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## Suspected toxicity by biological waste and air sac nematode infestation in a free-living peregrine falcon (*Falco peregrinus*)

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## Υποπτευομένη τοξίκωση αποδιδόμενη σε βιολογικό λύμμα και μόλυνση από νηματώδη των αεροφόρων σάκων σε έναν ελεύθερο πετρίτη (*Falco peregrinus*)

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**ABSTRACT.** This case report describes an uncommon complex of mortality in a free-living peregrine falcon (*Falco peregrinus*). The bird was found rinsed with biologic waste and the integument was mildly inflamed. No respiratory signs were detected at first. In addition, no alterations were detected during. During orthopaedic, neurologic, ophthalmologic and radiographic examinations. Parasitological pharyngeal and faecal examination revealed *Capillaria* spp. and *Serratospiculum* spp. eggs. The therapeutic scheme included stress reduction, supportive therapy with fluids, antifungals, antibiotics, anti-inflammatory, anti-parasitic agents and nutritional management. Cleaning with dishwashing gel to remove the biological waste took place, but its removal was partial. Despite the intensive care, the patient developed respiratory distress and succumbed on the fifth day of hospitalization. Pathological examination revealed adult *Serratospiculum tendo* parasites in the air sacs and *Capillaria* spp. in the large intestine. Bacteriological and mycological cultures revealed significant growth of *Klebsiella pneumoniae* isolated from the skin, heart, lung, liver, kidney and intestine and a mild presence of *Candida albicans* in the large intestine. Their probable role was judged as secondary. Mild multifocal haemorrhages with heterophilic and lymphohistiocytic infiltrates, fibrin and rod-like bacteria were detected in the skin histopathologically, confirming the clinical integument inflammation. Cause of death was asphyxia caused by oedema, haemorrhages and blood congestion of the lung. The contribution of *S. tendo* in the initial spill event and case evolution is discussed. This unusual case highlights the complexity of the wildlife cases, with which avian or small animal clinicians (consulting rehabilitation centres or falconers) might be confronted. Although the application of ancillary

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and advanced diagnostic tools, the in-depth knowledge of the species and the intensive monitoring are important, this might lead to an unrewarding outcome. Financial constraints in wildlife casualty cases urge the critical choice of diagnostic, therapeutic and post-mortem laboratory testing. *Serratospiculum tendo* should be considered in the differential diagnosis of acute respiratory distress in free-living peregrine falcons, presented in rehabilitation centres or in private practise by falconers in Greece and Europe.

**Keywords:** Biologic waste spilling, *Klebsiella sp*, peregrine falcon, *Serratospiculum sp*.

**ΠΕΡΙΛΗΨΗ.** Το παρόν περιστατικό περιγράφει μια ασυνήθιστη, επιπλεγμένη αιτία θανάτου σε έναν ελεύθερο πετρίτη (*Falco peregrinus*). Το πτηνό (γεράκι) παρουσιάστηκε βρεγμένο με βιολογικά απόβλητα, και το δέρμα του ήταν μετριώς φλεγμαίνον. Αρχικά δεν εμφάνιζε αναπνευστικά συμπτώματα. Η ορθοπεδική, νευρολογική, οφθαλμιατρική και ακτινολογική εξέταση ήταν ασυμπτωματικές. Η παρασιτολογική εξέταση, από τον οροφάρυγγα και τα κόπρανα, αποκάλυψε ωά από *Capillaria spp.* και *Serratospiculum spp.* Το θεραπευτικό σχήμα περιελάμβανε μείωση της καταπόνησης, υποστηρικτική αγωγή με υποδόρια υγρά, αντιμυκητιακά, αντιβιοτικά, αντιφλεγμονώδη, αντιπαρασιτικά φάρμακα και διατροφική διαχείριση. Επίσης πραγματοποιήθηκε έκπλυση του πτερώματος για απομάκρυνση του λύμματος, αλλά η απομάκρυνση ήταν μερική. Παρά την εντατική θεραπεία, ο ασθενής επέδειξε οξεία, αναπνευστική ανεπάρκεια και απεβίωσε, την πέμπτη μέρα της νοσηλείας του. Στην νεκροτομική εξέταση πλήθος ενήλικων νηματοδών παρασίτων του γένους *Serratospiculum tendo* ήταν παρόντα στους αεροφόρους σάκους καθώς και ωά *Capillaria spp.* στο παχύ έντερο. Η βακτηριολογική και μυκητολογική καλλιέργεια αποκάλυψε αυξημένη ανάπτυξη *Klebsiella pneumoniae* σε όλα τα δειγματοσιμμένα όργανα (δέρμα, καρδιά, πνεύμονες, ήπαρ, νεφρά και έντερο) και μέτρια παρουσία *Candida albicans* στο έντερο. Ο ρόλος τους θεωρήθηκε δευτερογενής. Μέτρια, πολυεστιακή αιμορραγία με ουδετεροφιλικές και λεμφοιστιοκυτταρικές διηθήσεις, ινική και κυλινδρικά βακτήρια αποτελούσαν τα ευρήματα της ιστοπαθολογικής εξέτασης του δέρματος, επιβεβαιώνοντας την δερματική φλεγμονή. Ως αιτία θανάτου καθορίστηκε η ασφυξία, προκαλούμενη από πνευμονική συμφόρηση, οίδημα και αιμορραγίες. Η συνεισφορά του νηματώδους *S. tendo* τόσο στην προσγείωση στο βιολογικό λύμα όσο και στην εξέλιξη του περιστατικού συζητείται. Παρά την αναποτελεσματική έκβαση, το περιστατικό ξεδιπλώνει την πολυπλοκότητα των περιστατικών άγριας ζωής με την οποία έρχεται αντιμέτωπο το κτηνιατρικό προσωπικό που εξετάζει πτηνά ή το προσωπικό των κέντρων περίθαλψης αγρίων πουλιών. Παρά την εφαρμογή απλών αλλά και εξελιγμένων διαγνωστικών τεχνικών το αποτέλεσμα μπορεί να είναι άκαρπο. Το οικονομικό κόστος επιτάσσει την κριτική εφαρμογή των κατάλληλων διαγνωστικών και θεραπευτικών μεθόδων. Η ανάγκη αναλυτικού διαγνωστικού σχεδιασμού και της εις βάθος γνώσης του είδους βοηθάει στην επιλογή των παραπάνω μεθόδων. Η σερατοσπικουλίωση θα πρέπει να συμπεριλαμβάνεται στη διαφορική διάγνωση της οξείας αναπνευστικής ανεπάρκειας σε ελεύθερους πετρίτες που προσέρχονται ως ασθενείς σε κέντρα περίθαλψης και ιδιωτικά ιατρεία στην Ελλάδα και την Ευρώπη.

