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Pathology and Veterinary Care

S. OSTROWSKI, A. GRETH and I. MIKAELIAN

8.1 Introduction

Diseases and pathology of the houbara bustard were poorly documented. This lack of knowledge posed a serious threat to the success of the captive-breeding project at the NWRC. Many pathological events occurred during the first years of captivity of this species, allowing the collection of data on virology, bacteriology, histology, parasitology, immunology and therapeutics. To follow the pathology, every sick bird under treatment has a record card, on which symptoms, loss of body mass and treatments are noted. A complete postmortem is performed on each dead bird, and if the carcass is found fresh, organs are fixed, frozen, and bacteriological, virological, histological, and parasitological samples are taken.

A summary of the causes of mortality, based on the records of 258 postmortems between 1987 and 1993, is presented in Fig. 8.1. Proportions of the various etiologies observed in 1989 were the same as in 1987. The increase in the number of deaths in 1989 compared to 1987 was mainly due to the increase in the total number of birds. This was followed by a decrease in mortality rate in 1990 and 1991. The large increase in the total number of birds in 1992 and 1993 explains the high number of deaths. Infectious diseases were the major cause, responsible for 50 per cent to 60 per cent of deaths.

After 1990, the decrease in infectious deaths was due to the eradication of chlamydiosis. In 1993, 'trauma-shock' became the primary cause of death in the captive breeding and rearing units, mainly occurring during visits of 'unknown' people and while birds were being handled. The houbara is very fragile, and must be handled with extreme care by immobilizing the

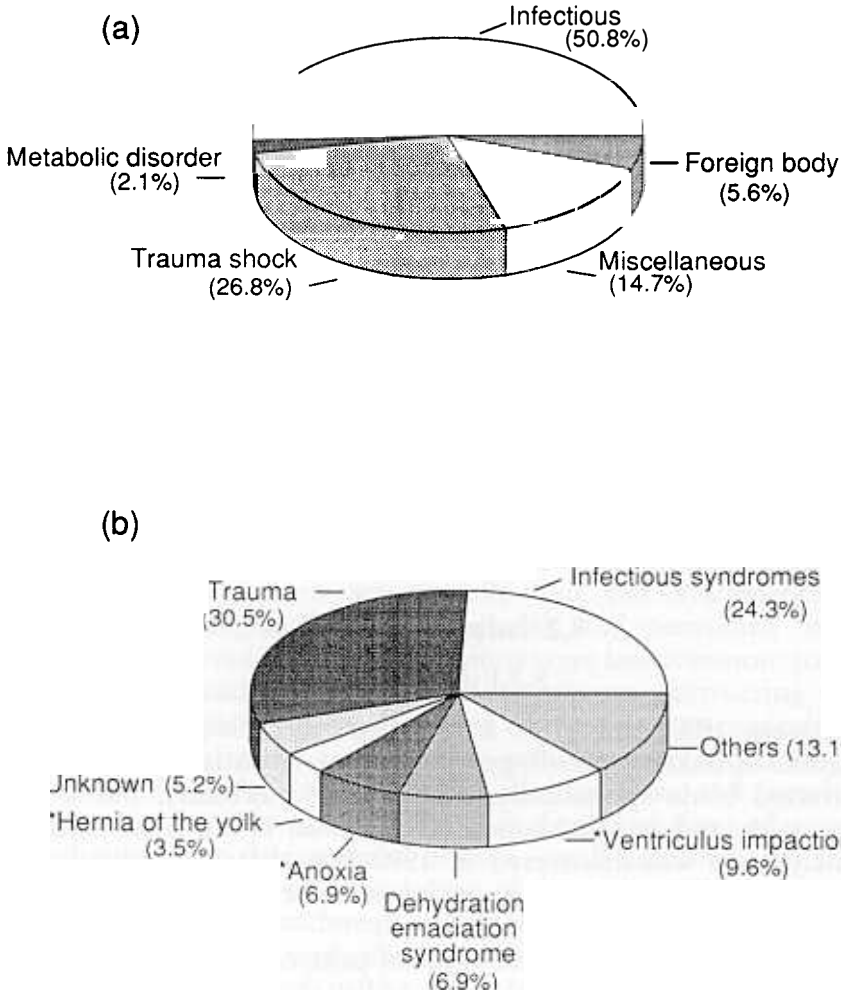

Figure 8.1 Causes of death among (a) houbara between 1987 & 1991 (n = 142), and (b) houbara in 1993 (n = 115)

entire body, with the wings and legs folded against the body (see Chapter 3). Houbara are also highly strung, and deaths or severe injuries can occur when a disturbance frightens the birds in their cages. Cranial traumas inducing death have been observed, as have various fractures and large, open wounds at the base of the neck, due to scraping along the wire mesh. Ingestion of foreign bodies (screws, pieces of wire, fragments of glass, rags) has been

recorded on several occasions, inducing severe wounds, and obstructing the gizzard or small intestine, causing death after several days. Particular care must be taken to remove such hazards, as they represent 10 per cent of the total causes of deaths. Other causes of deaths (aneurysm rupture, complete cloacal prolapse) occurred only infrequently.

Mortality of young birds (<1–yr-old) was higher than at other ages. Analyses revealed that mortality occurred mostly during the first month after hatching. Very early mortality (<8 days old) was most common; in 1993, 61 per cent of early deaths occurred before 8 days of age. Causes of these very early deaths were related to infectious and neonatal syndromes. After 1 month of age, most mortality was due to accidental traumas, with only a few birds dying from infectious diseases. Among other pathologies, the most frequent were infectious diseases, ventriculus impaction and secondary hyperparathyroidism.

8.2 Infectious Diseases

8.2.1 *Chlamydiosis*

Between 1987 and 1989, a so-called 'enteritis-peritonitis' syndrome of unknown etiology killed about 40 birds. This syndrome affected birds sporadically, with a low morbidity, but a high mortality and an acute course (Greth *et al.* 1990). After extensive analysis, it was discovered in 1990 that these mortalities were due to *Chlamydia psittaci*, an intracellular bacterium known to be responsible for chlamydiosis.

C. psittaci, the causative agent of psittacosis/ornithosis, occurs worldwide in avian species, generally as a latent infection but also as a clinical infection (Page & Grimes 1984; Eamens & Cross 1989). One reason for the latency seems to be a specific adaptation of this microorganism to the host species (Gylstorff *et al.* 1984), while retaining at the same time its infectiousness with regard to all other possible hosts. Therefore, clinical disease may either develop by passage into another host species or within the same host species, and is generally associated with stress factors (Eamens & Cross 1989). Chlamydial infections were reported by Burkhart and Page (1971) in 15 avian orders, including 140 species. Since then, more species have been shown to be susceptible to psittacosis.

In houbara the disease was of peracute to chronic course.

Morbidity rates among recently arrived birds reached 82 per cent, and the mortality rate was 12 per cent. The following description of the clinical signs should help the practitioner, as well as the breeder, to recognize the disease in houbara bustards.

