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DISSEMINATED MYCOBACTERIUM KANSASII IN A REINDEER (Rangifer tarandus).

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Abstract
This is the first report of a disseminated mycobacteriosis caused by Mycobacterium kansasii in a reindeer (Rangifer tarandus). A two-year-old female reindeer with a history of lethargy and dyspnea was diagnosed with multiple pulmonary abscesses radiographically. At necropsy the animal had severe diffuse granulomatous pneumonia and pleuritis with severe multifocal granulomatous myocarditis, lymphadenitis, nephritis, esophagitis, enteritis, and thyroiditis. Impression smears of necrotic debris from granulomas contained numerous acid-fast bacilli. M. kansasii was cultured from pulmonary, renal, hepatic, and lymph node sections. M. kansasii is a slow-growing photochromogenic non-tuberculous mycobacterial species that causes disease in severely immunocompromised individuals (3,4,9). Due to the popularity of reindeer as animals for exhibition in children's zoos, petting zoos, and holiday exhibitions, diagnosis of Mycobacterium kansasii in a reindeer is a significant public health concern.

Key words: Reindeer, Rangifer tarandus, Mycobacterium kansasii, atypical mycobacteriosis, granulomatous pneumonia, lymphadenitis, and acid-fast bacilli.

Introduction
Mycobacterium kansasii was first characterized in 1953 at the University of Kansas. It is a slow-growing photochromogenic mycobacterium (Runyon group 1) which is the second most common non-tuberculous mycobacterial species isolated in AIDS patients following mycobacteria in the Mycobacterium avium complex (MAC) (3). The most common disease presentation in humans is chronic pulmonary infection with cavitation, resembling classical tuberculosis (9). There are infrequent reports of disseminated infections in severely immunocompromised individuals as well as focal dermal and arthritic lesions. Tap water is believed to be the major reservoir in human disease although it has been cultured from lakes, ponds, swimming pools, sewage, and sphagnum moss (3,4,9). Interestingly, there are few reports of M. kansasii in animals in the United States and Europe. This is the first report of disseminated Mycobacterium kansasii infection in reindeer (Rangifer tarandus).

Case Description
A two-year-old female reindeer from a local zoo was noted to be lethargic and dyspnic for approximately two months. The reindeer did not respond to empiric antibiotic therapy. Thoracic
radiographs revealed multiple pulmonary abscesses in both lungs. Purulent material from a
prescapular lymph node was submitted for bacteriology. Culture was unrewarding. The animal
was euthanized and submitted to the Diagnostic Center for Population and Animal Health at
Michigan State University for a complete necropsy. This reindeer had been housed with one
other female reindeer from the time she was acquired as a calf, for display at the zoo.
There is no record of direct contact with any other animals, although the hoofstock at the zoo are
housed in a large barn with a common air handling system.
At necropsy the reindeer weighed approximately 80 kilos, was extremely thin and mildly
dehydrated. There were multiple subcutaneous nodules on the face and neck. The left
prescapular lymph node (fig. 1) was enlarged and cross sections revealed purulent to caseous
gritty material, suggestive of mineralization. There were multiple 0.5 – 3.0 cm subcutaneous
nodules with central areas of caseous necrosis. There were dozens of tan to yellow 0.2 – 2.0 cm
mineralized caseogranulomas randomly distributed over the diaphragmatic and thoracic pleura
(fig. 2) often arranged linearly, parallels to the ribs. Similar granulomas with caseous necrosis
were also found multifocally throughout the lungs (fig. 3), in the thoracic, mesenteric and
sublumbar lymph nodes, within the esophageal mucosa, the myocardium of both ventricles, the
small intestinal submucosa, both kidneys (fig. 4) and the thyroid glands.

Material and Methods
Sections of tissue were collected for histopathology, and immunohistochemistry. Tissues were
also submitted to the Michigan Department of Community Health and the bacteriology section at
the DCPAH for bacterial culture. Sections of lymph node were also submitted to the virology
section at the DCPAH to screen for bovine viral diarrhea.

Results
An impression smear of necrotic material from the left prescapular lymph node revealed large
numbers of acid fast positive staining organisms.
Histologic examination of tissues revealed variably sized multifocal to coalescing granulomas
with central caseation and mineralization throughout sections of lung (fig. 5), heart, liver, both
kidneys, both thyroid glands (fig. 6), thoracic, mesenteric, and sublumbar lymph nodes, and
within the submucosa of multiple sections of small intestine. Caseogranulomas had acid-fast bacilli with prominent banding (fig. 7) within the central necrotic debris, epithelioid macrophages,
and multinucleate giant cells.
Immunohistochemical staining of tissue sections was positive for Mycobacterium species and
negative for chronic wasting disease.
M. kansasii was isolated in both laboratories from sections of lymph node, lung, kidney, and
liver. Identification testing of growth indicated a Mycobacterium species other than
Mycobacterium tuberculosis complex. M. kansasii was subsequently confirmed based upon
growth characteristics, colony morphology, photochromogenicity, biochemical profile, high
pressure liquid chromatography and genetic probe testing. No virus was isolated from sections
of lymph node.

Discussion
Mycobacterium kansasii is a photochromogenic, slowly growing mycobacterium species that is
most commonly isolated from water, including tap water, ponds, lakes, swimming pools,
aquariums, and sewage, but rarely from animals and soil. There are individual case reports of M.
kansasii in a dog (canis familiaris) (8), a goat (Capra hircus) (1), a llama (Llama glama) (7), a
rhesus monkey (Macca mulatta) (6), and four squirrel monkeys (Saimiri sciureus sciureus) (2). A
survey of lymph nodes from cattle in the United States identified multiple strains of M. kansasii in
the lymph nodes of clinically healthy animals (5). The primates and the goat were identified
through routine tuberculosis testing. The dog was clinically ill with dyspnea and persistent pleural
effusion. Most importantly, all reports documented histologic lesions consistent with mycobacteriosis. This case of *Mycobacterium kansasii* in a reindeer is of significant public health concern, because reindeer are popular animals for exhibition in children's zoos, petting zoos, and holiday exhibitions. This young animal had disseminated granulomatous disease that was grossly and microscopically indistinguishable from *M. bovis*, a species included in the *M. tuberculosis* complex. A definite diagnosis requires bacterial culture. This reindeer had been screened for Johne's disease (*Mycobacterium avium* spp *paratuberculosis*) via a fecal culture. *Mycobacterium kansasii* had been identified in fecal material from this reindeer, and the animal had been shedding the organism in her feces before clinical disease was apparent. Unfortunately, *M. kansasii* was not considered a primary pathogen at the time of identification in the feces. The information was filed and forgotten until the diagnosis of mycobacteriosis had been made on post-mortem examination, thereby perpetuating potential transmission of *M. kansasii* to animal care staff and other animals in adjacent exhibits. Fortunately, there was no direct contact with the general public visiting the zoo.

The source of infection was not identified in this case. This is also quite common in the majority of human infections with *M. kansasii*. There are a few reported cases of focal dermal and arthritic infections that have a history of trauma (3). However, the most likely source of infection in this case is probably drinking water. *M. kansasii* has been cultured from municipal water sources (tap water), lakes, ponds, swimming pools, and rarely the soil. There is no evidence of human-to-human (3), animal-to-animal or animal-to-human transmission at this time.

Disseminated mycobacteriosis in animals and humans is typically associated with immunocompromised individuals. Human cases of *M. kansasii* have been reported in patients with AIDS, systemic lupus erythematosis, chronic pulmonary disease, and those undergoing immunosuppressive therapy (4,9). The majority of people with focal dermal or arthritic lesions also have a history of immunosuppressive disease or therapy, although there are case reports of immunocompetent individuals with *M. kansasii* (3). The severity of this reindeer’s disseminated disease and the fact that she was shedding the organism in her feces suggests immunosuppressive disease or compromised immune function. Virus isolation performed on sections of lymph node for bovine viral diarrhea, which causes immunosuppression in ruminants, was negative. Other factors considered as possible stressors and thus causes of immune suppression included the fact that this animal was behaviorally subordinate to the other reindeer she was housed with. How much stress is caused by captivity and display in a zoo is unknown. Increased cortisol levels have been demonstrated in numerous non-domestic species housed in captivity. There is also little information about this animal prior to her arrival at the zoo. It is not known if she was raised by her dam and received passive maternal immunity or if she was fed milk replacer, which would have had a marked effect on her immune status as a calf. No other animals housed at the zoo, including the other reindeer have been diagnosed with mycobacteriosis prior or subsequent to this animal’s demise. Based on the data reported here, *M. kansasii* should be considered a primary pathogen in animals with zoonotic potential.

**References**

Figures

Figure 1: Left prescapular lymph node, caseogranuloma.

Figure 2: Rib cage, severe multifocal granulomatous pleuritis.

Figure 3: Lung, severe multifocal granulomatous pneumonia and pleuritis.
Figure 4: Kidney, severe multifocal granulomatous nephritis.

