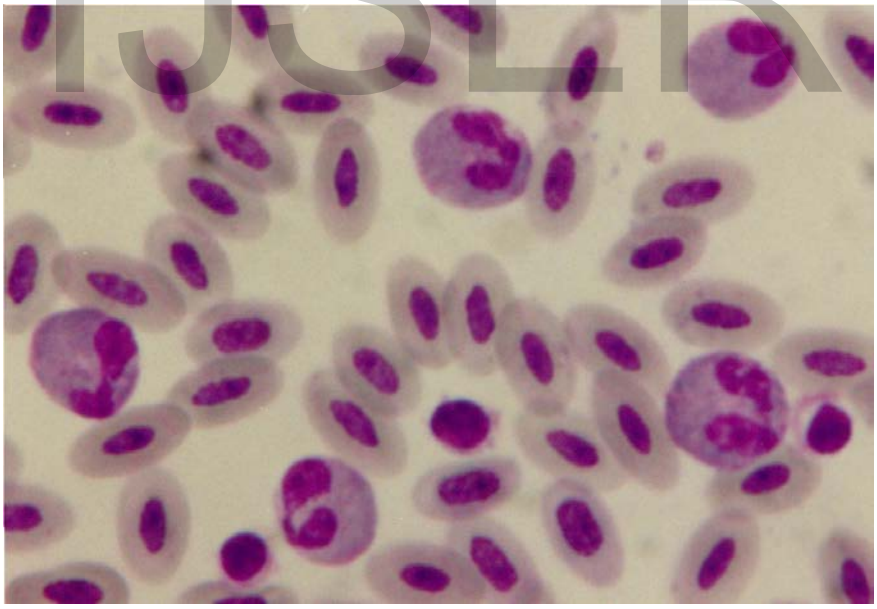


Fig. 4l.7. A blood smear field from a houbara bustard showing heterophils with severe (4+) toxic changes including severe degranulation (often, large fused granules also seen) and cytoplasmic vacuolation..

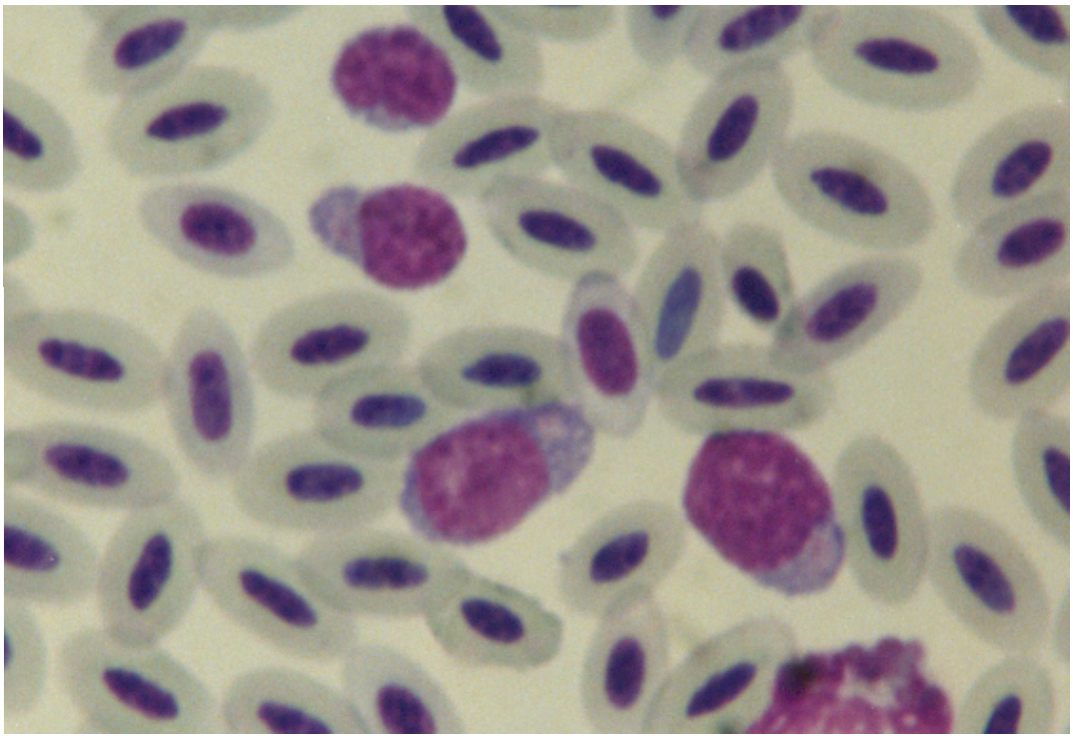


Fig.4l.8. A blood smear field from a houbara bustard showing elevated mature and immature lymphocytes due to lymphoild leucosis.

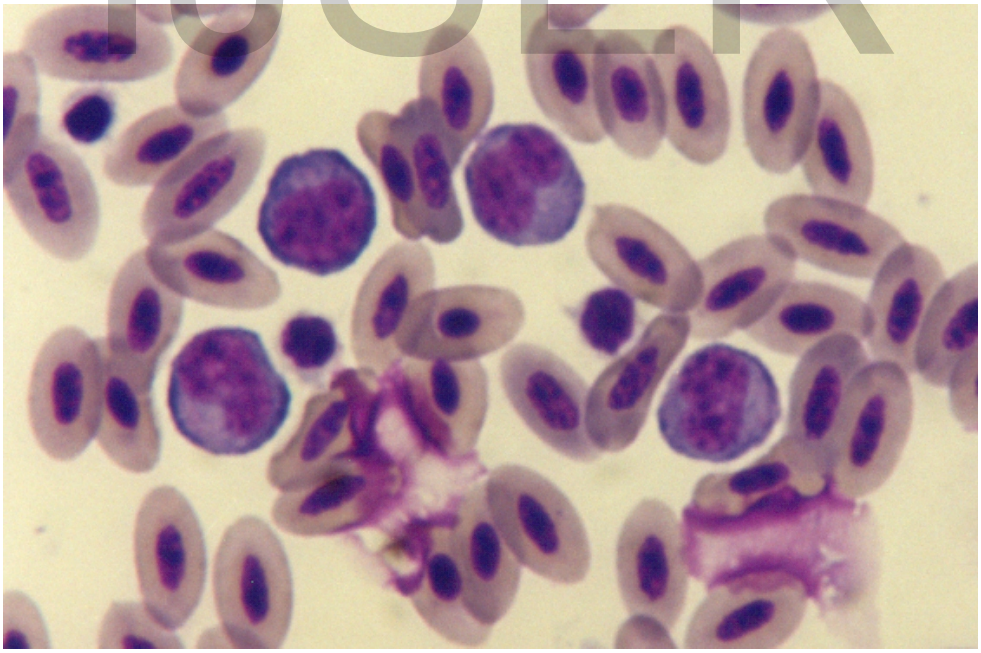


Fig.4l. 9. A blood smear from a peregrine falcon showing monocytosis due to mycobacterium tuberculosis.

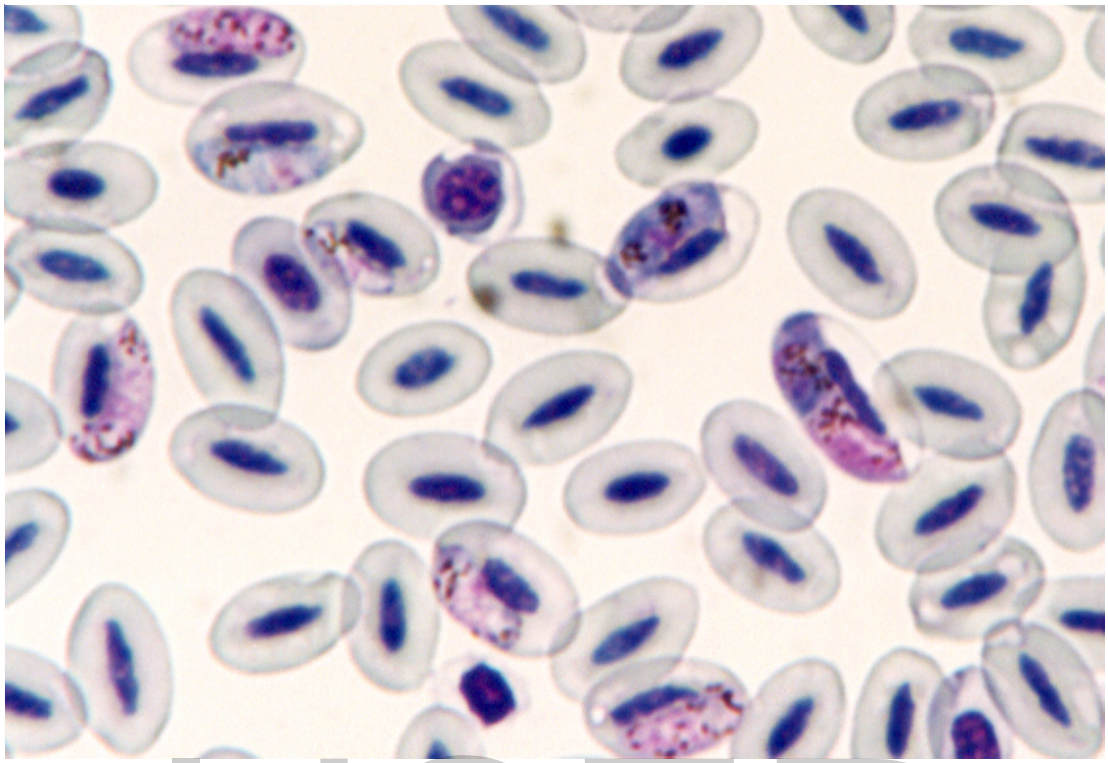


Fig. 4l.10. A blood smear from a houbara bustard showing *Haemoproteus tenderoi.*

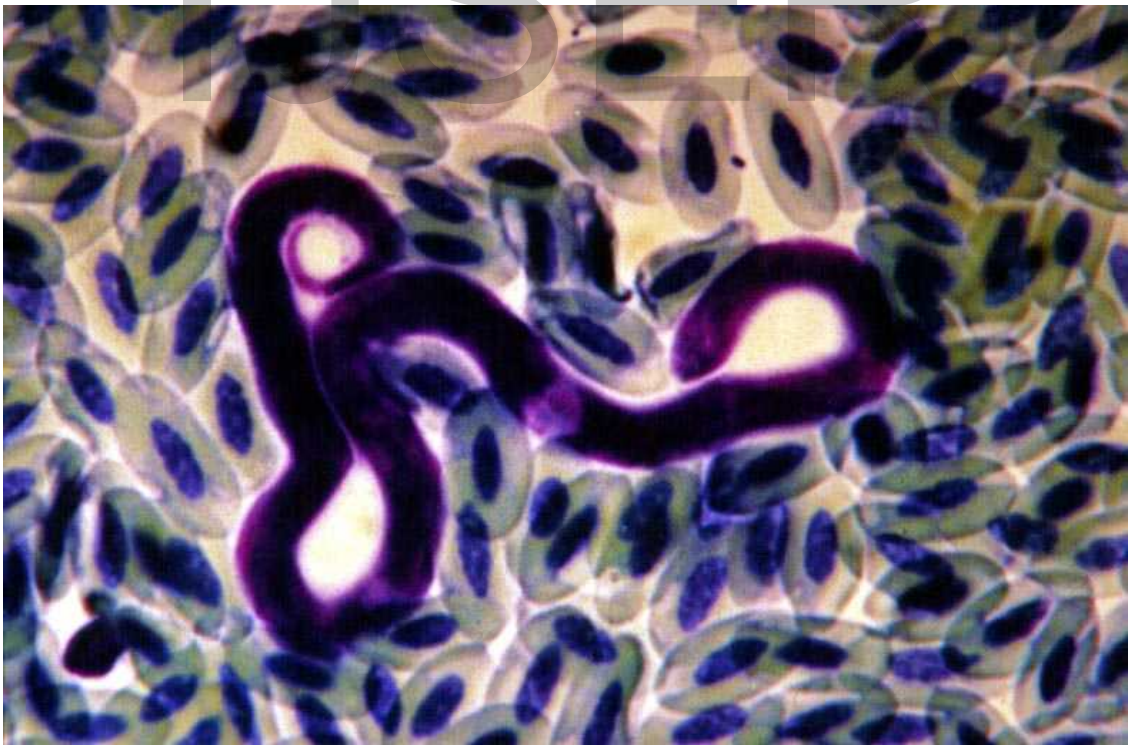


Fig.4l. 11. A blood smear from a saker falcon showing *Microfilariae* sp.

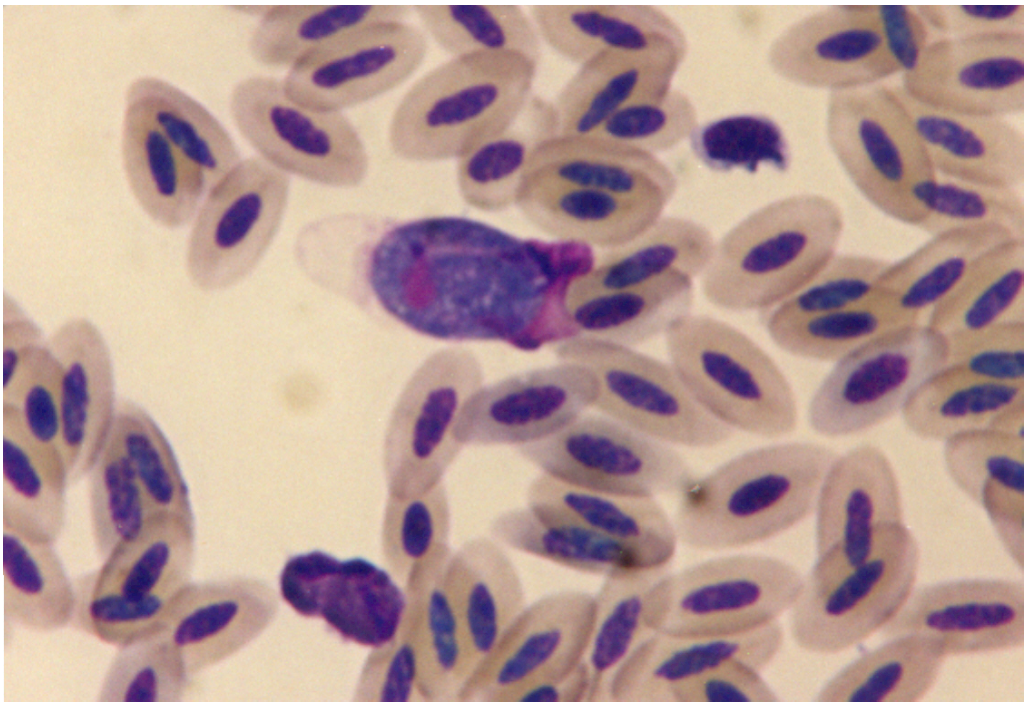


Fig 4l.12. A blood smear from a saker falcon showing *Leucocytozoon toddi.*

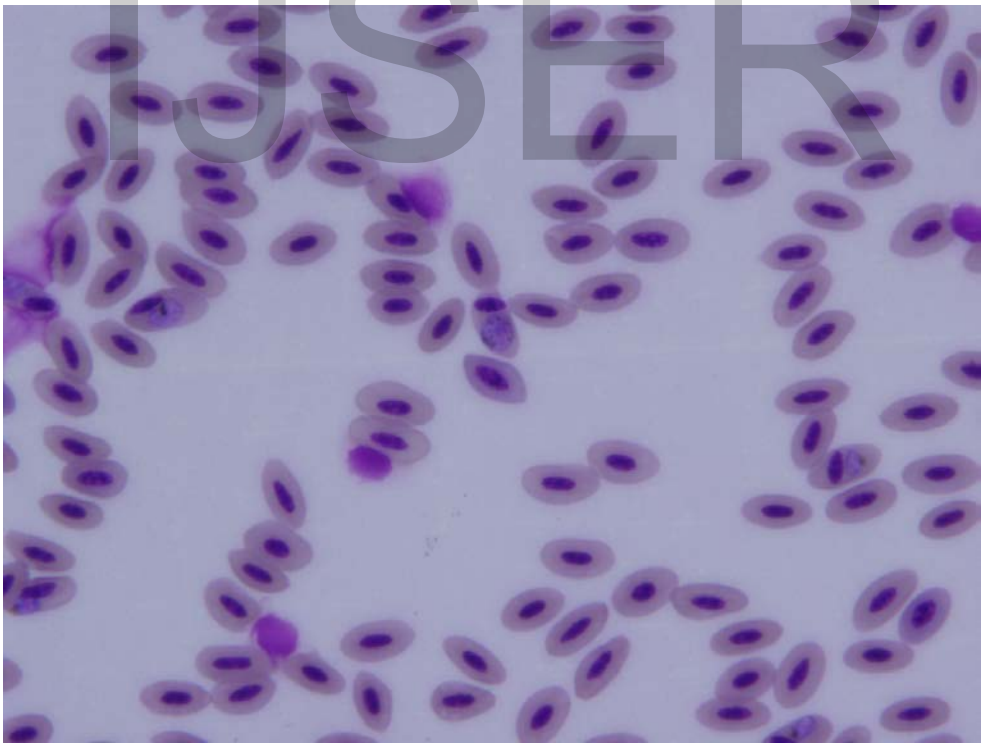


Fig. 4l.13. A blood smear from a gyr falcon showing *Plasmodium* sp (malarial parasite).

