

ENDOSCOPIC DIAGNOSIS, TREATMENT AND PATHOLOGY OF *ENTEROCYTOZOOM BIENEUSI* INFECTIONS IN FALCONS

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KEYWORDS

Falcons – Abscesses - *Enterocytozoon bieneusi* – Endoscopy -
Protozoal treatment - Pathology

ABSTRACT

Four falcons died due to liver and intestinal abscesses within 1 week. The cause of death was not clearly identified as all the falcons had visible yellow lesion on liver and intestines which appeared different to lesions of known diseases. The post-mortem was suggestive of a protozoal infection. Following a suspected disease transmission, all falcons of 3 different cages were caught and examined at the Abu Dhabi Falcon Hospital. Apart from other examinations, endoscopic evaluation was performed in 137 falcons. Individual treatment plans were established for all falcons with Emtryl® and homeopathic medicines for liver, kidney and intestinal disorders. Out of the examined birds, a total of 117 falcons survived and showed abscess regression in the follow-up endoscopies after treatment. Twenty of the examined birds died within 6 weeks due to advanced liver, intestinal and kidney abscesses. Samples of six dead falcons were further investigated and 5 months after the examinations, the diagnosis *Enterocytozoon bieneusi* genotype D was confirmed by PCR and sequencing at the University of Vienna. The presence of *E. bieneusi* in falcons is a completely new parasitic disease with zoonotic potential and needs to be taken into consideration from now on.

1 INTRODUCTION

Three falcons kept together in a large free-flight aviary died due to liver and intestinal abscesses within 3 days with an additional dead fourth bird from the same cage a few days later. Because the 4 dead falcons were kept together with 114 others in a large free-flight aviary and 2 other falcon cages were located close by, a disease transmission was regarded as possible and was further investigated. Therefore 137 falcons of 3 different cages were caught and presented for examination at the Abu Dhabi Falcon Hospital.

The gross post-mortem of the four dead falcons showed large multifocal abscesses such as structure resembling yellow nodules in intestines, liver and kidney. The death reason for those falcons were not clearly identified as all the falcons had visible yellow lesion on liver and intestines which did not resemble any of the known diseases e.g. tuberculosis, herpes virus infection or salmonellosis. Histopathology was performed in all cases in the Central Veterinary Research Laboratory, Dubai. However, the histopathology did not confirm the presence of tuberculosis or bacterial infections but was suggestive of a protozoal infection.

2 MATERIALS AND METHODS

2.1 Materials

A total of 137 Falcons, among them 47 gyr-peregrine hybrid falcons (*Falco rusticolus* x *Falco peregrinus*), 69 gyr-saker hybrid falcons (*Falco rusticolus* x *Falco cherrug*), 14 gyr falcons (*Falco rusticolus*), 6 peregrine falcons (*Falco peregrinus*) and 1 lanner falcon (*Falco biarmicus*) were examined during the time period of June 15th to July 9th 2005. The majority of the falcons were one and two years old.

2.2 Methods

For all falcons, the following examinations were performed: blood haematology and biochemistry, parasitological and microbiological testing of crop and faecal samples (including acid-fast stain), urate analysis, endoscopy and radiography. Furthermore, serological tests for detection of antibodies against Paramyxovirus 1 Virus, Avian Influenza Virus, Avian Reo virus, Avian Pneumo virus, Infectious Bronchitis virus, *Chlamydophila* and *Mycoplasma gallisepticum* were

performed as per request of the owner to get full information about the health status of the falcons.

Necropsy was performed from 24 falcons over a period of 6 weeks. Samples were taken for histopathology and bacteriology. Histopathology was performed using routine methods. Suspicious organ samples were also stained using Ziehl-Neelsen (ZN), PAS and Grocott.

Endoscopic laparoscopy

In all cases the following standard rigid endoscopy system was used: Wolf 2.7mm telescope with 0° angle, Olympus light source, matching Wolf canula and trocar and biopsy forceps. All endoscopies were recorded using the ADFH Endocap documentation system. The laparoscopy of the falcons were performed in left and right lateral recumbency with entry between the last two ribs.