Figure 5: Lung, granulomtous pneumonia with multiple multinucleated giant cells, H&E staining.
Figure 6: Thyroid, caseogranuloma with central mineralization., H&E staining.

Figure 7: Lymph node, acid fast organisms with prominent cross banding. Ziehl Nelson Staining.
CHLAMYDOPHILA ABORTUS AT PARIS ZOO: ARE WE ALONE?

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Abstract
Since 1999, all abortion cases at Paris Zoo have been routinely screened for abortive diseases such as Brucellosis or Chlamydiiosis. Between 1999 and 2003, several Chlamydiiosis-related abortions were reported among our hoofstock, including Springbok, Beisa Oryx and Sable Antelopes. These were diagnosed using both serology and immunofluorescence detection tests. Recently, using a highly sensitive PCR assay, a chlamydiaceae species was identified as being involved in Springbok abortion and Chlamyphila abortus, commonly known to be the causal agent of the “Ovine Enzootic abortion” in domestic ungulates was isolated. [Recently, using a highly sensitive PCR assay, a chlamydiaceae species was identified as being involved in Springbok abortion and Chlamyphila abortus, commonly known to be the causal agent of the “Ovine Enzootic abortion” in domestic ungulates was isolated.]

In an outbreak of C. abortus abortion, the first goal is to limit the spread of infection as much as possible. Treatment of clinically affected and pregnant animals with a long-acting oxytetracycline is the most commonly used method to decrease abortion, but this does not suppress bacterial shedding. Vaccination with killed, whole-cell bacteria before breeding can reduce the incidence of abortion but the infection and the bacterial shedding persists. In contrast, a temperature-sensitive commercially available live C. abortus vaccine gives strong, long-lasting protection and significantly reduces shedding. As the chlamydial strain of Paris Zoo has been isolated, the efficacy of this live vaccine against this strain will be tested on a mouse model, and then a vaccination program could be set up for some zoo animals.

Beyond prophylactic topics, the occurrence of this disease in Paris Zoo raises two different questions. Firstly, considering the epidemiology of chlamydiosis and the swapping of wild hoofstock between collections in Europe, Paris Zoo is probably not the only zoo facing C. abortus. Secondly, as it is not possible to predict the risk of chlamydial shedding of seropositive animals, does it change their value for conservation? Additionally, what about the opportunity for vaccination?

Key Words: Chlamydiiosis, Chlamyphila abortus, abortion, hoofstock

Introduction
Abortions in zoo collections are probably underestimated. Moreover, hoofstock abortions are even harder to record, as they may happen among large herds, with potentially non-identified animals, and in extensive enclosures. Hence, although the abortion rate can become very important, the cause of abortions is seldom looked for. Some infectious causes of abortion (e.g. Brucellosis) have a reduced incidence thanks to sanitary regulations, but others, like the genus Chlamyphila, are not covered by laws or routine screening, and could lead to chronic carriage of disease.
The *Chlamydophila* genus is well known amongst zoo vets for the species *C. psittaci*. However, another species within the genus *Chlamydophila* also causes disease and zoonosis: *C. abortus*, mainly involved in mammalian abortion. This is the agent of the “Enzootic abortion of ewes” (EAE) or “Ovine enzootic abortion” (OEA), which is classified as a List B disease by the O.I.E. This organism is known to infect many domestic mammals (sheep, goats, cattle), sometimes with a high prevalence [6;13], but also infects wild mammals [2;4;7].

**History**
Paris Zoo started to routinely screen all abortions for Brucellosis and Salmonellosis in 1998. In 1999, the lab found chlamydeous colonies in a dead Sable Antelope fetus during one of these routine tests. After this, *Chlamydophila* screening was also routinely carried out in cases of abortion, with additional serological testing in a few cases. A total of 10 suspect cases were found from 1998 to 2001; between 30 to 40% of all known abortion cases of the herbivores in the collection, including the following species: Sable Antelope (*Hippotragus niger*), Beisa Oryx (*Oryx gazella beisa*), Sitatunga (*Tragelaphus spekii*), Springbok (*Antidorcas marsupialis*), Pere David’s deer (*Elaphurus davidianus*) and Giraffe (*Giraffa camelopardalis peralta*) (table II).

Until 2001 the tests used on tissue (fetal, placental) were immunofluorescence and monoclonal antibody tests with low specificity. Hence, it was not possible to discover the species of *Chlamydophila* involved, nor whether it was really a pathogenic species. In 2001, the INRA team was contacted to enhance the specificity of the diagnosis, by screening using PCR. A few more cases were diagnosed and a strain of *Chlamydophila abortus* was clearly found to be the cause of abortion in a female Springbok and was also involved in Sitatunga and Impala abortions.

In our experience the only sign was abortion, sometimes associated with dark brown vaginal discharge and retained placenta. Additional signs reported in the literature include (in order of frequency): weak calves, vesiculitis in males, mastitis in females, cough, keratoconjunctivitis, arthritis and encephalomyelitis [12].

**Diagnostic difficulties and limits**

**New Taxonomy**
On April 1999, all the Chlamydiaceae taxonomy was reviewed and changed, so that, depending on the age of a publication, fact sheet or leaflet, “*C. psittaci*” may not mean the same organism before and after April 1999. [3;8]

<table>
<thead>
<tr>
<th><strong>Chlamydiaceae Family</strong></th>
<th><strong>Chlamydia genus</strong></th>
<th><strong>Chlamydophila genus</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chlamydia genus</strong></td>
<td><em>Chlamydia trachomatis</em> (Human strain)</td>
<td><em>Chlamyophila psittaci</em></td>
</tr>
<tr>
<td></td>
<td><em>Chlamydia muridarum</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Chlamydia suis</em></td>
<td></td>
</tr>
<tr>
<td><strong>Chlamydophila genus</strong></td>
<td><em>Chlamydia pneumoniae</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Chlamydia pecorum</em></td>
<td></td>
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<tr>
<td></td>
<td><em>Chlamydia caviae</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Chlamydia felis</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Chlamydia abortus</em> (previously <em>C. psittaci</em> serotype 1)</td>
<td></td>
</tr>
</tbody>
</table>

**Table I:** New taxonomy of chlamydiaceae
<table>
<thead>
<tr>
<th>Specie</th>
<th>Date</th>
<th>ID (A=abortion)</th>
<th>Imuno-Fluorescence Test</th>
<th>Monoclonal Antibody Test (Clearview&lt;sup&gt;ND&lt;/sup&gt;)</th>
<th>Serology (Complement fixation)</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervus eldii</td>
<td>28/02/95</td>
<td>Z83005 (T)</td>
<td></td>
<td>+ (av. strain)</td>
<td>Seronegative with bovine strain (serology)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>02/03/95</td>
<td>Z84294 (T)</td>
<td></td>
<td>+ (av. strain)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29/03/95</td>
<td>Z91305 (T)</td>
<td></td>
<td>+ (av. strain)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Z89021 (T)</td>
<td></td>
<td>+ (av. strain)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>All Females</strong></td>
<td></td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antidorcas marsupialis</td>
<td>25/04/2001</td>
<td>Z98166 (A)</td>
<td>+ placenta</td>
<td>+ placenta, nose, eye</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aepyceros melampus</td>
<td>06/12/2000</td>
<td>Z95172 (A)</td>
<td>+ eye, nose, liver</td>
<td>+ eye, nose</td>
<td>Egg inoculation =&gt; negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>14/03/2002</td>
<td>ZA0204 (A)</td>
<td>+/- lungs</td>
<td>+ eye, nose, lungs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giraffa camelopardalis</td>
<td></td>
<td>Z75137 (A)</td>
<td>+/- eye</td>
<td>-</td>
<td>PZP vaccination of the Dam</td>
<td></td>
</tr>
<tr>
<td>Elaphurus davidianus</td>
<td>27/01/2002</td>
<td>Z97071</td>
<td></td>
<td>+ placenta</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oryx gazella beisa</td>
<td>30/03/2000</td>
<td>Z94225 (A)</td>
<td>+/- Placenta</td>
<td>+ : placenta</td>
<td>Egg inoculation =&gt; negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10/04/2000</td>
<td>Z94225 (T)</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tragelaphus spekii</td>
<td>25/10/2001</td>
<td>Z94238 (A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippotragus niger</td>
<td>23/11/1999</td>
<td>Z94199(A)</td>
<td>+ liver, spleen</td>
<td>-</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>02/12/1999</td>
<td>Z94199(T)</td>
<td>-</td>
<td>+ nose</td>
<td>Vaccination</td>
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</tr>
<tr>
<td></td>
<td>13/04/2000</td>
<td>Z94199(T)</td>
<td>-</td>
<td>+ vagina, rectum</td>
<td>Vaccination</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Z94247(T)</td>
<td>-</td>
<td>-</td>
<td>Vaccination</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Z95155(T)</td>
<td>-</td>
<td>-</td>
<td>Vaccination</td>
<td></td>
</tr>
<tr>
<td>Tragelaphus strepticeros</td>
<td>30/10/2000</td>
<td>Z90107(A)</td>
<td>+/- eye</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kobus lechwe</td>
<td>14/11/2000</td>
<td>Z98190 (A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild feral carnivores:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vulpes vulpes</td>
<td>Since Nov 2000</td>
<td>3 foxes/5</td>
<td>(swabs)</td>
<td>(swabs)</td>
<td>Trapped within the Zoo</td>
<td></td>
</tr>
<tr>
<td>Martes foina</td>
<td></td>
<td>3 martens /3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table II:** Reported cases of suspected Chlamydial abortions in Paris Zoo since 1995
**Limits of bacterial diagnosis**

Stains, ImmunoFluorescence (IF), *ClearView*™ and Culture.