**Λέξεις ευρητηρίας:** *Klebsiella spp.*, *Serratospiculum spp.*, επίβρεξη με βιολογικό λύμα, πετρίτης

## INTRODUCTION

Avian species are of the most common free-living patients in rehabilitation centres, differing in all aspects from their mammalian counterparts. Therefore, extrapolations in diagnosis and management are dangerous and often catastrophic. Raptors are one of the most charismatic avian species, fascinating humans for their agility and speed and, therefore, used in falconry. Despite that admiration, raptors are victimized mostly by anthropogenic causes of mortality

(Komnenou et al., 2005; Kalpakis et al., 2009).

The peregrine falcon (*Falco peregrinus*) is a widespread common falcon species, distinguished in 19 subspecies, distributed on all continents, except Antarctica. (Ferguson-Lees and Christie, 2001). It is famous for its speed and its fearsome personality. In Greece it is widespread on the mainland and on the islands, with an estimated population of 300–500 pairs (Handrinos and Akriotis, 1997), and as such it is often presented to Greek rehabilitation centres. Additionally, the Hellenic Air Force uses expensive

peregrine falcons in the military airports to reduce aircraft-bird collisions. In Greece, although falconry is not as established as in central European countries, it is gaining ground among hunters. Peregrine falcons are often used by Greek falconers and, therefore, are presented more frequently to small animal clinicians consulting also avian patients.

Peregrine falcons are susceptible to a number of causes of mortality such as trauma, direct and environmental poisoning, viral, parasitic, bacteriologic, and neoplastic diseases that are well documented in the avian literature (Cooper, 2002; Wernery et al., 2004; Heidenreich, 2013). Oil spilling or spilling with other liquids is quite uncommon for raptors (Clark and Gorney, 1987) and falcons specifically (Clarke, 1977; Zuberogoitia et al., 2006). In these cases, they usually prey on oiled seabirds or fall into oil pits or ponds with oil products. The clinical presentation of oiled birds and the effects of oil in the avian organism have been reviewed (Fry and Lowenstine, 1985; Tseng, 1999; Mazet et al., 2002; Kammerer et al., 2004). Mechanical feather damage, hypothermia, haemolytic anaemia, hepatic and renal dysfunction due to toxicity, respiratory distress, ulcerative gastroenteritis and secondary bacterial and fungal infections pose the main threats of the avian oiled patient. In Greece with its many islands, extensive coastline and inland water surfaces, oil-spilled victims are admitted frequently in rehabilitation centres, although so far a massive incident of oil spillage has not been recorded.

Serratospiculiasis is a parasitic filarial disease mainly found in raptorial species and caused by nematodes of the genus *Serratospiculum* (subfamily of Dicheilonematinae, family of Diplostriaenidae), which is comprised of at least nine different species grouped according to the length of the spicules (Bain and Mawson, 1981; Green et al., 2006). An additional species, *Serratospiculum amaculata*, originally identified in North America from prairie falcons (*Falco mexicanus*) and peregrine falcons (*Falco peregrinus anatum*) (Wehr, 1938; Ward and Fairchild, 1972) has been reclassified as *Serratospiculoides amaculata* (Sonin, 1968). *Serratospiculum* spp. has

been reported in *Falco*, *Accipiter*, *Buteo*, *Haliaeetus*, *Aquila*, and *Gyps* genera (Green et al., 2006). The life cycle of the parasite requires an intermediate host (beetle) and a definite (bird). Samour and Naldo (2001), have described the life cycle, pathogenesis and treatment of *Serratospiculum seurati*. While the parasite is common in the Arabic Peninsula (Samour and Naldo, 2001; Tarello, 2006; Al Timimi et al., 2009), in Europe there are only two reports of *Serratospiculum* spp. in free-living birds (Honisch and Krone, 2008; Santoro et al., 2010). Falcons infested with *Serratospiculum* spp. may be clinically normal or exhibit reduced conditions after intense exercise (Samour and Naldo, 2001; Tarello, 2006; Al-Timimi et al., 2009). In severe infestations, respiratory signs might be observed due to bronchial obstruction or lower respiratory disease, such as airsacculitis and bronchopneumonia (Ward and Fairchild, 1972; Green et al., 2006). The parasite may predispose to secondary bacterial and fungal infections resulting in death (Bigland et al., 1964; Samour and Naldo, 2001)