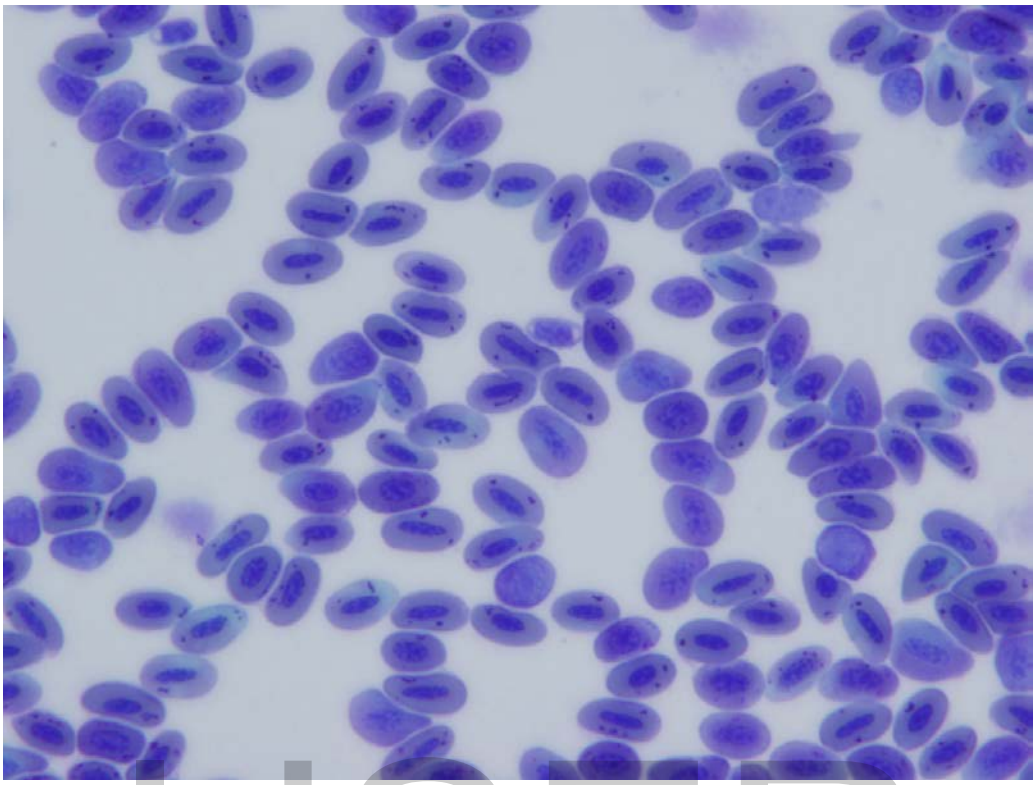
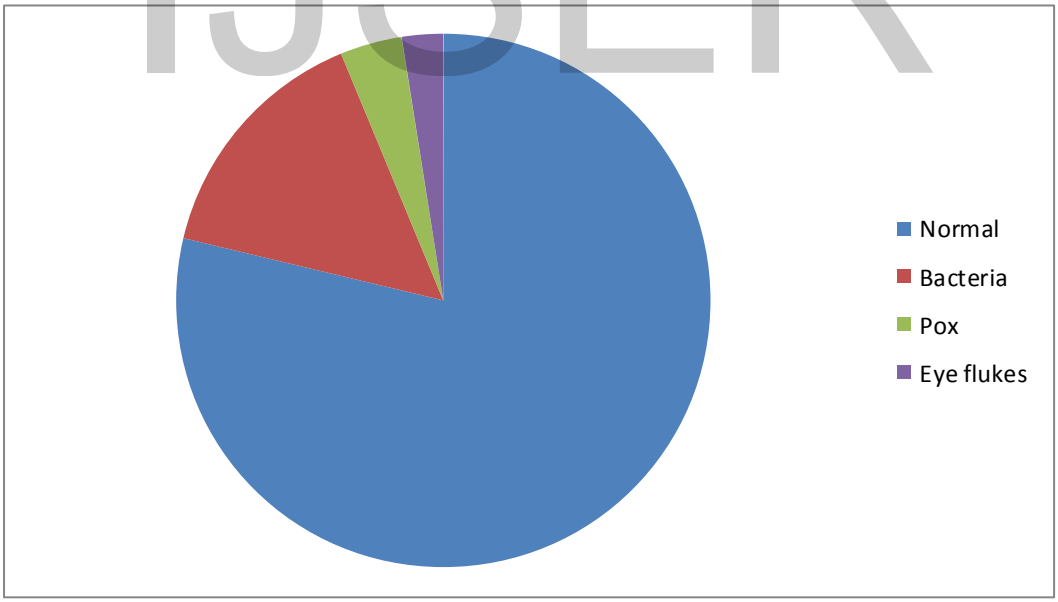


Fig. 4l.14. A blood smear from a saker falcon showing *Agyptinella* sp.

Chapter 5: Analysis

Incidence and prevalence of diseases and mortality of bustards and falcons are analyzing under this section. The habitat always plays a role in health and diseases. The falcons and bustards analyzed here are kept at captive and thus management and nutrition plays a big role in their health and diseases. Avian pox diseases are common in bustards and falcons but fungal diseases like aspergillosis are more common in falcons than bustards. This study makes clear that each disease has its own route of infection in birds like any other species. Aspergillosis is the diseases affect the respiratory tract and mostly in lungs and airsacs and occasionally seen in trachea. Aspergillosis leads to mortality in an alarm rate in falcons.

Fig 5.1: Pie chart showing bustard species with normal and abnormal findings in the conjunctiva of bustards.



Protozoal diseases are mainly caused by Trichomons sp in the upper alimentary tract of bustards and falcons but there is no role in the lower alimentary tract. Caryospora is a cyst forming coccidian protozoa which is common in the lower alimentary tract of falcons and heavy infection leads to diseases condition. Non infectious diseases such as amyloidoisis, gout and fatty liver are mostly related to nutrition and management. Figures 5.1 to 5.18 show the incidence of diseases in various sites and organs of bustards and falcons.

Fig.5.2. Pie chart showing falcons with normal and abnormal findings in the conjunctiva of falcons.

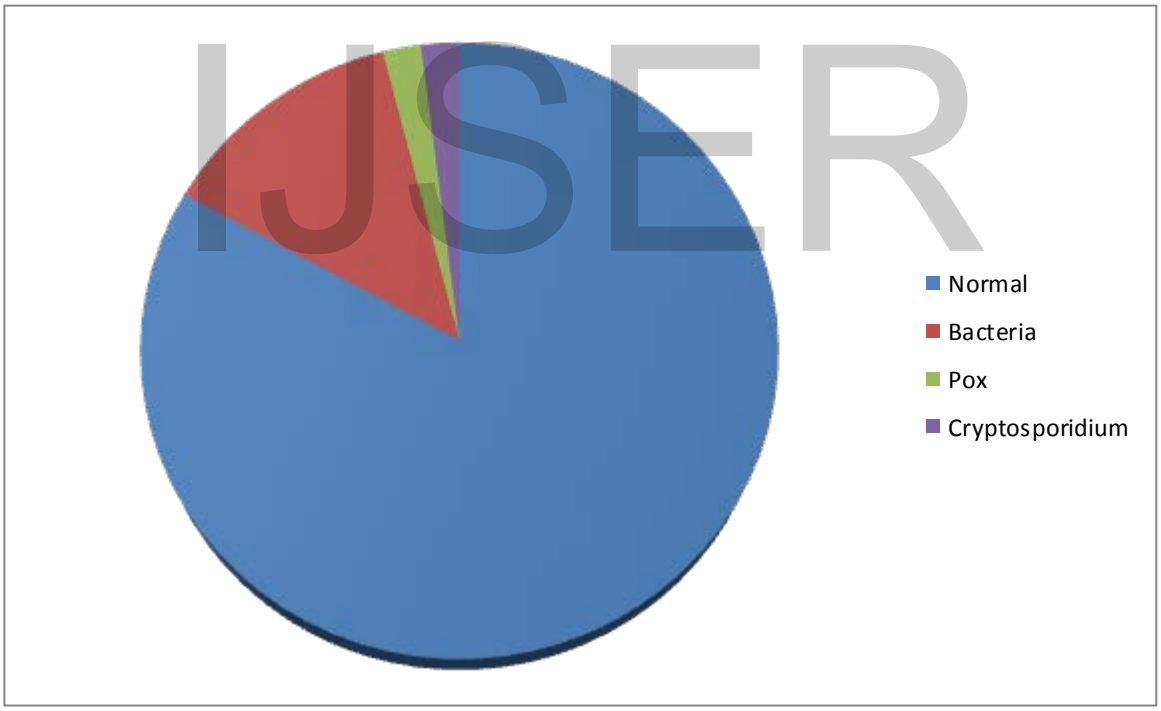


Fig.5.3.Causes of mortality in falcons and bustards in association with diseases of heart

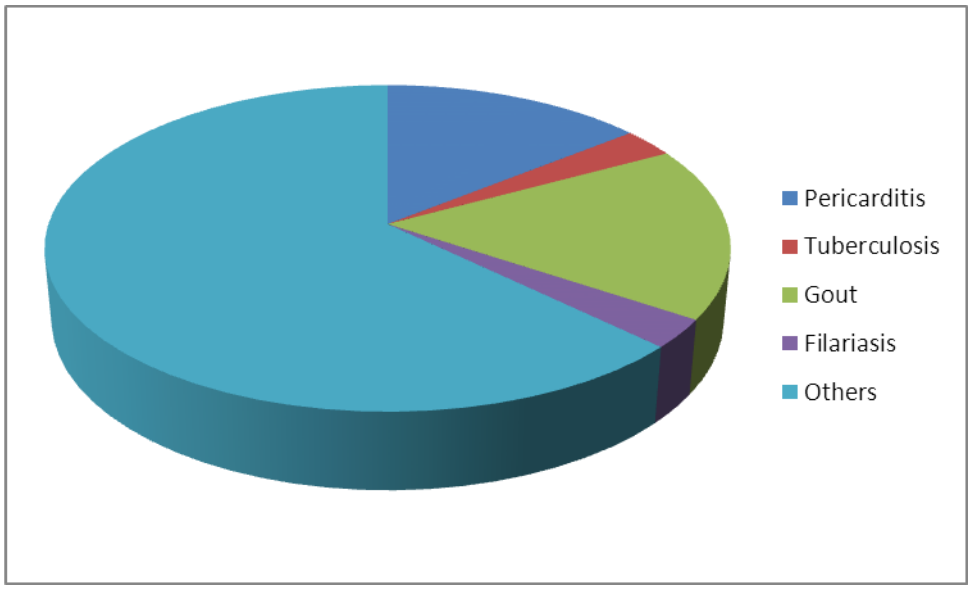


Fig.5.4. Causes of mortality in falcons and bustards in association with renal diseases.

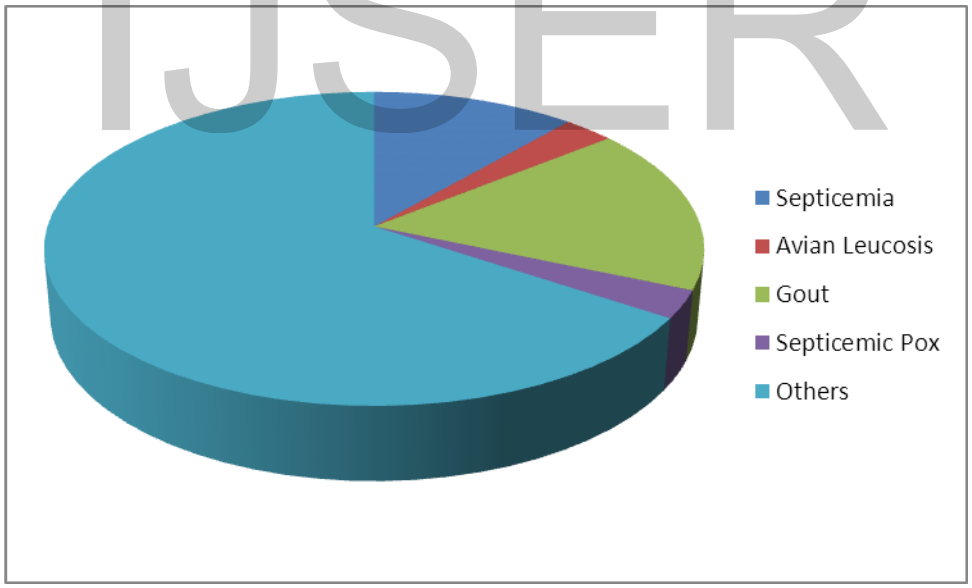


Fig.5.5. Pie chart showing bustard species with normal and abnormal findings in the liver.

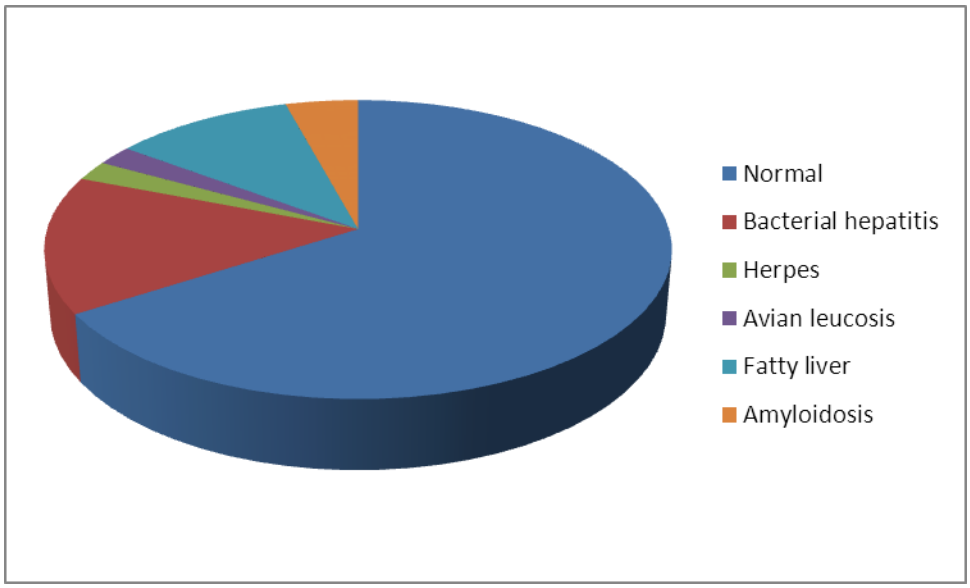


Fig.5.6. Pie chart showing falcons with normal and abnormal findings in the liver.