Initial laboratory diagnosis involves the staining of *Chlamydophila* (from placenta or fetus samples), but this requires an experienced technician, as stained *Chlamydophila* organisms look very similar to stained *Brucella* and *Coxiella* organisms.

Micro-immunofluorescence and *ClearView*™ tests both detect *C. abortus* antigens, but there are cross-reactions with other organisms (especially on intestinal-related samples), such as *C. pecorum*, widespread among ruminants and seldom pathogenic. IF with improved specificity exists, but these tests are too complex for routine screening. The screening at Paris Zoo was initially performed with these antigen-screening methods, but the lack of specificity was soon a matter of concern, especially on intestinal samples.

Culture (actually egg inoculation) is the only method giving 100% specific diagnosis, but it requires perfectly fresh samples to reach good sensitivity. Moreover, culture results take more than 30 days and thus delay diagnosis.

**Serology**

Serological diagnostics are only useful for a group, not individually. The first test to be used is the Complement Fixation test (CFT). CFT is useful in zoo animal medicine because it is not linked to a species-specific antibody; however, this test suffers from poor sensitivity and specificity because it often uses an antigen common with *C. pecorum*, which most herbivores harbor in their intestine.

Other tests include IF and ELISA, which share the same drawbacks as in tissue samples. Recently, some labs have developed a new ELISA test for sheep and goats with enhanced specificity (>99%), but still less than 75% sensitivity. Moreover, these tests have not been validated in wild hoofstock, regarding their antibody conformation.

Hence, serological information from zoo animals could only be informative. As in domestic hoofstock, a rising antibody titer between two CF tests (one from a sample taken at the time of abortion, the other 3 to 6 weeks later) could be relevant in aborting females, but the main use of serology is to know the group infection status. The antibody titer is poorly linked to the protection level of the animal; as the main protective immune response in Chlamydiosis is cellular; antibodies are directed against membranous Lipo-Poly-Saccharide and are generally not protective [11].

**Best solutions**

Polymerase Chain Reaction (PCR) on tissues and serology are the best solutions.

As in human medicine, PCR screening is now well developed and used in Chlamydiaceae diagnosis. Several primers are available and some are very specific, allowing distinction between *C. abortus* and *C. pecorum*. This technique was performed at the INRA laboratory to detect *C. abortus* involved in the Springbok abortion. In combination with a serological survey, this is a good diagnostic tool to encompass the development and issue of a *C. abortus* outbreak.

**Treatment and prophylaxis**

**Chemoprophylaxis: use of antibiotics**

Antibiotic therapy could be used during pregnancy to avoid abortion. At Paris Zoo, the Sable antelopes were treated twice using two doses of 20 mg/kg of long acting oxytetracyclin (T.L.A™) at mid-pregnancy and 15 days before the presumed birth date. This treatment allowed the females to produce two calves. One of them died within 10 months in poor condition but there was no evidence of *Chlamydophila* infection. This technique has been used in ewes, but doesn’t prevent the offspring from becoming infected and being a future carrier.
**Passive prophylaxis**

- Pest Control: more than 50% of the exogenous roaming Carnivores (mainly foxes and martens) trapped in the zoo since 2001 tested positive on swabs for *Chlamydophila* *spp*. Moreover, rodents and birds (pigeons; more than 50% of Paris pigeons are infected with *Chlamydophila sp.* [14]) are also a matter of concern as potential carriers of *C. abortus*, contaminated by eating aborted fetus or placenta in the litter, and excreting the bacteria via faeces.

- Population management: two levels of management could be used:
  
  - Interspecific level, with sanitary isolation of the infected species from other herbivores, to prevent spreading of disease through litter, keepers, or pest contamination.
  
  - Intraspecific level, by not swapping animals between the 'infected' sub-group and the 'non infected' one, thus managing them separately. However, this could be quite problematic in a zoo, where staff and facilities can’t be easily divided. At a minimum, pregnant herbivores should be segregated during calving seasons to reduce transmission.

**Active prophylaxis - vaccination**

- Killed vaccines: The first *C. abortus* vaccines were inactivated vaccines. These can reduce the incidence of abortion, but do not prevent either carriage nor shedding. We carried out a trial with this vaccine on three young Beisa Oryx from the Paris Zoo, since killed vaccine is less likely to cause vaccine-induced Chlamydiosis. Antibody titres were measured and revealed no significant titer elevation following vaccination. This failure to induce antibodies also occurs in domestic hoofstock.

- Live vaccine: Developed by INRA Tours (France), this is now available in many EEC countries (France, Greece, Italy, Spain, UK). This vaccine reduces the incidence of abortion and prevents carriage and excretion of the organism in sheep and goats. This commercial vaccine has already been used in some of Paris Zoo’s non-pregnant herbivores (*Damaliscus dorcas*, *Hippotragus niger*). No side effects (e.g. induced disease, local abscessation or hypersensitivity) were noticed. The efficacy of the live vaccine against a challenge with the strain isolated from one Paris Zoo Springbok abortion has been demonstrated in pregnant mice at INRA Tours: vaccinated mice inoculated with abortive doses of Paris *C. abortus* strain neither became infected nor aborted. Moreover, while vaccination of infected animals does not prevent excretion, it is not harmful, which could be beneficial for mass vaccination of extensively held herds.

**Conclusion**

The discovery of this disease within Paris Zoo collection was clearly an incidental finding. Hence, we started to seek… and found. Maybe things should just end here, as it is not a fatal disease and not subject to national sanitary regulations. However, *C. abortus* is an OIE list B classified agent. Hence, some legal obligations do exist, such as mandatory declaration and annual reports.

Moreover, *C. abortus* is a zoonotic agent, which may lead to important consequences regarding the zoo staff (e.g. keepers, vets) and even regarding members of the public in the children’s farm [7]. Some countries even run information campaigns aimed at pregnant women who work on farms about the risk of abortion at lambing season.
Finally, the real status of wild herbivores in captivity regarding *C. abortus* is almost unknown. Considering the large degree of movement of individuals of these species between zoos, and the asymptomatic carriage of this disease, a high prevalence (as in domestic animals) may be suspected. So, is the conservation value of infected herbivores affected? What about the releasing of positive animals in reintroduction programs? Do we have to consider that the perhaps the main sign (just one abortion) isn’t too threatening compared to other fatal diseases?

**References**


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Abstract
From 1992 until 2002 abortion in ruminants occurred 18 times at the Royal Zoological Society of Antwerp. No pathogen was found at necropsy. The seroprevalence over the same period of four major abortive agents, i.e. Brucella, Toxoplasma, Chlamydia and Neospora, was determined with agglutination tests, complement fixation test and DOT-ELISA. A total of 227 sera revealed a prevalence of 0 %, 31.3 %, 2.2 % and 1.1 % for brucellosis, toxoplasmosis, chlamydiosis and neosporosis, respectively.

Key words: seroprevalence, abortion, ruminants, Toxoplasma, Chlamydia, Neospora, Brucella

Introduction
During the period of 1992 – 2002, only 18 abortions were observed in the Royal Zoological Society of Antwerp (RZSA: Antwerp Zoo and Animal Park Planckendael). In spite of this low clinical prevalence, a seroprevalence study can suggest an underlying infectious problem. Major causes of abortion in domestic ruminants include Toxoplasma gondii, Neosporum caninum, Brucella spp. and Chlamydia (Chlamydophila) psittaci. Several reports describe these agents also in free-ranging wild animals, but reports in zoo animals are scarce. Some authors reported zoo ruminants dying from toxoplasmosis (2, 7, 9, 18), but only once toxoplasmosis was reported as a cause of abortion in muskox (3). Stillbirths by neosporosis are reported only two times, in antelope (16) and in deer (6). Until today, according to VETCD-ROM (1973-2003) of the CAB International database there are no reports of brucellosis or chlamydiosis in zoo ruminants. Nevertheless, under the Balai Directive, zoos are compelled to implement a disease surveillance for the OIE-listed diseases.

