CHAPTER 11

POST-MORTEM EXAMINATION AND BIOMEDICAL REFERENCE COLLECTIONS

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"The patient has been so completely taken to pieces that nobody is able to look on him again as a whole being. He is no longer an individual, but a jumble of scientific data."

Kenneth Walker (The Circle of Life)

11.1 Organisation of a Bustard Post-mortem Examination

11.1.1. Transport to laboratory

Freshly dead carcasses are the most suitable cases for post-mortem examination. Decomposition (autolysis) is a problem in the hot climates of the Middle East. If a dead bustard has to be transported over a long distance to a laboratory, it should be chilled at +4ºC and then submitted in an insulated box packed with ice. In this way it will stay fresh for several hours. Carcasses intended for post-mortem examination should not be frozen because freezing makes histopathological examination difficult by causing ice crystals, which disrupt tissue architecture. The problems associated with decomposition, however, can often be worse than the ice-crystal artefacts associated with freezing. When receiving a carcass the likelihood of these two problems has to be evaluated, and a decision made whether to freeze it or not. A markedly autolysed carcass is typically of less use than a frozen one. Note that frozen carcasses are still of great value for the evaluation of morphological abnormalities, toxicological conditions, and certain infectious conditions. Ideally, qualified staff should conduct post-mortem examinations on-site. If carcasses or samples need to be dispatched to a distant laboratory, it is important to consider local postage and customs regulations as well as CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and animal health legislation.

11.1.2. Humane killing

If a sick bird has to be euthanased for post-mortem examination, the most humane way is to kill it by lethal injection administered intravenously or intraperitoneally. Failing that bustards can be killed with a heavy blow to the back of the head and then bled out from severed carotid blood vessels. Bustards should not be euthanased by simply severing the head or dislocating the neck without stunning. This may cause severe and prolonged pain, because it has been found in domestic fowl that the brain remains responsive for up to 30 seconds after decapitation without stunning (Gregory and Wotton 1986, 1990). Ideally birds can be bled out under anaesthesia by the intra-cardiac route and given a lethal injection when sufficient blood for clinical investigations and banking has been collected.

11.1.3. Recording post-mortem findings

Unless all findings are recorded in a standard and retrievable format and archived materials accurately labelled, the post-mortem examination may provide a few short term answers but will be of little use in the long term. A customised post-mortem examination record form has been designed for this purpose (Chart 11.1). Well-organised records and archives of pathological material are of great assistance in furthering our

understanding of health and disease in poorly studied species such as bustards. It is standard practice to record findings as objectively as possible, with the examiner saving any subjective interpretations for a commentary and summary section at the end of the objective description. Following a standard technique is a useful process, but it is important that the examination does not simply become a dissection and collection of samples. Careful inspection is required during the examination. If the examiner is unsure of the significance of a finding a careful objective description, supported by a photograph, will enable others with greater experience and expertise to help resolve any uncertainties.

11.1.4. Bird identification

Each case should be given a unique case number. This can be simply a serial accession number, a collection identification number, or a number based on other coded information. An example of a coded number is SRC/HB/170403/3, for a houbara bustard examined at NARC on 17 April, 2003, the third bird examined that day. Any ring number or identification device on the bird should be read and noted on the form. The origin (e.g. captive-bred) should be noted if known, and the bird's location at death should also be noted for epidemiological purposes.

11.1.5. History

The clinical history of each bird should be obtained from the attending clinician or the bird's records prior to examination. This will allow the veterinarian conducting the investigation to decide which samples are of greatest importance and which organ systems deserve special consideration during the examination. A summary of the relevant clinical details should be added to the post-mortem record sheet.

11.1.6. Other procedures before post-mortem examination

It may be useful to carry out some measurements for biometric studies prior to dissection. In all cases it is essential to weigh the bird. Radiography of the carcass is a useful way of screening for nutritional bone disease, skeletal lesions, foreign bodies, or in the case of birds originating from the wild, gunshot. Photographs of external lesions or of wings for moult records may be taken at this stage.

11.2. Examination of the Carcass

11.2.1. External examination

The bird should be checked for external parasites, external trauma, general carcass condition and any external lesions such as discharges, fractures, wounds etc. A small magnifying lens can assist in the examination of the plumage, particularly if it is one of the smaller bustard species or a chick. If the bird is still alive, awaiting euthanasia, then a blood sample for serology, biochemistry, haematology, culture or parasitology can be taken as required. Frozen serum is useful for any retrospective surveys and should be collected if at all possible. If any skin lesions suggestive of dermatophytosis are found, the whole carcass can be examined under a Wood's lamp in a darkened room (Keymer 2000). Ectoparasites can be collected and stored in 70% ethyl alcohol. Feather plucks might be considered at this stage, as well as sampling of skin for histology.

11.2.2. Post-mortem technique

It is important to examine all dead birds thoroughly, even if the cause of death is known, to check for unexpected lesions and contributing disease factors. After the external examination the bird is weighed and dipped in a bucket of detergent or disinfectant (keeping the head above water to prevent entry of fluid into the oesophagus or trachea). This will keep dust and feather spread to a minimum, making the examination, cleaner, easier and safer, by reducing spread of aerosols. Ideally the bird should be examined in a microbiological safety cabinet (Class 2) to minimise the risk of transfer of zoonotic diseases to the pathologist.