3 RESULTS

3.1 Clinical findings

Out of the 137 examined falcons, 70 showed clinically apparent abscesses of intestines, liver and/or kidneys in the endoscopic examination.

In the cases of liver abscesses, they were directly visible after the entry in the thoracical airsac. The yellow intestinal abscesses were already visible through the abdominal airsac membrane and were better seen after puncturing the abdominal airsac. By doing this the kidney abscesses became visible as well. .

Table 1. Relation of total number of examined falcons and diseased falcons

Species	Falcons examined	Falcons with abscesses
Gyr-Saker falcon	69	36 (52.2%)
Gyr-peregrine falcon	47	28 (59.6%)
Gyr falcon	14	4 (28.6%)
Peregrine falcon	6	2 (33.3%)
Lanner falcon	1	0

A difference in the species distribution was observed regarding the presence of abscesses in liver, intestines and kidneys as mentioned below (table 2).

Table 2. Endoscopic findings and relation to species

Endoscopic finding	Total	Gyr-Peregrine	Gyr-Saker	Gyr	Peregrine
Intestinal abscesses*	38	16 (42.1%)	19 (50.0%)	2 (5.3%)	1 (2.6%)
Abscesses in liver	12	5 (41.7%)	5 (41.7%)	1 (8.3%)	1 (8.3%)
Abscesses in kidneys	20	7 (35.0%)	12 (60.0%)	1 (5.0%)	0 (0.0%)

* This includes all intestinal parts.

The complete examinations led to several findings, the most important was heavy coccidiosis. A total of 74 falcons suffered from a heavy *Caryospora* burden. Out of those, 39 gyr-saker hybrid falcons, 22 gyr-peregrine hybrid falcons as well as 9 gyr falcons and 4 peregrine falcons were tested positive for *Caryospora*.

Moreover, the examined falcons which had been diagnosed with abscesses suffered from aspergillosis in different stages (10%), hepatomegaly (14.3%) and renomegaly (25.7%) and bacterial infections such as *E. coli* which were identified by cloacal and faecal microbiological cultures (12.9%), as well as from other problems such as severe dehydration (22.9%) being other findings.

In none of the falcons salmonella, nor acid-fast rods or herpes virus were detected. Additionally none of the birds demonstrated antibodies against the above mentioned viruses.

3.2 Pathology results

Within six weeks after the first falcon had died, 20 more falcons out of the 137 examined falcons died. Therefore the total number of dead falcons amounted to 24 falcons including the four falcons that had died in the first week. Out of those 24 dead falcons, the disease was already in a very advanced stage in 18 cases. The dead falcons were examined for pathology and histopathology. Multiple yellowish plaques on the intestine occurred in 18 out of the 24 falcons. This focal diphtheroid enteritis and colitis involved all intestinal layers from the mucosa to the serosa. Numerous yellowish foci (1-5 mm in diameter) were also seen in the liver (15 out of the 24 cases) and kidneys (10 out of the 24 cases). Pancreas and spleen were also affected in one falcon each. The other 6 dead falcons died of other reasons such as advanced aspergillosis, clostridiosis, gout and amyloidosis.

3.3 Histology results

Histology of the liver lesions revealed large areas with foamy hepatocytes and bile duct proliferation, fresh necrosis and microabscesses in the adjacent areas. The kidneys showed severe diffuse degeneration with pyogranulative inflammation, most tubuli containing protein cylinders. Severe pyogranulative inflammation was found in one pancreas. The intestine showed severe focal pyogranulative to ulcerative enteritis. Special stains for acid-fast rods (ZN), fungi or parasites (PAS, Grocott) of the necrotic lesions were always negative. Due to these findings, the preliminary diagnosis of protozoal infection of unknown origin was made with suspected amoebiasis.