Material and Methods
From 1992 to 2002, twenty-nine serological tests for brucellosis were carried out for reasons of transport of ruminants from or to other zoos, i.e. 1 Dybowski deer, 1 Sitatunga, 1 Scottish Highlander, 2 Okapi, 2 Wildebeest, 8 domestic goat, 1 Savannah buffalo, 1 Soay sheep, 1 Reindeer, 2 Anoa, 1 Scimitar horned oryx, 1 Pere David’s deer, 2 European wisent, 1 Arabian oryx, 3 Cape buffalo, 1 Slender horned gazelle. The only veterinary laboratory in Belgium, authorised to provide import and export permits is the Veterinary and Agrochemical Research Centre in Brussels (VAR). This laboratory uses the standardised OIE tests, i.e. agglutination test, complement fixation test and Rose Bengal test for brucellosis.

In 2003, 227 ruminant sera of the same period were tested with commercial kits in the laboratory of the RZSA (Table 1), i.e. 40 Cervidae (Dybowski deer, Pere David’s deer, Wapiti, Reindeer, Red deer), 10 Giraffidae (Giraffe, Okapi) and 177 Bovidae (Savannah buffalo, Cape buffalo, European wisent, Yak, American bison, Watussi, Banteng, Dwarf forest buffalo, Scottish Highlander, Anoa, Slender horned gazelle, Blue duiker, Sitatunga,
Scimitar horned oryx, Common eland, Arabian oryx, Wildebeest, Bighorn sheep, Soay sheep, domestic goat, Ibex, Himalayan Thar, Rocky Mountain goat, Muskox).

Table 1: commercial serological tests used at the Royal Zoological Society of Antwerp

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Test</th>
<th>Commercial name</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucella</td>
<td>agglutination</td>
<td>Bengatest</td>
<td>Symbiotics Corporation</td>
</tr>
<tr>
<td></td>
<td>DOT-ELISA</td>
<td>Bovine Brucella</td>
<td>Biogal</td>
</tr>
<tr>
<td>Toxoplasma</td>
<td>agglutination</td>
<td>Pastorex Toxo</td>
<td>Bio-Rad</td>
</tr>
<tr>
<td></td>
<td>DOT-ELISA</td>
<td>Ovine Bispot Chlamydia &amp; Toxopl</td>
<td>Biogal</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>DOT-ELISA</td>
<td>Chlamydia Bovine</td>
<td>Biogal</td>
</tr>
<tr>
<td>Neospora</td>
<td>DOT-ELISA</td>
<td>Neospora Bovine</td>
<td>Biogal</td>
</tr>
</tbody>
</table>

Results

The 29 sera sent to VAR for detection of antibodies against brucellosis were all negative in the agglutination test, complement fixation test and Rose Bengal test.

Out of 227 ruminant sera, 15 (2 Dybowski deer, 4 Pere David’s deer, 1 Wapiti, 3 Reindeer, 2 Okapi, 1 Savannah buffalo, 1 Dwarf forest buffalo, 1 Common eland) were positive in the Rose Bengal test used in our laboratory. The same 15 sera were negative in DOT-ELISA.

Rapid agglutination with 227 sera revealed 71 positives for *Toxoplasma* (31.3 %), of which 28/57 (49.1 %) originating from *caprinae*. Using DOT-ELISA there were also 19/48 (39.6 %) of the same *caprinae* positive.

Antibodies to *Chlamydia* were found in 3/135 (2.2 %) sera of *bovinae, caprinae* and *giraffidae* with DOT-ELISA. Antibodies to *N. caninum* were detected with DOT-ELISA in only 1 serum out of 87 (1.1 %) sera of *bovinae, caprinae* and *giraffidae*.

Discussion

During the period of 1992 – 2002 a total of 18 abortions have been observed, i. e. 1 American bison, 1 Watussi, 3 Yak, 1 Anoa, 1 Sitatunga, 1 Slender horned gazelle, 1 Wildebeest, 2 Okapi, 1 Wapiti, 2 Reindeer, 2 domestic goat, 2 muskox. A necropsy did not reveal any infectious etiological agent. Although chlamydial elementary bodies and protozoal tachyzoites can be found in foetal tissue smears (5, 17), sometimes they are not present (3, 16). According to Thorne (19), reasonably clean and fresh tissues are needed for the detection of *Brucella* bacteria, which is difficult to obtain in wild animals. A serological survey was therefore carried out to assess the possible existence in the captive population of one or more major abortive agents.

In accordance with the recommendations of the OIE (14), sera are first tested by a buffered *Brucella* antigen test, i. e. Rose Bengal test, and then by an ELISA for confirmation. Furthermore, 29 sera were also tested with the complement fixation test in the Belgian national reference center (VAR) in Brussels. In spite of some false positives in the Rose Bengal test, the confirmatory tests were all negative. According to Thorne (19), clinical signs of *Brucella* infection in wild ruminants besides abortion can be retained placenta and enlarged testicles, none of which were observed in our captive population.

*Toxoplasma* has an unusually wide range of intermediate hosts and occurs worldwide. Prevalence in domestic sheep (21 %) and goat (25 %) is higher than in domestic cattle, in which it is believed to be low and unimportant (17). On the other hand, prevalence in free ranging wild ruminants in the United States varies between 2 %, 15 - 23 % and 30 - 60 % in bison, moose and white-tailed deer, respectively (5). Unfortunately, seroprevalence studies in captive ruminants are not available. In the captive population of RZSA *caprinae* have a higher seroprevalence than others, although only 4 abortions have been noticed. Since toxoplasmosis was suspected in the abortion of muskox in San Francisco Zoo (3) and two abortions occurred also in our herd, these animals will be followed more closely. *Chlamydia* causes abortion in sheep, goats and cattle (17). Apart from polyarthritis and keratoconjuntivitis in bighorn sheep, chlamydiosis probably remains usually inapparent in...
wild ruminants (20). One American bison, one domestic goat and one Soay sheep were seropositive in the present study (seroprevalence of 2.2 %). If wild ruminants shed these Chlamydiaceae also three weeks peri-abortively, as it happens in domestic sheep (17), environmental contamination should be so high that more animals of the respective herds would have antibodies. Presumably, the three abortions in bison, goat and sheep are not caused by Chlamydia.

Neosporosis is a major cause of abortion in cattle in many countries (4), including Belgium (12), with transplacental transmission being the main route of infection. In addition, the importance of N. caninum DNA in semen of bulls needs further research (15). The seropositive sample of a bull Yak may indicate the presence of this agent in the herd and needs close monitoring.

According to the OIE (14), the same serological techniques may be used in nondomestic species, but after each test being validated in the species concerned. A first attempt to achieve this purpose is the testing of binding capacities of immunoglobulins from nondomestic hoofstock species (11). The results of Kramsky et al. (11) indicate that immunoglobulins of many wild species recognise recombinant protein G, although they bind not uniformly in closely related species. Whether or not these immunoglobulins recognise an anti-bovine or anti-ovine conjugate in the same way remains to be determined.

Infectious bovine rhinotracheitis (IBR) causes abortion in cattle worldwide (17) and also merits investigation in captive ruminant populations. However, according to Anthony (1), IBR rarely produces clinical signs in wild animals and infected captive mouse deer were reported to remain clinical healthy (8).

References


APPROACHES TO CONTROL OF *Yersinia pseudotuberculosis* IN EUROPEAN ZOOS

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Abstract
A questionnaire sent to 78 European zoos aimed to collate information on the incidence of yersiniosis due to *Yersinia pseudotuberculosis* (*Y. pseudotb*) in the collections, methods of monitoring the organism in zoo populations and current approaches used in the treatment and prevention of yersiniosis. 38 zoos replied, 87% of which had confirmed outbreaks of *Y. pseudotb*.; 71% of outbreaks were in the winter months, mostly causing acute disease or sudden death, and 73% of outbreaks affected more than one animal. 34% of affected institutions monitored prevalence of the organism, mostly using faecal samples; other methods included serology and polymerase chain reaction (PCR). 55% of affected zoos implemented husbandry changes in response to an outbreak; of these only one did not consider the change to be effective. 33% of affected zoos vaccinated against *Y. pseudotb*, mostly in response to an outbreak.

Key words: *Yersinia pseudotuberculosis*, Yersiniosis, vaccination, disease control

Introduction
*Yersinia pseudotuberculosis* is a facultatively anaerobic Gram negative coccobacillus of the family *Enterobacteriaceae*. The organism has been isolated from a wide variety of domestic and wild animals with numerous reports of outbreaks in research institutes and zoological collections, most commonly in birds, rodents and primates (Baskin et al 1997, Welsh et al 1992, Buhles et al 1981, Parsons 1991, Wesche et al 2002, Zwart 1993). The disease also has a zoonotic potential. There are eight strains of *Y. pseudotb*, with serotypes 1A and 1B predominant in Europe, but no host specificity for the different serotypes is reported (Zwart 1993) or correlation between serotype and severity of disease (Carniel & Mollaret 1990).

