THE HEALTH STATUS OF THE

GREAT BUSTARD (OTIS TARDA LINNAEUS, 1758) IN HUNGARY

THESIS

1. Introduction, aims of the study

The Great Bustard (Otis tarda) is a strictly protected, „flagship” species and treated as a top priority by the Hungarian nature conservation authorities. During the last few years the population size of the country had a slight, but steady increasing tendency (over 1600 individuals), which had an obvious conjunction with the actions of the so-called „The protection of the Great Bustard in Hungary” Life Nature programme between 2004 and 2008.

Regarding the veterinarian antecedents it is important to stress, that the Dévaványa Great Bustard Rescue Station (DGBRS) started to function back in 1979. The main task of the DGBRS was to hand-raise and release the Great Bustard chicks which were transported to the station in egg rescue actions. Besides this activity rescued adult birds are also treated in the DGBRS.

It is a well-known fact that every conservation programme which has a captive phase needs a strong veterinarian contribution. These complex health issues (related to conservation efforts) inspired me to deal with this theme systematically, in more details. An important, additional aspect was that in the recent history of the 1866 founded Budapest Zoo and Botanical Garden some experts made thorough research on this species. Dr. Tamás Fodor made a doctoral thesis about the development during the incubation of the Great Bustard in 1968. He started his work in 1958 and his results greatly supported to set-up the animal husbandry technique in Dévaványa (DGBRS). This study was followed by Dr. Pál Mödlinger, who made detailed research on the species.

In my research (between 2002 and 2011) our rule and goal was to use non-invasive methods as much as possible, and to shorten the length of the manipulations in order to avoid any possible accidents in this strictly protected species. I made comprehensive examinations in clinically healthy specimens; moreover, I made a sum-up of those experiences which were gained during the rescue work of debilitated individuals. My intention was to get results
which would give practical information during the protection of the species (mainly during rescue or any kind of hands-on manipulation).

It is important to emphasize regarding the medical considerations of the species that the relevant (veterinarian) publications are very few, and the Hungarian ones are even scarcer. A number of papers deal with blood results and examinations, but these consisted small sample sizes, exclusively. Some Spanish researchers made mortality and toxicology studies. In contrary, when I made a thorough literature survey it became obvious rather early, that some related species, such as the Houbara Bustard (*Chlamydotis undulata*) were studied quite intensively.

**2. Materials and methods**

**2.1. Serology and virology**

Between 2003 and 2011 I collected blood from 53 birds (16 males, 6 females, 31 unknown sex); these were young birds, hatched in the same year (except for two, adult bustards). The samples were gained at the DGBRS each time. These birds were mainly originated from an egg rescue action and raised during the breeding season; the clinically healthy individuals were part of the study before the release phase. The blood was collected without sedation each time, the v. ulnaris was used, from where 5 ml blood was drawn with an open technique to heparin-filled tubes. I made a maximal precaution regarding the safety of the birds; therefore an important condition was to work quickly and effectively.

The birds are partly accustomed to people during the hand-raise procedure in the Dévavánlya technology, which they try to put aright during the following reintroduction phase. The timing of the blood collections took place always at the end of the hand-raise phase, before the bustards were moved to the six hectares big pre-release site; therefore, the catching of the birds did not take long. The drawn samples were cooled and transported to Budapest on the day of the collection.

The blood samples were examined and analysed in two laboratories. Between 2003 and 2006 the Sangui-Vet Veterinarian Clinical Laboratory made these, whereas between 2009 and 2011 the Praxislab Kft. carried out them following the general rules of processing blood samples.
The change of laboratory during the study was due to financial reasons. Both laboratories completed haematological and biochemistry measurements on the processed samples.

As the part of the veterinarian programme it was possible to run serological screenings on the blood samples of clinically healthy individuals in 2006, 2009 and 2011. In these years the blood collection took place in July-August, included 27 samples and avian influenza (HPAI), Newcastle-disease, West Nile virus and avian orthoreovirus infection were tested.