Clinical signs differ slightly from those that have been described in other birds. Prostration, depression, anorexia and ruffled plumage were the most common and earliest signs. Greenish droppings were observed in the capture pens, and birds had soiled vents due to diarrhoea. Respiratory signs were less often noted. Only upper respiratory symptoms were observed; some animals had a cough, and eleven animals had a translucent mucoid oculo-nasal discharge and signs of tracheitis. Two birds were affected by paralysis of both legs.

A complete postmortem was performed on each dead bird, and if the carcass was found fresh, organs were fixed and frozen. Most of the birds exhibited liver abnormalities: the liver was an unusually dark colour, as if it had been cooked, and of pulpy appearance. The spleen was usually but not systematically enlarged and congested. Colitis, jejunitis and peritonitis were frequently observed. Respiratory lesions were less common: some birds showed tracheitis with abundant mucous obstructing the glottis, and congested lungs. One bird displayed an aerosacculitis.

Further investigations of the histo-pathology and bacteriology focused on the multifactorial etiology of the disease, and revealed some interesting information. The liver displayed signs of a disturbed circulation (hyperaemia, oedema, small haemorrhages and bile congestion), as well as a degree of disseminated necrosis of hepatocytes with detachment from the basement membrane. Within the sinuses an increased number of Kupffer's stellate cells was observed, arranged in the form of a string of pearls. The spleen revealed a depletion of lymphocytes, mainly in the white pulp, and necrosis of some reticular cells (the type could not be established). Lymphatic follicles were rare or non-existent. The walls of many arterioles were either homogenized or invaded by mononuclear cells, in the latter case with activation of the endothelial cells. In a few cases, either hemosiderin was detected, or the number of heterophils in the tissue was markedly reduced. The heart showed a high degree of oedema which also affected the walls of the blood vessels, and a myodegeneratio cordis. In some cases there had been mild infiltration of mononuclear cells into the interstice. In one bird a purulent epicarditis was evident. In the kidney the signs of circulatory disturbance were prominent

(hyperaemia, oedema including the walls of the blood vessels, haemorrhages, thrombi). In addition, a necrosis of the tubular epithelium was seen (with or without detachment from the basement membrane or casts in the lumina).

In some birds lesions resembling glomerulonephritis were evident (activated layer of Bowman's capsule, and a non-identifiable eosinophilic staining material within the lumen of the same capsule), in addition to homogenization of the mesangium and widening and thickening of the capillary loops. The modified mesangium consisted mainly of collagenous fibers (van Gieson staining). The lungs showed hyperaemia and oedema, particularly in the atria, and small haemorrhages. There were thrombi mainly in the larger veins. The main lesion was probably an angiosis. The walls of the larger blood vessels displayed a cellular proliferation of mainly mononuclear cells. In the lumina of the larger bronchi there was detritus but no erythrocytes.

The trachea of one bird showed a haemorrhagic tracheitis with loss of the cilia and proliferation of mucous-producing cells. Other tracheae were difficult to evaluate because of mineralized tracheal rings. The mucosa appeared thin compared to other avian species, but cilia were present to some extent. No appreciable numbers of inflammatory cells and lymphatic follicles were demonstrable.

Finally, in the birds described here, and from others with antibodies against *C. psittaci*, it was noted that a peritonitis fibrinosa or fibrosa, particularly affecting the serosa of the intestinal tract, as well as parts of the intestinal wall, was regularly encountered. The disease process apparently began on the serosal side of the intestinal wall. The intestinal mucosa could not be thoroughly evaluated because of autolysis. However, there were no subacute or chronic inflammatory lesions.

From the clinical signs and pathological lesions described here, it is likely that *C. psittaci* contributed to the enteritis-peritonitis syndrome. The clinical, pathological, and histological lesions seen in chlamydiosis are highly variable according to strain and bird species (Gerlach 1986a). Although there are no pathognomonic macroscopic or histological lesions in chlamydiosis (Graham 1989), a comparison of the lesions described here with those of other bird species reveals some unusual features. Although aerosacculitis was observed in only one bird, there was subacute to chronic peritonitis, chronic glomerulitis, and clinically, a flaccid paralysis.

Table 8.1 Macroscopic Lesions in Houbara Showing Inclusion Bodies Resembling *Chlamydia*

	Pneumonia	Peritonitis	Aerosacculitis	Enteritis
A				
B				

	Pericarditis	Splenomegaly	Hepat. Invol	Tracheitis
A				
B				

A: Number of cases showing the lesion / total number of cases
B: % of cases showing the lesion / total number of cases

A mild fibrinous aerosacculitis is regarded as an indication for psittacosis/ornithosis in Psittaciformes and Columbiformes (Gerlach 1986a). In houbara, upper respiratory symptoms were prominent (as in Columbiformes), and a haemorrhagic tracheitis was seen in one case. The latter is not assumed to have been caused by *C. psittaci* alone, because there is no report in the available literature. Also, no decision can be made as to whether or not the fibrinous peritonitis was caused by *C. psittaci* and represents a distinctive form of serositis in this avian species. In the so-called enteritis-peritonitis syndrome, there was also no distinct inflammation of the mucosa, but, rather, an infiltration of the wall with inflammatory cells, starting from the serosal side. Another point in favour of the peritonitis not being caused primarily by *C. psittaci*, was that most of the birds died acutely, and the lesions appeared to be subacute to chronic. In any case, we need more information in order to evaluate some of our unusual findings. Either forms are specific to houbara, or they are caused by an incompletely understood multifactorial etiology.

We also observed that houbara were infected by a variety of other viral and bacterial agents: *Serratia marcescens*, *Staphylococcus sp.* (Greth *et al.* 1990). Thus it has to be assumed that the cases described here are caused by a multifactorial etiology, whereby *C. psittaci* is one of the more important agents. Andral *et al.* (1985) described an outbreak of rhinotracheitis in turkeys, with the involvement of *C. psittaci* and of viral agents (adenovirus - hemorrhagic enteritis virus- and paramyxovirus II).

Enterobacteriaceae were isolated in high numbers from the dead birds. Since the houbara does not have *Enterobacteriaceae* as a normal component of the gut flora (Greth *et al.* 1990),

bacteria of this family are considered potential pathogens, able to cause disease and death following generalization. Death may be due to septicaemia. *C. psittaci* infection was proved to modulate the host immune response (Lammert & Wyrick 1982), and thus to favour potential pathogenic bacteria. The *Enterobacteriaceae* in the cases described here may be the cause of purulent pericarditis, and of the development of thrombi during life. Gram-negative bacterial walls, particularly those of *Enterobacteriaceae*, can activate the alternative pathway of the complement cascade, resulting finally in thrombi (Tizzard 1987). The increase of body mass following treatment for *Enterobacteriaceae* indicates that these bacteria were part of the disease process. The therapeutics used were not particularly effective against *Chlamydia*.

Diagnosis and demonstration of *C. psittaci* in the bustard organs was done by cytology. Impression smears of organs (particularly the spleen) were repeated on the same slide, and on five different slides. A Köster Stamp staining (fuschin and methylen blue) allowed microscopic observations of many intracytoplasmic inclusions morphologically resembling *Chlamydia* (Giroud & Captoni 1964).