This case presents an unusual combination of mortality causes, which although previously reported in the literature, could add to the current knowledge. Oil spills are well described in sea and water birds, but only a few references exist for falcons. Additionally, there are no reports of falcons spilled by biological waste, which could potentially cause different symptomatology in comparison with crude oil. Serratospiculiasis is a well-described filarial disease in captive falcons, but only two reports exist in free-living peregrine falcons in Europe, and its prevalence is unknown (Santoro et al., 2010). Therefore, the authors believe that any reference of the parasite and its pathogenesis is worth reporting. Its possible contribution to the spill event and to the final outcome of the case are discussed. Last but not least, the Greek veterinary audience may be benefited in many ways; the complexity of the case offers the possibility to demonstrate a modern diagnostic work up of wildlife casualty cases and highlight the limitations; stimulate the small animal veterinarians/wildlife rescue personnel on the presence, diagnosis and therapy

of serratospiculiasis when presented with peregrine falcons and finally propose a therapeutic scheme for spilled raptors.

## CASE DESCRIPTION

On the 12th January 2012, an adult free-living peregrine falcon was found in a pond with biological waste and was unable to fly at that time. Members of the public authority collected the bird and presented it to the Clinic for Birds and Reptiles of the University of Leipzig, Germany. On the initial presentation the plumage was totally wet, with intense odour and jelly touch, while the shaft of two rectrices was bent and the barbules were split (Fig. 1). No ectoparasites were present. The weight (1.006 g) indicated a female bird, with good body score (4/5) and fully alert. A general examination revealed a small focal skin area (10 mm) on the right tarsometatarsus that was slightly bruised. The skin was largely intact except the right proapatagial and thigh areas, which were erythematous (Fig. 2). At initial presentation, no respiratory abnormalities were detected. In the orthopaedic, neurologic, and ophthalmologic examinations no abnormalities were detected as well.

## Diagnostic procedures

Whole-body radiographs (ventrodorsal and lateral views) including the head, wings and the legs



**Figure 1.** Wet and bent rectrices of the peregrine falcon.



**Figure 2.** Wet plumage and erythematic integument of the right thigh and proapatagium of the peregrine falcon.

revealed no abnormalities. Normal faeces and pellets were collected for further parasitological examination as routine procedure. A moistened swab taken from the crop, and examined within ten seconds on a preheated slide, was negative for *Trichomonas* spp. the day of admission. A wet mount parasitological examination was performed on the first day and revealed *Capillaria* spp. eggs in the faeces, while a 3-day faeces collection and complete parasitologic examination (pharyngeal swab, wet mount, flotation, sedimentation) revealed *Serratospiculum* spp. eggs in the pharyngeal swab and faeces on day 4 of hospitalisation. Flotation was performed with a saturated salt solution (specific gravity 1.18-1.2). Blood was collected from the left metatarsal vein (*Vena metatarsalis plantaris superficialis*) and a microcapillary hematocrit tube was filled. Packed cell volume value (PCV) was slightly increased (53%, normal range 39-51%) (Muller, 2009), indicating mild dehydration. A blood smear was prepared with the wedge technique. An estimation of the total white blood cell number (10 visual fields in 400×) indicated mild leucocytosis ( $1 \times 10^9$  cells  $L^{-1}$ , normal range:  $5.6 \times 10^9$ - $9.5 \times 10^9$  cells  $L^{-1}$ ) (Muller, 2009). The differential blood count revealed heterophilia (85%) with toxic changes (+2) in <25% of the leucocytes, indicating an early stage of infection. No haemoparasites were detected.

The next day, an effort was invested to specify



**Figure 3.** Hospitalization enclosure of the falcon. The door is covered, while the upper part allows sufficient light and airflow. The walls and floor of the enclosure are easy to be cleaned. On the floor a soft mattress (covered with newspaper), cartons on the side-walls, a perch (Astroturf) and an infrared bulb were added. The enclosure is situated in a quiet room, where temperature and light are controlled.



**Figure 4.** Washing procedure of the peregrine falcon with FIT® (dishwashing gel).

the nature of the biological waste. Contact was made with the staff of the biological plant station to identify the possible tank, pond and ultimately the ingredients of the waste. Due to mis-

information between the members of the public, which found the bird and the station staff confidential policy, this effort was unfruitful. A proposal to visit the location and sample the pond was rejected due to financial and time limitations by the finder. The university toxicology lab was contacted. A testing on the feathers was abandoned due to excessive cost of reagents and time-consuming feedback. A literature review was performed. Finally, other avian specialists were contacted to exchange views on therapy/management of the case.

### Treatment plan

#### Hospitalisation environment

A special, isolated enclosure was prepared for the falcon. No other birds were kept in visual or auditory contact. The photoperiod was 12-h light/12-h dark. The walls and floor of the enclosure were soft-padded (floor mattress and carton on the wall) to avoid further feather damage. A tail guard was applied. The perch was covered with Astroturf to avoid secondary pododermatitis. The enclosure door was covered to avoid visual contact (Fig. 3) with humans and further stress, which could potentially immunosuppress the falcon. The room temperature ranged from 23 to 28°C.