The above mentioned viral diseases were selected according to several aspects, including the known or suspected susceptibility of the Great Bustard and the epidemiological characteristics of the occurring bird species of the area (Dévaványa and its surroundings). The 27 samples were examined for avian influenza and Newcastle-disease at the Office of Agriculture, Veterinarian Diagnostics Directorate, Poultry and Swine Virology Laboratory. The West Nile virus screening was carried out at the Szent István University, Veterinarian Faculty, Epidemiological and Microbiological Department. The avian orthoreovirus serology was done at the Hungarian Scientific Academy, Rural Science Centre, Veterinarian Institute.

The detection of the avian influenza was done with a haemagglutination inhibition (HI) test (accepted by the 2006/437/EC Commission Decision and described in the relevant diagnostic manual).

In order to detect the Newcastle-disease HI was applied, specified in the OIE (Office International des Épizooties) 2009 diagnostic manual.

For the West Nile virus serological examination a competitive ELISA test (ID Screen West Nile Competition Screening Test, ID Vet, France) was used and the steps given by the manufacturer were followed.

In case of the avian orthoreovirus the ELISA analysis of the samples was done by the help of the IDEXX FlockChek Avian Reovirus Antibody test Kit (IDEXX Laboratories, USA), following the manual attached by the producer.
2.2. **Bacteriology**

During our work I was able to collect cloacal swabs from nine healthy birds in 2009 during the blood collection. Moreover, I got ten faecal samples from clinically healthy individuals in 2011 – in both occasions the aim was to detect the normal intestinal flora of the birds and to find possible pathogen bacteria.

Apart from these bacteriological examinations bacteriology was run in clinical cases, mainly in articular processes when a septic condition was suspected.

The swabs were smeared to blood agar and Drigalski-plates and the examination took place following a 24 hours, 37 °C incubation period. The exact identification of the bacterium species was possible according to the morphology of the colony, the growth characteristics and the biochemical specialities. The bacteriological examination and analysis was carried out by the Duo-Bakt Laboratory.

2.3. **Mortality**

I analysed different datasets between 2002 and 2011 in order to receive information about mortality in Great Bustards. These consisted the young individuals of the Kőrös-Maros National Park run DGBRS (originated from egg rescue actions), the examined birds in the Office of Agriculture, Veterinarian Diagnostics Directorate, Mammal and Bird Pathology Department, the specimens which went for necropsy at the Állategészségügyi Labor Kft. in Békéscsaba, the dead individuals on which post mortem examination was done at the Szent István University, Veterinarian Faculty, Pathology and Forensic Veterinarian Medicine Department and the rescued, but subsequently died individuals of the Budapest Zoo and Botanical Garden and the Szeged Zoo.

The post mortem examination was performed by a standard necropsy technique and in each case additional methods (histopathology, bacteriology, toxicology and virology) were used, if according to the pathological changes or the anamnesis it was necessary. Similar additional methods were used if the macroscopic examination did not reveal any obvious cause of the death. In the case of the rescued animals a radiological examination was also performed in order to detect any metal foreign bodies in the bird (for the exclusion of a possible shot pellet
or a swallowed foreign body). During the study period I analysed the data of total 95 individuals.

2.4. Anaesthesia

During my literature survey I was not able to find any relevant information regarding the anaesthesia of the species. In the study there was no need for sedation/anaesthesia in most of the examinations (e.g. screenings), but the anaesthesia is a must when the intervention is a painful one.

An anaesthesia related issue when according to the advice of the Great Bustard Working Group and the request of the Körös-Maros National Park surgical wing-pinioning was done on seven young birds in 2002. These birds (and an additional 12 in 2003) were moved to the so-called “Great Bustard Garden”, which was created in the neighbourhood of the DGBRS. This area is 406 hectares large, fenced, the predators are excluded, and its rigorously managed according to the needs of the species; the main goal was to use these wing-pinioned birds as “baits” to attract wild conspecifics and create safe breeding grounds for those. The „Great Bustard Garden” provides a suitable breeding place for 6-13 females since its creation, but the use of the wing-pinioned birds was a failure: these bustards could not realise that they were flightless and tried to escape in fly when eagles turned up, providing easy prey for them. Due this fact the remaining birds were collected from the area in the autumn of 2003. Nevertheless, the other part of the plan (to provide a safe breeding place for the species) works well from the beginning.