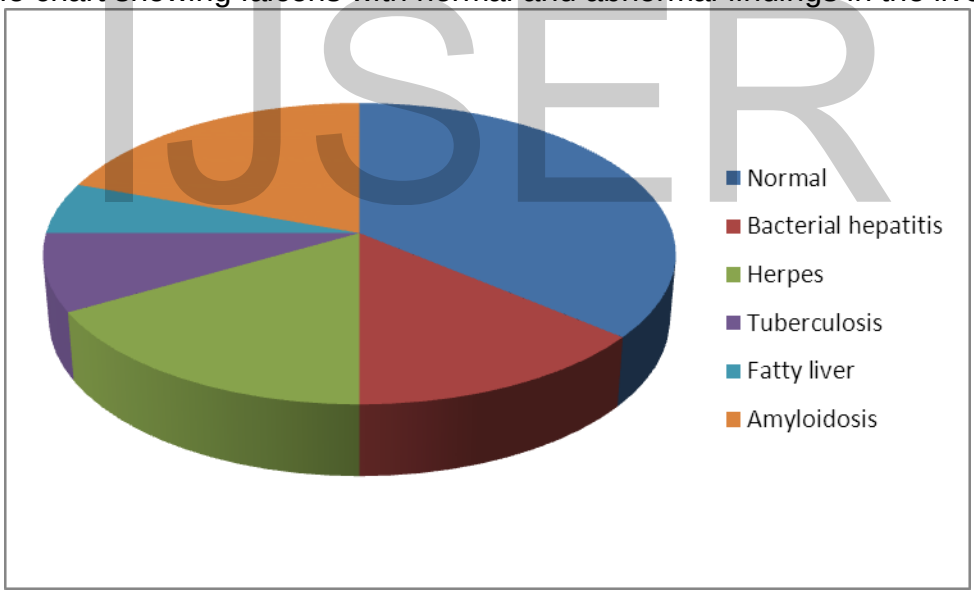


Fig.5.7. Causes of mortality in falcons and bustards in association with diseases of spleen

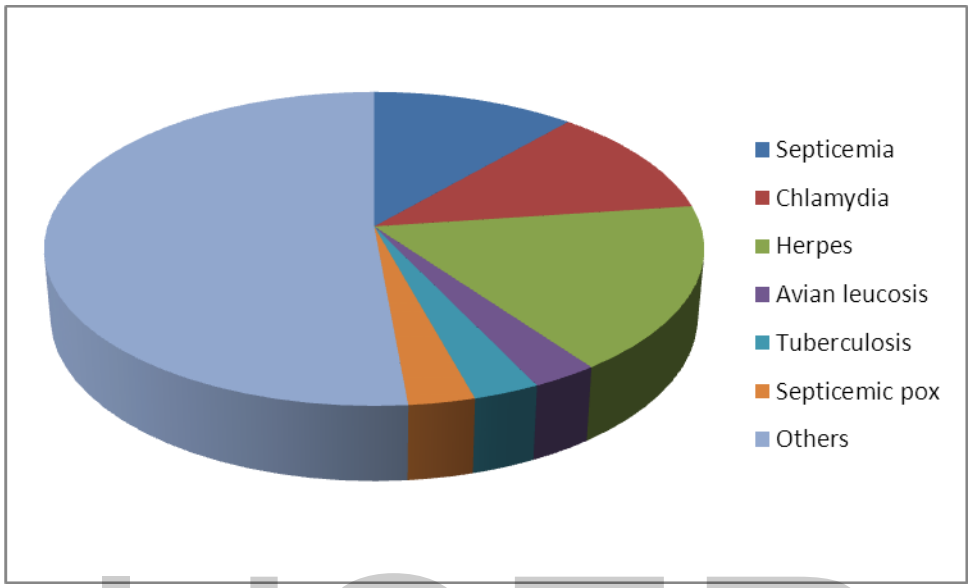


Fig.5.8. Comparative study of ectoparasites in falcons and bustards.

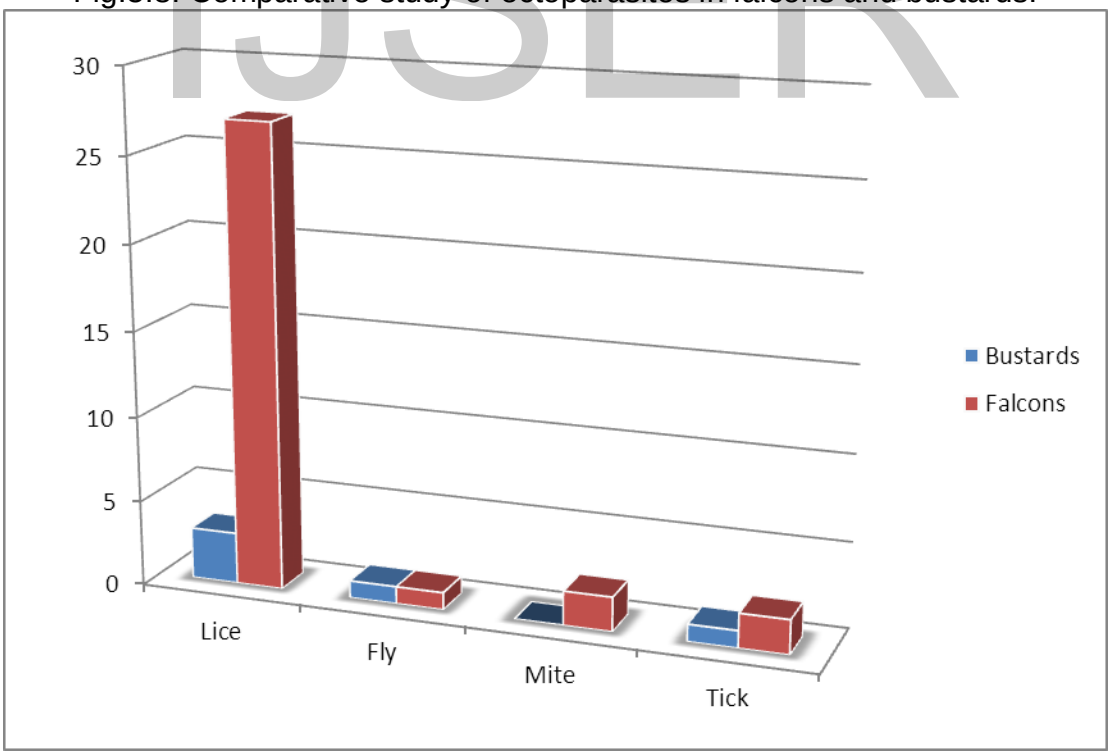


Fig.5.9. Pie chart showing findings associated with skin diseases in falcons.

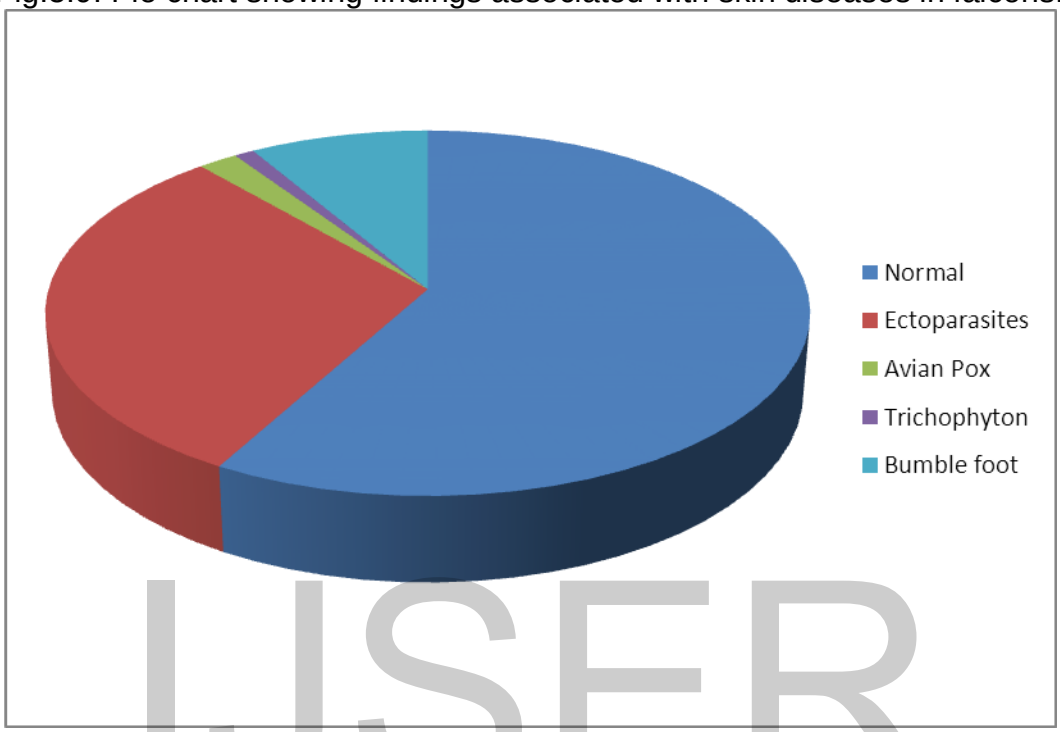


Fig.5.10. Pie chart showing findings associated with skin diseases in bustards.

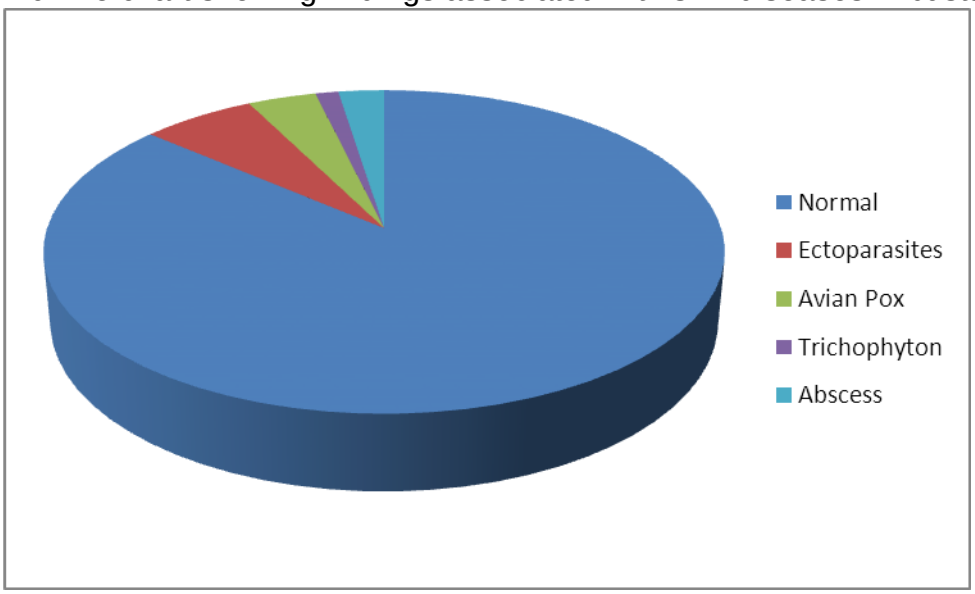


Fig.5.11. Pie chart showing bustard species with normal and abnormal findings in the lower respiratory tract.

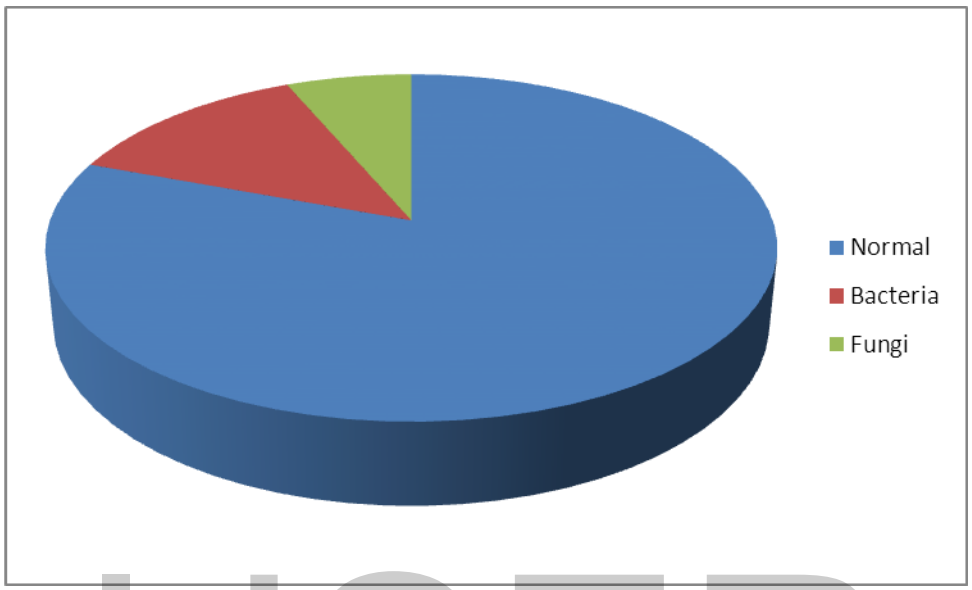


Fig.5.12. Pie chart showing falcons with normal and abnormal findings in the lower respiratory tract.

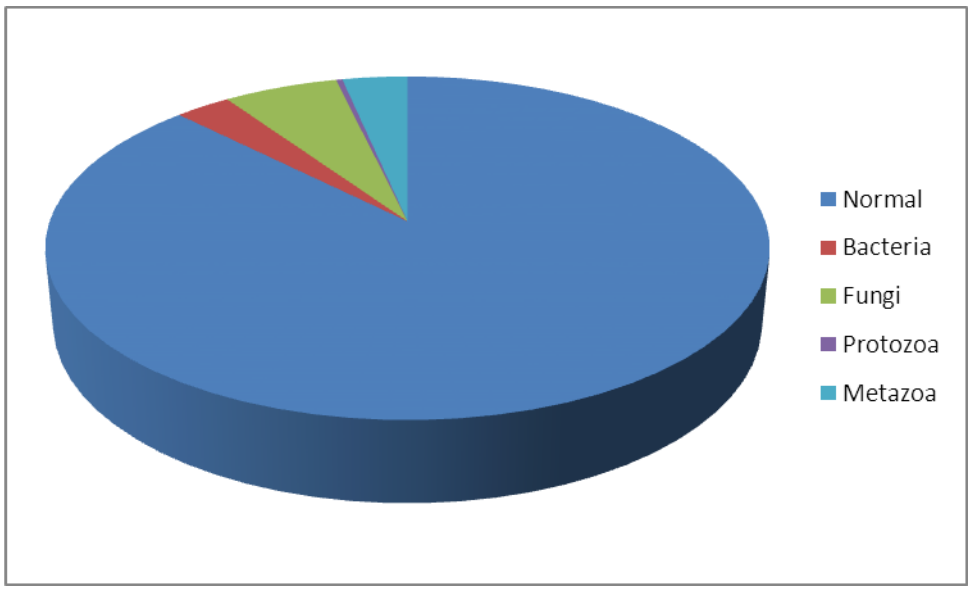


Fig 5.13. Pie chart showing bustard species with normal and abnormal findings in the upper respiratory tract.

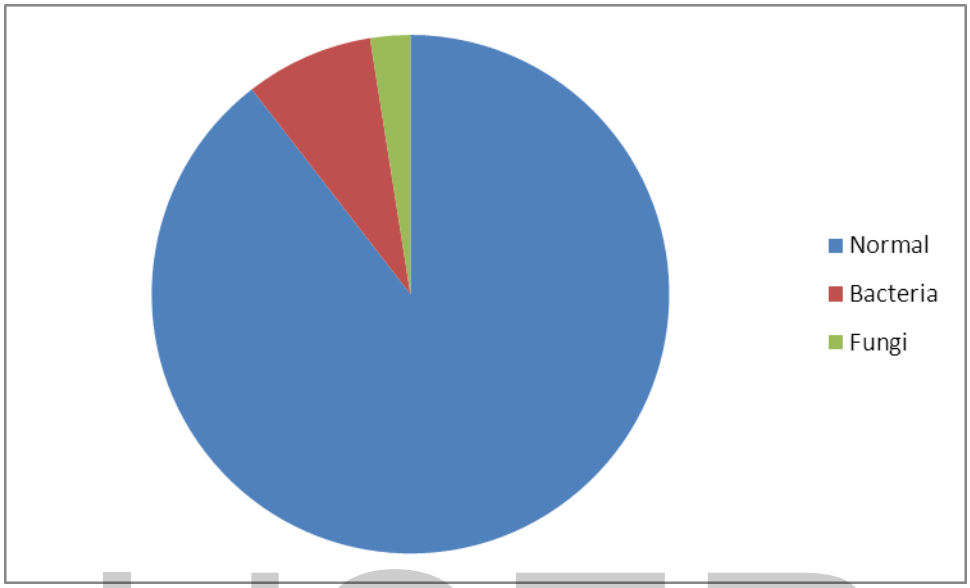


Fig.5.14. Pie chart showing falcons with normal and abnormal findings in the upper respiratory tract.

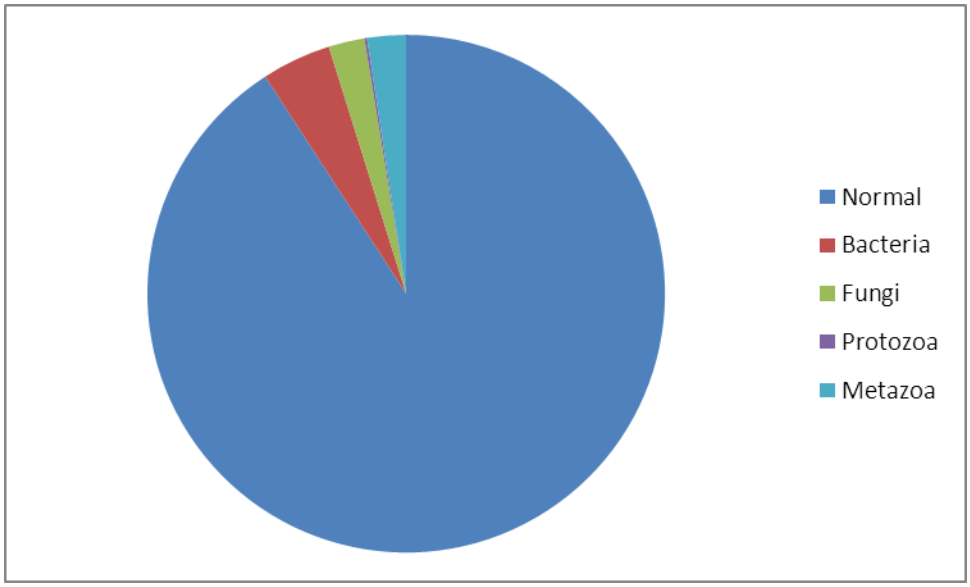


Fig.5.15. Pie chart showing upper alimentary tract diseases in bustard species with causative agents.