With the bird on its back, the skin is incised along the ventral midline from sternum to the vent and then stripped back manually to expose pectoral muscles and abdominal wall. The skin is pulled apart up the neck to expose cervical structures. The skin may be removed completely if bruising or other injuries are suspected. Any subcutaneous lesions are noted, together with condition of pectoral muscles. An incision is made in the abdominal midline and the body cavity inspected without disturbing the viscera. The pectoral muscles are incised down the left side of the bird and stout scissors are used to cut through the ribs cranially. Bone forceps are used to cut through coracoid and clavicle at the most cranial end. The sternum is then reflected over to one side, with incision of attached air sacs or muscle as necessary. The entire body cavity is now exposed with all the internal organs *in situ*. Figures 11.1 to 11.6 illustrate some post-mortem findings in bustards. Additional post-mortem images are presented in Chapters 16 to 22.

Chart 11.1. Example of post mortem form for bustards used at the National Avian Research Center. Page 1 of 2

Carcass Post Mortem Examination Form

Bird ID [_____]

Submission information (Please fill / tick √)

Species [_____] **Sex** [_____] **Ring code** [_____] **Ring colour** [_____] **Ring leg:** L ( ) R ( )
NARC site: AAZ ( ) SRS ( ) **Others** [_____] **PIT No** [_____]
Date of death [__/__/__] **Time found** [_____] **Circumstance of death:** Anaesthetic associated ( ) Euthanasia ( )
Restraint associated ( ) Environmental conditions ( ) Infection associated ( ) Injury from cage mate ( )
Predation ( ) Self-inflicted injuries ( ) Death in transit ( ) Other ( )

Laboratory use only

Post mortem data (Please fill / tick √)

PME date [__/__/__] **Carcass condition:** Fresh ( ) Refrigerated ( ) Frozen ( ) Decomposed ( )
Clinical summary [_____
_____
_____]

Post mortem condition data (Please fill / tick √)

Weight [_____g] **Pectoral muscle condition:** Obese ( ) Good ( ) Fair ( ) Poor ( ) Emaciated ( )
Feather condition: Good ( ) Poor ( ) **Ectoparasites:** None ( ) Lice ( ) Ticks ( ) Other ( ) specify [_____]
Condition comments [_____]

Post mortem systematic examination

(Circle number and describe lesions under notes below) Key : **NAD** (Nothing abnormal detected) **NE** (Not examined)

Number				Number			
	1. Body cavity	NAD	NE		7. Musculoskeletal system	NAD	NE
	2. Cardiovascular system	NAD	NE		8. Nervous system	NAD	NE
	3. Digestive system	NAD	NE		9. Reproductive system	NAD	NE
	4. Endocrine system	NAD	NE		10. Respiratory system	NAD	NE
	5. External examination	NAD	NE		11. Urinary system	NAD	NE
	6. Lymphoreticular system	NAD	NE		12. Other examinations [		]

No	Organ	Location	PM Findings
9	Testis ( ) Ovary ( )	L ( ) R ( )	**Gonadal activity:** Very active/cycling ( ) Active ( ) Inactive ( ) Immature ( ) **Gonad score:** 0 ( ) 1 ( ) 2 ( ) 3 ( ) 4 ( ) 5 ( )
1	Abdominal fat	Entire	

Chart 11.1. Example of post mortem form for bustards used at the National Avian Research Center. Page 2 of 2.

Post mortem systematic examination (continued)

Gross PME findings/diagnosis

Post mortem summary

Post mortem number [ ]

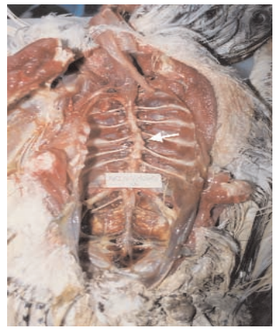
Not For Dbase Inputting

Reference samples taken: Code: Fo (Formalin / fixed) Fr (Frozen) AL (Alcohol)

Tissue/organ	FR	FO	AL	Tissue/organ	FR	FO	AL	Tissue/organ	FR	FO	AL
Eye				Pancreas				Brain			
Eyelid				Liver				Sciatic nerve			
Cere				Gall bladder				Brachial nerve			
Beak				Spleen				Heart			
Tongue				Bursa				Aorta			
Oro-pharynx				Thyroid				Pulmonary artery			
Crop				Parathyroid				Pectoral muscle			
Oesophagus				Adrenal				Bone femur			
Proventriculus				Kidney				Joint hock			
Gizzard				Ureter				Wing			
Duodenum				Trachea				Faeces			
Jejunum				Lung				Abdominal fat			
Ileum				Airsac				Endoparasites			
Caecum				Testis				Ectoparasites			
Rectum				Ovary							
Cloaca				Oviduct							

Reference comments [ ]

Veterinary surgeon [ ]



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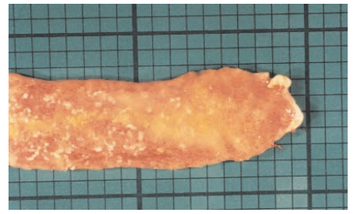