To confirm this preliminary diagnosis, liver and intestinal samples from 6 randomly selected dead falcons, samples were sent to the University of Vienna, Austria, for further investigation. Five of those selected falcons had clearly visible lesions and one was without visible lesion. However, it suffered from severe visceral gout. PCR for amoeba-DNA was negative. However, after 5 months, the presence of *Enterocytozoon bieneusi*, Genotype D was confirmed in all 6 falcons by PCR methods..

3.4 Therapy

Due to the unclear and unconfirmed diagnosis, the treatment plan was difficult to establish. However, following the preliminary diagnosis of "protozoa infection", a standard treatment for such advanced disease stage is not available in falcons and a tailor-made therapy was established for each falcon (table 3). All falcons were repeatedly endoscoped every two weeks until a complete regression of abscesses was reached. In those cases where the endoscopy showed partly regression of the abscesses, the treatment was continued and the Emtryl® course was repeated.

Table 3. Drugs and their application mode and time

Drugs	Application mode and frequency	Day 1-10	Day 11-17	Day 18-27	>Day 27
Emtryl®	50mg/kg p.o. 1 x d AM	Yes	no	yes	If required, after 10 d break another 10 d treatment Yes if required
Nux vomica®	1.0ml sc 1 x d AM	Yes	yes	yes	Yes if required
Mucosa compositum®	1.0ml sc 1 x d AM	Yes	yes	yes	Yes if required
Hepar compositum®	1.0ml sc 1 x d AM	Yes	Yes, if required	Yes, if required	Yes if required

Legalon®	1 tabl 5 x d AM, PM	Yes	no	no	no
Berberis compositum®	1.0ml sc 1 x d AM	Yes	Yes, if required	Yes, if required	Yes if required
Cantharis compositum®	1.0ml sc 1 x d AM	Yes	Yes, if required	Yes, if required	Yes if required
Hartmann's solution®	40-60 ml sc 1 x d AM	Yes	Yes	Yes, if required	Yes if required
NaCl and Glucose®	10 ml iv 1 x d AM	3-5 days	no	no	no
Baycox®	0.7ml/1kg BW, 1 x d AM	3 consec. d	If Caryo. still present, 3 more d	no	no
Spark®	1 spoon over the food 1 x d	yes	yes	yes	no
Probiotics®	1 spoon over the food 1 x d	no	no	no	yes

All falcons were treated with Emtryl® for 10 days. The treatment was not only performed for the falcons with endoscopic visible abscesses, but also for the apparently more healthy ones due to the fact that they were together in one cage and the transmission of the protozoal infection could have taken place already without being endoscopically manifest yet. Falcons with intestinal abscesses were treated after one week rest again with Emtryl® in the same dosage for another 10 days. If required in advanced cases, the Emtryl® course was repeated up to 3-4 times until the repeated endoscopic examination proved a regression of the intestinal abscesses. All falcons suffering from intestinal abscesses underwent additional homeopathic treatment with Nux vomica® and Mucosa compositum® daily for up to 4 weeks.

Those falcons suffering from liver abscesses received initially Hepar compositum® (Heel) 1.0ml daily for 1-4 weeks depending on the severity of the diseases as well as Legalon® 1 tablet for 5 days. Falcons affected by kidney abscesses were treated additionally with the homeopathic medicines Berberis® 1.0ml and Cantharis® (Heel) daily for 1-3 weeks depending on the case.

All falcons were rehydrated with Hartmann's solution® daily until they were fully rehydrated. Spark® powder was given over the food day to add electrolytes for the dehydrated falcons. The massive coccidiosis was treated with Baycox® given for 3 days although usually two days treatment is sufficient. After one week another 2 days treatment were given if *Caryospora* was still prevalent in the faeces. The intestinal flora was restored with Probiotics® (Vetafarm) given daily over the food after the Emtryl® therapy was finished. Adjacent diseases such as aspergillosis and bacterial infections were treated accordingly.

The sand layer of the complete aviary and all adjacent aviaries were removed up to 20 cm depth and replaced by new sand. A cleaning and disinfection took place with Terminator™ for all cages.