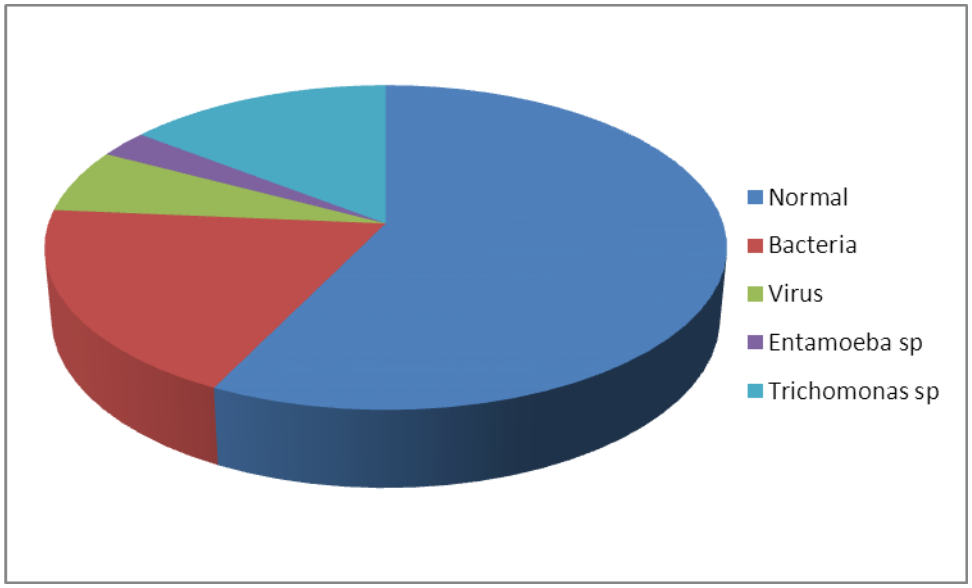


Fig.5.16. Pie chart showing upper alimentary tract diseases in falcons

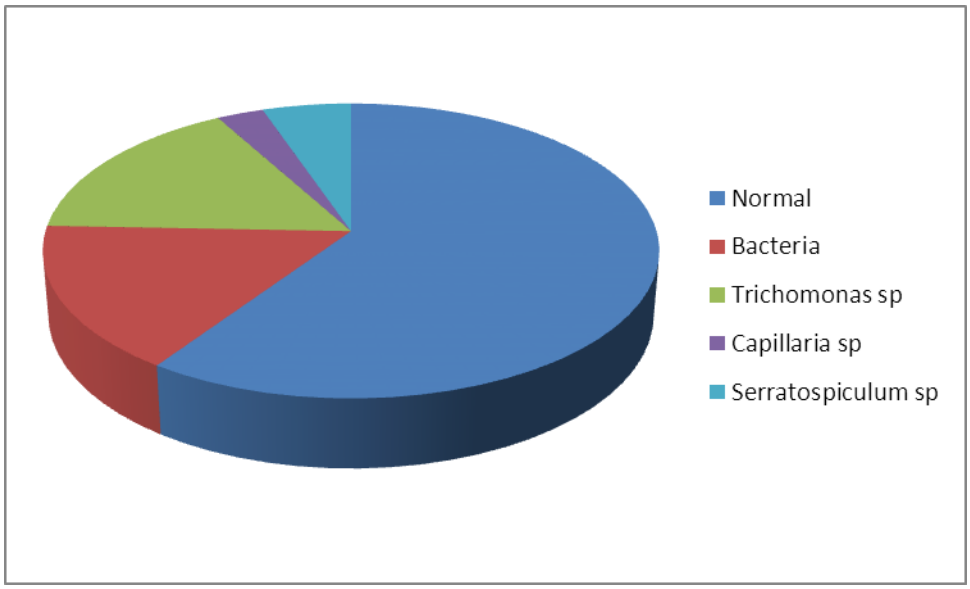


Fig.5.17. Pie chart showing bustard species with normal and abnormal findings in the lower alimentary tract.

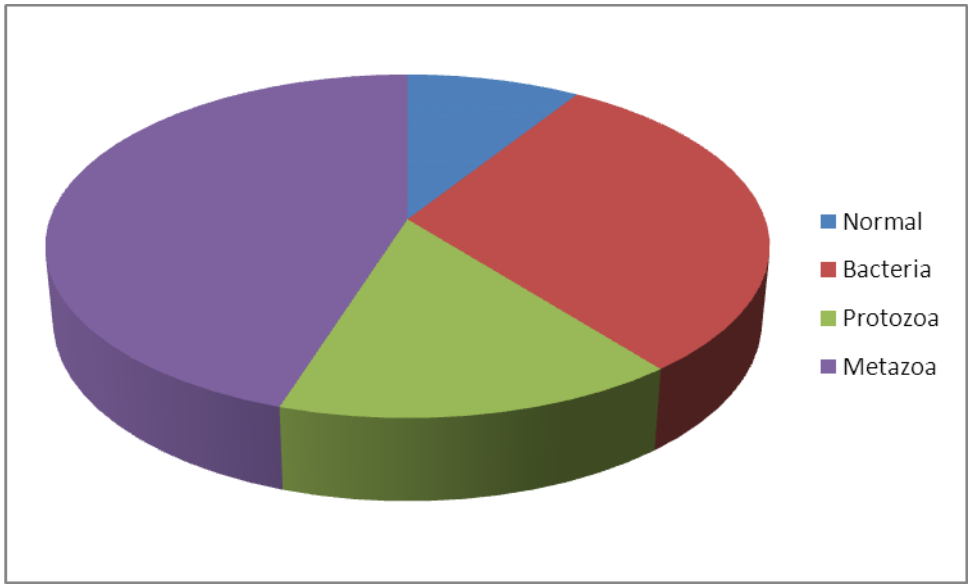
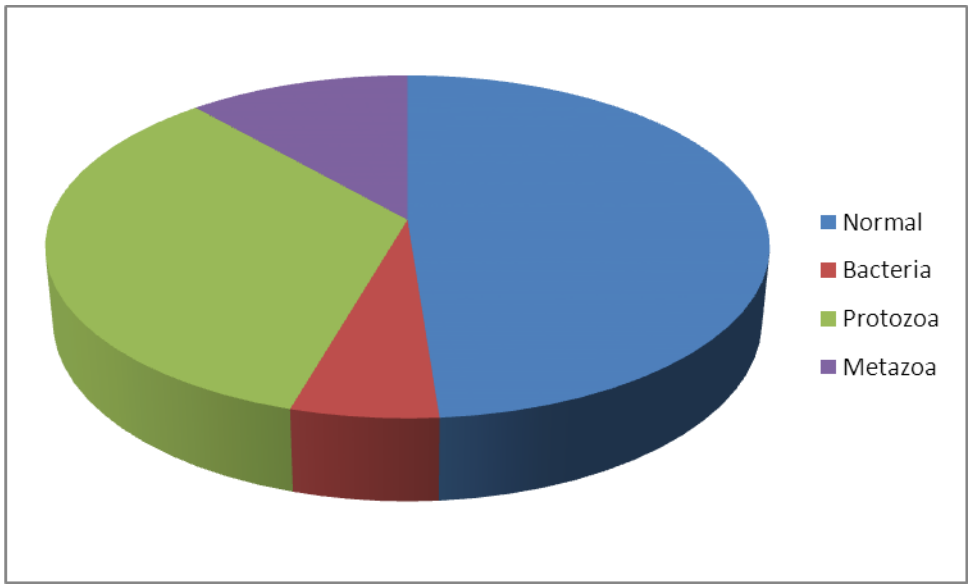


Fig.5.18. Pie chart showing falcons with normal and abnormal findings in the lower alimentary tract.



Chapter 6: Discussion

MGG stain is widely using for cytological evaluation of dry smears, which provides nucleus and cytolplasmic details. So MGG is the one of the top choice stain in this list. If any further details special stains are recommended. To get confirmation of Chlamydia inclusion, Giminez or Machivello stain is recommended. Chlamydial inclusions are obvious in MGG stain and may not be seen in rapid stains. Similarly, viral inclusions are seen MGG stain and it may not appear in rapid stains. To demonstrate AFB rods Z-N or Kinyoun stain is required and will appear in MGG as negatively stained rods, usually referred as ghost cells.

Gram stain is rapid stain to differentiate gram-positive and gram-negative bacteria which narrow down the pathogen and thus efficient treatment can start at the earliest. Rapid stain can perform within one minute and thus emergency situation, it is preferred and another smear can preserve for MGG or special stain. Direct smear can prepare from lesions and any sites. Fluid samples may contain less number of cells and thus cyto-centrifuge is used to prepare a good cellular smear. If cyto-centrifuge is not present in any lab, samples can centrifuge at low speed 2000 rpm/min and sediments can use to prepare smear.

Cellular responses are categorised as inflammation, hyperplasia, metaplasia and neoplasia (Teachout 2005). Inflammatory responses are probably the most common type encountered in birds. Inflammation is due to aetiological infectious agents. Metaplasia is due to long-term infections or toxic actions to the cells by infectious agents. Acute inflammation is primarily characterised by heterophils (more than 70% of inflammatory cells). Chronic active inflammation is characterised by a mixture of heterophils (50% of the inflammatory cells) and mononuclear cells including macrophages, lymphocytes and plasma cells. Chronic inflammation is marked by the presence of predominantly mononuclear cells (more than 50% of the inflammatory cells). Tissue hyperplasia is uncommonly seen in birds and represents an increased growth rate of normal cells in response to an insult (Pinches, 2005). Metaplastic lesions

can be diagnosed by nuclear cytoplasmic ratios and may show cellular pleomorphism. Neoplastic lesions can be diagnosed by cellular criteria including nuclear cytoplasmic ratios, prominent nucleoli, multiple nuclei, mitotic figures, cellular pleomorphism, cytoplasmic vacuolation and basophilia. The important neoplasias seen in birds include carcinomas, adenocarcinomas, sarcomas and lymphoid neoplasm. Carcinomas are characterised by round to oval cells, arranged in patterns. Adenocarcinomas (ovary) are characterised by giant cell formation and secretory granules. Sarcomas are characterised by spindle shaped cells, poor exfoliation, increased cell size and nuclear cytoplasmic ratio. Lymphoid neoplasm is characterised by discrete cells, round or oval; increase in lymphoblasts; pleomorphism, basophilia, mitotic figures and multiple nucleoli (Neil, 2000).

Synovial joints are composed of articular cartilage and an articular capsule, which is composed of a fibrous layer and a synovial membrane. The synovial membrane in turn is composed of flat cells that produce the synovial fluid that lubricates the joint. A few cells including macrophages, lymphocytes and synovial lining cells are usually seen in normal smears (Campbell, 1995). Synovial fluid with increased numbers of leucocytes and alternation in colour, turbidity and viscosity is considered abnormal. An increase of heterophils is seen in smears of inflammatory joint diseases (Cooper 2006). Non-inflammatory joint disorders show a normal cytology picture. The amount of granular back- ground particles indicates the amount of mucin present in the fluid (Campbell, 2007). The presence of erythrocytes usually indicates contamination from the peripheral blood (Silvanose 2006). Macrophages with erythrophagocytosis may be indicative of haemarthrosis (Campbell 2007). Articular gout can often be diagnosed by the gross appearance of the affected joint. Aspirated fluid can be collected from the lesion to detect the presence of needle shaped monosodium urate crystals (Cooper 2008).

The peritoneum produces a small amount of lubricating fluid to facilitate organ movements, but little or no fluid can be collected from the abdomen of normal birds. The cytology of abdominal fluid from a normal bird shows the occasional mesothelial cell

and a few macrophages. Mesothelial cells are round or oval and variable in size, and have a homogenous basophilic cytoplasm and a centrally positioned round nucleus. An abdominal effusion can be classified as transudate and exudate according to the cellularity. Transudates are characterised by low cellularity and exudates are characterised by high cellularity (Bailey 2006).

Exudate and transudate effusions in the abdominal cavity (ascites, peritonitis and haemoperitoneum) are a result of inflammatory processes within the peritoneal cavity. Acute inflammatory effusions are characterised by a predominance of heterophils. Septic exudates are characterised by macrophages with bacterial phagocytosis. Malignant exudates have a haemorrhagic character and contain malignant cells. It is difficult to conclude the origin of undifferentiated malignant cells in peritoneal effusion. Bacteria or degenerated heterophils may be present in purulent exudates. Reactive mesothelial cells may show cytoplasmic vacuolisation (Silvanose 2006). Plasma cells frequently occur in chronic inflammatory exudates. An egg-related peritonitis (yolk peritonitis) can be recognised by the presence of yolk drops, which are homogenous, round and basophilic in stained smears (Campbell 1993). In advanced aspergillosis, lesions can be seen on the peritoneal surface resulting in an inflammatory picture of the abdominal effusion. Transudates accumulated in the serous cavities of falcons are noticed in association with filarial nematode infection (*Serratospiculum* sp.) and its eggs and larvae were noticed in fluids collected from such cases.

Accumulation of pericardial exudates (pericarditis) is due to cardiac inflammatory response and systemic disease that could be due to various infectious agents. *Clostridium perfringens* and *Streptococcus bovis* have been isolated from the pericardial fluid. Non-infectious pericarditis is seen due to the deposition of urates called gout (Silvanose 2006). Cytology smears from such lesions are characterised by urate crystals. Transudates can occur with heart failure and hypoproteinemia. Inflammatory cells are indicative of pericarditis.

The conjunctival mucosa is composed of stratified columnar epithelium. Normal smears made from the conjunctiva are usually poorly cellular with a few epithelial cells present in a clear background. The conjunctival cells have an eccentric, round to oval nucleus with coarse nuclear chromatin and abundant weakly basophilic cytoplasm with distinct margins. The cornea is composed of stratified squamous epithelium. Normal smears from the cornea are poorly cellular with noncornified squamous epithelial cells (Campbell, 2007).

Abnormal cytology of the conjunctiva, (conjunctivitis and keratoconjunctivitis) caused by bacteria reveal numerous inflammatory cells and the inflammatory cells may show phagocytosis of bacteria and degenerative epithelial cells. The degenerative changes include cytoplasmic vacuolation, karyolysis or karyorrhexis (Campbell 1993, Campbell, 1995). Acute inflammatory lesions are represented by an increase in heterophils. Chronic inflammatory lesions are represented by an increase in mononuclear leucocytes. Conjunctival goblet cells may also be present in chronic cases (Cooper 2008, Harrison 1994)). Chronic active inflammatory lesions are represented by heterophils, macrophages and goblet cells. *Staphylococcus aureus*, *E. coli, Chlamydia* sp., *Pseudomonas aeruginosa, Pasteurella multocida*, *Mycoplasma* sp., *Haemophilus* sp,and are pathogens commonly isolated with conjunctivitis (Bailey, 2006). The increase in cornified squamous epithelial cells may be due to chronic irritation, hypovitaminosis A or contaminations of cornified squamous epithelial cells from the surrounding skin. Pox lesions are seen in bustards on the eyelid and 3rd eyelid. Cytology smears reveal swollen corneal cells with Bollinger bodies (Silvanose, 2006).

A survey was carried out by Silvanose (2001) to describe the normal aerobic bacterial flora of the conjunctiva and nasal cavity of captive houbara bustards (Chlamydotis undulata), kori bustards (Ardeotis kori), and white-bellied bustards (Eupodotis senegalensis) maintained at the National Avian Research Center, Abu Dhabi, United Arab Emirates. A total of 58 samples were examined from the nasal cavity and 55 samples from the conjunctiva of healthy bustards. There was no bacterial growth in 45% of conjunctival samples. Bacteria isolated from the conjunctiva of healthy birds included

Micrococcus spp., Staphylococcus auricularis, Staphylococcus xylosus, Staphylococcus capitis, Staphylococcus warneri, Bacillus spp., and Enterobacter amigenus. Bacteria isolated from the nasal cavity of healthy birds included Bacillus spp., Micrococcus spp., S. auricularis, S. xylosus, Staphylococcus simulans, Staphylococcus saprophyticus, Staphylococcus hyicus, Staphylococcus cohnii, Staphylococcus sciuri, Aerococcus spp., and Providencia rettgeri. These findings were compared with bacterial isolates from bustards with clinical signs of ocular or upper respiratory tract diseases. Mycoplasma spp., Pseudomonas aeruginosa, Pseudomonas stutzeri, Proteus mirabilis, Escherichia coli, Klebsiella spp., Aeromonas hydrophila, and Staphylococcus aureus were the pathogenic bacteria isolated from the conjunctiva of 34.3% bustards with ocular discharges. Mycoplasma spp., P. aeruginosa, Pseudomonas spp., P. mirabilis, E. coli, Klebsiella pneumoniae, and S. aureus were the pathogenic bacteria isolated from the nasal cavity of 74% bustards with upper respiratory tract diseases (Silvanose, 2001).