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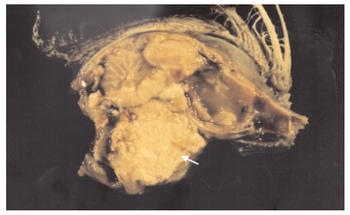

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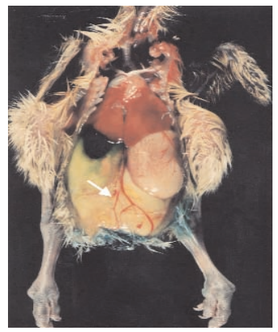


11.6

11.1 Kyphosis (arrow) in a wild caught adult houbara bustard (Photo credit Tom Bailey).

11.2 Catarrhal enteritis in a segment of small intestine with an endoparasite burden (Photo credit Tom Bailey).

11.3 Large granuloma (arrow) caused by *Trichomonas gallinae* in the roof of the oropharynx of a houbara bustard (Photo credit Tom Bailey).

11.4 Diphtheritic lesions (arrows) in the roof of the buccal cavity of a houbara bustard. This is the wet or diphtheritic form of avipox and can be confused with other causes of oral lesions including trichomonosis, hypovitaminosis A and bacterial stomatitis (Photo credit Tom Bailey).

11.5 Spinal cord deformity (arrow) in a white bellied bustard chick that failed to hatch (Photo credit Tom Bailey).

11.6 Yolk sac infection (arrow) in a 3 day old kori bustard chick. *Staphylococcus* sp. was isolated from the yolk sac and samples of liver. Reprinted with kind permission of the Association of Avian Veterinarians, *Journal of Avian Medicine and Surgery*. 11: 166-174, 1997. (Photo credit Tom Bailey).

11.2.3. Musculoskeletal system

The musculoskeletal system is examined by noting general carcass condition, incising joints and tendon sheaths as indicated. Radiology is very useful for examination of the skeletal system. Muscles should be incised and examined.

11.2.4. Sensory system

In bustards the openings of the external auditory meatuses are covered by feathers and are located caudal and just below each eye. The nares and the eyes should be examined for signs of any macroscopic lesions. If the eyes are required for histopathology they should be fixed without delay. Although special fixatives such as Bouin's or Davidson's fixatives are often suggested for fixation of eyes, prompt fixation in 10% neutral buffered formalin (the routine fixative) is perfectly adequate in most instances.

11.2.5. Cardiovascular system

The heart is examined for size, shape and pericardial fluid and then removed by cutting through the major blood vessels, leaving long vascular stalks attached to the heart. The amount of fat in the coronary groove of the heart is assessed as an indicator of the birds state of nutrition. The pericardium and epicardial surfaces are inspected and the heart is opened by making a transverse incision halfway down to obtain a cross section of the ventricles. The right ventricle is then examined by inserting scissors and cutting up and out of the pulmonary artery, checking the valve en route and checking the pulmonary arteries for nematodes. The right atrium is exposed in a similar manner. The heart is then turned over and opened by cutting up into the atrium and also into the aorta. Epicardium, myocardium, endocardium and valves are now available for inspection. At this stage the thyroids and parathyroids should be located and saved since they may be difficult to find later on in the dissection.

11.2.6. Endocrine system

The thyroids are paired, red ovoid structures located at the base of the neck in close association with the jugular veins. The parathyroids can be hard to find, but are small yellow structures closely associated with the thyroids. Enlargement of the parathyroids can occur with metabolic bone disease in many avian species. The thymus glands are bilateral, pale multilobular structures situated at the base of the neck at the cranial aperture of the thorax located near to the jugular vein. The adrenal glands are yellow, paired organs situated at the anterior end of each kidney.

11.2.7. Respiratory system

The infraorbital sinus is also opened from the nostril on each side and the upper beak is cut off with a pair of scissors, exposing the nasal cavities in cross section. The remaining respiratory system is examined by inspecting the larynx and then cutting the trachea open along its length down to the level of the tracheal bifurcation. The thoracic and abdominal air sacs are inspected. Airsacs collected for histopathology should be pressed on a piece of card to ensure that the airsac remains straight during fixation and that it can be easily found amongst the other organs. The lungs are removed by using the blunt end of a scalpel to gently dissect them away from the thoracic wall in a cranial to caudal direction. Transverse slices are made through the lungs.

11.2.8. Digestive system

The alimentary tract of all species of bustard consists of an oropharynx (cavum oralis and pharynx), oesophagus, proventriculus, ventriculus, small intestine (duodenum, jejunum and ileum), large intestine (paired caeca and a rectum) and cloaca. The oropharynx and choanal slit are common sites for lesions caused by a variety of agents. The oesophagus is then opened along its length from mouth to thoracic inlet using scissors. It is important to note that the cavum oralis of males of some species of bustard (e.g. kori bustard and great bustard) have an inflatable oesophageal diverticulum, the saccus oralis, which acts as a resonating chamber during courtship displays. The large V-shaped opening to the saccus oralis is ventral to the radix lingua between the rami of the mandible. The saccus oralis is absent in houbara, white-bellied, and buff-crested bustards. This can also be a site where lesions can be found (e.g. trichomonosis), and so should be carefully inspected. The oesophagus is mobile and has a markedly expansible diameter, presumably because in the absence of a crop it serves as a food storage organ (Angel et al. 1996). Bustards do not possess a crop and the oesophagus connects the pharynx to the gastric region, and consists of a proximal pars cervicalis and a distal pars thoracica. A transverse incision of the oesophagus is made at the level of the tracheal bifurcation to allow removal of the rest of the tract by traction from the proventriculus and gentle dissection to remove the proventriculus, gizzard (or ventriculus) intestines, pancreas, liver and surrounding fat as a unit.