3.5 Treatment results

Apart from 20 more falcons which died shortly after the initial examinations due to advanced disease stages, all other falcons responded well to the individual treatment plan, survived and recovered well. The repeated endoscopic examinations showed a good response to the treatment until full recovery in the surviving falcons. Falcons with smaller abscesses showed a continuous regression of the abscesses up to a full disappearance. The recovered falcons could be used for falconry again in the winter season of 2005.

4 DISCUSSION

The microsporidia *Enterocytozoon bieneusi* was detected for the first time in France in 1985 in an immunosuppressed AIDS patient with diarrhoea and isolated from the small intestine, especially the jejunum (DESPORTES et al. 1985). Other risk groups are travelers especially from tropic countries (LOPEZ-VELEZ et al. 1999) as well as elderly people and children due to their reduced immune system (LORES et al. 2002 b) It is the smallest microsporidia spore of 1.2-1.7µm long (CANNING 1993). An infection of humans might arise through inhalation, direct contact with mucosa and ingestion of the microsporidial spores (HARO et al. 2005). Moreover, in humans a significant symptom of this microsporidial infection is diarrhoea accompanied by slow weight loss (CANNING 1993).

Detection in the faecal is difficult due to the small size of the protozoa (CANNING 1993). One method is the simple salt flotation followed by cytospin and Giemsa staining to improve the background in order to easier detect the small *E. bieneusi* spores (VAN GOOL et al. 1990). The *E. bieneusi* spores are Gram positive and stain with Ziel-Neelsen acid-fast stain. Moreover PAS (Periodic Acid-Schiff) stain can be used to show the polar cap, whereas Giemsa stain is helpful to identify the proliferative stages and show the nuclei of the spores especially after hydrolysis (CANNING 1993). Another identification method from a faecal smear is the Weber's chromotrope and Gram chromotrope-based stains (WEBER et al. 1992, MOURA et al. 1996). Despite several trials to identify the suspected protozoal organism by different staining methods, no

identification of the pathogen could be made. Another method is the detection of *E. bienersi* by PCR with species-specific primers EBIEF1/EBIER1 (DA SILVA et al. 1996).

E. bienersi is known to be present in domestic animals (LORES 2002a) such as rabbits, goats, pigs and dogs (DEL AGUILA et al. 1999). Infections of birds with *E. bienersi* have been for the first time detected in chicken in 2002 (REETZ et al. 2002) and recently in a second avian species, urban pigeons (HARO et al. 2005). In 20% of the pigeons tested, the prevalence of *E. bienersi* was confirmed by PCR. This finding is even more interesting as falcons are birds of prey which are frequently fed with pigeons. The lack of a transmission barrier of *E. bienersi* might lead to a possible zoonotic potential (DENGJEL 2001) In 6 dead falcons from the here presented group, *E. bienersi* has been detected by PCR methods. The identified genotype D has not been found in birds so far. Infections with other microsporidial agents such as *Enterocytozoon hellem* are much more known to infect birds such as lovebirds (SNOWDEN et al. 1999), ostrich (SNOWDEN and LOGAN 1999), budgerigars (BLACK et al. 1997), parrots (PULPARAMPIL et al. 1998) and a finch (CARLISLE et al. 2002). To the author's knowledge this is the first report about endoscopic diagnosis, treatment and pathology of an *Enterocytozoon bienersi* infection in falcons.

The presence of *E. bienersi* in falcons is a completely new parasitic and zoonotic disease which needs to be taken into consideration from now on. It might be possible that the captive-bred pigeons which live in the same farm as the falcons and are used as their food might have infected the falcons. Another possibility is free flying pigeons which might have flown over the falcons' aviary and transmitted the microsporidiosis with their droppings into the cage. If a transmission from an infected human to the falcons might be possible, is not researched yet. However, many caretakers of this particular falcon group originate from Asian countries. In travelers from tropic countries the presence of *E. bienersi* infections is known (LOPEZ-VELEZ et al. 1999). Therefore a transmission of possible infected staff to the birds following poor hygienic measures can not be ruled out, especially as the genotype D is reported in humans. The falcons suffered from immune suppression caused by predisposing factors such as overcrowding in the aviary and adjacent diseases such as aspergillosis. The massive *Caryospora* burden led to a severe damage of the intestines thus may have paved the way to an invasion of the microsporidial spores. Moreover, the falcons of all 3 cages had been infected as identified by the PCR method thus leading to the assumption that a disease transmission had taken place from one cage to another.