The second step of the diagnosis was performed through serological analyses. Blood was taken by alar venipuncture and the extracted sera were kept at −30°C. Serological examination was performed with the commercially available competitive enzyme immunoassay Chlamydia-psittaci-AK-EIA (Röhm Pharma GmbH, Darmstadt, Germany). It was used to detect *C. psittaci* antibodies in sera of birds according to the instructions of the manufacturer. Results revealed that the antibody titre was high, and a significant seroconversion was observed in newly imported birds.

Although *C. psittaci* was not identified by culture, the demonstration of intracytoplasmic inclusion bodies morphologically different from the ones caused by *Rickettsia*, the occurrence of antibodies against *C. psittaci*, and the epidemio-clinical findings provided safe grounds for the diagnosis of *C. psittaci*. The antigen used showed cross-reactions only with *C. trachomatis*, an infectious agent which does not occur in avian species.

To maximize the survival chances of houbara being released as part of the reintroduction programme, and to avoid the risk of spreading *C. psittaci* to the indigenous wildlife (Brand 1989), it was decided to attempt to eradicate *C. psittaci* from the breeding flock by means of therapeutic and sanitary measures (see

sanitary measures). Tetracyclines are recommended, or even prescribed by law for the treatment of *C. psittaci* infections in several countries. Since psittacosis is a zooanthroponotic disease, and may be transmitted from Psittaciformes to humans, much information has been collected from the Psittaciformes as well as from the more widely kept species. Tetracyclines are considered cross-sensitive with regard to bacterial sensitivity, but in terms of pharmacology, many differences have been observed between the various derivatives. The superior lipid-solubility of doxycycline (DC) provides a good bio-availability and diffusion into the cells, where the initial bodies of *C. psittaci* develop (Gylstorff 1987b). When administered intramuscularly (IM; only possible with the European and Canadian preparation) at 75–100 mg./kg. body mass (BM), efficient blood levels are reached, and sustained for six to seven days (Jakoby 1979a; Jakoby 1979b; Flammer 1989). In contrast to the other tetracyclines, DC is excreted in the faeces without impact on the physiological intestinal flora (Huber 1988). Another side-effect of all antibiotics and, in particular the tetracyclines, is immunosuppression. The possible influence on the immune system should not be ignored, particularly since the microbiological agent is only inhibited by the treatment, but still has to be eliminated actively by the immune system (Gerbermann & Pauels 1982). Before treating all the birds systematically with doxycycline, a pharmacokinetic study was performed to adapt the dosage and to be sure that the minimal therapeutic blood concentration was reached (Greth *et al.* 1993). The mean concentration of doxycycline in the blood was measured after the first injection during the initial seven-day time period, and then again after the seventh injection (each injection administered one day apart), and during seven days following this (Figs. 8.2 & 8.3). Conclusions of the trial are that, due to a rather high plasma peak level, the dosage should be about 80 mg./kg. BM, and SC administration might be generally more desirable. Seven injections (100 mg./kg. BM) are given over a period of 38 days at the following intervals: seven, seven, seven, six, six, five days. Following this treatment, epizootie of Chlamydiosis in the Center has stopped.

Newly arrived birds at the Center have certainly acted as carriers and triggered outbreaks of Chlamydiosis. No mortality attributed to infectious agents occurred from the beginning of 1989 until September 1990. A latent infection had perhaps existed for years in the breeding unit, and may have conferred a premun-

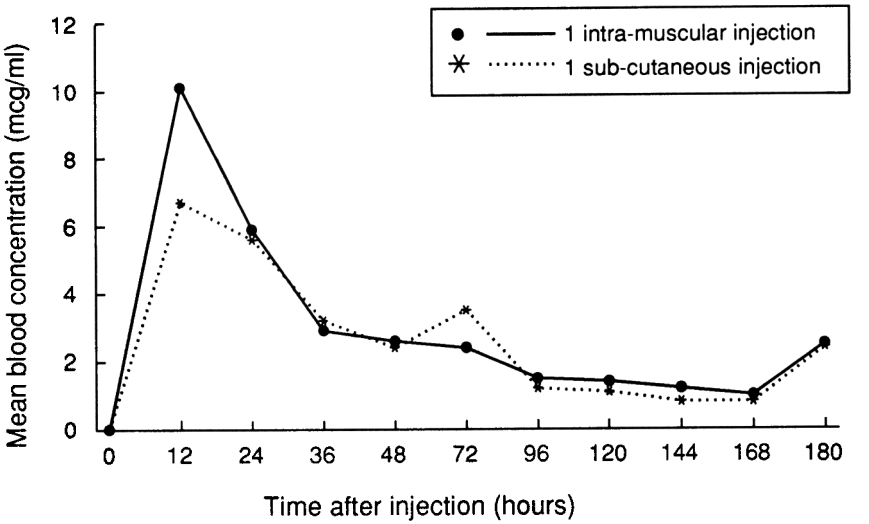

Figure 8.2 Mean doxycycline blood concentrations versus time after the first injection, during the initial seven-day period; intra-muscular (n = 10) and sub-cutaneous (n = 4)

ity status to the birds (Gerlach 1986a). Since a latent infection of *C. psittaci* can be triggered into a manifested clinical disease by various stressing agents, the transport of birds to protected areas (transport itself, change of environment, feed and temperature), should be considered as a significant stress factor.

Houbara are opportunistic feeders that peck the ground frequently, even though food is presented on plates at the NWRC. This behaviour may enhance the transmission of disease in dust and dry particles contaminated by faeces (Brand 1989). Chicks are bred from artificially incubated eggs, in separate rooms without contact with birds of the breeding unit; therefore, it is possible that vertical transmission of *C. psittaci* in the houbara may occur. Such transmission has been shown in ducks, parakeets, gulls and turkeys (Rüffle 1962; Gylstorff 1987a; Eamens & Cross 1989; Olson 1990).

As some bustards are destined to be released in protected areas of Saudi Arabia, an important question is whether or not these birds are suitable for life in the wild. While clinically sick birds can be treated successfully (Meyer & Eddie 1955; Gylstorff *et al.* 1983; Wachendörfer *et al.* 1985), an intact immune system is essential (Gerbermann & Pauels 1982), and latent infections cannot be eradicated by medication, as has been shown by Sch-