The bustard stomach consists of a cranial glandular compartment or proventriculus and a caudal muscular compartment or ventriculus. The proventriculus is cone-shaped and the caudal extent is marked by a constriction, the isthmus gastris. Raised papillae, papillae proventricularis, are present on the mucosal surface of the proventriculus. The ventriculus lies in the left dorsal and ventral regions of the thoraco-abdominal cavity. The ventriculus is oval-shaped and consists of a body with two tapering ends, the saccus cranialis and saccus caudalis. The thick muscular wall consists of the crassus caudodorsalis and crassus cranioventralis muscles. A much thinner muscle layer, consisting of the tenuis craniodorsalis and caudoventralis muscles is present in the saccus cranialis and the saccus caudalis respectively. The inner aspect of the ventriculus is lined by a hardened membrane, the cuticula gastrica, which frequently appears green due to regurgitation of bile. Grit is commonly found at post-mortem in all species.

In young chicks the yolk sac and its attachments to the umbilicus and to the small intestine are examined, before the yolk sac is removed. The abdominal air sacs are examined as the viscera are removed. A cut is made around the vent to allow complete removal of the alimentary tract. The removed organs are examined completely starting with incision into proventriculus and gizzard. The horny (koilin) lining of the gizzard is stripped back to expose the underlying surface for inspection. The duodenal loop is inspected and the entire intestinal tract opened for inspection along its length. The pancreas is a pale-yellow organ with a finely lobulated surface situated between the two limbs of the duodenum and it is frequently hidden by fat. It consists of two lobes lying dorsally (lobus pancreatis dorsalis), and ventrally (lobus pancreatis ventralis). Pancreas can be saved with duodenum as a unit if part of the duodenal loop is saved.

11.2.9. Liver

The liver consists of left (lobus hepaticus sinister) and right (lobus hepaticus dexter) lobes which are joined cranially at the midline by an interlobar portion The liver is examined for size, shape, consistency and colour. The gallbladder should be examined and checked for patency and consistency of contents. Abdominal fat is weighed.

11.2.10. Lymphoreticular system

The spleen is a dark red, round to oval organ situated between the proventriculus and ventriculus on the right side. It is an important organ and must be examined during every post-mortem. The Bursa of Fabricius is situated in the dorsal wall of the cloaca and can only be seen grossly in young bustards because it involutes with age. The tibiotarsus is the best bone from which to collect bone marrow in bustards. Bone marrow can be collected for histological examination by breaking the bone with bone forceps and either by removing a small piece of bone plus marrow intact and placing direct in formol saline, or by removing a tube of bone marrow. Cytological preparations of marrow should be made by rolling marrow core over a slide, or by making the usual squash / smear preparations.

11.2.11. Urinary system

In bustards the kidneys are lobulated and situated in bony depressions of the synsacrum. The kidneys extend from the posterior edges of the lungs to the end of the synsacrum. The appearance and colour of the kidneys should be noted. The kidneys can be dissected out using careful blunt dissection with some cutting, being careful not to damage the nerves near the sacral plexus.

11.2.12. Reproductive system

The gonads are examined, dissected out with the adrenals and saved as necessary before examination of the kidneys.

Females – Only the left ovary is present in bustards. The appearance of the ovary and stages of follicular development should be recorded. The oviduct should be opened and checked for lesions or impactions with egg material.

Males – The testes are paired and situated just anterior to the kidneys. They are ovoid and variable in colour, ranging from whitish grey to black. The appearance of the testes and stages of development, including size (measure length and width) should be recorded.

11.2.13. Nervous system

Examination of the nervous system includes the brain, brachial and sacral nerve plexuses and peripheral nerves such as intestinal, vagal and cervical nerves. The peripheral nerves (brachial and lumbosacral plexuses) can be examined at various stages of the post mortem. Keymer (2000) recommends that any nerves collected for histopathology should be pressed on a piece of card to ensure that the nerve remains straight during fixation. The head should be skinned in preparation for removal of the brain. Haemorrhages can be observed in the boney substance of the skull, but these are usually agonal (Keymer 2000). Haemorrhages may be pathological if they are associated with contusions, with intracranial haemorrhages or with haemorrhages on the skin overlying the skull. The brain can be removed by sawing or cutting the skull in half longitudinally, or by careful cutting away of the roof of the cranium using forceps and removing the brain intact. When neurological lesions are suspected it is recommended to sample the brain as rapidly as possible during necropsy because of the fast autolysis of this organ (Ostrowski pers. comm.).