Mammals and birds are warm blooded and they have four chambered heart, with complete separation of oxygenated and de-oxygenated blood. The right ventricle pumps blood to the lungs, while the left ventricle pumps blood to the rest of the body. Because the left ventricle must generate greater pressure to pump blood throughout the body (in contrast to the right ventricle that pumps blood to the lungs), the walls of the left ventricle are much thicker & more muscular (J.T. Lumej).

The red and white blood cells are formed in the spleen. A bird's red blood cells are unique in that they are nucleated (there is a nucleus) whereas a mammal's are not. Significant diseases of the cardiovascular system in poultry meat inspection include pericarditis and ascites**.** Pericarditis is an inflammation of the fibroserous membranous sac called pericardium, which encloses and lubricates the heart. Ascites is an increase of fluid in one or more of the abdominal spaces.

Accumulation of fluid is occasionally seen in the pericardium and the fluid is called pericardial fluid. Pericardial fluid can be obtained by aspiration. Cytology imprint smear of heart is performed to assess the presence of blood parasites in dead birds (Campbell

1993). The gross appearance of heart is reported on post-mortem to support the cytological diagnosis. Normal impression smear from the heart shows primarily blood cells and it is valuable in dead birds to screen samples for blood parasites and to assess the level of parasitemia.

Accumulation of pericardial exudates (pericarditis) is due to cardiac inflammatory response and systemic disease that could be due to various infectious agents. *Clostridium perfringens* and *Streptococcus bovis* have been isolated from the pericardial fluid (Cooper 2008). Non-infectious pericarditis is seen due to the deposition of urates called gout Cytology smears from such lesions are characterised by urate crystals (Silvanose 2006). Transudates can occur with heart failure and hypoproteinemia. Inflammatory cells are indicative of pericarditis (Campbell 2007, Campbell 1995). *Paronchocerca filariae* have occasionally been seen in the cardiac arteries of bustards (Bailey 2006).

Viseral gout is a common post-mortem finding in bustards. Articular gout has only occasionally been observed in bustards. Gout should not be considered s a disease entity, but as a clinical manifestation of any severe renal dysfunction that results in a persistent hyperuricaemia and very often gout can be attributed to renal diseases (Lumjej 1994). If uric acid crystals crystals precipitate in the kidney, an acute obstructive uropathy occurs and the secretion of uric acid is severely compromised. This will result in the elevation of plasmauric acid concentration and the precipitation of urates on visceral surfaces, rapidly leading to death (Lumeij, 1994).

The most common clinical sign of gout in bustards is sudden death (Bailey 2006). In other species clinical signs are vague and include anorexia and polyuria (Lumeij, 1994). Gross post-mortem examination reveals the characteristic accumulation of white uric acid tophi on the serosal surface of viscera. Gout is caused by any disease that results in hyperuricaemia. In bustards causes include dehydration and bacterial renal disease (Bailey 2006). Cytology imprint smears from normal kidneys show primarily tubular

epithelial cells. These cells have an abundant cytoplasm and round to oval slightly eccentric nucleus (Campbell 1995, Campbell 1993). Irregular swollen kidneys with white foci are seen in bustards and falcons; and are due to urate crystal deposition (renal gout). Cytology smears from such cases reveal urate crystals (Silvanose 2006, Cooper 2008, Campell 2007). In certain cases, iron pigments (haemosiderin) are seen in cells, indicating haemoglobin catabolism. Chlamydia sp., Clostridium perfringens, Streptococcus bovis and Salmonella sp. are the pathogenic bacteria isolated from the kidney of bustards (Bailey 2006). Irregular swollen kidneys with multifocal abscessation may be due to bacterial infection. Cytological smears showing bacteria should be differentiated from contaminants. Cytology smears from septicemic pox cases are characterised by vacuolated renal cells. Plasma cells, multinucleated giant cells are also often seen.

There is no neoplasm reported in this study which may reported in birds include carcinomas, adenocarcinomas, sarcomas and lymphoid neoplasm. Carcinomas are characterised by round to oval cells, arranged in patterns. Adenocarcinomas (ovary) are characterised by giant cell formation and secretory granules. Sarcomas are characterised by spindle shaped cells, poor exfoliation, increased cell size and nuclear cytoplasmic ratio. Lymphoid neoplasm is characterised by discrete cells, round or oval; increase in lymphoblasts; pleomorphism, basophilia, mitotic figures and multiple nucleoli (Neil, 2000).

Imprint cytology smears from the cut surface of the liver show primarily hepatocytes and a variable numbers of blood cells. Hepatocytes are large cells with round or oval nuclei and an abundant cytoplasm. Hepatocytes occur singly, in sheets or in clusters. These cells are easily damaged, resulting in free nuclei. Lymphocytes, macrophages and spindle-shaped cells are occasionally seen in normal smears (Cooper 2008).

Abnormal cytology of liver includes non-infectious diseases (fatty liver, amyloidosis, hepatoma and gout) and infectious diseases (Silvanose 2006). Fatty liver syndrome is often seen in captive bustards and is characterised by fat deposition in hepatocytes.

Cytology smears are characterised by vacuolated hepatocytes, including micro-vesicular vacuolation, macro-vesicular vacuolation and lipocytes. Micro-vesicular vacuolation is less common and is characterised by many tiny vacuoles. Macro-vesicular vacuolation is more commonly seen and characterised by two to three big vacuoles. Lipocytes are abnormal hepatocytes with a very big fatty vacuole that pushes the nucleus to the periphery and nuclear changes may be observed. Fat deposition is less often seen in Kupffer cells. A small amount of fat is normally present in liver smears from adult birds and it is high in neonatal chicks. The scoring of the amount of fat in hepatocytes is important to diagnose fatty liver syndrome. When more than 50% of hepatocytes are vacuolated and have nuclear changes it is considered significant. Degenerative changes also result in the vacuolation of hepatocytes. Sudan II stain is used to differentiate between degenerative changes and fatty liver (Harrison 1994). Degenerative hepatocytes are noticed in many hepatic diseases and also in post-mortem autolysis. Urate crystal deposition is often seen in the liver of bustards and cytology smears are characterised by crystalline aggregates of variable sizes. Kupffer cells often contain iron pigment (haemosiderin), indicating haemoglobin catabolism. Excessive iron pigment within many macrophages can be associated with haemolytic anemia or iron storage caused by chronic inflammatory disorders. Bile pigments are seen in the cytology smears of bustards with chronic diseses and liver disorder. Amyloid is a fibrillar protein derived from immunoglobulins (Campbell 1993). Amyloidosis is a pathological tissue change due to the deposition of amyloid proteins. Amyloid is insoluble and almost resistant to proteolysis, so when it is deposited in tissues it cannot be eliminated, resulting in tissue destruction. Primary and metastatic neoplasia of the liver can occasionally be detected. Cells from neoplastic lesions have cellular features including pleomorphic nuclei (variable size and shape), variable N:C ratios, abnormal nucleoli, multinucleation, irregular chromatin and mitotic figures. Hepatomas are benign hepatic masses with cellular features of normal-appearing hepatocytes (Canpbell 1995).

Hepatomegaly seen in falcons is mainly caused by fatty liver, amyloid accumulation, bacterial hepatitis caused by tuberculosis, chlamydiosis, salmonellosis, viral hepatitis caused by herpes and adeno virus infections. While in bustards, hepatomegaly is

associated with fatty liver, hepatitis, chlamydiosis, salmonellosis and leucosis. Inflammatory responses involving the liver (hepatitis) usually showed numerous heterophils. Septic inflammatory lesions are indicated by the presence of bacteria within leucocytes. Extracellular bacteria must be differentiated from bacterial contamination of the sample or from the cadaver bacteria when the sample is obtained during necropsy. Chlamydiosis is a common disease of bustards, frequently involving the liver, spleen and air sacs. The hepatic cytology in chlamydia cases shows inflammatory cells primarily of macrophages, lymphocytes and plasma cells (Campbell 2007). Tuberculosis is occasionally seen in falcons and is caused by Mycobacterium sp. that produces granulomatous lesions in the liver. Cytology smears are characterised by macropages. Ziehl-Neilsson stain is useful to demonstrate Mycobacterium sp. Salmonella sp., Clostridium perfringens, Streptococcus bovis, Streptococcus milleri and toxic E.coli are the pathogenic bacteria isolated from the liver of bustards. While Salmonella sp., toxic E.coli, Clostridium perfringens, Clostridium histolyticum and Streptococcus bovis are the pathogenic bacteria isolated from the liver of falcons.

Avian leucosis is a viral disease in poultry that is occasionally seen in bustards (Bailey 2006). Liver imprint smears are characterised by mature and immature lymphocytes. Hepatocytes with cytoplasmic basophilia are seen in association with avian leucosis. Herpes and adeno virus infections are reported from falcons and can cause liver disease. Adeno virus infection was seen in a falcon that showed massive hepatic necrosis and pale yellow (discoloration) of liver. Liver imprint smears from this case were characterised by cytomegaly and karyomegaly. Intranuclear inclusion bodies were found in hepatocytes. Herpes virus infections in falcons show hepatomegaly with focal areas of necrosis and cytology smears are characterised by intranuclear inclusion bodies (Wernery 2004).

Occasionally, gametocytes of Hemoproteus sp. are seen in hepatic smears. These parasites are considered contamination from red blood cells. In bustards and falcons schizont stages are usually not seen in internal organs, but an exceptional case was

seen in a houbara bustard that showed schizont stages of Hemoproteus sp. In other avian species, the schizont stages are reported from the liver.

The functions of the little-studied avian spleen are reviewed and compared with those of its better known mammalian counterpart, which is generally larger in proportion to body size than in birds (John JL, 1994). A role in immunity similar to that in mammals is evident, but the organ's contribution to oxygen supply seems less extensive; splenic storage of erythrocytes, for example, is unrecorded for birds. The spleen is a principal organ of systemic immunity, and its importance in disease resistance is presumably accentuated by the scarcity of aan lymph nodes. The striking intraspecific viariation in size partly refelcts seasonal changes in spleen morphology and activity. Several explanations, principally based on changing oxygen demand, have been proposed previously for these periodical cycles. But seasonally small spleens could sometimes simply stem from a combination of (1) a cessation of active splenomegaly as seasonally patent infections recede, and (2) a seasonal lymphoid involution, occuring even if an individual has not recently responded to, and recovered from, and infection. Possible determinants for these and other processes are discussed from evolutionary and ecological perspectives. There is a pressing need for a thorough investigation of both hematological and immunological functions, using a phylogenetically and ecologically broad range of species, as well as modern histological and experimental techniques (John JL, 1994).

The spleen is a blood-forming and blood-destroying organ. Therefore cytological imprints of normal spleens reveal a marked number of haemic cells. Many lymphocytes (predominantly small mature lymphocytes) are present since the spleen contains lymphoid tissue. Other cells including a few prolymphocytes, lymphoblasts and plasma cells may be seen in normal smears with less than 10% of the cell population of normal lymphoid tissue.

The evaluation of the lymphoid elements in the spleen provides an indication of the overall status of the bird’s immune system and lymphoid elements. Excessive iron

storage within macrophages and erythrophagocytosis may be associated with haemolytic anemias (Campbell 1993, Campbell 1995, Campbell 2007). Systemic infections may be confirmed by the presence of the aetiologic agent in spleenic samples. Salmonella sp., Chlamydia sp. and herpes virus infections cause splenomegaly in falcons. A reactive spleen is characterised by a marked increase of plasma cells and mild to moderate increase in immature lymphocytes. Reactivity of spleenic lymphoid tissue suggests systemic stimulation of the immune system. Chlamydia sp., Streptococcus bovis and Salmonella sp. are pathogenic bacteria isolated from the spleen of bustards. Cytology preparations from bacterial septicemia cases are characterised by macrophages with bacterial phagocytosis. Granulocytopoeisis (elevated immature granulocytes) is occassionaly seen in the spleen imprint smears from bustards and could be the result of granulocytopoiesis in the bone marrow (Cooper 2008).

Septicemic pox is seen in bustards and cytology smears from the spleen are characterised by vacuolated plasma cells with karyorrhexis (Bailey 2006, Silvanose 2006). Viral infections are characterised by numerous reactive lymphocytes.

The skin of bird is composed of keratinized, stratified squamous epithelium. The normal cytology findings from skin include anucleated, cornified squamous epithelial cells, nucleated cornified squamous cells and variable amount of debris. Lesions caused by bacterial infections are represented by large number of inflammatory cells with bacterial phagocytosis and cellular debris. Non- pathogenic bacteria are often associated with the surface of squamous epithelial cells. Bumblefoot lesions are very common in falcons and Gram stain helps to provide more information about bacterial morphology. Avipox viruses causes cutaneous forms of lesions that appear as scab-like lesions around the eyes, beak, nares, hock joint, tarsometatarsus and feet. Cytology findings of pox lesions show swollen squamous epithelial cells with one or more large cytoplasmic vacuoles that push the cell nucleus to the margin of the cell. The large cytoplasmic vacuoles are called Bollinger bodies and contain tiny, round, pale eosinophilic inclusions that are called Borrel bodies (Campbell 2007). The presence of inflammatory cells in pox lesions

may be caused by superimposed bacterial or fungal infections. Lymphoid leucosis is occasionally seen in aspirate from the subcutaneous lesions is characterised by a marked number of immature lymphocytes with inclusions and mitotic structures. Fungal lesions are occasionally observed skin scraping samples, including *Trichophyton sp., Trichosporon sp. and Blastomycetes dermatitidis* from the beak and from the skin. Skin scraping smears may contain whole ectoparasites and eggs of lice, mites, ticks and flies

Normal cytology of the nares and choana of bustards and falcons are lined by stratified squamous epithelium. The trachea is lined with pseudostratified ciliated columnar epithelium with goblet cells. The columnar respiratory epithelial cells have prominent cilia at the large end of the cells that have staining affinity for eosin (Campbell 2007, Campbell 1993). The respiratory cells exfoliate singly or in clusters. Goblet cells are columnar cells that resemble ciliated respiratory epithelial cells with abundant cytoplasm, but no cilia. The normal cytology from the nares show superficial squamous epithelial cells with background debris and the choana shows intermediate squamous epithelial cells. The normal tracheal washing or swab cytology is poorly cellular with ciliated columnar respiratory epithelial cells and little background debris (Cooper 2008). The normal bacteria isolated from the nasal cavity of bustards includes Bacillus sp., Micrococcus sp., Staphylococcus simulans, S. saprophyticus, S. hyicus, S. auricularis, S. xylosus and S. cohnii. The normal bacterial flora isolated from the nasal cavity of falcons include Bacillus sp., Micrococcus sp., S. saprophyticus, S. hyicus, S. xylosus, Streptococcus sp., Corynebacterium sp. and Acinetobacter sp. The normal bacteria isolated from the trachea of falcons include Nocardia sp., coagulase negative Staphylococcus sp. and Pseudomonas diminuta (Silvanose CD 2006). Cytology smear from the trachea of normal bird shows low numbers of bacteria per field and elevated bacterial presence is considered to be abnormal.

Abnormal cytology of the upper respiratory tract (sinusitis, tracheobronchitis) shows inflammatory cells and infectious agents. Squamous cells from the choana may show nuclear changes associated with inflammation. Many plasma cells, macrophages and

lymphocytes are seen in smears of chronic inflammation. Macrophages often have a highly vacuolated cytoplasm, indicating increased phagocytic activity, and they often contain phagocytised material. An increase in heterophils is seen in acute inflammation. Septic sinusitis is indicated by the presence of intracytoplasmic bacteria within leucocytes. Chlamydia sp. commonly occurs as small intra-cytoplasmic inclusions within macrophages and epithelial cells (Harrison 1994). Tracheal cells with degeneration show loss of cilia, cytoplasmic vacuolation and karyolysis and may be seen in severe tracheobronchitis. An increase in the number of goblet cells and mucin background is often seen with tracheobronchitis. Mycotic involvement can be detected by the presence of fungal elements in the smear. Sinus or choanal aspirates that contain numerous eosinophils are suggestive of an immune-mediated inflammation. A foreign body upper respiratory tract disease (aspiration tracheobronchitis or pneumonia) may be revealed by the presence of large macrophages that form giant cells containing phagocytised foreign material (Campbell 1995).