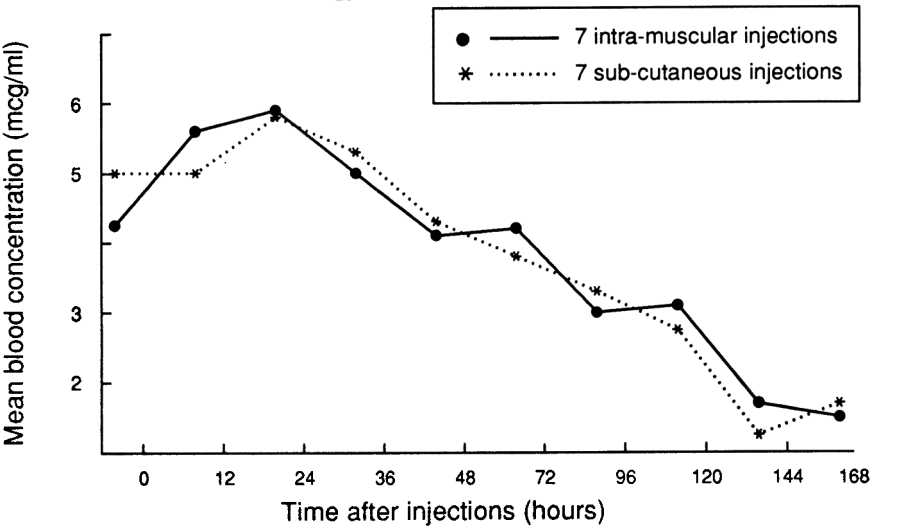

Figure 8.3 Mean doxycycline blood concentrations versus time after the seventh injection, and during the next seven days: intra-muscular (n = 10), sub-cutaneous (n = 4)

achter *et al.* (1978) and Gerbermann *et al.* (1990). Some authors (Shewen 1980; Gerbermann *et al.* 1990) consider a latent infection with avian strains of *C. psittaci* to be normal; they found *C. psittaci* antigens in 31 per cent to 41 per cent of cloacal swabs and faecal samples taken from 119 hawks (Accipitriformes), falcons (Falconiformes), and owls (Strigiformes). Antibodies were demonstrated in 71 per cent of birds kept in a large aviary and used for demonstrations of falconry. Another indication for continued latency is the fact that antibody titres remain high following treatment (Schmeer 1983; Janeczek 1989).

Surveys for chlamydial infections (Burkhart & Page 1971; Ruppaner *et al.* 1984) in wild birds have shown many species to be infected, but with major differences in prevalence between species and regions. Since latency is dependent on the activity of the immune system and environmental stressors (Brand 1989), an outbreak of sub-lethal effects (Brand 1989) could occur in a newly introduced population. However, a serological survey of common avian species in the area, such as ravens (*Corvus ruficollis*) and house sparrows (*Passer domesticus*), has shown that free-living birds have antibodies against *C. psittaci*. This suggests that there is no reason why houbara should not be released into the wild in Saudi Arabia.

8.2.2 Other Bacteriological Findings

Analyses of faecal samples show that the aerobic intestinal flora consists mainly of Gram-positive organisms, and that *Enterobacteriaceae* are not normally a component of the faeces. With respect to Gram-negative rods other than *Enterobacteriaceae*, we do not have enough data yet. All isolates of *Enterobacteriaceae* represent facultative pathogens indicating colonization, but also secondary infection or disease and death. Various beta-haemolytic and non-haemolytic *Bacillus* species, as well as various alpha-haemolytic and non-haemolytic *Streptococcus* species, mainly belonging to Lancefield Group D, are isolated from normal intestinal contents. Other specimens found in faecal samples are *Citrobacter amabonaticus* and *Acinetobacter calcoaticus*. Their pathogenicity is not certain.

Escherichia coli, *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter*, *Proteus* and *Citrobacter* are considered as secondary invaders, and can be the cause of septicaemia followed by death. This seems to have occurred during a chlamydiosis outbreak. *E. coli* is the most common secondary invader isolated at the Center. Three types have been identified, all fully sensitive to polymixin B, enrofloxacin and furazolidon; O_1K_1, O_2K_1 and $O_{78}K_{80}$. Chloramphenicol, neomycin and tetracyclins were less efficient. *Klebsiella pneumoniae* strains were usually also fully sensitive to polymixin B, enrofloxacin and furazolidon. Only one salmonella, *Salmonella mbandaka*, an African species, has been found, but in a bird killed by a raptor and without any macroscopic lesions at postmortem. Other bacteria isolated on single occasions are: *Pasteurella multocida*, *Citrobacter freundii*, and *Moraxella*. *Pseudonomonas aeruginosa* is also a frequently isolated secondary invader that can cause death, probably because of toxin production. Type P_3 was frequently isolated: this extremely resistant type is dangerous in nosocomial contamination. None of the numerous micrococcaceae isolated demonstrated any clumping, confirming that they are secondary invaders. During the outbreak of Chlamydiosis, many bacteria which were thought to be secondary invaders were isolated. *Serratia marcescens* was frequently isolated and was sensitive to erythromycin and to neomycin. Bacteriological cultures of the major organs revealed *Escherichia coli* $O_{78}K_{80}$ in two birds; *E. coli* O_1K_1 in one bird, and *Klebsiella pneumoniae* in two birds.

To evaluate the epidemiological environment and exposure of

houbara to *Mycoplasma gallisepticum* (MG), *Mycoplasma synoviae* (MS), and *Salmonella pullorum* (SP), a serological survey was carried out on the entire flock. No antibodies against MG were detected; serological prevalence to MS and to SP were respectively 2.8 per cent and 3 per cent.

8.2.3 Avian Poxvirus

Poxvirus infection in birds is a slowly spreading viral disease, inducing cutaneous lesions on the unfeathered parts of the skin, and/or diphtheric lesions in the digestive and upper respiratory tracts (Tripathy & Cunningham 1984). Natural poxvirus infections have been reported in more than 60 species of wild birds from about 20 families (Clubb 1986).

Avian pox has always occurred in the breeding unit as a mild latent infection in three different clinical forms: cutaneous, diphteric and coryza. Expression in adults was mostly cutaneous, in the form of nodules on the unfeathered parts of the body; legs, toes, or around the oral cavity.

Epidemio-clinical observations showed that three different forms of the disease were present at the Center: in spring 1989, eight chicks died with severe diphteric lesions of the buccal cavity. Consistent clinical signs were anorexia, weakness, and death occurring within a few days (one to 10 days after the discovery of the lesions). Morbidity and mortality rates reached 45 per cent. Chicks seemed to be particularly affected by the disease between the fifth and sixteenth week. The diphteric lesions were white opaque nodules, slightly elevated, developing on mucous membranes. In some of the chicks the nodules coalesced and became large proliferative caseous lesions, with diphteric membranes and pus, invading most of the mouth and covering the tongue. At the end of the progression of the lesion the necrotic membranes could be removed, leaving bleeding erosions. One chick had 21 nodules in the buccal cavity, including the tongue, as well as 14 ulcerations on the oesophagus, and a few circular opaque white nodular lesions on the proximal part of the trachea. Small hemorrhagic spots were also present on the walls of the trachea. Houbara with the cutaneous form had dry scabs around the nares or on the skin. One bird had small ulcers on the legs and the toes, was limping, and had swelling around the left eyelid; prognosis was poor. All the dead birds were frozen in order to carry out virus isolation.

Seven birds of the 1992 generation (between 10 and 14 months old) and three older birds exhibited cutaneous lesions (tibiotarsotarsometatarsal joint, carpal joint and toes). Lesions were elevated, closed, warm and relatively smooth when palpated. Development of the disease was subacute to chronic, with progressive swelling of nodules. After two weeks, the necrotic tops of the nodules could be removed, leaving bleeding and swelling erosions. After one month, lesions were reduced to dry scabs. Morbidity in the breeding unit remained low during the season, and only the cutaneous form was observed. A significant weight loss ocurred. Prognosis was very good, although one bird displaying a complete fibrosis of the joint had to have this amputated.