#### Washing procedure

The bird was assigned a washing candidate based on his PCV value and general condition. Despite the effort invested, it was not possible to determine the waste quality and analyse its potential toxicity in order to apply a specific antidote or a specific cleaning detergent. A quick cleaning action,

as described below, took place to remove the waste or some of it as soon as possible. A dishwashing gel (Fit®, Fit GmbH, Hirschfelde, Germany) was diluted (1–2%) in warm water (39–41 °C). Two persons were assigned for the washing procedure (one handler and one washer), while a third person took photographs and helped along. The bird was kept hooded to reduce stress (Fig. 4). All feathers and body parts were handled as previously reported (Tseng, 1999). As the hardness of water in Leipzig is increased (> 2.5 mmol CaCO<sub>3</sub> L<sup>-1</sup>, grade > 4 °dH), the bird was rinsed with warm (39–41 °C) distilled water used commonly in steam ironing. The plumage cleansing was repeated as quickly as possible three times in this first attempt. The bird was dried at first with soft towels and then left to dry under a commercial infrared bulb (100 W, 240 V; OSRAM® Siccatherm, Osram Licht Ag, München, Germany) kept 80 cm above the falcon. Removal of the waste was only partial. No further baths were taken on the first day as the bird showed excitation, and more cleansing attempts have been associated with increased mortality in oiled seabirds (Mazet et al., 2002).

#### Supportive care

Fluid therapy was initiated to rehydrate and assist the cardiovascular/renal/hepatic functions to cope with a possible parental intoxication, due to waste ingestion. Warmed normosaline fluids (Tutofusin H G5®, Boxtex, Unterschleißheim, Germany) were administered subcutaneously in the precrural fold (50 mL kg<sup>-1</sup>, *sid-bid* for five days). Amino acids (Aminin®, Merial, Hallbergmoos, Germany), vitamins A, D3, E, C and B-complex (Ursovit®, Serumwerk Bernburg, Bernburg, Germany, 5000 IU kg<sup>-1</sup>, sc, every two days; Vit B-Komplex® 2.5 mg kg<sup>-1</sup>, sc every two days) were mixed in the fluids before application to correct any possible deficiency of these elements, assist the integrity of the gastrointestinal/respiratory mucosa and act as an anti-oxidant. Calcium (Calcium gluconate® 10%, B. Braun Melsungen, Melsungen, Germany, 100 mg kg<sup>-1</sup> for five days) and glucose (5%) were also mixed in the fluids to supply energy

reserves and avoid hypocalcaemia and hypoglycaemia. To reduce the skin inflammation, meloxicam (Metacam®, Boehringer-Ingelheim, Basel, Switzerland, 0.2 mg kg<sup>-1</sup>, *bid*, per os for 5 days) was administered. Since it is known that oil-spill birds suffer commonly from secondary mycotic infections (e.g., *Aspergillus* spp.), itraconazole (Itrafungol®, Provet AG, Lyssach b. Burgdorf, Switzerland, 15 mg kg<sup>-1</sup>, *sid*, per os, for 5 days) was administered. The mild elevated total white blood cell count indicated an emerging inflammation and, therefore, a broad-spectrum antibiotic with good penetrability in skin, lung and gastrointestinal tissues was administered (enrofloxacin, Baytril®, Bayer AG, Leverkusen, Germany, 15 mg kg<sup>-1</sup>, *sid*, im, for five days). The population status of the species, its exciting personality, the progressive nature of the skin inflammation, the possible ingestion of waste, the risk of secondary infections and the knowledge that *Escherichia coli* and *Klebsiella* spp. are often isolated from oiled birds (Steele et al., 2005) led us to use this reserve antibiotic, with no prior culture and sensitivity testing, an otherwise strictly followed rule for exotic/captive patients in the authors' clinic. Another factor for this decision was the cost reduction because the clinic itself finances the wildlife casualty cases and receives several hundreds of wildlife patients per year. The authors strongly recommend that in cases of sufficient funding, antibiotic applications should be preceded by sampling, culture and sensitivity testing. Additionally, the bird was started on febendazole (Panacur®, MSD Tiergesundheit, Unterschleißheim, Germany, 50 mg kg<sup>-1</sup>, *sid*, per os for 3 days) due to the finding of nematode eggs. *Capillaria* spp. was considered a secondary pathogen, profiting from the impaired general condition of the bird, which could further interfere with the overall recovery of the bird and, therefore, warranted treatment. Finally, laxatives (paraffin oil, 2 mL kg<sup>-1</sup>) were administered to ease the passage of any possible ingested waste.

#### Nutritional management

The weight was monitored daily at the same time (morning). The bird was offered day-old cock-

erels the first evening that it did not eat voluntarily until the next morning. The second evening force-feeding was started since the weight started to decline (885 g). Day-old cockerels were cut into small pieces, while the skin and legs were removed to ease digestion. The pieces were offered with forceps in a quiet, semi-darkened environment. The force-feeding was not well accepted and the day-old cockerels were vomited the next morning. The weight declined further and the falcon received metoclopramide (MCP® Ratiopharm, Ulm, Germany, 0.5 mg kg<sup>-1</sup>, per os, once) and carnivore critical care formula (Oxbow® Critical Care Carnivore diet, Oxbow Animal Health, Murdock, NE, USA) with a rubber gavage tube, which were well accepted. Force-feeding (20 mL kg<sup>-1</sup>, *sid-tid*) was performed twice daily until the third day. Parallel the falcon was offered day-old cockerels on a daily basis through the enclosure floor. Fresh water was at all times available.

The following two days the general condition (mental, activity, posture) of the bird was stable and allowed more washing procedures (as described above) to try to remove more of the waste (in total three cleaning attempts were made in five days). The weight of the bird decreased gradually despite the force-feeding (873 g). On the fourth day, the fluid administration was doubled and the force-feeding tripled to halt the weight loss. The next day (day 5), the bird exhibited acute respiratory distress (open-beak breathing, dropped wings, tail-bobbing and mixed dyspnoea), skin inflammation that was expressed with wet dermatitis and possible pulpitis and vasculitis, with oily excrement (Fig. 5). The bird received



**Figure 5.** Dermatitis with suspected pulpitis and vasculitis in the left thigh and left propropatagium of a peregrine falcon rinsed with biological waste.