Animals are infected via faecally contaminated water and food sources and ingestion of infected prey. Although there are reports of the isolation of *Y. pseudotb* from wild-living mammals and birds (Mair 1973, Fukushima et al 1990, Fukushima & Gomyoda 1991, Hayashidani et al 2002) the relationship between infection with *Y. pseudotb* and the prevalence of *Y. pseudotb* in wild-living animals is not well understood. The relationship between the presence of *Yersinia* species in environmental substances and in wild-living animals is also unclear (Brice 1995). Wildlife reservoirs, predominantly rodents and birds, are widely assumed to play an important part in the organism’s epidemiology but there is little evidence to directly support this and some that appears to directly contradict the theory (Pocock et al 2001). *Y. pseudotb* is reported to be widely spread in the environment where it can survive and replicate for months to years in substrates...
such as soil, water, faeces and vegetation due to minimal nutritional requirements and tolerance of temperature extremes from 5°C to 42°C (Brubaker 1991, Carniel & Mollaret 1990). This makes it unpredictable and difficult to assess the effectiveness of control measures.

Outbreaks may be precipitated by stressors such as cold and wet weather, decreases or changes in food availability, overcrowding or capture. There is a well-established seasonal occurrence, with increased incidence associated with the colder temperatures of late autumn, winter and early spring (Mair 1973, Zwart 1993). The physiologic effects of stress may cause disease in animals already carrying *Y. pseudotb*; almost all susceptible animals may become carriers (Mair 1973) or yersiniosis may occur as a consequence to exposure to the bacteria concomitant with stressors or simply as a sporadic infection. In cold weather the organism survives in faecal deposits or on vegetation for longer (Mair 1973) and *Y. pseudotb* infection in wild mice may more readily occur in newborns and spread into the population during the cold season (Fukushima *et al* 1990).

The virulence of *Y. pseudotb* depends on the ability to produce two plasmid-encoded antigens thought to provide the pathogen with a resistance to phagocytosis and allow intracellular multiplication within macrophages (Gemski *et al* 1980). These antigens are also associated with calcium dependency of the organism (Gemski *et al* 1980). A low-calcium environment leads to increased secretion of Yops; antiphagocytic proteins that are key factors in the virulence process (Pettersson *et al* 1996). This could have implications in the nutritional state of animals influencing their susceptibility to yersiniosis. Ingested bacteria invade the epithelium of the small intestine and are transported into the lamina propria. Following an intracellular stage in which different virulence factors are expressed, pathogenic strains may be disseminated through the host. Bacteria spread via lymphatic vessels to distal lymph nodes and invade the liver and spleen where pathology is characterized by multiple abscessation. Rapid invasion of the bloodstream leads to septicemia and death (Carniel & Mollaret 1990).

*Y. pseudotb* presents a problems to zoos due to the often peracute nature of disease with a potentially high mortality rate, its broad host range and a degree of unpredictability. Its persistent nature and multifactorial epidemiology, with the roles of wildlife and environmental reservoirs or sub-clinical infection still not completely understood, precipitate recurrent outbreaks so that it may threaten the success of captive breeding programs of endangered species.

**Results**

**Incidence and outbreak characteristics**

Of the 38 zoos that replied, 87% had had confirmed outbreaks of *Y. pseudotb*. The replies confirmed the wide host range of the organism and varying species susceptibility as shown in Table 1. 73% of outbreaks were classified as acute, with the vast majority occurring in the winter months. The disease often resulted in heavy losses with the main clinical sign being sudden death; in the few cases where clinical signs had been observed they included weakness, lethargy, diarrhoea and weight loss, the latter two in the more chronic cases. The percentage of affected animals that died varied from 1 – 100%, as shown in Figure 1. (This information was provided from 27 zoos; the remainder did not estimate losses).
Table 1. Species reported in *Y. pseudotb* outbreaks

<table>
<thead>
<tr>
<th>Frequent</th>
<th>Less frequent</th>
<th>Occasional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Callitrichids</td>
<td>Lemurs</td>
<td>Other primates: (Diana monkey, gibbon, colobus, macaque)</td>
</tr>
<tr>
<td>Birds esp turacos and toucans</td>
<td>Guenons</td>
<td>Bison</td>
</tr>
<tr>
<td>Capybara</td>
<td>Fruit bats</td>
<td>Banteng</td>
</tr>
<tr>
<td>Agouti</td>
<td>Squirrels (var. spp.)</td>
<td>Patagonian hares</td>
</tr>
<tr>
<td>Mara</td>
<td>Deer (var. spp.)</td>
<td>Sloth</td>
</tr>
</tbody>
</table>

71% of outbreaks affected more than one animal. There is no apparent relationship between the species and the number of animals affected eg callitrichid populations incurred single losses (35%), group losses (47%) and epizootics (18%). 33% outbreaks were classified as recurrent; 82% of these were in callitrichids.

**Control of outbreak**
The majority of zoos’ approach was to treat affected animals and all in-contacts with broad spectrum antibiotics; there was some success with prophylactic treatment but little in clinical cases. Often in conjunction with this, affected animals were isolated, their environment thoroughly disinfected and pest control measures increased. Euthanasia of groups of animals was necessary in some cases. A number of zoos used vaccination to control an outbreak, either alone or with other treatment. One zoo also used probiotics.
Monitoring
12 of the 33 zoos affected by *Y. pseudotb* carried out regular monitoring for the organism, mostly using faecal samples. One institution also used serology and one used solely PCR. The extent of an outbreak seemed to influence whether a zoo monitored for *Y. pseudotb*; 4 of the 5 experiencing epizootics monitored populations regularly. Experiencing recurrent outbreaks did not seem to encourage zoos to monitor populations as only 5 of the 11 zoos experiencing recurrent outbreaks carried out regular monitoring. All replying zoos routinely post mortem their dead animals.

Prevention
12 institutions vaccinate against *Y. pseudotb*; 10 of these use *Pseudovac* from Utrecht, one makes its own vaccine and one uses a vaccine made in Nuremberg. Most started vaccinating in response to an outbreak; 10 had had recurrent outbreaks. 3 of the 12 combine vaccination and regular monitoring, 3 combine vaccination and husbandry changes and 1 zoo undertakes all three approaches to control. There is no apparent relationship between the severity of the outbreak and the decision to vaccinate; some zoos with an isolated outbreak where only one animal has been lost now vaccinate, and not all of those having experienced epizootics vaccinate. One zoo has been vaccinating for over 40 years, although many have started more recently, within the last 3 years. Most users thought vaccination to be effective but one zoo reported problems with vaccine reactions causing lethargy and pain at the injection site and another had stopped vaccinating due to subsequent pseudotuberculosis in vaccinated animals. 18 of the 33 affected zoos implemented husbandry changes to try and control the disease, 5 in conjunction with regular monitoring. Measures taken included rodent control, disinfection, changing/removing substrate, population control to decrease stocking density, quarantine and prevention of contact with wild birds or their droppings by placing mesh or polycarbonate over the enclosure. Only one zoo did not consider the changes they made to have significantly reduced the incidence of disease.

Discussion
Results confirm that yersiniosis due to *Y. pseudotb* is still widespread and causes mortality in a range of species. There is the potential for high mortality in a single outbreak, although this will be influenced by species susceptibility and husbandry methods. Most of the outbreaks described were acute with non-specific, generally minimal clinical signs, providing little opportunity for intervention in such events, consistent with previous reports (Zwart 1993). Observation of clinical signs will be easier in some species than others, but as an indication of disease onset it is clearly of limited use. Recurrence was also an important characteristic of outbreaks; this could be related to a reservoir of disease or to population factors such as overcrowding; an increased population would lead to increased competition for mates, space and food, increased contamination of food and environment and decreased individual attention per animal from keepers. Stress is often put forward as a factor in many conditions affecting animals; it is widely reported to be a predisposing factor in Yersiniosis and is inextricably tied in with husbandry. Furthermore, intervention with the various methods described is rarely successful in the long-term, treatment being unrewarding with 100% affected animals being lost in the majority of outbreaks. All these results point towards prevention as the most effective strategy in order to minimise losses. Having established that prevention is the best weapon against *Y. pseudotb*, which approaches do zoos adopt and why?
The simplest way to try and control the disease is through changes in husbandry aimed at decreasing contamination and stress, considering issues such as accommodation, enclosure design, hygiene of food kitchens as well as enclosures, rodent control, nutrition and group dynamics; zoos with an isolated outbreak of yersiniosis were most likely to opt for this method. This makes sense as both vaccination and monitoring programmes require a greater investment of time and cost which would be unjustified as a reaction to a single outbreak. This control method was the most popular and nearly all zoos considered their changes to have significantly reduced the incidence of disease. However, zoos with a recurrent problem looked to other methods, reflecting the supposition that although husbandry changes can decrease the incidence of disease, if there is an established problem then such measures are unlikely to eradicate it.

The decision to vaccinate seems to be driven by recurrent losses rather than the number of animals lost; 10 of the 11 zoos with recurrent outbreaks opted to vaccinate and only 2 zoos with isolated outbreaks did so. This may be because it is perceived as the closest to a guarantee against disease that can be obtained; it has been advocated as the way forward (Parsons 1991, Bielli et al 1999). Most of the zoos used Pseudovac, a formal killed vaccine containing different serotypes collected from clinical outbreaks and produced in Utrecht, Netherlands. It is administered subcutaneously in late summer or autumn, as most of the outbreaks occur in winter or spring. The primary course consists of 2 doses 6 weeks apart and subsequent annual boosters. The optimal protection range seems to be about 9 months, although no challenge studies have been done to support this, and a couple of zoos noted they needed to revaccinate every 6 months to prevent disease from returning.