I made 24 Great Bustard anaesthesia during the study period. The interventions took place at the DGBRS and at the Budapest Zoo and Botanical Garden. After Ketamin-HCl (CP-Ketamin 100 mg/ml, CP-Pharma Handelsges, Germany) premedication (15-20 mg/kg) isoflurane (Forane 100 ml, Abbott Laboratories, Hungary) was used. For the induction 1 L/minute gas flow and 5 vol% inhalation gas was given. For the maintenance 1,5-2,5 vol% isoflurane was administered. For the induction a mask was used, which was changed to an uncuffed endotracheal tube for longer procedures (e.g. bone surgeries). The insertion of the endotracheal tube is not difficult due to the lack of the epiglottis, but compared to other species the position of the opening of the trachea is deeper in bustards, therefore the intubation and the opening of the bill is only possible in properly sedated birds (which is an additional reason for the need of premedication). According to my experiences the anaesthesia of the Great Bustard is not differing considerably from other, similar sized birds.
2.5. Intensive therapy, rescue work

Amongst Hungarian circumstances it rarely occurs that injured, adult, live Great Bustards are transported to rescue stations. These cases are always almost emergency ones. The prognosis is frequently poor/guarded, since these cases are often trauma originated; it is also important to mention that the Great Bustard is a stress sensitive species, which cannot or hardly can cope with human manipulations; moreover, to keep the bird properly in captivity during the possible repatriation phase is also a challenge. Between 2002 and 2011 according to my best knowledge only two rescue centres in Hungary worked with injured individuals of the species, namely the Budapest Zoo and Botanical Garden and the Szeged Zoo.

3. The results and their evaluation

The following Table provides information about the results of our haematological and biochemical examinations between 2003 and 2011.

**Table.** Blood examinations in the Great Bustard between 2003 and 2011 (total results)

<table>
<thead>
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<th>Unit</th>
<th>N</th>
<th>average</th>
<th>deviation</th>
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<td>G/l</td>
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<tr>
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<td>T/l</td>
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<td>1,21</td>
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<tr>
<td>Hb</td>
<td>g/l</td>
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<td>121,15</td>
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<tr>
<td>PCV</td>
<td>%</td>
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<tr>
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<td>fl</td>
<td>21</td>
<td>161,68</td>
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<td>g/l</td>
<td>14</td>
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<td>G/l</td>
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<td>G/l</td>
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<td>lymphocyte</td>
<td>%</td>
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<td>total protein</td>
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<td>g/l</td>
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<td>A/G ratio</td>
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<td>1,01</td>
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Table. Blood examinations in the Great Bustard between 2003 and 2011 (total results, continued from the previous page)

<table>
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<tr>
<th>Table</th>
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<th>105.91</th>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALKP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGT</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>bile acids</td>
<td>μmol/l</td>
<td>16</td>
<td>78.36</td>
<td>77.43</td>
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<tr>
<td>CK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>mmol/l</td>
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<td>glucose</td>
<td>mmol/l</td>
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<td>3.86</td>
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</tr>
<tr>
<td>creatinine</td>
<td>mmol/l</td>
<td>33</td>
<td>34.68</td>
<td>15.09</td>
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<td>uric acid</td>
<td>μmol/l</td>
<td>32</td>
<td>255.83</td>
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<tr>
<td>natrium</td>
<td>mmol/l</td>
<td>16</td>
<td>148</td>
<td>4.69</td>
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<tr>
<td>potassium</td>
<td>mmol/l</td>
<td>10</td>
<td>6.06</td>
<td>0.74</td>
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<td>Na/K ratio</td>
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<td>3.12</td>
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<tr>
<td>magnesium</td>
<td>mmol/l</td>
<td>16</td>
<td>1.53</td>
<td>0.23</td>
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<tr>
<td>Ca</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>P</td>
<td></td>
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</tbody>
</table>

When evaluating my results it is important to stress that the total sample size was decreased in the case of several parameters due to the haemolysis in the blood. The mild haemolysis can slightly elevate the GGT, CK, LDH and potassium values. The moderate haemolysis can raise the GGT, CK and LDH values considerably, whereas the AST and phosphorus values can raise moderately; in this case the total protein, albumin and cholesterol values can elevate slightly; the value of the calcium can decrease. The strong haemolysis can affect most of the examination results considerably.