11.2.14. Archiving tissues

A thorough post-mortem examination is also essential for the collection of frozen and buffered formalin preserved archive tissues. This archived material will be of great value for future studies on bustard health. Details of the range of tissues archived at NARC are presented in Table 11.1.

11.2.15. Sample Collection

Blood - Blood samples should be obtained from live birds before euthanasia. After death blood can be collected from the heart. If the bird has been dead for a while and the blood in the heart has coagulated, small quantities of uncoagulated blood can be squeezed from the lung capillaries.

Samples for microbiology - Bacteriology and mycology samples are best taken from the organs in situ, before they have been handled and contaminated. Sterile

instruments are required for this and if not available, post mortem scissors and forceps should be immersed in formalin and then air dried or heated in the flame of a Bunsen burner before they are used for sampling.

Samples for cytology – Impression smears of the liver, spleen, lung and kidney should routinely be collected along with impression smears from any abnormal organs or lesions (Chapter 7).

Samples for histopathology - Small blocks (ideally no more than 1x1x1cm) should be taken for histopathological examination and immersed in 10% buffered formalin solution. There should be 10 times as much liquid as the mass of the organ pieces to be preserved.

Samples for parasitology - Parasites are best preserved in 70% ethanol, but can be stored in 5% formalin. Ideally helminth endoparasites should be washed in saline before they are transferred to storage bottles (Gibbons 2000).

Foreign bodies - It is useful to collect and clean foreign bodies that are found in the alimentary tract of bustards.

11.2.16. Disposal of the carcass

The carcass should be disposed of appropriately (ideally incinerated), being aware of possible infectious hazards. Check that all necessary tissues have been collected prior to disposal of the carcass.

Table 11.1. Post-mortem examination protocol summary sheet for bustards.

Bird identification	Give the bird a unique accession number.
History	Record any observations or known previous history in the record sheet.
Radiography	Take lateral and ventrodorsal radiographs of the whole bird prior to post-mortem examination. Take radiographs of any areas that warrant specific radiography e.g. fractured wing or suspected fractured neck.
Blood samples	If the bird is still alive awaiting euthanasia take blood samples, EDTA for haematology, plasma for biochemistry and serum for serology.
Archived tissues	Unless the bird is very decomposed tissues must be retained in formalin or frozen for the reference collection. As a minimum the following tissues must be retained from each carcass;
Formalin	Adrenal, brain, bone marrow, gonad, heart, kidney, large intestine, liver, lung, pancreas, parathyroid, small intestine, spleen, thymus, thyroid,
Cytology	Liver, lung, spleen, kidney, bone marrow.
Frozen	Brain, liver (whole, after smears and formalin sample taken), abdominal fat (entire), kidney, lung, small intestine, large intestine Any abnormal tissues must be collected and stored in formalin and cytology preparations made Record the tissues retained in the PM form
Microbiology (bacteriology and mycology)	Collect samples for microbiology (e.g. swab in Amies transport media) from abnormal tissues, or whole tissue/lesions in a sterile containers (e.g. petri dish)
Parasitology	Collect specimens in 70% ethanol or 5% formalin.
Virology	Collect fresh, uncontaminated tissue samples and refrigerate for virus isolation (preferable to a swab in MEM transport media) from abnormal organs, or freeze whole tissue/lesions in a sterile containers. Freezing destroys the viability of some viruses, so check with the virology laboratory for the best type of sample for the virus in question.
Archived carcasses	Any carcass remains or whole carcasses to be frozen must be logged into the freezer reference book.
Laboratory samples	Record what samples have been taken and ensure that any samples taken are labelled adequately.

11.2.17. Labelling post-mortem samples

All samples collected at post-mortem must be stored properly and labelled. The bird ID and the date the sample was taken must be written legibly on the label. To ensure that the label does not detach or smudge, put an additional clear sticky tape over the ID label and use pencil or non-water soluble ink to write labels.

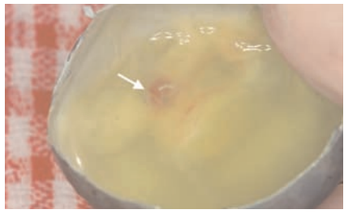
11.3. Examination of Eggs

11.3.1. Examination of unhatched eggs and dead-in-shell chicks

The problem of fertile eggs that fail to hatch is frustrating for aviculturalists and veterinarians and it is important that all unhatched eggs are examined (Figure 11.7). While examination of unhatched eggs may not always be diagnostic, as many problems are management related, egg post-mortem is the most likely way of identifying infectious diseases, nutritional deficiencies and toxins.

The majority of fertile eggs investigated for post mortems will fall into two categories or age. First, eggs with embryonic deaths within 3-5 days of incubation (first mortality peak), and second, eggs with embryonic death at the end of incubation (Joyner and Abbott 1991). The causes of embryonic death are often hard to determine and ideally eggs should be examined as soon as possible after development arrests as autolysis progresses rapidly (Figures 11.8 to 11.12).