Finally, six birds translocated to a reserve for reintroduction in spring 1993, exhibited respiratory symptoms in summer 1993. During the first five days symptoms were only in the upper respiratory tract, i.e. acute sinusitis, conjunctivitis, respiration with a slightly open mouth. Weight loss was progressive. Despite antibiotherapy associated with a symptomatic treatment, the disease reached the lower respiratory tract; dyspnea and open mouth respiration was evident, and rattling and aerosacculitis sounds were noted during auscultation. Endoscopy of one bird revealed an aerosacculitis. Sinusal injections of antibiotics as well as fumigations had variable results. After six days four birds had died.

Electron microscopy confirmed the diagnosis of an avipoxvirus of the family Poxviridae (Joubert 1985) in sampled chicks. For viral culture, samples were inoculated after filtration on the chorio-allantoic membrane of 10–day-old embryonated chicken eggs (Cunningham 1973). Negative staining on both samples, and chorio-allantoic membranes of inoculated eggs, showed typical pox viral particles. A poxvirus was also isolated from two dead embryos, one dying at eight days' incubation and the other of unknown age. This raises the question of the possible transmission of the virus to the eggs. Viral cultures as well as microscopy confirmed the presence of avipoxvirus in the cutaneous lesions of subadult and adult birds sampled at the Center. The strain was isolated and cultivated, and the virus was identified as a member of the Poxviridae family. Further investigations revealed that the virus was not related to known poultry strains. Seroagglutination analyses indicated no antigenic proximity with chicken and turkey pox strains. A noticeable agglutination occurred with a canary pox strain.

Histology also yielded interesting results. In one chick, Bol-

linger bodies were found in the epithelium of the buccal cavity, towards the larynx. In three other chicks the epithelium of the oesophagus showed a ballooned degeneration with Bollinger bodies. Examination of the spleen in the eight chicks revealed a severe depletion of lymphocytes of the white pulp. Cutaneous biopsies of subadult and adult birds showed a ballooned degeneration and Bollinger bodies.

In the case of the translocated birds, a severe necrotizing pneumonia was found. The tracheal epithelium was very irregular, and sometimes a cellular inclusion body was seen. Small vesicles, most likely the beginning of the ballooning process, had also formed. A focal acute necrosis of tubular cells was observed in one kidney sample. Despite observing no Bollinger bodies, histological signs as well as epidemiology were strongly in favour of a poxvirus origin. The secondary infection seemed to have partially masked the primary causative agent.

In a serological survey done in December 1989 on 33 birds, 18.2 per cent of the birds (six individuals) had antibodies. Of 180 birds tested (in January 1990), only 4.4 per cent (eight individuals) showed antibodies against fowlpox. In the serological survey at the end of January, none of the six birds that had antibodies in December had them in January. In 1990, out of the 45 egg yolks of non-fertile eggs collected, four (8.9 per cent) had antibodies against fowlpox. Avian poxvirus does not produce humoral antibodies to any great extent, which is why the few birds with antibodies against fowlpox are not significant. Serological surveys of the flock are not useful, as seroconversion is not systematic, and the virus strain is not cross-reacting.

No deaths could be directly attributed to avian pox in the adult flock, despite adults dying with severe proliferative lesions in the buccal cavity, and numerous diphtheritic membranes on the oesophagus. Instead they were affected by an enteritis-peritonitis syndrome or a necrotizing pneumonia, due to other infectious agents.

Of the four chicks dying of septicaemia or enteritis, three certainly had a concurrent disease. Intranuclear inclusions due to an unidentified virus were found in the pancreas, the liver or the spleen. Moreover, as shown by histological examination of the spleen, all eight chicks seemed to have severe immunosuppression. It is thus difficult to know the real significance of the pox infection in relation to the other virus. Deaths were the result of secondary bacterial infections, as shown by the bacteriological

cultures obtained from the organs. Pneumonia due to *Pseudomonas* was the most usual complication of poxvirus infection. *Escherichia coli* ($O_{18}K_{80}$) as well as *Klebsiella pneumoniae* were described as causal agents of associated enteritis.

There is no specific therapy against avian pox (Gerlach 1986b), although supportive treatment can be carried out. Administration of vitamin A (2000 UI/kg) and antibiotics can be given to support epithelium growth and prevent secondary bacterial infections. Application of iodine and glycerin on the diphteric lesions is ineffective.

In 1993, all sick birds had been previously vaccinated with chicken and turkey vaccinal strains, proving the low efficiency of these strains. Furthermore, despite a good local post-vaccinal reaction (vaccinal take), results showed only a low seroconversion (two birds out of 12 vaccinated became positive). Although immunity against pox is known to be mostly of cellular nature, and poor seroconversion of little prognosis value, the poor result of vaccination against the virus strain of houbara was confirmed by immunotyping (seroagglutination) processed on the isolated strain. According to our present knowledge, adequate immunization would require the use of an autovaccinal strain, or a canary pox strain. Further studies should be carried out in this direction.

Poxvirus infections may occur in stable, captive avian populations if the disease is regularly brought in by free-ranging wild birds or insect vectors (Clubb 1986). Avipoxvirus is not capable of penetrating intact epithelia (Gerlach 1986b); other vectors such as biting insects are necessary. Mosquitos are present during the period of the year when an outbreak occurred in the houbara chicks (June-July), and could be responsible for transmission from bird to bird (Tripathy & Cunningham 1984). It has been shown that *Culex nigripalpus*, once infected by a donor bird, can remain infected for several weeks and thus mechanically transmit the virus (Akey *et al.* 1981). The breeding unit was also visited daily by hundreds of house sparrows, which fed from the houbaras' plates. Many were found dead in the cages and in the alleys with obvious poxvirus lesions in the mouth and around the beak. Since houbara are newly introduced into the area, and since no virological study of the 'houbara strain' has been performed, it is possible that sparrowpox strain is involved. To reduce the risk of sparrows being disease carriers, the entire breeding unit was covered with fine mesh wire to prevent them from entering the cages. The subsequent decrease in the incidence of pox could be

explained by the isolation of the houbara from the sparrows, as the vaccination programme of the females and chicks was not fully effective. In 1992 and 1993 only cutaneous forms were observed at the Center. The coryza form of the disease found in young translocated birds seems to be linked to a stress-related immunomodulation (new environment, different weather conditions, different food). Interference of an immunosuppression agent is also suspected.

8.2.4 Other Viruses

As in other birds, houbara can be hosts to a number of viruses. Cultures of viruses from frozen samples, as well as electronic microscopy on ultra-thin sections of organs, demonstrated one Herpesvirus, one poxvirus, one Gumboro Disease Virus, three Paramyxovirus (Newcastle Disease Virus, PMV-Pigeon virus and PMV-2) and probably Enterovirus. The strains were sent to specialized laboratories for typing. It is difficult to assess the pathological significance of these findings. Most of the birds showed severe cellular immunosuppression on histological examination of the spleen and blood smears, perhaps due to the Herpesvirus.