**Figure 6.** Necropsy findings of the peregrine falcon. Nematode parasites filled both caudal thoracic air sacs (arrows), while the air sac wall was thickened.

slowly bolus fluids (10 mL kg<sup>-1</sup>, iv *Vena ulnaris*) and was placed in an oxygen cage (5 L min<sup>-1</sup>) for 40 min. Skin swabs were taken for cytological examination and culture. Despite the treatment, the falcon passed away on noon of day 5 after an acute decline of its general condition (respiratory distress, weakness, and reduced mental state).

### Necropsy and pathologic examination

#### Macroscopic post-mortem findings

Necropsy was performed directly after death. The bird weighed 866 g. The integument had a diffuse red colour and slippery touch. Its feathers were wet, oily and their vane edges spilt, curled or broken. The barbules and barbs were divided, entangled and curled in the areas of intense inflammation. The shaft was bent in a few rectrices and remiges. The nutritional status was defined as cachectic (body score 2/5), accessing the absence of fat deposit of the coronary groove. The air sacs showed a high number (> 60 worms) of adult nematode worms ( . 6), while the wall was thickened and the air sacs were adhered to the visceral organs. Separation of the thickened air sacs from the underlying visceral organs was accompanied by detachment of organ tissue. The nematodes were thin, elongated and of white to yellowish colour and measured between 16 cm (males) and 20 cm (females). The left ovary was inactive. No other abnormalities were detected.

Wet mount samples were prepared from the crop, proventriculus, small and large intestines and examined microscopically (10×). Cytological samples

obtained from the skin, crop, proventriculus, liver, spleen, lungs and intestines were examined using a quick stain (Diff Quick®, Dade Behring, Düdlingen, Switzerland). The skin, liver, heart, kidney, lungs, small and large intestines, as well as any organs with gross lesions, were sampled with individually packed sterile microbiological swabs (Applied, Châtel-St-Denis, Freiburg, Switzerland). As initial isolation media, columbia blood agar (Oxoid, Wesel, North Rhine-Westphalia, Germany), MacConkey agar (Oxoid) and brilliant green agar (Oxoid) were streak inoculated with the samples and then incubated at 41 °C for 24 hours under aerobic conditions. A mycological culture was performed on Sabouraud dextrose agar (Oxoid) as well as Potato dextrose agar (Oxoid) and incubated at 41 °C for up to 120 hours. A combined sample of spleen, liver, lung, kidney and intestine was taken and examined for *Chlamydia* species using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) (Chlamydia ClearView®, Unipath Limited, Bedford, UK). For detection of *Salmonella* species, samples of liver and intestine were inoculated into Rappaport Vassiliadis enrichment broth (Oxoid) and Selenit enrichment broth (Oxoid), incubated at 41 °C for 48 h followed by culture on XLT4 agar (Oxoid) and brilliant green agar (Oxoid) at 41 °C for 24 h. Samples of skin, thyroid, parathyroid, heart, aorta, spleen, liver, pectoral muscle, oesophagus and crop, proventriculus, gizzard, small and large intestine, cloaca, pancreas, kidney, adrenal gland, ovary and oviduct, lung, air sac, brain and bone marrow were obtained for histopathology and fixed in 4% neutral-buffered formalin. Fixed samples were embedded in paraffin, and 5 µm sections were stained with haematoxylin and eosin for histological examination by light microscopy. Finally, nematodes were also collected, fixed in 4% formalin and sent for further identification (Institute of Parasitology, Veterinary Faculty, University of Leipzig).

#### Laboratory findings

The wet mount examination of the contents from the large intestine revealed a mild presence



of *Capillaria* spp. eggs. The wet mounts from the crop, proventriculus and small intestine revealed no parasites. Cytological examination of the skin, spleen and lungs revealed polymorphic, rod-like bacteria and cocci partly inside macrophages as well as lymphocytes and heterophiles. Abundant heterophile granulocytes, lymphocytes and macrophages were detected in the liver. Cytological examination from other organs (crop, proventriculus, intestines) revealed no abnormalities. The bacteriological examination recovered high-grade *Klebsiella pneumoniae* in all sampled organs and *Candida albicans* out of the small intestine. Testing for *Chlamydia* and *Salmonella* spp. yielded a negative result. Due to the absence of histopathological findings, no further molecular testing against *Chlamydia* spp. took place. Histological examination of the air sacs exhibited a significant presence of nematodes in sections, eggs, fibrin and diffuse haemorrhages. The lung tissue was characterised by haemorrhage, blood congestion and parabronchial oedema. The skin had mild multifocal haemorrhages with heterophilic and lymphohistiocytic infiltrates of fibrin and rod-like bacteria. Histopathology from other organs was unremarkable. The nematode from the air sacs was identified as *Serratospiculum tendo* based on the morphometric keys of Skrjabin (1991). Toxicology from the feathers or other organs to determine a possible toxicity or toxin-related pneumonia due to the biological waste was not performed due to financial constraints.

The immediate cause of death in this falcon was asphyxia caused by oedema, haemorrhages and blood congestion of the lung.