11.7



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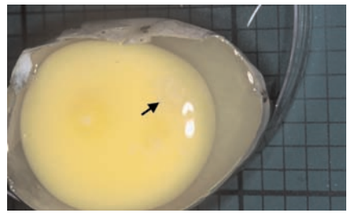

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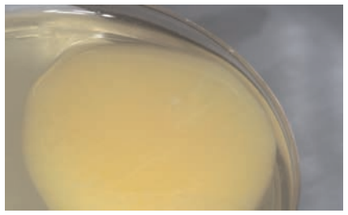

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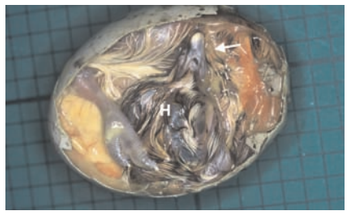

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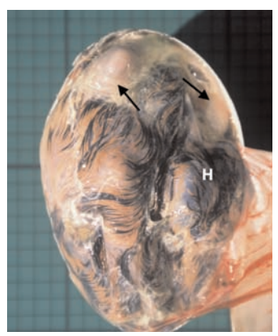


11.12

11.7 Examination of unhatched eggs is important in breeding projects (Photo credit Tom Bailey).

11.8 Early embryonic death (arrow) in a white-bellied bustard egg (Photo credit Tom Bailey).

11.9 Bacterial contamination of a white-bellied bustard egg (Photo credit Tom Bailey).

11.10a/b a) Early fertile houbara bustard egg, showing a distinct ring (arrow) consisting of a white peripheral region (area opaca) surrounding a clear central region (area pellucida) and b) The same area in the infertile egg is small in size and lacks cellular organization. In other avian species the diameter of the fertile blastodisc is four to five times that of the same area in an infertile egg (Photo credit Tom Bailey).

11.11 Malposition in a houbara bustard embryo, the head is over the right wing (arrowhead) (Photo credit Tom Bailey).

11.12 Malposition in a kori bustard, the head (H) is between the legs (arrows) (Photo credit Tom Bailey).

11.3.2. Examination technique

Examination must be accompanied by an adequate history and detailed information on candling history and incubation technique. A flow chart developed by Francois Lampen for the examination of bustard eggs is presented in Chart 11.2. An egg post-mortem submission form is presented in Chart 11.3, sketches and photographs are useful additional ways of recording findings. Before opening the egg, the external characteristics such as the amount of dirt present on the shell, egg shape, size, excess calcium deposits on the shell, cracks, or thinning of the shell should be noted.

It is necessary to follow sterile procedures until microbiological samples have been collected from embryonic fluids and tissues. Alcohol swabs should be used to clean the blunt end of the egg. Eggs are opened with scissors over the air cell, which is usually the rounded, blunt end of the egg. The eggshell should be carefully perforated to avoid contaminating the cleaned area. Forceps can be used to enlarge the hole to about 0.5 cm by carefully removing excess shell. The relative size of the air cell should be assessed and recorded at this stage. Shell membranes are examined for abnormalities and then carefully peeled back to expose the internal egg contents. Internal egg membranes and structures should be evaluated before destruction caused by manipulation and sample collection. The colour, consistency and clarity of the albumen, allantois and yolk sac inside the remaining eggshell along with the presence or absence of abnormal odours and a circulatory system should be noted. If exposed the albumen and amnion can be sampled at this stage.

Enlarging the hole over the round end of the egg is performed by careful removal of the eggshell fragment with thumb forceps. The membranes should be peeled from the inside of the shell to examine for potential spots of bacterial and fungal growth under the outer membrane. If no chick or a very small chick is present, the contents of the egg can be carefully poured into a sterile container.

11.3.3. Infertile eggs

Sterile swabs of the contents of infertile eggs can be submitted for microbiology culture and the contents can be frozen for any subsequent investigations such as toxicology, serology and nutritional assessment. The shell can also be dried and retained for studies. Assessing whether incubated bustard eggs are fertile could be problematic in the early stages of incubation. In some cases a large donut-shaped blastodisc can be seen in the fertile egg, similar to other avian species (Joyner 1994). However, it is not possible to accurately differentiate between infertile eggs and fertile eggs that suffered early embryonic death.

11.3.4. Fertile eggs

Where dead embryos are present, their size gives an indication to their age at death, which can help pinpoint the stage in the incubation cycle where problems are occurring. If embryos died at the point of internalising the yolk sac into the abdominal cavity, it is important to look for signs of inflammation of the umbilical area and the yolksac. The crown-rump length of embryos should be recorded to build up a database to enable embryos to be accurately aged. Where chicks are well-developed the position of the appendages and head should be observed in relation to the general position of the chick so that any malpositions can be documented. Table 11.2. presents the different malpositions observed in bustards at the National Avian Research Center.

Dead-in-shell chicks should also be carefully examined and a hand lens or magnifying lamp can be helpful for small chicks. It is important to keep the yolk sac membrane intact, to pay attention to the size of the hatching muscle, signs of oedema and haemorrhage, skin colour and haemorrhage, musculoskeletal deviations. Similarly, a full set of tissues should be archived for further investigation (formalin fixed and frozen).

If abnormalities in the eggshell are noted, these can also be cleaned, dried and stored correctly identified for reference.

11.3.5. Malpositions

Table 11.2. Classic malpositions in chick embryos observed in bustards at the National Avian Research Center (from Joyner 1994).