To evaluate the epidemiological environment and the exposure of birds to 10 selected viral diseases, as well as to implement an efficient vaccination programme, a serological survey was carried out on a randomly selected group for the following pathogens: paramyxovirus 1 -fowl strain- (NDV), paramyxovirus 1 -pigeon strain- (PMV1P), paramyxovirus 2 (PMV2), paramyxovirus 3 (PMV3), Hemorrhagic Enteritis Adenovirus (HE), celo-adenovirus (CA), Herpes virus -Marek disease- (MDV), Pigeon herpes virus (PHV), Birnavirus -Gumboro disease- (IBD), and Fowlpoxvirus (FP). The serological techniques used were agar gel precipitation tests for HE, CA, GD, MDV, FP and antibodies and hemagglutination inhibition texts for myxoviruses antibodies.

Antibodies to HE, MDV, PHV, FP were detected, but at extremely low prevalences (Table 8.2). Evidence of ND, PMV1P, PMV2, PMV3, CA and GD was found with low but significant prevalence.

In order to understand the incidence of antibodies against Marek's disease virus, pigeon Herpesvirus, Birnavirus, or the Adenovirus strains, it is necessary to isolate the respective virus strains or find the cause of probable cross-reactions. Some birds

Table 8.2 Prevalence of antibodies for 10 Microbial Pathogens in Houbara

Pathogen	n	%
Newcastle disease	180	
Paramyxovirus I Pigeon	180	
Paramyxovirus II	110	
Paramyxovirus III	128	
Hemorrhagic Enteritis Adenovirus	163	
Celo Adenovirus	180	
Marek Disease 180	2.2	
Pigeon Herpes Virus	180	1.1
Gumboro Disease	180	7.2
Fowl Poxvirus 180	4.4	

n = total number of sera tested
% = number of positive sera as a percentage of total tested

showed antibodies against diseases that are specific to poultry, such as Marek's disease and Gumboro disease. Antibodies against the latter have also been found in pheasants (Louzis *et al.* 1979) and guineafowl (Adewuyi *et al.* 1989). This suggests that houbara at the NWRC may have been exposed to the virus from poultry farms in the vicinity, but that they did not express any clinical signs.

Prevalence for all pathogens was lower than 10 per cent. In contrast, prevalences for NDV, PMV1P, PMV3 and PHV were significantly higher. The high occurrence of NDV, PMV1P, PMV3 and PHV suggested that the birds had recently been in contact with other birds, probably pigeons, during transportation. It is not surprising that houbara can be infected by avian Paramyxovirus I, because this virus has a wide host spectrum (Gerlach 1986b), and has been reported frequently in countries where the disease is endemic (Alexander 1988). No deaths were directly related to this pathogen, but three birds showed signs of a nervous disorder during this period: i.e., loss of balance, and when flushed they were unable to fly. Two years later two of these birds still exhibited marked nervous disorders, whereas the other one appeared to have recovered completely. The birds may have been infected with a paramyxovirus lentogenic strain, with minimal pathogenicity. This study is the first to report antibodies against NDV, PMV1P, PMV2, PMV3, HE, CA, MDV, PHV, GD, FP, in houbara bustards.

8.2.5 Parasitology

Cestodes were found during postmortems, and a thickening of the duodenal walls was frequently noticed. After histology, sections of cestodes were noticed in the duodenal lumen or within the mucosa as a proliferation of the mucosal epithelium of the small intestine. Granulomas, sometimes containing parasitic bodies, were found under the serosa of the intestine and on the mesentery. At least two kinds of cestodes were found in the intestine: the largest was identified as *Raillietina paroniella*, a species that has ants and flies as its intermediate hosts. The smaller species was probably *Idiogenes otidis*, and occurred in huge numbers.

Four nematode species (*Hartertia rotundata*, *Histiocephalus choristidis*, *Subulura brumpti* and *Heterakis gallinarum*) have been found, although they were encountered less frequently than were the cestodes. Subcutaneous larvae of *Ascaridia* or *Spirurida* were also found on one occasion.

According to previous work (Mikaelian 1993), haematozoa of the *Haemoproteus* genus have been found in houbara, as well as a haemoparasite of the *Babesia* genus. In 1993, we observed for the first time an infestation of four young birds with *Trichomonas sp.* Treatment was successfully performed with Carnidazole. The origin of this very localized infection (four birds in the same enclosure), is not known, although sparrows, which are rare but still present in the unit, could be carriers.

Cestodes remain a problem in the breeding unit because infestation can be heavy, particularly in the chicks, and may result in weakness and deficiencies of essential nutrients. Houbara eat insects in their cages, which may serve as intermediate hosts. The incorporation of drugs against these parasites into the houbara food would seem the most practical solution.

8.3 Non-infectious Diseases

8.3.1 *Shock and Traumas*

Shock and traumas are the main causes of death in the breeding flock and in captive-reared birds, accounting for 60 per cent of deaths among old (> 1 year) birds in 1993. It is often difficult to prove the traumatic origin of these deaths, and complete epidemiological and pathological analyses are frequently required.

Birds less than 1 month old rarely die from traumas: the youngest bird dead of trauma was 44 days old. After 1 month of age all birds are vulnerable to traumatic accidents. Particularly susceptible are stressed or poorly domesticated birds, and those which have not had their wing feathers cut. Events that can often precede traumas are visits of 'unknown' people, attempts to catch birds, and handling of birds.

Necropsical analyses can help to diagnose a traumatic origin, but can also be very disappointing. Carcasses are always in good condition, and frequently have a significant layer of abdominal fat. Death is always sudden (no symptoms, no decrease in food intake), and necropsy rarely yields obvious indications of the cause of death. Evidence of cerebral congestion or even haemorrhages are not specific to traumas, and can be found following other etiologies. Sometimes concomitant lesions (fractures, luxations) can lead to a traumatic-origin conclusion. It is important to note the position of the dead bird, and to search for evidence of struggling and convulsions; struggling usually occurs when death is not immediate.

The following lesions have been observed: (1) vertebral fracture; (2) vertebral luxation; (3) cerebral haemorrhages; (4) comminutive fracture of the legs and wings; (5) luxation of the femoropelvic joint; and (6) soft tissue injuries. Diagnosis can be very difficult, especially if symptoms of decrease in food intake were observed previously. When associated traumatic lesions are present, diagnosis is easier. The most common traumatic events are cerebral damages: nystagmus, head uncoordination, abnormal gait and leg uncoordination are frequently observed in these cases. Sometimes ataxia or even paralysis is evident. When birds are found dead or paralysed, differential diagnosis must be done to discriminate between nervous manifestations of some acute infectious diseases, and intoxication.