## DISCUSSION

In this case of biological waste spilling and *Serratospiculum tendo* infestation in a free-living peregrine falcon, the primary cause of death was not identified. It is not clear why the falcon was in the biological waste pond. Possible explanations would have been flight inexperience, miscalculation during a hunting manoeuvre, ocular pathology, trauma, debilitation and reduced endurance due to

underlying respiratory pathology. As this bird was an adult, flight inexperience seems less probable as this occurs mainly with fledglings or young birds (Stenkat et al., 2013). Trauma, emaciation and ocular pathology were excluded from the clinical and radiographic examinations. The authors hypothesize that the most probable cause of landing in the pond was a miscalculation of a vertical dive during hunting. In addition, the moistened plumage prevented the bird from escaping, and bruising might have resulted during these escape efforts. It remains unknown if the *S. tendo* worms played a role in endurance reduction and abnormal flight, since the published data is controversial. Some authors report that even with high numbers of nematodes, birds remained asymptomatic and their flight and hunting capacity were not influenced (Samour and Naldo, 2001; Tarello, 2006; Al Timimi et al., 2009), while others associate the nematode directly or indirectly with pneumonia and air sacculitis, resulting in the onset of respiratory signs (Ward and Fairchild, 1972; Green et al., 2006), which subsequently may affect the flight endurance. It is also suggested that even mild infestations, in the absence of clinical signs, may cause reduced speed and strength in flight (Tarello, 2006). Interestingly in two studies, asymptomatic falcons with positive faecal examinations for *Serratospiculum* spp., showed improved fitness and flight endurance after treatment (Tarello, 2006; Al Timimi et al., 2009).

The biological waste spill was perceived as the main life-threatening condition in the present report and, therefore, the management plan focussed on tackling the direct (mechanical feather damage, hypothermia, intoxication) and indirect consequences of the waste (secondary mycotic and bacterial infections, anaemia, immunosuppression), based on the relevant knowledge in oil-spilled water birds.

The common types of liquid biological waste, their compounds and treatment methods have been extensively reviewed in the past (Eckenfelder and O'Connor, 1961; Ward et al., 1978; Rushton, 2003; Barbusiński, 2005; Biswal, 2013). The authors focussed their survey on municipal sewage or livestock waste, although other types (e.g., biomedical

liquid waste) could not be excluded from affecting the falcon. These biological wastes are commonly treated under three main processes of aerobic stabilization; lagoons, activated sludge and bacterial beds. One third to one half of the organic matter is treated by anaerobic digestion of the liquid sludge (Eckenfelder and O'Connor, 1961; Rushton 2003). In comparison to petroleum products, the liquid wastes may harbour various components, which could affect a living organism. Such components include heavy metals (copper, zinc and in to a lesser extent cadmium and lead), viruses, *E. coli*, *Shigella* spp., faecal coliforms, antibiotic-resistant enterococci, spores of sulphite-reducing bacteria, yeast and fungi, protozoan cysts and ova of helminths, surfactant and systemic lubricants, endocrine-disrupting chemicals (EDCs) and finally high concentrations of nitrogen and phosphorus (Burge and Marsh, 1978; Ward and Ashley, 1978; Barker, 1996; Hassen et al., 2001; Lewis and Gattie, 2002; Chipasa, 2003; Vilanova and Blanch, 2005; Stamper and Montgomery, 2008; Bolong et al., 2009).

The topical and systemic effects of oil and other pollutants on living organisms, including birds, have also been reviewed (Rossi et al., 1970; Leighton, 1991; Frink and Miller, 1995; Fry, 1995; Briggs et al., 1997; Stephenson, 1997; Tseng, 1999). A variety of organic contaminants can affect the surface tension of feathers and negatively influence the insulation in ducks (Stephenson, 1997). The ulcerative dermatitis in the peregrine falcon was probably the effect of irritant compounds of the waste (e.g., nitrogen, phosphorus, acidifiers) and is consistent with the ulcerative effect of swine water waste in fish (Burkholder et al., 1997; Austin, 1998) and dermatitis and eczema in humans (Hoek et al., 2005). Nitrogenous compounds were also found to raise the susceptibility to infections (*Aeromonas hydrophila*) in fish (Austin, 1998), altering the probability of secondary local or systemic infections also in our case. Industrial products (e.g., phenol and formaldehyde compounds, suspended solids, nutrient and faecal coliforms) proved toxic causing massive deaths (Burkholder et al., 1997; Tišler and Zagorc-Končan,

1997), while high levels of polyhalogenated aromatic hydrocarbons had various reproductive implications in birds (Fry, 1995; Murk et al., 1996). It is suggested that different biological waste products could have similar implications to the health of living organisms. In our report, systemic intoxication could not be excluded, but appears less likely, despite the presence of some relevant clinical signs (e.g., vomiting once, lethargy the last day). The immediate rinsing of the plumage and the absence of further intense clinical signs (i.e., head tilt, opisthotonus, paresis, incoordination, persistent vomiting or diarrhoea, crop stasis, advanced depression and persistent lethargy) could suggest limited waste uptake to cause systemic intoxication. Haemolytic anaemia was also not detected in the blood films, supporting the aforementioned hypothesis. Additionally, in an experimental study in American kestrels (*Falco sparverius*), crude oil posed little acute hazard (Pattee and Franson, 1982), in contrast to studies in marine birds (Leighton, 1991; Briggs et al., 1997).

Regarding the systemic and local treatment of biologic waste lesions, as well as its removal from feathers, little is described in the literature. There is limited specific data on the quality of spill liquid properties and their detergents (Kummerfeld and Thielking, 2013) on feathers, thereby making it difficult to determine the optimal match between spill liquid and detergent to be used. A study that tested different cleaning substances in oil-spill birds concluded that a dishwashing gel (Dawn®, Procter & Gamble) gave the best results (Bryndza, 1991). However, it is reported that sludge from livestock waste contains a high percentage of undegradable lignin and cellulosic fibre (Barker, 1996), while some liquid wastes (e.g., low concentration of oily wastewater) are mineralized even in the presence of abundant substrates such as synthetic water, containing vegetable oil, detergent and gelatin (Stamper and Montgomery, 2008). This could suggest that biological wastes may contain compounds different from oil wastes on which the dishwashing gel may not be effective. Such products could possibly justify the incomplete cleaning of the biological waste from the

feathers or avian skin of the peregrine falcon.