The haemolysis can occur during the collection of the blood (e.g. the massage, pumping of the vein), during the transport of the sample or while it is processed in the laboratory. In my case the haemolysis of some samples most probably took place during the transport (longer the time lapse between the time of the blood collection and the analysis of the sample more the chance for the disintegration of the red blood cells). The existing distance between Dévaványa and Budapest and the necessary transportation of the samples in between was obligatory in the study; this problem could be largely eliminated in the future with “at the spot” centrifugation of the samples.

Regarding the results of the serological examinations the avian influenza, Newcastle-disease and avian orthoreovirus screenings were all negative, whereas the competitive ELISA test gave positive results of the West Nile virus infection each year when screening was performed (total 14 samples).
The highly pathogenic avian influenza had a cumulative occurrence in the Great Plain (mainly the Kiskunság region) in early 2006; this area is not far from one of the most important Great Bustard habitats of the country. Nevertheless, it is crucial to point out, that the chance of the infection is slim, as the species does not form common gatherings with waterfowl and do not use the same wetland habitats (except for the drinking spots) where the most important and relevant species related to the disease (e.g. Anseriformes) make big flocks (due to this fact the chance for the spread and passage of the virus is much higher in this group). My results support that epidemiological role of the Great Bustard regarding the HPAI is negligible.

The Newcastle disease causes high mortality in related bustard species; relevant and comprehensive information is available especially about Houbara Bustards. Such examinations regarding the Great Bustard have not been performed previously in Hungary, hence the success of the conservation programme requires regular screenings which is awfully important related to its success.

The occurrence and the disease causing potential of the West Nile virus has been proven in various bird species earlier; due to the zoonotic issue the epidemiological role of the birds was already studied. The sensitivity of the affected species and the virulence of the viral strains differ a lot for the West Nile virus. The occurrence of both existent lineages (the lineage-1 and lineage-2) was proven previously; according to our experience mainly falconers can suffer considerable losses. The Goshawk (Accipiter gentilis) is the most susceptible and affected species amongst the bird species used for falconry purposes. Today it is still not known, whether the Great Bustard has any epidemiologic significance regarding this disease, but the species occurs as a resident bird in an infected territory, where the important mosquito species spreading the virus and the occurrence of the virus is well-known. Until now my results support that in certain years the infection can turn up in large scale (e.g. all birds tested in 2009 [n=9] were positive), but as it did not manifest in serious clinical signs, the susceptibility against West Nile virus is presumably low in the species.

The avian orthoreovirus usually causing disease in crowded poultry populations. At the DGBRS the species is kept more densely compared to the natural situation, but the serological results suggest that reoviruses either do not occur or did not concentrate on such a level which could cause infection at the station. According to our current data the significance of the avian orthoreovirus infection in the Great Bustard cannot be evaluated at this stage.
The results of the serological screenings are favourable as the serious, notifiable, high mortality causing viral diseases (avian influenza, Newcastle disease) did not affect the tested population; moreover, the avian orthoreovirus infection was not detected which is often linked with captive conditions. Nevertheless, the strong seropositivity against the West Nile virus raises the attention about the fragility of the population towards the emerging diseases of the environment. Therefore, the precautions regarding epidemiologic rules, regular screenings and detection and follow-up of possible viral diseases are crucial.

During several blood collection occasions in Dévaványa I noticed skin alterations which were clinically reminiscent to avian pox cutaneous lesions. These usually turn up as dry papules on the bare, featherless parts (feet, base of the bill), but sometimes bleeding, ulcerative processes were observed. The biopsies from these lesions did not reveal the virus by histopathological methods (cytoplasm inclusions). One post mortem case revealed a generalised form of avian pox as the cause of the death.