Viral infections in the upper respiratory tract may show ciliated fragments of respiratory cells with a mononuclear leucocytic inflammatory response in cytology smears. Influenza virus may produce an accumulation of necrotic debris in the sinuses and trachea. Bacterial infections are commonly reported from the upper respiratory tract of bustards and falcons. The common bacterial pathogens identified from the upper respiratory tract of bustards include Pseudomonas aeruginosa, Proteus mirabilis, Streptococcus sp., Klebsiella pneumoniae, E. coli, Mycoplasma sp. and Chlamydia psittaci. The pathogenic bacteria identified from the upper respiratory tract of falcons include Staphylococcus aureus, Streptococcus bovis, Pasteurella multocida, Pasteurella haemolyticum, Pseudomonas aeruginosa, Bordetella avium, E. coli, Proteus mirabilis, Klebsiella pneumonia, Chlamydia psittaci, Mycoplasma anatis and Mycoplasma columborale. Aspergillosis is a common fungal disease in falcons caused by Aspergillus fumigatus, A. flavus and A. niger that may be isolated from the trachea of affected falcons (Silvanose CD 2011, Wernery R 2004). This disease is also occasionally reported from bustards (Bailey 2006). Syngamus trachea and Serratospiculum sp. are

nematode metazoa observed in the trachea of falcons. Metazoa infections have not been seen in the upper respiratory tract of captive bustards.

Normal cytology of the lung air capillaries and air sacs are lined by simple squamous epithelium. The normal lung imprint cytology reveals a marked amount of peripheral blood contamination and an alveolar-like pattern. Normal air sac cytology is poorly cellular with occasional non-cornified epithelial cells seen in a clear background. Abnormal cytology of the lungs and air sacs (pneumonia and air sacculitis) are characterised by numerous inflammatory cells and the causative agents. The air sacs are poorly vascularised and have no clearance mechanism (mucociliary blanket). Respiratory abscesses in the air sac of falcons can be produced by Aspergillus sp., Serratospiculum sp. and Mycobacterium sp. Smear preparation from some granulomata can be difficult and such lesions should be cut using a scalpel blade and then cytology preparations can be made for direct microscopy, staining, KOH preparation or lactophenyl aniline blue preparation.

Viral infections: Paramyxoviruses can cause respiratory signs in bustards and falcons. Influenza and herpes viruses are also reported from falcons and can cause respiratory signs (Samour 2000). Bacterial infections in the lower respiratory tract of bustards and falcons are caused by Pasteurella multocida, Pseudomonas aeruginosa, E. coli, Klebsiella pneum. pneumoniae, Staphylococcus aureus, Mycobacterium sp., Mycoplasma sp. and Chlamydia psittaci. Bacterial pneumonia and airsacculitis are indicated by the presence of many inflammatory cells and increased background material. Smears of acute inflammatory response due to bacterial infections show numerous heterophils while a chronic inflammatory response shows predominantly macrophages. Septic lesions show leucocytic phagocytosis of bacteria. Smears of chronic bacterial or chlamydial air sacculitis predominantly show macrophages. Plasma cells and lymphocytes may also be seen with chlamydial infections (Campbell 2007).

Aspergillosis and aspergillomas are the common fungal infections in the lower respiratory tract of falcons and are occasionally seen in bustards (Bailey 2006, Wernery 2004). Aspergillus fumigatus and A. flavus are the pathogenic fungi that are commonly isolated. A. nidulans, A. versicolor, and A. niger are also occasionally isolated from the air sacs and lungs of falcons. Cytological evidence of aspergillosis includes the presence of branching septate hyphae, conidiophores and spores. The cellular findings may be associated with chronic aspergillosis including plasma cells, heterophils, toxic heterophils, macrophages and giant cells. Metaplasia of air sac squamous lining cells is noticed in association with chronic aspergillosis. Acute aspergillosis is characterised by a predominance of heterophils and the smear does not show giant cell formation. Aspergillus sp. often produces granulomatous lesions called as aspergilloma and the cytology shows mixed inflammatory cells including heterophils, macrophages and macrophage giant cells (Silvanose 2006). Histoplasmosis is a fungal disease that caused by Histoplasma capsulatum and has been reported from falcons with airsacculitis. The clinical signs are similar to aspergillosis (Bailey 2006). Leucocytozoon toddi has been observed in the air sac of a falcon with air sacculitis and it is not documented in the literature as a cause of air sacculitis (Cooper 2008). This bird had a heavy parasitemia of L. toddi in the peripheral blood. L. toddi is a hematozoa reported from falcons and there is a dearth of knowledge about the pathogenicity. Cytology preparation from the air sac of this bird showed vacuolated air sac lining cells and gametocytes of L. toddi. So it is possible that it could have contributed to the air sacculitis of this falcon.

Serratospiculosis is an air sac disease caused by filarial nematodes called Serratospiculum sp. and Diplotriaena falconis. S. seurati is a common parasite identified in the Arabian penisula and North Africa, S. guttatum is restricted to Australia, S. congolensis is found in Equatorial Africa, S. chungi is found in China and S. tendo is found in Europe. Filarial worms are long, threadlike organisms that can be found bunched up like spaghetti inside the falcon's air sac. Filarid nematodes are difficult to

treat successfully because medication may cause dead worms to remain in the respiratory system and could cause problems as they decay or produce abscess. As a result, secondary bacterial infections (Pseudomonas aeruginosa, E. coli and Staphylococcus aureus) and fungal infections (aspergillosis) can occur. Highly vacuolated (net like), swollen air sac lining cells and Serratospiculum sp. eggs are commonly noticed in such air sac cytology preparations. Metaplasia of air sac squamous lining cells and squamous giant cells are seen in falcons with chronic serratospiculosis. Inflammatory cells are often seen in association with secondary bacterial and fungal infections (Silvanose 2006, Harrison 1994). Aspiration pneumonia can occur due to foreign body inhalation and the cytology smears show macrophages and macrophage giant cells that have engulfed foreign bodies (Campbell 1995, Campbell 1993).

The oral cavity of bustards and falcons is lined with cornified squamous epithelium. The outer layer of the epithelium is lined by anucleated squamous cells. In healthy birds, the normal exfoliation of the superficial cells of the epithelium that lines the upper alimentary tract reveals normal, mature squamous epithelial cells. Superficial cells usually exfoliate singly, and the cells are polygonal in shape, show varying degrees of cornification, often possessing angular or folded cytoplasmic margins, and with condensed pyknotic nucleus. The cells under the superficial layer are called intermediate cells. Intermediate squamous epithelial cells may be oval to polygonal, have a low nuclear to cytoplasmic ratio (N:C), and possess an oval, centrally located vesicular nucleus with finely granular chromatin(Cambell, 2007). A few intermediate epithelial cells can be observed in smears from the oral cavity of normal individuals. The oesophagus of bustards and falcons is lined with non-cornified stratified squamous epithelium. Normal cytology smears from the oesophagus show intermediate epithelial cells. They often occur in sheets and do not have folded cytoplasmic margins. The cells under the layer of intermediate cells are called basal cells. Basal cells are smaller than intermediate cells and are round with a deeply basophilic cytoplasm and high N:C ratio. Only a few basal cells may be seen in oesophageal smears of normal individuals. High numbers of basal cells indicate inflammation. Bustards not have a crop. The crop of falcons is lined with

intermediate squamous cells. Samples collected from the crop of falcons may shows superficial squamous cells that are contaminants from the oral cavity. A variable number of extracellular bacteria are present in normal cytological samples from the upper alimentary tract (Cambell, 1993, Cambell 1995). The normal bacterial flora isolated from the upper alimentary tract of bustards include Escherichia coli, Enterobacter sp., Klebsiella sp., Citrobacter sp., Staphylococcus sciuri and Staphylococcus cohnii. Escherichia coli, Nocardia sp., Pseudomonas diminuta and coagulase negative Staphylococcus sp. are the common bacteria isolated from the oral cavity of falcons. Thus, normal cytology smears from bustards and falcons may reveal a mixed bacterial background.

Inflammatory changes caused by viruses, bacteria, fungi and protozoa have been detected in the upper alimentary tract of bustards and falcons, while in falcons, metazoa eggs are also commonly detected. In certain cases, inflammatory lesions are noticed in the upper alimentary tract (stomatitis, oesophagitis) with a mucous discharge or white - yellow lesions. Cytology of inflammatory lesions reveals an increased number of inflammatory cells, and a variable number of squamous epithelial cells with degenerative changes. Cytology of nodular lesions often shows an increase in the amount of background debris. Septic lesions are indicated by the presence of leucocytic phagocytosis of bacteria. Basal cells are indicative of inflammatory lesions with ulceration of the epithelium. Acute inflammatory lesions are associated with a predominance of heterophils (70% of the inflammatory cells) with degenerative changes such as basophilia, vacuolation, degranulation, nuclear karyolysis and karyorrhexis. Chronic inflammatory lesions are associated with predominance of macrophages, and chronic active inflammatory lesions are associated with heterophils mixed with variable number of lymphocytes and macrophages. Cytological samples with inflammatory cells and large number of bacteria are considered abnormal. Mucous background or lesion debris was commonly found in such cases. Phase-contrast microscopic examination will help the rapid screening of inflammatory responses caused by bacteria, candida, protozoa and metazoan.

Diphtheritic pox lesions in the oral cavity of bustards are caused by virulent strains of Avipox virus and smears show pleomorphic vaculated squamous cells with peripheral nuclei. The large cytoplasmic vacuoles called Bollinger bodies, contain tiny, round, pale eosinophilic (Giemsa/Wright's stain) inclusions called Borrel bodies are observed. Cutaneous pox lesions are most commonly seen in falcons caused by Avipox falconi.

Bacterial infections are commonly reported from the upper alimentary tract of falcons and bustards. Staphylococcus aureus, Streptococcus sp., Pseudomonas aeruginosa, Pasterulla multocida, Salmonella sp., Campylobacter sp. and Chlamydia psittaci are the common pathogens reported from bustards and falcons. Inflammatory cells and epithelial cells are common findings in a mucus background associated with bacterial infections. Septic lesions reveal inflammatory cells containing intracytoplasmic bacteria. Septic inflammatory lesions reveal degenerate leucocytes, primarily heterophils. Mycobacterium sp. has been detected from the granulomatous lesions crop of a falcon.

Candidiasis is a common fungal disease of falcons and bustards that is usually confined to the upper alimentary tract. This disease is caused by fungus of the genus Candida. Candida albicans and C. tropicalis are the pathogenic species isolated from clinical specimens from bustards and falcons. This disease is usually associated with malnutriton, inhibition of normal bacterial flora caused by prolonged use of broadspectrum antibiotics and poor hygiene. Infected birds were listless and had a diminished appetite. Candida infections usually present with lesions in the oral cavity and oesophagus, characterised by white, circular ulcers with raised surface scabs that produce thickening of the mucosa. Very often no lesions may be noticed and cytology will help to diagnose candidiasis. Glossitis was also observed in an individual with chronic candidiasis. Direct phase-contrast microscopic examination of a sample suspension in normal saline is a quick and easy method to detect the presence of Candida sp . Samples can also be examined by direct light microscopy with the help of

lactophenol aniline blue stain. Candida albicans is an oval, budding yeast that produces a pseudohyphae (elongated budding cells resembling hyphae) both in tissue and exudates and in broth culture. Eosin and methylene blue stain (Neat stain, Rapi-Diff) are also used to stain to enable detection of exfoliated epithelial cells and Candida sp. in smears. In Gram-stained smears Candida sp. appears as Gram-positive, oval, budding and elongated budding yeast. Candidiasis is detected by the presence of many oval, thin-walled, narrowly based budding yeasts measuring 3 - 4µm in diameter, stained deeply basophilic with MGG. A marked amount of background debris is often present with candidiasis, but few inflammatory cells are present, unless mucosal ulceration has occured. The presence of pseudohyphae and blastospores indicate tissue invasion by Candida sp. Cytology preparations from the oral cavity of bustards with candidiasis showed numerous budding and hyphe formation of yeasts, epithelial cells including superficial, intermediate cells with inflammatory changes such as karyorrhexis, cytoplasmic vacuolation and multinucleation (giant cells) in a mucus background with scanty inflammatory cells.

Trichomoniasis is a common protozoal disease in the upper alimentary tract of bustards and falcons caused by Trichomonas gallinae. The organisms are best detected on a saline wet mount preparation under phase contrast microscope. They appear as motile piriform, flagellate protozoa with anterior four flagella and an undulating membrane. Lesions caused by Trichomonas sp. often reveal an increase in inflammatory cells and background debris. Some of the T. gallinae positive clinical cases were associated with secondary bacterial infections, white-yellow caseous nodular lesions, mucous discharges, exfoliation of epithelial cells and the presence of inflammatory cells (Harrison 1994, Teachout 2005).

Entamoeba sp. resembling to E. anatis has been found in bustards with clinical signs, including secondary bacterial infections, white-yellow caseous nodular lesions, mucous discharge, exfoliations of epithelial cells and the presence of inflammatory cells. The

clinical signs of infected birds varied according to the level of parasitism, duration of infection and associated bacterial or fungal infections. Endolimax sp. and non-pathogenic Entamoeba sp. were detected in the oral cavity without any clinical signs. While Naegleria sp were detected in the oro-pharynx of bustards with clinical signs including discharges and increased exfoliation of epithelial cells.

Metazoa infection caused by Capillaria sp. is reported from the oesophagus and crop of falcons. Metazoa eggs detected in the oesophagus and crop of falcons include Capillaria sp., Syngamus sp. and Serratospiculum sp. Adult worms of Serratospiculum sp. are found in the lungs and air sac, and the eggs are usually coughed from the lungs and swallowed to the alimentary tract. Adult worms of Syngamus sp. are found in the trachea of falcons and the eggs are coughed into the alimentary tract. Capillaria sp. produces oro-pharyngeal and oesophageal infections resulting in visible lesions or a thickening of mucosa. Mild oro-pharyngeal infection of Capillaria sp. produces yellow deposits on the mucosa of the pharynx, around the base of the tongue or at the inner corners of the mouth. Advanced oro-pharyngeal Capillaria sp. infections produces yellowish-brown, bean sized granulomas. Oesophageal Capillaria sp. infection causes thickening of mucosa and in advanced stages the lesion can ruptures through the skin of the neck, revealing accumulations of malodorous caseous exudate. Direct phase-contrast microscopic examination is a rapid evaluation of metazoa and it is more helpful than stained smears to identify metazoa eggs. Hypovitaminosis A has been detected in the oral cavity of falcons. Cytology of lesions from hypovitaminosis A cases showed large numbers of highly cornified squamous epithelial cells with little background debris and no evidence of inflammation, candida or protozoa.

Normal cytology of the intestine and cloaca of bustards and falcons are lined with columnar epithelial cells. The vent (cloacal opening) is lined by cornified, stratified squamous epithelial cells. The normal cloacal cytology is poorly cellular with a predominance of squamous epithelial cells. Many basal cells and erythrocytes are seen

in smears after traumatic injury. A variable quantity of bacteria including cocci and bacilli, urate crystals and background debris are also present in normal smears (Campbell 2007, 1993). The normal bacterial flora isolated from the lower alimentary tract of bustards include E. coli, Klebsiella oxytoca, Enterobacter sp., Citobacter sp., Serratia marcesens, Enterococcus casseliflavus, Enterococcus faecalis, Enterococcus faecium, Enterococcus durans, Staphylococcus sciuri, Aerococcus viridians, Micrococcus kristinae, and Proteus mirabilis. Staphylococcus aureus has been isolated from the faeces of bustards with heavy parasitism of T. gallinarum and C. gallinarum. Escherichia coli, Enterococcus sp, Micrococcus sp., coagulase negative Staphylococcus sp. are isolated from the cloaca of normal falcons. Thus the normal cytology smear shows a mixed bacterial flora including rods and cocci.

Inflammatory lesions in the intestine and cloaca show a predominance of inflammatory cells, erythrocytes and elevated exfoliation of columnar cells. Smears from the intestinal contents and lesions of dead birds should be submitted for cytological evaluation, while in live birds, faecal preparations in saline are used to assess intestinal cellular exfoliation and to determine the presence of parasites. Abnormal cellular exfoliation may be due to intestinal injury caused by parasitic or bacterial enteritis. Numerous erythrocytes are usually found in association with heavy trematode infection, amoebiasis and bacterial enteritis caused by Salmonella sp., toxic E. coli and Clostridium sp. Biliverdinuria may be noticed in falcons in association with aspergillosis, chlamydiosis and mycobacterium infections. Often greenish, semisolid or liquid faeces are noticed in association with these diseases. Metallic greenish faeces and biliverdinuria is seen in birds with lead toxicity. The presence of blood or mucus in the faeces should be considered abnormal.