Routine diagnosis of *Serratospiculum* spp. is usually made upon repeated pharyngeal and faecal examinations, as a single wet mount was not a reliable indicator of the severity in one study (Al-Timimi et al., 2009). The presence of the embryonated eggs in the faeces, however, does not necessarily indicate the presence of air sac nematodes because other nematodes also produce embryonated eggs of a similar morphology (Sterner and Cole, 2008). According to the latest authors, the only clear identification criteria include the confirmation of the nematodes' presence in the air sacs and the subsequent determination based on their morphology. The presence of embryonated eggs in pharyngeal swabs has been proposed as diagnostic criterion by some authors (Ward and Fairchild 1972; Al-Timimi et al., 2009). It should, however, be taken into account that the shedding may be intermittent, although the data is controversial. Based on the life cycle of the parasite [when eggs reach an adequate number in the lungs, they are coughed up to be swallowed and excreted from the faeces (Sterner and Cole, 2008)], the authors tend to support the intermittent shedding hypothesis and therefore propose the combination of repeated pharyngeal and faecal samples as adequate diagnostic tool for possible *Serratospiculum* infestation. In some case reports, the adult parasites were identified in the radiographs (Ward and Fairchild, 1972; Ackermann et al., 1992; Green et al., 2006). The authors, however, believe that only suspicion can be made upon radiographs including the parasite in the differential diagnosis of linear or irregular lung/air sac opacities, together with air sacculitis and haemorrhage. In captive falcons, *Serratospiculum* is commonly found during routine abdominal endoscopy (Samour and Naldo, 2001; Tarello, 2006), but usually this technique is not readily applicable in free-living falcons due to lack of infrastructure in rehabilitation centres and the less invasive management of these patients. Free-living peregrine falcons, lanner falcons (*Falco biarmicus*) and saker falcons (*Falco cherrug*) are considered to be more susceptible since they prey on wild birds, which could have eaten infested arthro-

pods and invertebrates (Tarello, 2006; Al-Timimi et al., 2009). Different treatment protocols with drugs, such as febendazole, ivermectin, moxidectin and melarsomin, and/or endoscopic removal have been described (Samour and Naldo, 2001; Tarello, 2006; Al-Timimi et al., 2009).

Avian species are often timid and hide their symptoms as a natural protective mechanism from predators. Free-living patients under captivity are more prone to stressors that could further compromise their immune status. Stressors include a variety of physical, psychological, chemical or infectious causes that are modified by intrinsic and extrinsic factors (e.g., stressor severity, duration, novelty, host genetics and immune status) (Dohms and Metz, 1991). The sources of stress in captivity could be abiotic or environmental (e.g., artificial lighting, exposure to loud noises, arousing odour, uncomfortable temperature and substrate, restricted movement, reduced retreat space, forced proximity to humans, abnormal grouping and reduced feeding opportunities) (Morgan and Tromborg, 2007). Captivity of wild passerines led to the immunoredistribution in the avian skin, which might affect the immune-cell trafficking to the avian skin (Kuhlman and Martin, 2010). Although stress-induced immunosuppressive resistance in domestic fowl is poorly defined, there are four possible pathways, which include the autonomous nervous system, the hypothalamic-adrenal axis, extra-adrenal pathways and neuropeptides, neurotransmitters and neuroimmunological mediators (Dohms and Metz, 1991). Inadequate nutrition may also affect the immunocompetence of birds. Force-feeding had a short-term effect on indicators of humoral immunity. Low mineral levels (sodium and chloride) also reduced the humoral immunity. Reduced vitamin A levels were associated with a reduced cellular immune response, while reduced levels of antioxidants (vitamin E and selenium) reduced the cellular integrity (Latshaw, 1991). Ingestion of methylmercury by adult American kestrels (*Falco sparverius*) revealed immunotoxic effects (Fallacara et al., 2011). Last but not least, the washing process was rather stressful for the excited falcon, even with

the use of a hood and may have contributed to its immunosuppression. The authors presume that the combination of the irritant compounds of the waste and the immunosuppression could have set the basis of the intense inflammatory skin reaction on the fourth day of hospitalization of the peregrine falcon as well as its weight loss.

Based on the previous results, a reduction of stress in the free-living patient is crucial. Therefore, the free-living bird should be housed in an isolated, quiet area. Enclosures should permit daily monitoring/grooming/feeding activities without direct visual contact to the bird and imitate the bird's natural habitat as much as possible. Pre-planning and synchronization of all activities (daily medical check, drug administration, sampling, diagnostic testing, force-feeding) is imperative to minimize the handling duration and avoid multiple captures daily. Experienced nursing and medical personnel should handle potentially aggressive species. A balance should always be considered between the gain of an action (diagnostic, prognostic) to the related stress. Due to this captivity-induced stress and subsequent immunosuppression, the avian veterinarian should be constantly alert to prevent, detect and treat secondary complications in time.