In Hungary during the last ten years most of the post mortem examinations of Great Bustards took place at the Office of Agriculture, Veterinarian Diagnostics Directorate, Mammal and Bird Pathology Department and the DGBRS, whereas the other institutions made necropsies only sporadically.

The strong bias amongst the examining institutions is due to the fact that the birds come into “human hands” only related to the captive technology; the chicks or juveniles from the Köröș-Maros National Park were examined either at the DGBRS or were sent to a special pathologic institution.

During the study period the examined birds originated almost solely from the Dévaványa region. The 92,63% ratio is linked with the work of the DGBRS; the other regions – though possess considerable bustard populations – turned up only sporadically, with 1-2 specimens in the data set of the last 10 years. Amongst the origin of the birds the only exception was an individual from the south of Budapest, which is outside of the currently known national distribution of the species; this bird was found as an injured specimen, died subsequently and could be linked with an accident of a vagrant bird.

Regarding the full mortality pattern the omphalitis (n=17) was the most common one. The omphalitis is a collection term, includes the incomplete and late closure of the umbilicus and the late and incomplete resorption of the yolk sack. This is more precisely the inflammation of
the umbilicus, which is caused by non-infectious and infectious agents; this condition can be in conjunction with other infectious diseases (e.g. lung- and air sac mycosis) as well. One of the post mortem examinations revealed an Aspergillus fumigatus caused lung and air sac mycosis and E. coli sepsis in a 100 grams Great Bustard chick with omphalitis.

The development abnormalities (n=6) include all cases when the death of the birds occurred after a longer period of a chronic condition. The six cases contained three rachitis, and in the other three the main clinical and pathological findings were the anaemia and the emaciation. This disease group can be related with the husbandry and nutrition (e.g. not sufficient amount, not proper ratio of calcium, phosphorus and magnesium), but in the background it can be an absorption problem or an insertion problem as well. The exact cause is often difficult to find.

The bacteria causing sepsis (n=8) were the E. coli five times and the Pseudomonas sp. three times.

The bacterial gastrointestinal diseases (n=11) mainly caused death amongst the day-old and young birds of the DGBRS. It is also likely, that the bacterial, non-fatal diseases occur during the hand-raise of the birds. In four cases salmonellosis-like inflammatory lesions were found in the caecum, but the culturing of the bacteria gave negative results.

The respiratory bacterial diseases (n=6) consisted two E. coli, and four Pseudomonas cases.

Confirmed viral disease as a cause of death (n=1) occurred only once. A 642 grams young bird had a towsy plumage in all over its body; the right lower eyelid and the lateral part of the right tarsal joint were covered with lentil-sized, red papules. Over the right hip joint a complex, cherry-sized lesion was seen (with reddish papules and crusts). The cause of the death was the generalised avian pox, which is unusual as the disease regularly occurs amongst the hand-raised youngsters of the DGBRS, but usually has a benign course and is not fatal.

The parasitic gastrointestinal diseases (n=2) caused death in young female birds. In December 2002 two birds died 2-3 days apart, without any previous symptoms. Both birds suffered serious emaciation and had Raillietina sp. cestode infection. The occurrence of the Raillietina nyrai was previously described in Houbara Bustards as well. These females also had mild Capillaria hepatica infestation, too.
The parasitic pulmonary diseases (n=4) consisted syngamosis cases. The syngamosis can cause serious health issues in several bird species; for example in the Budapest Zoo and Botanical Garden young Crowned Cranes (*Balearica pavonina*) died in different years.

Fungal disease (n=1) was caused by *Aspergillus fumigatus*. The diagnosis of aspergillosis is often post mortem; the clinical diagnosis is frequently difficult and only possible with the help of diagnostic imaging techniques.

The occurrence of renal disease (n=7) could be associated with animal husbandry, since in 2004 cumulative cases were found (this year for the sake of a more successful repatriation a new development was made in order to model the dry steppe circumstances; therefore ad libitum water was not provided).