Bacterial enteritis in the lower alimentary tract of bustards has been caused by Salmonella enteritis, Salmonella typhimurium, Salmonella reading, Salmonella arizonae, Salmonella kentucky, Clostidium perfringens, Clostridium botulinum, Enterococcus

avium, Salmonella albany, Vibrio cholera, toxic E. coli and Chlamydia psittaci. Pathogenic bacteria causing diseases in the lower alimentary tract of falcons include Salmonella enteritis, Salmonella typhimurium, Salmonella munster, Clostridium perfringens, toxic E. coli, Streptococcus bovis, Vibrio cholera, Chlamydia psittaci and Mycobacterium sp. Mycobacterium sp. produces intestinal granulamatous lesions. Smears should be made from the cut surface of granulamatous lesions for routine and AFB staining and culture in L.J media. Clostridium perfringens, toxic E. coli and certain Salmonella sp. cause intestinal haemorrhages. The cytological smears from such lesions are characterised by numerous columnar cells and predominantly uniform bacteria. The Gram stain is a useful procedure to differentiate Gram-positive and Gram-negative bacteria. Clostridium sp. is the Gram-positive bacteria associated with intestinal haemorrhage in falcons and bustards.

Intestinal Candidiasis is rarely seen in falcons and may due to invasion from the upper alimentary tract. Candida sp. budding cells may be present in normal faeces, but numerous Candida sp. hyphae and budding cells in fresh faeces should be considered abnormal. Long term broad spectrum antibiotic treatment may cause suppression of normal bacterial flora resulting invasion of Candida sp. Poor body conditions and unhygienic food will also results invasion of Candida sp. Cryptococcosis is not clearly defined in falcons and bustards. Cryptococcus sp. is found in the lower alimentary tract of bustards and falcons.

Protozoa are important causes of digestive tract disease in birds. However, the presence of protozoa in the digestive tract of birds does not always constitute a disease process and depends on host resistance, immune status, level of parasitism, pathogenicity of protozoa and concurrent bacterial, viral and fungal infections. Protozoa detected from the lower alimentary tract of bustards include Trichomonas gallinarum, Chilomastix gallinarum, Giardia sp., Caryospora sp., Isospora sp. and Eimeria sp. Low levels of T. gallinarum and C.gallinarum are found in the lower digestive tract of healthy

bustards without clinical signs. Protozoa detected from the lower alimentary tract of falcons include Caryospora falconis, Caryospora neofalconis, Caryospora megafalconis, Caryospora kutzeri, Isospora sp., Eimeria sp., Giardia sp. and Entamoeba sp. Coccidiosis is a common parasitic disease in falcons caused by Caryospora sp. Oocysts of Caryospora sp. are excreted through the faeces of infected birds and are easily identified in saline wet mount preparation or zinc sulphate flotation of faecal smears under phase-contrast microscopy. Caryospora sp. affected birds usually show weight loss and the faeces are initially soft, changing liquid brown, eventually becoming foul smelling and bloody. Giardiasis is caused by Giardia sp. resulting in diarrhea; and Entamoeba sp. causes amoebiasis resulting in bloody faeces and diarrhoea.

Metazoa including larva and eggs of nematodes; segments (proglottids) and eggs of cestodes; eggs of trematodes and acanthacephala are commonly found in the faeces of bustards and falcons. Serratospiculum sp., Diplotriaena falconis, Ascaridia sp., Capillaria sp. and Syngamus sp. are the nematode eggs detected from the faeces of falcons. Serratospiculum sp. and Diplotriaena falconis are most commonly identified from lungs and air sacs, often partially attached to serosal membranes. The thin-shelled, embryonated ova are coughed out of the trachea, then swallowed and excreted in the faeces. Syngamus sp. is usually found in the trachea and the eggs are coughed out of the trachea, then swallowed and excreted in the faeces. Capillaria falconis can infect the small intestine of falcons, resulting in inflammatory changes and destruction of the mucosa. Initial clinical signs may be mild and limited to diarrhoea. The nematodes reported from the bustards include Allodapa sp. from the caeca and Hartertia rotundata from the duodenum and jejunum. Hymenolepis exilis, Plagiorchis elegans and Claotaenia sp. are the common cestodes found in the alimentary tract of falcons. Strigea falconis is a trematode and Centrorhynchus cylindraceus is an acanthocephala identified from falcons. Cestodes are very common intestinal metazoa in bustards include Otiditaenia conoideis, Raillietina neyrai, Idiogenes sp., Ascometra vestita, Ascometra choriotidis, Hispaniolepsis falsata and Otiditaenia macqueenii. Mediorhynchus sp. is the acanthocephala identified from bustards (Bailey 2006).

Pathological changes of erythrocytes include hypochromasia, elevated polychromasia, microcytic/macrocytic anisocytosis, poikilocytosis, erythroblasts and basophilic stippling. Hypochromic erythrocytes are red blood cells with low haemoglobin content; and Romonowsky stained smears show pale coloured or colourless inner cytoplasm and yellowish-pink peripheral cytoplasm (Campbell 2007). According to the degree of staining intensity and percentage of hypochromic erythrocytes this can be classified as mild (5 to 10 cells/1000X), moderate (10 to 20 cells/1000x) or severe (more than 20 cells/1000x). Polychromatic erythrocytes are red blood cells with more basophilic cytoplasm than that of mature erythrocytes and the nuclear chromatin appears loosely packed (Campbell 1995). Reticulocytes are early stages of mature erythrocytes with more basophilic cytoplasm and have nucleus with loosely packed chromatin (Harrison 1994). In mature erythrocytes, the nuclear chromatin is tightly packed and stains darkly. The cytoplasm of reticulocytes contains stippling or reticulum and new methylene blue or brilliant cresyl blue can be used to demonstrate this bluish stippling or reticulum. One percent solution of new methylene blue in normal saline is mixed 1:1 with whole blood and incubated (37°C or room temperature) in a closed test tube for 10 minutes. One drop of this mixture can be examined as a wet preparation or dry smear. More than 5% polychromatic erythrocytes is considered significant and may due to regenerative anaemia. If significant numbers of polychromatic erythrocytes are present with a variable nuclear chromatin pattern, it is called erythropoiesis. Erythropoiesis can be classified as mild, moderate or severe according to the degree of polychromasia and nuclear immaturity. Above 5% and less than 10% of erythrocytes with polychromasia is classified as mild erythropoiesis, 10 – 20% of erythrocytes with polychromasia is classified as moderate erythropoiesis and more than 20% of erythrocytes with polychromasia is called severe erythropoiesis. In moderate erythropoiesis there may be a few rubriocytes seen. In severe erythropoiesis there may be many rubriocytes and a few rubrioblasts (Samour 2000). Poikilocytosis is a descriptive term for erythrocytes with variable shapes, including oval cells mixed with round, eliptical and tear-shaped cells (Bailey 2006). The tail region of a normal smear may show a few poikilocytes which are artefacts due to the pressure applied during the smearing technique. In such smears an

increased number of smudge cells are seen. Poikilocytosis can be classified as mild, moderate and severe according to degree and percentage of shape irregularity. Poikilocytosis is seen in iron deficiency anaemia and haemolytic anaemia. Anisocytosis is a descriptive term for erythrocytes of variable size and a blood smear shows normocytes with microcytes, or/and macrocytes (Wernery 2004). Microcytes are small sized erythrocytes and macrocytes are large sized erythrocytes. Anisocytosis can be classified as mild, moderate or severe according to degree and percentage of size irregularity. Macrocytic anisocytosis is noticed due to lead toxicity and bacterial toxaemia in bustards and falcons. Microcytic anisocytosis is seen in chronic diseases and malnutrition. Basophilic stippling is the description for basophilic dot like inclusion bodies that are present in the cytoplasm of erythrocytes. These inclusion bodies may be fine and numerous or scanty. Basophilic stippling in erythrocytes is observed in cases of lead toxicity in falcons.

Increased erythrocyte count is seen in primary and secondary polycythaemia. Relative polycythaemia mostly occurs due to dehydration. Absolute polycythaemia is further separated into primary and secondary. Primary polycythaemia is caused by an abnormal proliferation of stem cells (polycythaemia vera) and is poorly documented in birds. Secondary polycythaemia occurs due to decreased blood oxygen levels such as chronic respiratory diseases due to pulmonary infections or pulmonary hypersensitivity.

Anaemia is a decrease of the erythrocyte count and is due to blood loss (haemorrhage), increased erythrocyte destruction or decreased erythrocyte production. In general, all anaemias can be divided into two types, regenerative and non-regenerative. The presence of increased reticulocytes or polychromatic erythrocytes in the peripheral blood smear indicates regenerative anaemia. Regenerative anaemias are those in which there is active erythropoiesis to replace the lost cells, which results in a higher percentage of immature erythrocytes in the peripheral blood. If there are few or no reticulocytes in the peripheral blood smear, the anaemia is non-regenerative. Regenerative anaemia may be due to extra vascular haemorrhage or intra vascular haemolysis. Regenerative anaemias are characterised by the presence of polychromasia, reticulocytosis, macrocytosis, anisocytosis in the peripheral blood smear

(Campbell 2007). Haemolytic anaemia (increased erythrocyte destruction) may occur due to *Plasmodium* sp. infection, bacterial septicaemias, acute aflatoxicosis, and toxaemia. Severe lead toxicity and bacterial endotoxaemia also results in haemolytic anaemia. Haemolytic anaemias are often regenerative, and result in elevated polychromasia, macrocytosis, anisocytosis and reticulocytosis. Haemorrhagic anaemia can result from trauma, blood sucking ectoparasites, coagulopathies, gastointestinal parasitism or rupture of internal organs. Severe acute and chronic regenerative anaemias are characterised by anisocytosis and poikilocytosis. Non-regenerative anaemia (depressed erythrocyte production) can be associated with a number of chronic infectious diseases, such as tuberculosis, chlamydiosis, aspergillosis, trichomoniasis and chronic liver diseases. Certain nutritional deficiencies including iron and folic acid may cause hypochromic microcytic anaemia.

Leucocytosis is an increase in the numbers of white blood cells above normal range. Mild leucocytosis occurs due to stress, injury, tissue necrosis, acute bacterial infection, acute aspergillosis and acute trichomoniasis. Moderate leucocytosis occurs in yolk peritonitis, granulomatous disease and in some phases of septicaemia. Severe leucocytosis occurs with active chronic diseases including chlamydiosis, aspergillosis, trichomoniasis, tuberculosis and lymphoid leucosis. Leucocytopaenia is a decrease of white blood cells below normal range. Leucocytopaenia is seen in bustards in association with long term medication of doxycycline, overwhelming septicaemias and viremias (Bailey 2006).

Estimation of white blood cells count can be assessed from the blood smears to double check the total wbc count. Scan the stained blood smears on low power to check the distribution of cells and focus under 1000X (oil immersion) in an area where the cells are evenly distributed. Then, count the white blood cells found in 20 evenly distributed fields. As a general rule, do not use fields where clumps of white blood cells or large numbers of ruptured or smudged blood cells are found. Divide the total number of WBC found in those twenty fields by 20 thus finding the average number per field. For

example, the upper limit of normal WBC in saker falcon is 10 x 11 10^9/L and an average of one WBC (0 – 2) per 1000 X field is considered within normal limits. A WBC count more than the normal limits and up to 50% elevation is classified as mild leucocytosis and such blood smears (cells evenly distributed fields) may contain 1 – 3 leucocytes (average two) per field (1000X) throughout smear. A WBC count 50 to 100% elevation above normal limits is classified as moderate leucocytosis and a blood smear from such case may contain 2 – 4 leucocytes (average three) per standard field (1000X). A WBC count more than 100% elevation from the normal limits is classified as severe leucocytosis and blood smears from such cases may show four or more leucocytes per standard field (1000X). This estimation is dependent on the quality of the smear and will not be correct if smears are too thick or too thin. This is dependent on the amount of blood, spreader pressure and speed used for smear preparation. So each laboratory should standardise their own evaluation criteria.

Heterophilia is an increase in the number of heterophils in the peripheral blood circulation. Physiological heterophilia may occur because of stress, injury or tissue damage. Pathological heterophilia may be seen in acute bacterial, chlamydial, fungal and *Trichomonas gallinae* infections in bustards and falcons (Silvanose 2006). Heteropaenia is a decreased number of heterophils in the blood circulation. This is due to depressed bone marrow as part of a syndrome of pancytopaenia (decreased numbers of WBC's, RBC's and thrombocytes) or depression of granulocytosis. Bone marrow suppression or damage and viraemia may cause heteropaenia. Shift to the left is classified according to the loss of nuclear lobulation and loss of cigar shaped cytoplasmic granulation. In a normal smear the proportion of heterophils is less than 10% with band forms and non-lobulated nuclear forms. When the non-lobulated and band form heterophils exceed more than 10% and is less than 25%, it is called a mild shift to the left, 25 – 40% is called a moderate shift to the left and when it is more than 40 % it is called a severe shift to the left. In a moderate shift to the left few metamyelocytes (mesomyelocytes) may be seen (Fig 16.19). In a severe shift to the left myelocytes and metamyelocytes may be seen. The main function of heterophil is first line defence against foreign materials. The shift to the left occurs in severe infections,

since new cells are released into the circulation from the marrow. So, in severe infections there may be metamyelocytes and myelocytes in the peripheral blood circulation. In active chronic infections, including aspergillosis, tuberculosis, chlamydiosis in bustards and falcons there is often a moderate to severe heterophilic shift to the left and a mild to moderate monocytosis seen. The presence of myelocytes and metamyelocytes in the peripheral blood indicates a severe condition (severe active chronic infections) and is usually associated with excessive peripheral utilisation of mature heterophils.

Toxaemia will cause loss of nuclear lobulation and toxic cytoplasmic granulation. Due to the toxic changes the cytoplasm becomes swollen, vacuolated and basophilic; and cytoplasmic granular dissolution will occur and leaving the round basophilic granules. Such heterophils are called toxic heterophils (Cooper 2008). The degree of heterophil toxicity is reported as 1+, 2+, 3+ and 4+ according to the cytoplasmic basophilia, vacuolation and degranulation. Heterophils with a 1+ toxicity are indicated by a slight cytoplasmic basophilia. Heterophils with a 2+ toxicity show a darker cytoplasmic basophilia and partial degranulation. Heterophils with a 3+ toxicity show a darker cytoplasmic basophilia, moderate degranulation and cytoplasmic vacuolation. Heterophils with a 4+ toxicity show deep cytoplasmic basophilia, moderate to severe degranulation (often showing large, fused granules), cytoplasmic vacuolation, karyorrhexis or karolysis. Toxic heterophils are seen in bustards and falcons with lead poisoning, infections caused by toxic *Escherichia coli, Clostridium perfringens, Aspergillus flavus, A. fumigatus, Mycobacterium avium* and *Chlamydia psittaci*. The presence of immature heterophils (shift to the left) should not be confused with toxic heterophils. In association with toxin producing bacterial infections a normal or mild leucocytosis with toxic heterophils may be found and this may be due to the destruction of leucocytes by endotoxin (Samour 2000). Refrigerated samples more than 24 hrs old also shows degranulation of heterophils. Long term administration of certain drugs also results vacuolation and degranulation. Eosinophilia is an increase of eosinophils above the normal range. Heavy parasitic infections, especially cestodes may cause eosinophilia. Degranulation of granulocytes is often seen in smears as a cytological

artifact due to granule dissolution caused by prolonged methanol fixation or when there are impurities of the fixative. The ideal time to fix smears in methanol is less than 10 minutes.

Lymphocytosis is an increase in the number of lymphocytes above the normal range. In chicks and young bustards lymphocytes will be higher than in adults (Fig 16.24). Viral infections in bustards and falcons may cause lymphocytosis. Paramyxovirus and avipox virus infections may cause mild to moderate lymphocytosis. Severe leucocytosis with severe lymphocytosis is noticed in bustards with lymphoid leucosis. Reactive lymphocytes are large lymphocytes with reactive changes including deep basophilic (blue) cytoplasm, cytoplasmic blobbing and vacuolisation. Reactive lymphocytes are seen in birds with viral infections including avipox and Newcastle disease. Lymphocytes transform into reactive lymphocytes in the presence of antigenic stimulation. This is often associated with tuberculosis, chlamydiosis and aspergillosis. Azurophilic granules (magenta granules) are often seen in the cytoplasm of lymphocytes of normal bustards and their elevated presence is considered abnormal. Plasma cells are immature large lymphocytes with large eccentric nuclei with deep blue (basophilic) cytoplasm. Plasma cells are usually not seen in peripheral blood smears and are rarely seen in some infectious diseases. The presence of lymphoblasts in the peripheral blood is considered abnormal. Lymphoblasts are seen in bustards with lymphoid leucosis. A relative lymphocytopenia occurs with a severe heterophilia. In fact, the heterophils are present in such large numbers that the relative lymphocyte count appears low. Monocytosis is an increase of the numbers of monocytes above the normal range and it indicates either chronic infections or active chronic infections. Monocytosis is obvious in birds with tuberculosis, chronic aspergillosis and chlamydiosis. Mild monocytosis is seen in bustards with chronic pox in association with lymphocytosis. Thrombocytosis is an increase of the numbers of thrombocytes above normal range. In some infections, there is thrombocytosis with megathrombocytes.