Malposition	Description	Cause in other species
Malposition 1	Head between the thighs. Failure of the chick to lift and turn its head to the right in the middle of the last third of incubation. Completely lethal.	Incidence increased by high incubation temperature.
Malposition 2	Head in the small end of the egg. Chick is upside down in the egg. Hatchability reduced by 50% in domestic species.	Incidence increased by incubator egg position and low temperature.
Malposition 3	Head is under the left wing. Chick rotates its head to the left as opposed to the right. Usually lethal.	Incidence increased by incubator egg position, temperature and parental malnutrition.
Malposition 4	Beak is away from the air cell. Upward turned aspect of maxilla and egg tooth is not near the air cell; however, the rest of the embryo is normally positioned. Slightly reduced hatchability in domestic species.	Incidence increased by incubator egg position.
Malposition 5	Feet over head. Usually lethal.	
Malposition 6	Head is over the right wing. Normally the head is under the right wing in domestic species. Reduces hatchability slightly in domestic species.	Incidence may be increased by parental malnutrition.

11.3.6. Interpretation of egg post-mortem findings

Interpretation of egg post-mortem findings is not easy because of all the contributing genetic, management, maternal, behavioural and nutritional factors involved in embryo development. The history must include aspects of incubation management, the age of the egg, the duration and type of storage, and the egg disinfection protocols. Additionally, the health and nutrition of the breeder flock is important. Exposure to environmental toxins may be of relevance for eggs examined from captive and free-living bustards. It is important to realise that bacteriological findings from such eggs may be hard to interpret, as pathogens can be overgrown and replaced by other bacteria (Cooper 1993b). Similarly, failure to isolate organisms does not rule out infection, because albumin inhibits bacterial growth and for this reason Gerlach (cited in Cooper 1993b) recommends culturing the vitelline membrane. A complete clinical examination, including endoscopy and microbiology screening of the reproductive tract of the female bird may be a necessary part of any investigation of dead-in-shell chicks. Samples should be frozen for toxicology, antibody and nutritional studies.

Chart 11.2. Egg post-mortem form used at the National Avian Research Center.

Chart 11.2. - An example of the standard form used to record egg post-mortem data at NARC.

Egg Post Mortem Examination Form

Post-mortem examination [_____]

Egg number [_____]

Submission information (Please fill / tick)

Species [_____] Date laid [___/___/___] Date of submission [___/___/___]

Specimen : Egg infertile ( ) Early fertile ( ) Fertile / dead in shell ( ) Exploded ( ) Hatched ( )

Postured : No ( ) Yes ( ) Period [_____] Artificially incubated : No ( ) Yes ( ) Days [_____]

Date taken out of incubator [___/___/___]

Was the egg stored prior submission : Period [_____] Conditions [_____]

Candling observation description [_____

_____

_____]

Provisional diagnosis [_____

_____

]

Avian vitalist [_____]

For Laboratory use only

Analysis information (Please fill / tick)

Date received [___/___/___] Time received [_____]

Laboratory : SE3 ( ) AA2 ( ) Other [_____]

External egg shell description [_____

_____]

Internal observation description [_____

_____

_____]

Embryo description [_____

_____

Laboratory samples taken : Histopathology ( ) Bacteriology ( ) Fungal culture ( ) Other [_____]

Laboratory findings [_____

_____

_____]

Comments [_____]