The impaction of the gastrointestinal tract occurred several times (n=9). A young bird suffocated due to an impacted mouse in its oesophagus. In other eight cases the cause of death was a gastric, non-digested trichobezoar which was formed by plant fibres. In a male bird, which had a good condition and died on the 6th of January, 2006 the following alterations were found: a man-fist sized trichobezoar partly blocked the gizzard; acute enteritis in the small intestines, acute tubulonephrosis and shock. *E. coli* was isolated from the intestines.

Heart diseases are extremely rare (n=1), or at least extremely rarely diagnosed. On the 26th of February, 2003 a female suffered the rupture of the right ventriculus and subsequent bleeding out; histopathology was not made from the altered organ. Similar cases were described in Capercaillies (*Tetrao urogallus*), in which the degeneration of the heart muscle fibres was the real reason of the disease.

Death due to a chronic disease (n=6) was associated with bumblefoot or the complications of a former bone surgery. All cases involved subadult or adult birds, which died after a few months of disease course and emaciation.

Mortality due to traumas (n=16) occurred always amongst subadult or adult birds. In this category not only the Dévaványa birds are included, but all the other bustards fall into it from the other parts of the country. Both the Budapest Zoo and Botanical Garden and Szeged Zoo rescued and subsequently died birds are belonging to this group. Collision with power lines was a frequent cause, and in other cases predators were involved (for the latter several examples were found in Dévaványa, in one case a raptor (presumably a White-tailed Sea Eagle [*Haliaeetus albicilla*] attacked a bustard, but the bird survived and was treated...
successfully). In these cases the post mortem examination revealed fractures, severe blood loss and shock; out of the birds which died due to an attack by a carnivore four birds had moderate and one bird had a severe tapeworm infestation. It can be assumed that a weaker general health status caused by the parasites could have played a role to become a victim of a predator.

According to the 24 anaesthetic events during the study period it can be stated that the ketamin-HCl (CP-Ketamin 100 mg/ml, CP-Pharma Handelsges, Germany) premedication (15-20 mg/kg) and the subsequent isoflurane (Forane 100 ml, Abbott Laboratories, Hungary) anaesthesia is safe and provided uneventful interventions in this species; moreover, the anaesthesia of the species does not differ considerably from other, similar-sized birds.

Amongst Hungarian circumstances the rescue work with live Great Bustards occur very rarely. My own (limited) experience suggests a poor-guarded prognosis; during the care of these birds the intensive therapy is mandatory.

4. New scientific results

1. The author and his co-workers described first the West Nile virus serological conversion in Great Bustards, which was strong in each case.

2a. The author and his co-workers examined first the possible occurrence of the avian orthoreovirus in the species, but the virus was not detected; according to the current data it is still not possible to judge the significance of the avian orthoreovirus in the Great Bustard.

2b. The author and his co-workers were not able to detect the avian influenza in the species, which suggests that the Great Bustard is not important regarding the spread and the epidemiology of the disease.

3. The author described first the full spectrum of haematological and biochemistry values from healthy birds.


5. The author described first an anaesthesia protocol which can be used practically for invasive, painful procedures.
5. Publication list

5.1. Publications regarding the Great Bustard

Written publications:


Presentations:


Posters:


2. Bakteriológiai vizsgálatok túzok (Otis tarda) állományokban. Állatkerti- és egzotikus állatok emésztőszervi megbetegedései konferencia, Fővárosi Állat- és Növénykert, Budapest, 2011. március 30.- április 1. (in Hungarian)

9.5. 2. Other veterinarian (medical management of exotic and zoo species, wildlife health) and zoological publications


Mycobacterium bovis subsp. caprae in captive Siberian tiger. Emerging Infectious Diseases, 9 (11), pp. 1462-1464.


26-28.) konferencia kiadványa. Fővárosi Állat- és Növénykert, Magyar Vad- és Állatkerti Állatorvosok Társasága, Budapest, pp. 45. (in Hungarian)