Treatment of paralysed and ataxic birds is hopeless, and the prognosis for birds in shock is poor. Emergency treatment consists of injections (IV and IM) of short-acting corticosteroids every two hours during 36 hours, and after 12 hours the administration t.i.d of a cerebral vasodilator molecule. Antibiotics are recommended to prevent bacterial invasion (Redig 1993). Symptomatic treatment can be employed as well. Supportive treatment consists of the administration of a vitamin B complex, and of strychnine sulphate during five days. The bird should be force-fed, and attempts to make it defaecate are essential, as cloacal

sphincter paralysis can occur. In some cases administration of fluids is also necessary (Redig 1993). Among paralysed birds, recovery rates were low, with a mortality rate of 93.4 per cent in 1993. Treatments of concomitant lesions are carried out according to previously described techniques. Broken wings are systematically amputated. When the humerus is concerned, total amputation is necessary, and we recommend a prophylactic amputation of the other wing to avoid imbalance. Leg fractures are difficult to repair successfully. Simple pinning (direct or indirect) is often useless, as it rarely prevents rotation of bones. Use of neutralization plates is not often possible, considering the size of the bone and its pneumatized structure. Fractured bones often appear at the end of the laying season when the birds' ossature is decalcified. We used external skeletal devices with variable success. Double external fixation using external plastic supports instead of metallic ones, which reduces the weight of the device, has given the best results with closed, diaphyseal fractures of the femur.

A few measures can greatly reduce deaths caused by traumas. Preventing stress is strongly recommended: high stress levels as well as the presence of visitors seem to cause most accidents. Between 1992 and 1993, 40 per cent of deaths due to traumas among old (>1–yr-old) birds occurred during such visits. It is essential that visitors are fully informed of the possibility of disturbance to the birds, and are closely supervised while inside the units.

Modification of the fenced environment is often necessary. The use of 'soft' fences is essential; no deaths due to traumas occurred inside the enclosures with walls made of shade-cloth. Therefore, enclosures should have walls made of shade-cloth, with no posts inside the enclosures or close to the walls, and a tensioned net ceiling at 1.5 m height to prevent birds from attempting to take off. Finally, appropriate management techniques are essential; panic is communicative, therefore there should be no more than two birds per breeding enclosure. Feathers of both wings should be cut at least twice a year, and should be checked when the birds are being handled for other reasons. Attempts should be made to limit the physiological decalcification of laying females. Careful handling of birds is necessary; the bird should wear a hood at all times, and the handler should not attempt to restrain the bird from kicking its legs.

8.3.2 Hernia of the Yolk and Anoxia

These syndromes are only found in very young chicks (mortality at 0 – 3 days of age). Hernia of the yolk is a neonatal syndrome directly linked to hatching, which is easy to treat when the hernia is small. Treatment consists of ligature of the yolk, followed by excision and disinfection. Antibiotherapy and rehydration by subcutaneous injections of normal saline are always performed. It is necessary to use sterilized equipment throughout the procedure. After the suture is performed, subcutaneous injections of warm and sterile lactated Ringer's solution is necessary. These injections help supply the chick with fluid lost by removal of the yolk sac. They should be done into the inguinal web of the leg or under the lateral skin of the thigh. Approximately 0.4 ml. of rehydrating fluid, which creates a water 'blister' in the fold of the leg, can be administered. The 'blister' will disappear within a few hours. Discontinue the injections when the chick has regained its vigour and is eating normally. Survival of chicks with partial unretraction is much higher than for chicks with total unretraction. This treatment is less effective when the hernia is voluminous, and septicaemia may occur less than 24 hours after hatching.

Anoxia (or long-hatching syndrome) is also linked to the hatching period, and occurred at the beginning of the hatching season. Newly hatched chicks' eyes failed to open, they were unable to stand, and suffered from lethargy and anoxia. Even if force-fed, these chicks inevitably died. The incidence of this syndrome was reduced when oxygen was added to the atmosphere of the incubators.

8.3.3 Ventriculus Impaction and Stomacale Perforation

These occurred in chicks and young birds: death resulted from accidental ingestion of foreign bodies (pieces of wire, nails and screws) or by over-ingestion of sand (Plate 8.1). In very young chicks treatment is hopeless, as ventriculostomies are unsuccessful at this age. Foreign bodies usually perforated the ventriculus, and peritonitis followed the perforation. Medical treatment using mineral oil was successfully performed on birds showing a low level of ventriculus impaction. Gizzard impaction is known to cause high mortality during the first three weeks of life in turkey flocks (Riddell 1991). Affected turkeys are usually emaciated

owing to an empty intestinal tract, but gizzards are full of a solid mass of interwoven fibrous material. Houbara also have a well-developed gizzard and require grit in their diets for the grinding of seeds and hard foods (Arnall & Keymer 1975). In houbara chicks the gizzard is mainly impacted with fine gravel, which often extends into the first part of the duodenum and the lower intestine. This impaction results from eating litter which the gizzard is unable to handle. If the grit is too fine, impaction rate is increased. Prevention is aimed at discouraging the eating of litter by young chicks. Chicks are gradually introduced to gravel floors, or reared on gravel-free spaces and moved to normal ground only after meals. Further studies concerning the nutritional requirements of houbara chicks, and their qualitative choice of food and grit will certainly improve rearing efficiency.

8.3.4 Secondary Hyperparathyroidism

Calcium is needed for bone mineralization by the eighth day of embryonic life: it is derived initially from the yolk and then from the egg shell (O'Connor 1984). Bone metabolic disorders are a common pathological problem in growing chicks, both in wildlife practice (Redig 1993) and in avian pet practice (Fowler 1980; Wallach & Flieg 1967). Calcium deficiency leads to secondary hyperparathyroidism syndrome (increased secretion of PTH as a compensatory mechanism induced by nutritional imbalances, in this case, low calcium content of the diet). Between 1991 and 1993 we observed chicks with calcium deficiency symptoms, which appeared at three weeks of age; i.e. lameness varying from a slight limp to inability to walk and folding fractures of the long bones. Lesions are typical of a fibrous osteodystrophy. We successfully treated these chicks with intensive calcium and vitamin D3 therapy.

8.4 Therapeutics

The drugs listed below are not intended to represent a complete pharmacological arsenal for houbara; however, their choice is based on their observed effectiveness and safety (Table 8.3). No particular intolerance to drugs was found. Use of antibiotics must be preceded by a culture and a sensitivity test. However, because

it is often necessary to initiate treatment very quickly, broad-spectrum drugs are recommended. A synthesis of the results from all the antibiograms showed that the most efficient antibiotics *in vitro* were quinolones and/or furan derivatives, or aminoglucosides. Baytril (Enrofloxacine) is presently considered to be one of the safest most effective and broad spectrum of all avian antibiotics, and is an excellent 'first choice' before a diagnosis is made (Remple & Riddle 1991).

Short-acting steroids are occasionally used to combat shock and stress. Vitamin and mineral preparations are used to supplement deficiencies and act as tonics. Gaseous anaesthesia with halothane is certainly one of the safest and most efficient methods. Induction time is short, and recovery fast and of very good quality.