Additionally, as the wildlife casualty cases are often subjected to limited financial resources, the most cost-effective diagnostic tools should be used first. Simple techniques such as weight/appetite/droppings and behaviour monitoring, parasitology, cytology, haematology, monocular ophthalmoscopy and radiology offer in most cases an effective triage, saving human and financial resources. In the authors' clinic, parasitology (at initial presentation and repetition after three days), radiology and ophthalmologic examination are cornerstone procedures for wildlife cases, as well as necropsy of all diseased or euthanised free-living birds. A standard necropsy procedure is always followed (Latimer and Rakich, 1994). Familiarization of the medical personnel with the species-specific behaviour and early disease signs, susceptibility to diseases and causes of admission could reduce the differential list and the input of

more sophisticated diagnostics. Nevertheless, the authors suggest that if medical indications exist and the resources and infrastructure allow, then more sophisticated tools (e.g., ultrasonography, computed tomography, specific microbiology or laboratory testing) can provide the wildlife casualty victim the best possible medical service to recover.

The sudden onset of respiratory distress (e.g., accelerated breathing, open-mouth breathing, increased respiratory effort, and flight inability) has been described by many authors (Ward and Fairchild, 1972; Ackermann et al., 1992; Green et al., 2006; Tarello, 2006; Al-Timimi et al., 2009). Most authors support that the respiratory distress and on-going death is due to secondary infections (*Klebsiella* spp., *Pseudomonas* spp., *Aspergillus* spp.) or bronchial obstruction. Ackermann et al. (1992) suggested that death can also occur secondary to lung and air sac rupture, by free air in the coelomic cavity (pneumo-coelom), which aggravates the respiratory distress in an already compromised patient. In most cases, the distress started two to five days before death, consistent also with the current case. In addition, the onset of respiratory distress in this bird could be attributed to multiple pathogenic sequences, e.g., aspiration pneumonia due to biological waste and collapse due to suffocation, toxin-related pneumonia attributed to the biological waste, a polystemic failure/respiratory paralysis due to intoxication from the biological waste, an anaphylactic shock and immunosuppression attributed to extensive skin irritation by the irritant waste compounds, a bronchial obstruction/pulmonary oedema and haemorrhage due to death of *S. tendo* adult worms after treatment or respiratory failure and septicaemia due to secondary infection with *K. pneumoniae* predisposed by the presence of *S. tendo* in the air sacs or biological waste in the lungs.

Various combinations of the aforementioned sequences could lead to death. The skin barrier loss and absorption of toxic compounds could have contributed to a generalised immunosuppressive/anaphylactic shock, resulting or adding to the respiratory distress of the compromised falcon. The culture of

*K. pneumoniae* from multiple organs, including the skin, indicated a peracute septicaemia and multisystemic failure, which could lead to heart, liver and lung congestion, secondary lung oedema and suffocation, as is described in advanced cases of serratospiculiasis (Samour and Naldo, 2001). The mild presence of *C. albicans* was evaluated as a minor secondary infection with a minor contribution to death. Despite the fact that both *Serratospiculum* spp. infestation and biologic waste exposure predispose to the growth of *Aspergillus* spp., none was isolated in the mycologic cultures. This diminishes the probability that the air sac lesions or the respiratory distress could have been a result of chronic aspergillosis or an acute event of mycotoxicosis. Indirectly, this could suggest that despite the stress of cleaning/therapy/captivity and weight loss, the immune system of the falcon could still combat a possible mycotic growth. Weight loss resulting in cachexia is consistent with previous cases in which peregrine falcons were infested with more than ten *S. tendo* worms (Santoro et al., 2010), although this could also be attributed to the overall stress/immunosuppression. The authors' opinion is that the biological waste with its direct inflammatory reaction triggered the decline of general condition and disrupted the probable balance between host-parasite. The authors tend to affiliate the sudden onset of respiratory distress either directly (parasite number and fibrinous air sacculitis) or indirectly (*K. pneumoniae* secondary infection predisposed by the *S. tendo* infestation or the biological waste) with *S. tendo*, as a similar symptomatology has been described in other species of the parasite (Ward and Fairchild, 1972; Samour and Naldo, 2001; Green et al., 2006; Tarello, 2006), rather than with a toxic-related anaphylactic shock (also due to the absence of haemolytic Heinz body anaemia). Possible further toxicological tissue analysis would have contributed to rule out this scenario.

### CONCLUDING REMARKS

Determination of the inciting cause of mortal-

ity in free-living patients might be a complex investigation, despite an extensive diagnostic work-up. Wildlife casualties may harbour simultaneously different pathogens and identifying the primary pathogen may be time and cost consuming. Post-mortem laboratory examinations (parasitology, cytology, microbiology, virology, toxicology, histology, molecular biology) are invaluable in filling in the gaps of the cause of mortality puzzle. In the present case, the falcon died from acute respiratory failure. This might have been the result of adirect waste compound immunotoxicity, *K. pneumoniae* septicaemia predisposed either from the immunosuppressive effect of the biologic waste and captivity or from the *S. tendo* infestation and/or *S. tendo* fibrinous, air sacculitis and bronchial obstruction, due to host-parasite imbalance, promoted by the immunosuppressive effect of the waste and captivity. As it is evident that stress and immunosuppression play a key role in disease escalation and death, minimization of captivity stressors should always be addressed in avian wildlife casualties. Disease escalation should be recognised as soon as possible and therapeutically addressed, and simple, cost-effective diagnostics should be the first choice. The effects of the different types of biological waste and their compounds in avian health, as well as their removal from the avian plumage require further investigation. The effect of *S. tendo* infestation in the flight performance of the falcon and the waste spilling remains unclear. Serratospiculiasis should be considered in the differential diagnosis of acute respiratory distress in free-living peregrine falcons (*Falco* spp.) in Europe.

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### CONFLICT OF INTEREST STATEMENT

None of the authors of this article has any conflict of interest. ■

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