Although some zoos have been using this vaccine for decades and nearly all of those using it thought it effective and problem-free, there is no actual data on the efficacy of this vaccine. No challenge trials have ever been performed (G. Dorrestein, Utrecht University, pers. comm.) and owing to the sporadic incidence of disease it is difficult to assess protection in field trials. There may be reason to question the efficacy of the vaccine; Jersey Zoo conducted a study to measure antibody titres following vaccination, which showed scant response to the vaccination course (unpublished data). The situation seems to be similar in toucans, where neutralising antibody titres appear to be zero (J. Lewis, International Zoo Vet Group pers. comm.). A further drawback is the stress involved in capturing and handling the animals for injection, although the effectiveness of the vaccine would ideally override any increased predisposition to disease caused. It is also a time-consuming, costly exercise. The development of oral vaccination may eliminate some of these problems (Thornton and Smith 1996). Maybe vaccination is best reserved for use only after other control methods have failed. Improved knowledge of epidemiology with more widely available monitoring programmes would decrease the need for vaccination and enable zoos to really tackle the disease rather than mask the complexities of an ongoing problem.

Monitoring populations for Y. pseudotb was the least popular method of control. The least invasive method is to isolate and identify the organism from faecal samples, but this can be insensitive despite using enrichment techniques (Greenwood & Hooper 1988); at its best it will not detect carriers, only clinical cases (W. de Meurichy, pers. comm.). The organism is more usually isolated from lymph nodes, liver or spleen; in vivo, the bacterium is located mainly in organs and not in the intestinal lumen. This also raises the question of how accurately faecal samples reflect the clinical state of the animals. One zoo used serology; the classical agglutination test is not reliable and gives many false positive and / or false negative results (E. Carniel, pers. comm.). Serological diagnosis is complicated by the number of serotypes and limited by cross-reactions with other organisms such as Brucella abortus, Salmonella, Campylobacter (Carniel &
Mollaret 1990). An ELISA test has been developed for human serological diagnosis using Yops as antigens which seems to be much more specific and sensitive, but this would need to be adapted and validated for each animal species. PCR would provide a more sensitive technique, but may be too sensitive; where there is a pseudotuberculosis problem we can be fairly sure the organism will be present in the population at some level, even if it is insufficient to cause clinical signs. We would need to know what levels to look for in a population to prevent excessive use of prophylactic antibiotics. Monitoring is important as where there is a pseudotuberculosis problem the organism will persist; identification of increasing levels could provide forewarning of an outbreak providing time to give prophylactic antibiotics. Greater sensitivity than current techniques allow may be needed to detect all carriers. Despite discouraging reports (Brice 1995) it is worth pursuing refinement of the technique of isolation from faecal samples as this is the most practically applicable method.

Yersiniosis is still a poorly-characterised disease with much of its epidemiology still largely based on assumption. For zoos holding susceptible species funded research will hopefully provide some much needed answers.

Acknowledgements
Thank you to Tony Allchurch and Ann Thomasson at the Durrell Wildlife Conservation Trust, where this work was carried out as a student project, and to all participating zoos.

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EPIDEMIOLOGICAL INVESTIGATIONS OF LEPTOSPIROSIS IN ZOOLOGICAL GARDENS

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Key words: Leptospirosis, zoo animals, PCR, serology, epidemiology

Abstract
A total of 890 blood samples from zoo animals (106 species) was tested for antibodies against Leptospira by micro-agglutination-test (MAT). Antibodies with titres of at least 1:400 occurred in 3.2 % of the samples from Wilhelma zoo and in 10.2 % of the samples from some other German zoos. The following serovars were tested: australis, autumnalis, canicola, grippotyphosa, copenhageni/icterohaemorrhagia, pomona, hardjo, sejroe, saxkoebing and tarassovi. Additionally kidney tissues of 920 free roaming rodents and other potential excreters from zoo areas were tested for leptospiral DNA by PCR. The brown rat (Rattus norvegicus) was found to be the most relevant host with 11.4 % positives. Epidemiological aspects and risk factors associated with Leptospirosis in zoological gardens are discussed.
AN OUTBREAK OF LEPTOSPIROSIS IN SEALS (*Phoca vitulina*) IN A ZOO POPULATION

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**Abstract**
In Ouwehand Zoo in the summer of 2003, 5 of the 10 seals died due to a leptospirosis epidemic. Nutrias (*Myocastor coypus*) that were housed in the same water stream have been investigated for a leptospirosis infection. Seals and nutrias had serologic responses to serovar Icterohaemorrhagiae and Copenhagi. In nutrias the *L. interrogans* serovar has been isolated also belonging to the serogroup Icterohaemorrhagiae. The nutrias have been moved to another exhibit away from the seals.

**Keywords:** seals, nutrias, leptospira

**Case report**
Ouwehand Zoo houses harbor seals (*Phoca vitulina*). In the summer of 2003, 2 males and 8 females were present in a 1000 m³, fresh water basin. The basin water is filtered through a sand filter and water flows out to a connected water stream with approximately 25 m³ water. This pond is inhabited with several species of fish, waterfowl and 20 nutrias (*Myocastor coypus*). The quality of the water is monthly checked and has to conform to the level of drinking water for poultry. In autumn of 2002, numerous brown rats (*Rattus norvegicus*) were seen surrounding the premises. But elaborate efforts were made to eradicate those animals.

In June, a death seal was found on the edge of the pool. The next day the animal was offered for necropsy at the Department of Pathobiology, Section Diseases in Special Animals and Wildlife, Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

At necropsy, the animal was very autolytic. Hemorrhagic foam flowed out of the nostrils. The fat was yellow. Multifocal ecchymotic and petechial hemorrhages were present in the thoracic cavity, the mesenterium, and around the kidneys. The lungs were hyperemic and edematous, and there was hemorrhagic foam in the trachea and bronchi. The heart, the liver and the kidneys were pale. The enlarged spleen showed hyperplasias of the white pulp. Further work up was performed.

In the following days 4 other seals died. At necropsy they all showed signs of acute septicemia, consistent with acute leptospirosis. In general multiple hemorrhagic lesions: blood around the mouth and nostrils, in the trachea, serosal membranes, around the kidneys. The fat was yellow. The lungs were hyperemic and edematous, with hemorrhagic foam in the trachea and bronchi. The livers were pale and soft. The kidneys were pale with varying degrees of hemorrhages.

2 juvenile seals did not show previous described signs.
Based on the jaundice and hemorrhages on pathology, infection with leptospirosis was suspected.
The basin was emptied, and the remaining 5 seals were all treated with oxytetracycline i.m. Blood was collected from each animal for hematological and biochemical examination. Serum of seals that had died and from ill seals was screened for leptospirosis with the microscopic agglutination test (MAT). In one seal that had died response to serovar Icterohaemorrhagiae and Copenhagi 1:640 were determined. Both belonging to the serogroup Icterohaemorrhagiae.

Clinically, the animals deteriorated while being out of the water at the base of the basin. They were lethargic, anorectic, and some vomited blood. After 3 days the basin was filled again and the seals were treated with antibiotics in the food or by dartgun during another week. During this episode no brown rats were observed. The decision was made to screen the nutrias for leptospirosis. 11 nutrias were anesthetized. Blood and urine samples were taken, and serologically and with the polymerase chain reaction (PCR) evaluated for the presence of leptospira. 8 animals were euthanised.

In 7 nutrias response to serovar Icterohaemorrhagiae and Copenhagi 1:20 up to 1:5120 were determined. In 3 nutrias the urine was positive for L. interrogans serovar Copenhagi also belonging to the serogroup Icterohaemorrhagiae. In 1 nutria the kidney was positive for L. interrogans serovar Copenhagi.

The remaining nutrias were treated with doxycycline in the food during 2 weeks and moved to another exhibit away from the seals.

The 5 surviving seals did not have problems so far. After the epidemic the seals have not been vaccinated for leptospirosis.

References

AN OCULAR DISEASE OF UNKNOWN ETIOLOGY IN HAWAIIAN MONK SEALS
(Monachus schauinslandi)

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Abstract
Eleven of 12 female wild Hawaiian monk seal (Monachus schauinslandi) pups captured at French Frigate Shoals, Northwestern Hawaiian Islands, and brought into captivity for rehabilitation developed clinical signs of an eye disease of unknown etiology characterized by three clinical categories. Most seals progressed through a sequence of clinical signs beginning with conjunctivitis and blepharism, followed by corneal opacities, and culminating in bilateral cataracts and blindness. Results regarding this eye disease suggest an infectious etiology; while many pathogens have been ruled out, the cause of this clinical eye disease and the site where it originated remain unknown.