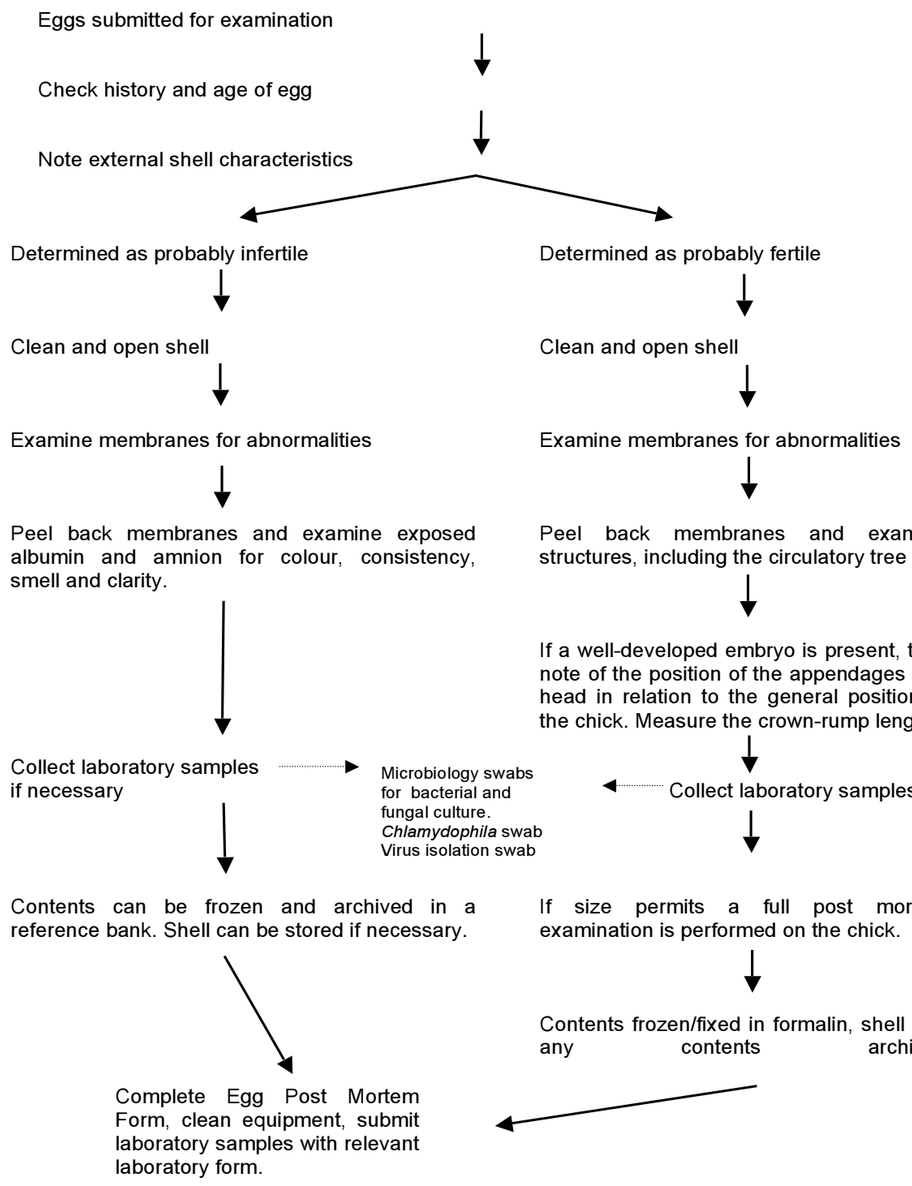
Shell kept for : Whole shell reference collection ( ) Studies on thickness/porosity ( ) Toxicological studies ( ) Chemical studies ( )

Examined by: _____

Eggpme1.doc

Chart 11.3. Flow chart for the examination of bustard eggs post-mortem.

Chart 11.3. Flow chart for the examination of bustard eggs post-mortem.



11.4. Biomedical Reference Collections

11.4.1. The need for reference collections

Monitoring the causes of morbidity and mortality of both free-living and captive bustards is essential to minimise the transfer of novel diseases between captive and free-ranging populations through re-introduction programmes (Hutchins et al. 1991, Cooper 1993a, Kirkwood 1993, Munson and Cook 1993). Disease monitoring protocols of animals maintained in zoological collections should include provision for serum and tissue banking (Cooper 1989a, 1989b, 1993; Munson and Cook 1993, Jacobson 1993, Munson and Woodford 1995). Banks of biomedical tissues derived from both clinically normal and abnormal individuals are indispensable in retrospective investigations to determine the historical prevalence of diseases, exposure to infectious organisms within populations and also as a source of normal material for comparative studies. Collection of material during post-mortem examinations provide an important source of samples for reference collections and Table 11.1 shows the range of samples that were incorporated into the NARC reference collection.

11.4.2. Uses of reference collections

The establishment of a reference collection and integration with a computerised database described by Bailey et al. (2003b) allows the co-ordination of research work on bustards. Retrospective analysis of biomedical records and material of captive breeding projects has a dual role; first to assess husbandry and biomedical management practices and secondly to target research projects at specific 'problem areas' with the aim of allowing management to adapt and improve. Such research projects often need to access material that has been retained in a reference collection. For example, in 1995 it became apparent that fatty liver disease was a problem in bustards maintained in some private collections in the UAE. A research project was undertaken to establish the incidence and severity of fatty liver disease by examining formalin-preserved livers and retrospective analysis of clinical and post-mortem records stored in the database. The project established risk factors for the disease and offered preventive and therapeutic alternatives (Nicholls et al. 1997).

Future research projects can access material and data stored in reference collections in order to conduct; genetic studies (frozen blood, frozen tissues or feather samples); virological surveys (frozen tissue/organ samples); toxicological/pesticide surveys (frozen fat/organ samples); histopathological studies (formalin samples); biochemistry analysis (frozen serum/plasma); serological surveys against other avian infectious diseases (frozen serum samples); eggs from free-living and captive birds may be used for comparative studies on egg-shell thickness, porosity, mineral analysis, pesticide studies and studies of the micronutrients and trace elements of stored tissues. Note that an increasing number of tests can be undertaken on formalin-fixed paraffin-embedded tissue (histology wax blocks), even after they have been preserved for many years, or even decades. Such tests include electron microscopy, immunohistochemistry, and PCR detection of nucleic acids (such as viral or bacterial agents). For this reason, such wax blocks form a very useful archive indeed.

In the few instances where reference collections have been established they have provided valuable material for investigations of species maintained in captivity. Examples of other collections include the Mascarene reference collection established at the Royal College of Surgeons of England (Cooper and Jones 1986) and the Edward Elkan Collection of Lower Vertebrate Pathology (Williams 1994). Comprehensive banks of biomedical data and material are essential for retrospective research projects allowing those managing bustard collections to build up accurate biological profiles of their species in health and disease. Depending on the resources available to projects, such collections may be of a wide or narrow range of tissues.