Owing to the lack of muscle mass, oral administration of drugs is most desirable for young chicks. With respect to adult birds, medicating food on a 'free choice' basis is not always effective, as consumption is unpredictable. However, for the parenteral administration of drugs, the risks associated with handling and the resulting induced stress on the birds have negative effects that could interfere with the efficacy of the treatment. Incorporating drugs into pellets could be effective, and should be carried out when general treatment or prophylaxis of the entire flock is required, but associated problems may be manufacturing delays, and the homogeneity of the drug's distribution in feed when small quantities are ordered. Intramuscular injections into the breast musculature are always preferred for individual treatment because they are more accurate, and therefore the treatment is more effective. Intravenous administration may be appropriate for delivering fluids or drugs, or to draw diagnostic blood samples. The most easily accessible vein in a houbara bustard is the *venal basilica*, which runs along the inside of the elbow joint. It is easily visible once the feathers have been wet with alcohol. Access to the vein requires two people, one to immobilize the bird on its back. The syringe operator then grasps the clenched wing of the bustard with the left hand and guides the needle into the distended vein with the right hand.

8.5 Sanitary and Prophylaxis Plan

Young birds are vaccinated at 2 months of age with a living strain (Hitchner B1) of Newcastle Disease by eye or nares instillation. They are injected one month later with an inactivated strain (Texas strain) and re-instillation with the living strain. Vaccination revealed significant seroconversion and no adverse side-effects. Adult and sub-adult birds receive a booster instillation of this vaccine once a year (Plate 8.2).

Until recently, chicks were vaccinated at 2 months of age against fowlpox with a turkey and chicken vaccinal strain. However, many vaccinated birds develop the disease despite vaccination, and seroconversion is sporadic, with no statistical significance. Therefore, we discontinued systematic immunization, and are trying to develop an autovaccinal strain.

Deworming is performed twice a year on adult birds: in June and December (after and before the laying season). Young birds are dewormed four times a year. Different drugs have been used alternately to prevent parasites from becoming resistant to drugs (see Therapeutics).

It is very important that sanitary procedures are carried out carefully. Regular examinations of the birds are necessary: before and after the laying season houbara are checked, dewormed and blood-sampled. A basic haematological examination is carried out on certain individuals (blood smear, PCV, Red Blood Cell Count). Whenever houbara die, a necropsy is performed within six hours to collect reliable data on the cause of death, and to be able to prevent outbreaks of potentially contagious diseases. A normal dissection procedure is followed that ensures no contamination or destruction of material.

After numerous deaths due to infection in 1989, the breeding unit was isolated from the outside by a finer covering of mesh. A changing room at the entrance of the breeding unit, a hygiene protocol rotating the usage of the cage blocks, as well as regular disinfection with caustic soda were implemented. Subsequently, infection sources were better controlled, and a greater proportion of chicks were successfully reared without any losses as a result of infectious diseases.

A strict quarantine period was established for any bird entering the NWRC. The purpose of quarantine is to prevent the propagation of parasitic, bacteriologic and viral diseases. These diseases may show no clinical signs in healthy birds that survived

Table 8.3 Drugs and Dosages Used for Houbara

Generic Name	Route	Dose	Frequency	Notes
		100 mg/kg		Effective drug against bacterial diarrhoea and infected injuries
Carnidazole	Oral	20 mg/kg	SID (4 days)	For trichomoniasis
Dexamethasone	IM	2 mg/kg up to 50 mg/kg	BID	Life-threatening shock, trauma (see 8.3.1)
Dimetridazole	Oral	0.5 ml powder in 10 ml water/kg	SID (5 days)	For trichomoniasis
Doxycycline	SC Oral	10 mg/kg	SID	See treatment of Chlamydiosis
Enrofloxacine 5%	IM/SC	10 mg/kg	BID	Broad spectrum antibiotic. Antibiotic of choice for *mycoplasma*
Fenbendazole 2.5%	Oral	25 mg/kg 25 mg/kg	Once	Prophylatic drug against round worms
	Oral	25 mg/kg	SID (3 days)	Treatment
Flumequin	Oral	10 mg/kg	BID	Good antibiotic against opportunistic bacteria
Ivermectin	Oral	Dilute the bovine preparation 1:4 with propylene glycol and give 0.15 ml/kg	Once	Effective against most gastro-intestinal worms (not tape worms)
Kaopectate solution	Oral	2 ml/kg	TID	As a soothing effect on the gut
Mineral oil	Oral	1–3 ml/kg	Once	Aid in removal of small foreign objects from the ventriculus
Niclosamide	Oral	200 mg/kg	Once	For tape worms
Oxytetracycline (LA)	IM	200 mg/kg	SID	
Praziquantel	Oral IM	1/4 tab./kg 10 mg/kg	Once Once	For tape worms
Trimethoprime 40 mg and Sulphonamide 200 mg (Sulfamethoxypyridazine)	IM	0.2 ml/kg	BID	Prolonged use can cause kidney damage
Rifampicin	Oral	20 mg/kg	BID	Effective against bacterial diarrhoea
Tylosin	IM	20 mg/kg	TID	For *mycoplasma*
Vitamin A, D3, E	IM	0.2 ml/400g	One every 5 days	For A, D3, E hypovitaminosis

Intramuscular (IM); once daily (SID); once every 12 hours (BID); once every eight hours (TID)

the infection, but can cause disastrous consequences in birds that

have not been in contact with the pathogens. Any bird entering the Center is blood-sampled, vaccinated for Newcastle Disease, dewormed and serologically checked. Birds are not released from quarantine until their serological results are available. Birds positive for Chlamydiosis are allowed to join the flock only after Doxycyclin treatment for seven weeks.

Because it is often very difficult to confirm the appearance of discrete infectious agents in the breeding or rearing units, we follow a selected population sample (based on position in the unit, age, sex, sub-species) serologically to detect possible outbreaks of a subclinical disease. Sera are screened once per month against a panel of poultry diseases, including Chlamydiosis.

8.6 Conclusions

Mortalities diagnosed as resulting from Chlamydiosis, as well as positive serological results indicate that *C. psittaci* was widely distributed in the Center. Our eradication programme contributed substantially towards increasing the level of hygiene in the flock.

The clinical signs and microscopic pathological lesions observed in the houbara during avian pox outbreaks are similar to those described for fowlpox in other avian species (Tripathy & Cunningham 1984). The virus has been isolated, but the level of mortality observed suggests this strain is particularly lethal (Clubb 1986).

Regular serological analyses revealed that well-known poultry pathogens can still appear in the flock. Efforts must continue to halt the introduction of contagious infectious agents into the Center. Infectious diseases are no longer the primary cause of death, indicating that we have achieved a high level of hygiene. The next challenge must be to prevent deaths from traumas. Avian species are often extremely vulnerable to stress, and frequently die of stress-related traumas. When contemplating an intervention, the practitioner should consider from a medical point of view whether 'the end justifies the means'.

The systematic collection of samples during houbara postmortems or blood sampling has allowed us to gather a wide range of data in various pathological fields. But this survey of disease problems raises more questions than it yields exact diagnoses or solutions. As no data were available previously on this species,

many other fields should be investigated. The analysis of resulting data will provide unique information on this poorly known bird, and is necessary for the success of houbara captive-breeding and reintroduction programmes in Saudi Arabia.