Furthermore, one falcon with *E. bieneusi* infection did not show any histopathological symptoms thus raising the question of possible disease carriers. It is still unclear for how long the *E. bieneusi* infection was present in the falcon flock.

The endoscopic picture of *E. bieneusi* infections appears to be a bit different from, but reminds to the picture of tuberculosis, salmonella or herpes virus infection although the clinical picture is different for those diseases. However, from now on *E. bieneusi*-infection should be included as a differential observing the described lesions. The treatment of these sick falcons posed a major challenge as the cause for the disease could not be clearly identified at the time of treatment. Therefore an individual treatment plan was established for each single falcon following the complete clinical findings based on the suspected diagnosis "protozoal infections".

The *E. bieneusi* infections in humans might be treated with albendazole which has been successful in AIDS patients (BLANSHARD et al. 1992). The effect of albendazole seems to be in a damage of the developmental stages of the *E. bieneusi* spores in the small intestines thus partially inhibiting the parasite reproduction (BLANSHARD et al. 1993). Contrasting these findings, albendazole did not work effectively in other AIDS patients with *E. bieneusi* infections who responded well to the new broad-spectrum antiparasitic medicine nitazoxanide (BICART-SÉE et al. 2000). Nitazoxanide being effective for *E. bieneusi* infections is a nitroheterocyclic drug, same such as dimetridazole (RAETHER and HAENEL 2003). However, this drug is not approved for veterinary use in protozoal infections (RAETHER and HAENEL 2003). Even in the 9th Conference on Retroviruses and Opportunistic Infections, MOLINA et al. (2002) stated that currently no treatment is available for *E. bieneusi* infections. They tested oral fumagillin successfully for the treatment of chronic *E. bieneusi* infections in humans (MOLINA et al. 2002). Another treatment approach in humans is the use of furazolidone which has changed the faecal sporocysts of *E. bieneusi* in clinical tests (DIONISIO et al. 1997).

In birds, a treatment for *E. bieneusi* infections has not been clearly specified yet. Despite in vitro research for *Encephalitozoon cuniculi* showing that drugs such as itraconazole, toltafuril, metronidazole and ronidazole are not effective (FRANSSEN et al. 1995), there seem to be a difference to *E. bieneusi* infections. For veterinary use, another nitroheterocyclic drug for treatment of protozoal infections is dimetronidazole (1,2-Dimethyl-5-nitroimidazole) (RAETHER and HAENEL 2003). The drug Emtryl® (dimetronidazole) used in this case seemed to be effective against *E. bieneusi* due to the regression of the abscesses and high survival rate. Whether human

drugs such as nitazoxanide can be used for an efficient treatment of *E. bieneusi* infections in birds is not investigated yet.

The homeopathic treatment of animals even as supportive therapy is very helpful as they respond well to it (WOLTER 1989). Medicines such as Nux vomica®, Mucosa compositum®, Hepar compositum®, Berberis compositum®, Cantharis compositum® have been successfully used in intestinal, hepatic and renal disorders in birds (DORENKAMP 2000).

5 CONCLUSIONS

The detection of *E. bieneusi* in falcons as third confirmed susceptible avian species leads to the question how many more falcons might have got infected unnoticed from this disease especially as the genotype D is so far not reported in birds, but in humans, macaques and pigs. However, despite the fact that a diagnosis was only available 5 months after the infection occurred, the comprehensive treatment led to the survival of the majority of falcons and even regression of the abscesses. Further research is required to which extent other medicines against microsporidia can be used in falcons.

The zoonotic potential of *E. bieneusi* is an issue that should be taken into consideration as falconers who live in close contact with their falcons might be at risk of a disease transmission from their birds. Further research is required to identify further affected avian species as well as the different transmission way.

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