Key words: Cataract, corneal opacity, eye disease, Hawaiian monk seal, Monachus schauinslandi

Introduction
The Hawaiian monk seal (Monachus schauinslandi) is the only marine mammal located entirely within U.S. waters. The species is endangered of extinction and has been declining since the first range-wide surveys in the late 1950s. Currently there are approximately 1,300 to 1,400 seals, but abundance will likely decline further due to high juvenile mortality and low reproductive recruitment at French Frigate Shoals (FFS), the site of the largest colony of this species. Conceivably, the Hawaiian monk seal could be on the verge of extinction in 20 years. Several natural sources of mortality have been identified or suggested including ciguatera poisoning, starvation, shark predation, disease, and trauma/mobbing, but the relative significance of these factors and their effect on population trends are unknown.

In accord with the National Marine Fisheries Service (NMFS) recovery plan, a rehabilitation program was initiated in 1984 to further enhance recovery. Undersized, weaned female pups were taken from FFS to Oahu, where they were held in captivity for 8-10 months to increase their weight. At the end of the captive period, pups were transported back to the Northwestern Hawaiian Islands for release into the wild.

Case Report
In 1995, 12 Hawaiian monk seal female pups were captured for their rehabilitation in FFS; however, were not released as anticipated due to a persistent eye condition of unknown etiology. In cooperation with numerous outside scientists and laboratories, NMFS has conducted an extensive effort to diagnose this eye condition since 1995. The condition has not been previously
reported in wild or captive monk seals. Reinstatement of rehabilitation efforts at Oahu will partly depend on the characterization of the etiologic agent(s) responsible for the ocular syndrome, relocation of affected seals to another facility and proper rehabilitation and quarantine protocols. We provide herein a descriptive overview of the clinical course of this disease process, the epidemiologic associations, laboratory testing and diagnostic efforts performed to date.

Methods and Results
Twelve female pups were captured sequentially at FFS between 27 May and 31 July 1995. At FFS, each seal was held temporarily (generally less than two weeks) in a shoreline enclosure, and then transported (singly or in pairs) via aircraft to Sea Life Park (SLP), Oahu. The first two pups to be captured and transported (YC03 and YC04) were noted to have eye problems (described as redness, conjunctivitis, and discharge) during the holding period at FFS. Within a few weeks after arrival on Oahu, nine of the 12 female pups were reported to have initial clinical signs associated with conjunctivitis, red eyes, blepharism, blepharospasm, and photosensitivity. The nine pups were YC03, YC04, YC16, YC21, YC22, YC26, YC28, YC33, and YC35. The initial clinical signs seemed to resolve within two to seven weeks of onset. Three pups sampled during this period presented a neutrophilia, but other cell blood counts (CBC) and serum chemistry values were considered normal. Appetite and activity levels were also normal, and no other clinical signs were observed. Approximately 14 weeks after arrival (ca. four months after capture), these nine pups developed corneal opacities associated with transient conjunctivitis, ocular discharge, photosensitivity, "blue eye", edema, and bulloss keratitis. The corneal opacities developed centrally and progressed outward to the limbus. In seven of the nine seals, opacity and edema developed first in the right eye. Through the course of the disease process, however, opacity and edema progressed similarly and to the same extent in both eyes. Conjunctivitis and photosensitivity occurred transiently after the entire cornea became opaque with a bulloss keratitis. At this stage of the disease, vision was severely impaired for approximately one week. Gross examination indicated that the opacities began to recede from the limbus about 50 days after initial detection. Approximately one year after the development of corneal opacities, seven of the nine seals developed rapid formation of cataracts. To date, these seals appear to be functionally blind. Of the three seals that did not follow the observed pattern of eye disease, only one (YC14) has remained asymptomatic. Seal YC24 developed mild opacities 10 months after collection (and following a conjunctival biopsy and aqueous humor aspiration), but the opacities have almost completely resolved and this seal has not developed cataracts or conjunctivitis. Seal YC32 did not display eye disease until 20 months after collection, when it acutely developed bilateral cataracts. Two of the nine seals exhibiting the common pattern of eye pathology have died; YC33 died in mid-February 1997 and YC26 died in late March 1997. Necropsies and laboratory investigations indicated Clostridium endotoxosis as the cause of death for YC33 (NMFS unpubl. data 1997). YC26 was diagnosed with fulminating hepatic sarcocystosis.

After arrival on Oahu, all 12 seals were evaluated clinically and blood samples were collected for hematology and serum biochemistries. Consequently specimens were collected approximately 6 times (once every 6-8 weeks) a year. Clinically these animals have remained stable and healthy during the sampling period. This stable state was reflected in hematologic and biochemical data parameters that usually fell within previously determined reference ranges. Serology was also completed upon arrival for canine distemper, canine parvovirus, Dirofilaria immitis, Leptospira spp., and Toxoplasma gondii. Fecal samples were evaluated for intestinal parasites and Salmonella spp. Results from these initial tests indicated that three seals
presented a leukocytosis and neutrophilia; however, blood samples from these seals were taken within 24-48 hours after a stressful transport to Oahu. Feces of seven seals cultured positive for *Salmonella sandiego*, *S. orientburg*, and *S. reading*, however, commonly, feces from 40% or more of the pups brought into captivity for rehabilitation have yielded positive cultures in the past (NMFS unpubl. data, 1984-1994). Fecal flotation tests to observe parasites were negative for all twelve pups. Thus, none of these tests were indicative of disease or an abnormal condition.

Extensive ocular tests were completed and unsuccessful attempts were made to culture aerobic bacteria, fungus, *Chlamydophila* (culture/antigen) and *Mycoplasma*. During the bullous keratitis phase, the roughened cornea retained fluorescein stain, demonstrating pathology of the corneal epithelium. Before and after this period the stain was not retained. Conjunctival swabs and brushings were evaluated by light and electron microscopy. Particles of about 40 nm and 80 nm were noted; these dimensions are consistent with the size of certain viruses. Inconclusive attempts were made to isolate a virus using cells from other species. New cell lines of monk seal dermis, conjunctiva, kidney, liver, spleen and brain have been developed, and attempts were negative to culture possible viruses from the eye specimens.16,17 Aqueous humor collected during eye examinations tested negative for *T. gondii*. Serum vitamin A levels were determined for seals with and without clinical signs and the results did not indicate a significant difference between the two groups. The observed levels (19-34 mcg/dl) were similar to values from other pinniped species, and likely represent normal serum levels of vitamin A. Serum neutralizing antibody tests for Calicivirus and human herpes simplex II virus were positive for seals with and without clinical signs, as well as for stored serum from seals rehabilitated in previous years (i.e., asymptomatic). Caliciviruses were detected in a previous study using a monoclonal antibody probe and direct electron microscopy but could not be cultured in vitro from swabs (rectal and nasal) and blood of healthy and debilitated seals at FFS in 1992.18

Five of the twelve pups were sedated with oral diazepam and then anesthetized using isofluorane for complete ocular examinations by both a veterinary and a human ophthalmologist on 24-25 February 1996. The examinations were documented by fundic, macro, still, and video photography. Intraocular pressures of anesthetized seals were similar in seals without clinical signs and seals with receding opacities (14-21 mmHg). Corneal opacities and associated vascularization were limited to the anterior one-third of the corneal stroma. The corneal endothelial surface and posterior two third of the corneal stroma appeared normal. The anterior eye chamber, iris, and lens were normal. There was no evidence of filtration angle closure or synechia. Pupillary dilation was achieved with 10% neosynephrine applied topically and an intraocular injection of 0.5 ml of 1/10,000 epinephrine. Fundic examination revealed a normal posterior compartment including optic disc, tapetum, and retina. Aqueous humor (0.5 ml) was aspirated from each eye for further evaluation.

Four thin-layer corneal biopsies were obtained by superficial keratectomy. Three were preserved in 10% buffered formalin and one in glutaraldehyde. Five conjunctival biopsies were also collected. Four were preserved in 10% buffered formalin and one in glutaraldehyde. Histopathology of the formalin-preserved biopsies revealed mild to moderate inflammation with corneal edema and some protein deposition; all pathology was limited to the anterior one-third of the corneal stroma. No cells were found in the aqueous humor. No viruses were observed during scanning electron microscopy of the aqueous humor. Virus isolation attempts from the aqueous humor yielded negative results (no virus was isolated), although cytopathic changes occurred in some cell lines of other species. Virus isolation will be attempted using stored aqueous humor samples and the newly developed Hawaiian monk seal cell lines.16,17 In January 1997, two previously examined seals were anesthetized for a second comprehensive examination. YC04 had recently developed cataracts and the examination indicated that the cataracts were mature
and likely to result in permanent blindness. Intraocular pressures (21-29 mm) were higher than observed in the previous examination. Pathology, including edema and vascularization, was limited to the anterior one-third of the cornea. Conjunctival biopsies were obtained for future diagnostic evaluations and for development of a new cell line. The second seal (YC24) had been examined in February 1996 as our normal or asymptomatic reference seal. Notice that YC24 developed slight opacities ten months after collection (ca. seven months after the first nine seals developed opacities), but that those opacities modulated toward resolution since June 1996. Her eyes never developed many of the clinical signs (e.g., microvesicles, bullous keratitis, blindness or cataracts) described for the nine seals originally affected by this condition. On re-examination, the corneas of this seal were thin and non-edematous, and appeared to be scared. These observations indicated a different disease process or different manifestation of the same disease, but were decidedly different symptomatically and macroscopically from observations of the other seals examined.