Haemoproteus tendeiroi, *H. telfordi* and *Leucocytozoon* sp. are the haematozoa found in bustards (Figs 16.30 to16.32). Low numbers of *Haemoproteus* species are seen in

healthy birds without any clinical signs. Gametocytes of *Haemoproteus* sp. are mostly observed in erythrocytes. *H. tinnunculi*, *Leucocytozoon toddi*, *Babesia* sp., *Plasmodium* sp., *Trypanosoma* sp. and *Microfilariae* sp. are the blood parasites observed in falcons. Parasitemia with *Leucocytozoon toddi*, *Trypanosoma* sp. and *Microfilariae* sp. should be reported per smear and the unit is expressed number of hematozoa per μl (quantity of blood used for the smear). *Babesia* sp., *Plasmodium* sp. and *Haemoproteus* sp. should be reported as the percentage of infected erythrocytes. *Haemoproteus* sp. are halter-shaped intra-erythrocytic gametocytes that encircle the nucleus, appear pigmented and occupy more than 50% of the cytoplasm. *Haemoproteus* sp. are non-pathogenic to bustards and falcons with low parasitemia. Gametocytes of *Leucocytozoon* sp. are elongated intra-erthrocytic forms that deform the host cell and are usually non-pathogenic to falcons and bustards with low parasitemia. High parasitemias of *Leucocytozoon* sp may be pathogenic to falcons*. Plasmodium* sp. cause avian malaria, and are elongated or round intra-erythrocytic gametocytes that often displace the nucleus, appear pigmented and occupy less than 25% of the cytoplasm. Trophozoites are small and round intra-erythrocytic stage with 'signet ring' appearance. Round intra-erythrocytic schizont stages containing darkly stained merozoites. *Trypanosoma* sp. and *Microfilariae* sp. are pathogenic if organisms are very numerous (Harrison 1994).

Diagnostic pictures of peripheral blood smears in mild lead toxicity (<20 μg/dl in blood), RBC's are normochromic and normocytic. In moderate to severe lead toxicity RBC's are hypochromic and may show anisocytosis with macrocytes. Polychromatic erythrocytes are present with erythropoiesis and basophilic stippling. Heterophils shows toxic changes. Leucocyte count may be normal/mild elevated with heterophilia.

In acute aspergillosis, RBC's are normochromic and normocytic. WBC's shows mild to moderate leucocytosis with heterophilia. Few reactive lymphocytes are seen. In chronic aspergillosis, RBC's are normochromic to mild hypochromic; show mild anisocytosis

and mild poikilocytosis. Polychromatic erythrocytes are present within normal limits or slightly elevated and may shows mild erythropoiesis. WBC shows moderate to severe leucocytosis with heterophilia. Mild monocytosis may be seen in chronic cases. Heterophils may show mild to moderate toxic changes. Few to many reactive lymphocytes are present.

Diagnostic pictures of peripheral blood smears in bacterial infections (Salmonellosis, Pasteurllosis, bumblefoot infections caused by enterobacteriaceae and *Staph. aureus*, conjunctivitis, respiratory infections caused by *Bordetella* sp and *Pseudomonas aeruginosa*) show RBC's are normochromic or mild hypochromic and normocytic or shows mild anisocytosis. Polychromatic erythrocytes are present within normal limits or elevated and may show mild erythropoiesis. (Chronic infections may associate with hypochromasia, polychromasia and anisocytosis). WBC's show mild (acute) to moderate leucocytosis (chronic) with heterophilia. In Chlamydiosis mild to severe leucocytosis with heterophilia and heterophils with toxic changes may be seen. Enteritis caused by toxic *E. coli* and *Clostridium perfringens* infections may show mild hypochromic erythrocytes with anisocytosis (macrocytes). WBCs show mild to moderate leucocytosis with heterophilia and toxic heterophils. Often, a normal WBC count with heterophilia and toxic heterophils is observed in association with Clostridiosis. As a result of endotoxemia (Clostridiosis and toxic *E. coli* infections) necrotic leucocytes may seen. A shift to the left occurs in the peripheral blood smear in chronic infections. Often, a normal WBC count with heterophilia is observed in association with overwhelming bacterial septicaemias.

Tuberculosis response shows RBC's are mildly hypochromic and show mild anisocytosis with few to many microcytes and mild poikilocytosis. Polychromatic erythrocytes are present within normal limits or are elevated and may show mild erythropoiesis. WBCs show moderate to severe leucocytosis with heterophilia and monocytosis. Heterophils show toxic changes. A few reactive lymphocytes may be present.

Acute Trichomoniasis response may show are normocytic and normochromic erythrocytes. In bustards, WBC show mild to moderate leucocytosis with heterophilia. In falcons, a normal WBC count or mild leucocytosis is seen in association with acute trichomoniasis. Chronic trichomoniasis in bustards may show normocytic erythrocytes with mild to moderate hypochromic. WBC's show moderate to severe leucocytosis with heterophilia and mild monocytosis. A shift to the left may be seen in chronic cases. In falcons, normocytic and normochromic erythrocytes are seen. WBC's show mild to moderate leucocytosis with heterophilia. In falcons, less haemoresponse is produced by trichomonasis compared to bustards.

Viral diseases (Avipox and Newcastle diseases): The WBC count may be normal or decreased (except in lymphoid leucosis in bustards) and show lymphocytosis and reactive lymphocytes. Mild monocytosis and many reactive lymphocytes may be present in chronic pox cases. Lymphoid leucosis: WBC's show moderate to severe leucocytosis with severe lymphocytosis. Few to numerous reactive lymphocytes and lymphoblasts are present. Heterophils show toxic changes.

Chapter 7: Conclusion

MGG is the first choice of stain to study the morphology of the cell and special stains should be done if required. Gram stain helps to differentiate the bacterial pathogen and Z-N will help to AFB rods which cause tuberculosis.

Arthritis is the one of the common synovial disease in falcons and bustards. Inflammatory cells, fibrin strands and bacteria are the findings in inflammatory conditions caused by *Mycoplasma* sp, *Staphylococcus aureus* and *Streptococcus* sp. Pox lesions are associated with Bollinger bodies in squamous cells. Gout is characterized by urate crystals. Conjunctivitis is the common conjunctiva disease in falcons and bustards caused by bacteria, pox virus, cryptosporidia and eye flukes. In cytology smears, numerous inflammatory cells noticed in association with bacteria, chlamydia and cryptosporidia. Pox lesions are associated with Bollinger bodies in squamous cells.

Pericarditis and gout are the common cardiac diseases reported from birds. Pericarditis characterized by inflammatory cells and bacteria, while gout is characterized by urate crystals. Major renal diseases found in bustards and falcons were bacterial septicemia, septicemic pox, avian leucosis and gout. Reactive renal cells were found in infectious diseases, while urate crystals are found in cases of gout. This study concludes the gross pathology of kidney in relation with diseases and cytology. So this study is a useful guideline to diagnose disorders in spleen of avian patients during endoscopy procedure. Hepatitis causes mortality in bustards and falcons, which is caused by mainly virus and bacteria. Inflammatory cells including heterophils and kupffer cells are characteristics of hepatitis. Non-inflammatory diseases such as fatty liver and amyloidosis are also commonly seen in captive birds. Spleenomegaly was found in association with bacterial septicemia, herpes, septicemic pox, avian leucosis, chlamydiosis and tuberculosis. Lymphoid cells are found in normal spleen imprints while reactive lymphoid cells and plasma cells are seen in reactive spleen. In septicemic

cases, bacteria also present along with reactive cells. This study concludes the gross pathology of spleen in relation with diseases and cytology. So this study is a useful guideline to diagnose disorders in spleen of avian patients during endoscopy procedure.

An infection in the upper respiratory tract always shows exfoliation of lining cells with mucous which is produced by goblet cells. Inflammatory cells including heterophils and macrophages are characteristics of all infectious response but giant cells also seen in fungal infections. Cytology is a rapid diagnostic method which provides the information about the causative agents and thus proper effective treatment can start immediately to avoid further lower respiratory complications such as airsacculitis and pneumonia. Aspergillosis is a common fungal infection seen in the lower respiratory tract of falcons. Inflammatory cells including heterophils and macrophages are characteristics of all infectious response but giant cells also seen in fungal infections. Lower respiratory tract diseases cause mortality in bustards and falcons. Thus cytology is a rapid diagnostic method which is useful for the differential diagnosis to save the birds from lower respiratory complications such as airsacculitis and pneumonia.

Diagnostic cytology is an easy tool for differential diagnosis of acute and chronic inflammatory responses. Acute inflammatory response includes predominant of heterophils while chronic inflammatory response includes predominant of macrophages. Diagnostic cytology provides the information about the causative agents and thus proper effective treatment can start immediately. It is is an easy tool for differential diagnosis of bacterial enteritis and parasites of lower alimentary tract. Heavy infection of parasitic infection only cause pathology mainly blood and mucous in the feces. Diagnostic cytology provides the information about the causative agents and thus proper effective treatment can start immediately.

Bumble foot diseases are not seen in bustards. Bumble foot infections are common in captive falcons. Cytology evaluation is helpful for differentiation of infectious and non-infectious bumble foot cases. Pox lesions and bacterial abscess can be differentiated by the presence of Bollinger bodies and bacterial colonization respectively.

Ectoparasites are seen in sick falcons and bustards and among them lice are common in sick falcons.

Bibliography:

1. Bailey TA (2006) Bacterial Diseases. In: Bailey TA (ed) Diseases and Medical Management of Houbara Bustards and other Otididae, Ch 18. National Avian Research Center, Abu Dhabi.

2. Bailey TA (2006) Viral Diseases. In: Bailey TA (ed) Diseases and Medical Management of Houbara Bustards and other Otididae, Ch 18. National Avian Research Center, Abu Dhabi.

3. Bailey TA, Silvanose CD (2006) Parasitic Diseases. In: Bailey TA (ed) Diseases and Medical Management of Houbara Bustards and other Otididae, Ch 18. National Avian Research Center, Abu Dhabi.

4. Campbell TW, Ellis CK (2007) Avian and exotic Hematology and Cytology, 3rd edn. Blackwell publishing, USA.

5. Campbell TW (1993) Cytodiagnosis in raptor medicine. In: Redig PT, Cooper JE, Remple JD, Hunter DB. Raptor Biomedicine, pp. 11–14. University of Minnesota Press, Minneapolis.

6. Campbell TW (1995) Avian Hematology and Cytology, 2nd edn. Iowa State, University Press, Ames, IA.

7. Cooper JE (1994) Biopsy techniques. Seminars in Avian and Exotic Pet Medicine 3: 161–165.

8. Cooper JE, Silvanose C (2008) Avian Medicine, 2nd edn. Mosby, Elsevier, 3: 72 - 78.

9. Dorrestein (2008) Book Review: Cytology, an issue of veterinary clinics. Exotic pet, Veterinary Pathology.

10. Lierz, M (2003). Avian renal disease: pathogenesis, diagnosis, and therapy. Vet Clin Exot Anim 6 (2003) 29–55.

11. Lumeij JT. (1994). Nephrology. In: Ritchie, BW, Harrison, GJ and Harrison LR (eds). Avian Medicine: Principles and Application: 539-555. Wingers Publishing Inc, Lake Worth, USA

12. Harrison Gj, (1994) Cytology, Avian Medicine: Principles and application, wingers publishing.

13. Nabipour A, E. Alishahi, M. Asadian (2009), Some Histological and Physiological Features of Avian Kidney, J. Appl. Anim. Res. 36 : 195-198

14. Neil AF (2000). Neoplasms of Birds of Prey, Raptor Biomedicine III 127-145, Zoological Education Network.

15. Pinches M (2005). First steps in cytology; non inflammatory cell types. UK vet 10(5): 78-81.

16. Pinches M (2005). First steps in cytology; the examination, and beyond. UK vet 10(4): 89-92.

17. Pinches M (2005). Increaseing information yield in cytology. UK vet 10(3): 96-98.

18. Pollock C (2006), Diagnosis and Treatment of Avian Renal Disease. Vet Clin Exot Anim 9:107–128

19. Reece, W.O. 2004. Dukes' Physiology of Domestic Animals. Twelfth Edition, Cornell University Press, Ithaca, PP: 107-113.

20. Silvanose CD, Bailey TA (2006) Bustard diagnostic cytology. In: Bailey TA (ed) Diseases and Medical Management of Houbara Bustards and other Otididae, Ch 7. National Avian Research Center, Abu Dhabi.

21. Teachout DJ (2005) Cytological sample analysis and interpretation. National Wildlife Rehabilitator's Association, USA. Topics in Wildlife Medicine. Clinical Pathology 1: 43-45.

Appendix: 1

Table 1 Cytology stains.

Stain	Results
MGG	Cytoplasm of erythrocyte – yellowish pink, Nucleus of erythrocyte – bluish violet, Nucleus of leucocyte and epithelial cells - purple to violet, Cytoplasm of superficial cell - pinkish blue, Cytoplasm of intermediate and basal cells - blue, Heterophil granules- red-lilac, Eosinophil granules - red orange, Basophil granules - dark purple, Cytoplasm of monocyte - light blue, Cytoplasam of lymphocyte - medium blue, Micro-organisms - blue to violet, Protozoa - cytoplasam blue to violet and chromatin pink.
Wright Or Wright-Geimsa	Erythrocytes-yellowish red cytoplasm with dark violet nucleus, Heterophils – pale to dark violet nucleus and colourless cytoplasm with red-orange, rod shaped granules and lymphocytes-dark purple nucleus with pale blue cytoplasm.
Z-N stain	Acid-fast bacilli – pink and non acid fast bacteria and cellular materials-blue.
Kinyoun stain	Acid-alcohol fast bacilli appear red against a blue background.
Indian ink	Indian ink visualises the large capsules of *Cryptococcus neoformans.*
Gram stain	Gram positive- violet colour Gram negative - red/pink colour
Machiavello's stain	Chlamydial elementary bodies (0.2 - 0.3µm) stain red and the larger initial bodies (0.2-1µm) stain blue.
Giminez stain	Chlamydial inclusions (0.2 - 0.3µm) are circular and stain red against green background. Chlamydia may be found both

	intracellularly and extracellularly.

Appendix: 2

Table2. Gram stain interpretations

Staphylococci sp.	Gram-positive cocci in groups.
Streptococci sp.	Gram-positive cocci in chains.
Pneumococci sp. and *Enterococcus* sp	Gram-positive cocci in pairs with lanceolate appearance.
Micrococci sp.	Gram-positive large cocci in singles, pairs and small clusters.
Bacillus sp.	Gram-positive bacilli with endospore. Bacillus anthrax appears in large chains and other bacillus species are in short chains.
Listeria sp.	Gram-positive short, thin coccobacillary to diptheroidal forms
Erysipelothrix sp.	Gram-positive, short, thin coccobacillary to dipthreoidal. May form long filaments.
Kurthia sp.	Gram-positive rods in long chains.
Clostridium sp.	Gram-positive bacilli with terminal or sub terminal spores.
Nocardia sp.	Gram-positive or gram variable pattern with beaded appearance of long filamentous bacteria with or without branching. Substrate mycelium on ageing fragments in to bacillary and coccoid forms.
Nocardiopsis sp.	Gram-positive or variable long filamentous forms. Aerial hyphae produce long chains of spores.
Streptomyces sp.	Gram-positive aerial mycelium with long chains of spores.
Rhodococcus sp.	Gram-positive substrate mycelium that undergoes rapid fragmentation into bacillary and coccoid forms.
Oerskovia sp.	Gram-positive fragments of substrate mycelium.
Actinomadura sp.	Gram-positive aerial mycelium with short chain of spores.
Dermatophilus sp.	Gram-positive rudimentary substrate mycelium fragments longitudinally and transversely to forming packets of eight coccoid or cuboid shaped cells.
Nesseria sp.	Gram-negative cocci in pairs or small groups.

N.elongata sp.	Gram-negative coccobacillary forms.
Branhamella catarrhalis	Gram-negative diplococci with adjacent sides are flat.
Veillonella sp.	Gram-negative cocci in pairs, short chains or small groups.
Haemophilus sp.	Gram-negative pleomorphic bacilli.
Pasterulla sp., *Yersinia* sp., *Francisella* sp. and *Bordetella* sp.	Gram-negative short bacilli.
Brucella sp.	Gram-negative rounded or oval cocco-bacilli.
Actinobacillus sp.	Gram-negative small bacilli.
Moraxella sp.	Gram-negative short bacilli in singles, pairs, and some times in short chains.
Enterobactereaceae sp. and *Pseudomonaceae*	Gram-negative straight bacilli.
Compylobacter sp.	Gram-negative curved/ 'S' shaped/ long spiral/gull winged bacilli.
Helicobacter sp.	Gram-negative helical or spiral bacilli.
Flexispira sp.	Gram-negative spiral bacilli.
Listonella sp.	Gram-negative curved bacilli.
Vibrio species sp.	Gram-negative curved rods.
Shewanella sp.	Gram-negative curved or straight rods.