Seals with opacities and associated clinical signs were treated with antibiotics, corticosteroids, glaucoma inhibitors, hypertonic solutions, miosis inhibitors and pupil dilators. During the initial phase of the condition, four of the nine affected pups were treated with topical triple-antibiotic ointment for 35 days. When the corneal opacities were observed, enrofloxacin (Baytril; 20 mg/kg) was administered orally and chloramphenicol 1% ophthalmic ointment was administered topically twice daily (BID). Chloramphenicol 1% ophthalmic ointment was then replaced by a hyperosmotic sodium chloride 5% ophthalmic ointment, which had no discernible effects. Gentocin ophthalmic ointment was then applied twice daily for five days. After examination by other veterinarians, and because no positive results were observed from the initial therapy, Chloramphenicol was administered orally (20 mg/kg BID) and topically (1% ophthalmic ointment BID), in conjunction with prednisone given orally (0.50 mg/kg BID). A single pup (YC22) that was just beginning to display corneal opacities was given prednisone orally (0.50 mg/kg BID). None of these therapies appeared to alter the progression of the disease process. Topical treatment (BID) with the nonsteroidal anti-inflammatory drug suprofen (Profenal 1%), greatly reduced blepharospasm, photophobia, and self-trauma to the eye. The use of timolol (Timoptic), apraclonidine (Iopidine), and dichlorphenamide (Daranide) did not affect the progression of the corneal opacities.

Epidemiological information was collected on the time course and expression of ocular disease, patterns of social affiliation, and medication history. Descriptions of seal affiliations are restricted to associations prior to the onset of ICS. By the time corneal opacities emerged, seal associations were so mixed that no meaningful patterns were discernible (Fig. 1).

**Discussion**

Conjunctivitis, keratitis, corneal edema, corneal ulceration, and cataracts have been reported as common ocular diseases in other species of pinnipeds, especially in captivity. Examination of the eyes of 1501 wild seals by Stoskopf et al. resulted in no evidence of corneal edema/ulceration or keratitis, although corneal scars were common. A field examination with binoculars (at distances of 215 m) or following capture with a hoop net performed by two veterinarians (Aguirre and Braun) in 25-31 August 1996, 8-21 March 1997 and 7-17 July 1998 at FFS concluded that from 158 observed, mostly adult monk seals (87 females and 71 males) observed, 21 presented ophthalmologic problems. Ocular disease included evidence of unilateral or bilateral ocular discharge in 19 seals, ‘puffy’ eyes in two seals, diffuse blue-gray opacities in six seals with evidence of trauma. Corneal opacities were observed in 14 seals and ranged in location and nature. Diffuse maculae and nebulae were described in four seals, and
central well defined leukomas in six seals. Most of these opacities appeared to be trauma related. The opacities in four seals were similar to those of the captive seal pups, however, the cause could not be determined at the time of observation. Two seals demonstrated a condition similar to ‘cherry eye’ with dark purple coloration resembling melanomas and one seal presented papilloma-like lesions on eyelids.  

Transient, dense, and extensive corneal edema has been described in pinnipeds associated with ultraviolet light in bright sunshine and the miotic pupil causing endothelial inflammation. The pathogenesis of these theories is speculative at this point.  

The temporal and spatial distribution of the ocular condition described herein in Hawaiian monk seals can be characterized as an infectious outbreak implying defined clustering of diseased individuals. Although the development of ICS was defined as the onset of the ocular condition; opacities and cataracts occurred during consecutive, well-defined time frames. Epidemiologically, there are three well-defined groups of clinical signs related to ocular disease manifested in these captive seals (Fig. 1). One can speculate on the origin or primary etiology or insult triggering the ocular problems. It was not until February 1996 (8-9 months following ICS) that a full examination and collection of specimens were performed for microbiologic identification. It is very possible that the replication phase for isolation of the primary agent was not detected in time. Many infectious agents are known to cause ocular disease in mammals. Phocine herpes virus, morbilliviruses, Toxoplasma gondii, Chlamydophila psittaci, caliciviruses, and canine adenovirus, to name a few. Multiple serum specimens from these seals were tested for a wide range of infectious agents. A phocine herpes virus has been serologically found widespread among phocid populations around the world. For example, a recent study reports that 78% of 324 harbor seals (Phoca vitulina) presented antibody titers to the virus. PHV-1 is known to cause systemic disease and transient conjunctivitis in harbor seals. The infection has been demonstrated in neonatal seals supporting the possibility of transplacental transmission. It is accurate to say that Hawaiian monk seals at FFS have been previously exposed to a herpes virus-like agent similar to human herpes simplex II virus. The presence of group-reactive antibodies to herpesvirus in Hawaiian monk seals utilizing an enzyme linked immunosorbent assay (ELISA) has been recently identified. The assay was established utilizing a pathogenic isolate of PHV-1 as antigen. While the Hawaiian monk seal sera were positive by ELISA, lack of virus neutralization using this isolate demonstrated the exposure was to a virus different from PHV-1. Currently, polymerase chain reaction (PCR) techniques are being validated for the identification of diverse herpesviruses, using a recently developed harbor seal model. This technique could identify the presence and genotypes of herpesviruses in monk seal samples.  

The cause of the cataracts in these seals has not been determined. There are three primary etiologies of cataracts in animals including congenital, post-inflammatory as the secondary result of a primary eye injury, and idiopathic. Cataract extraction as a treatment was discussed but never pursued. Results to date suggest an infectious etiology of this eye condition; however, comprehensive testing, perhaps too late, did not identify the causative agent. While we have ruled out many pathogens, the cause of this problem and the site where it originated are unknown. Unfortunately, given our lack of understanding of this disease process and the inability to isolate the pathogen, these 10 seals will remain in captivity in a public oceanarium and will never return to the wild. We have increased basic understanding of the anatomy of the monk seal eye and the pathology of this disease process. Cell lines developed in this investigation will be an important future diagnostic tool. The diagnostic efforts have identified many tests, laboratories, and scientists as important resources for disease investigation in captive and wild Hawaiian monk seals.
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References


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Figure 1. Time course of initial clinical signs (ICS), corneal opacities and cataracts from each Hawaiian monk seal (*Monachus schauinslandi*) date of capture.
MALIGNANT CATARRHAL FEVER IN REINDEER (*Rangifer tarandus*)

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Abstract
Two female reindeer from a local petting zoo with a history of poor appetite, polydipsia and elevated body temperature, and poor body condition were euthanized due to deteriorating conditions. At necropsy there were multifocal ulcers along the mucocutaneous junction of the vulva and throughout the epithelium of the upper portion of the respiratory and digestive tract. The principal microscopic lesion was multifocal lymphoplasmacytic and necrotizing vasculitis affecting primarily small to medium sized muscular arteries in the respiratory, digestive and urogenital tract. Additional lesions included multifocal lymphoplasmacytic interstitial nephritis and ulcerative lesions in the esophagus, trachea, and abomasum. Sections of esophagus, spleen, lung, kidney and placenta were positive for malignant catarrhal fever virus (MCFV) by PCR and additional PCR testing and sequencing of the PCR products confirmed 99% homology with ovine herpes virus type 2 (OvHV-2). Additional testing with *in-situ* hybridization (ISH) and immunohistochemistry (IHC) demonstrated the presence of OvHV-2 DNA and protein in lymphocytes in perivascular infiltrates and focally in degenerate epithelial cells and endothelial cells of inflamed vessels. OvHV-2 is considered the causal agent of sheep-associated malignant catarrhal fever (SA-MCF), an acute lymphoproliferative disease of cattle, bison and exotic ruminants. This disease occurs sporadically in cattle and bison in the USA. Epizootics have been described in domestic cattle Wyoming and Colorado, and MCF has been reported in farmed deer, elk and bison. MCF in reindeer was suspected, but not confirmed, in one outbreak of SA-MCF in a petting zoo, but the disease has not been previously confirmed in this species. The epidemiology of SA-MCF is unclear and the disease has been reported most frequently after direct contact of susceptible animals with suspected carrier sheep. In the case presented here, the reindeer shared a pasture with six recently purchased lambs that were clinically unremarkable. We speculate that these sheep carried the virus and infected the reindeer on this farm.

Key words: Reindeer, *Rangifer tarandus*, Malignant catarrhal fever, SA-MCF, ovine herpes virus type 2.

Introduction
Sheep-associated malignant catarrhal fever (SA-MCF) is an acute lymphoproliferative disease of cattle, bison and exotic ruminants. Ovine herpes virus type 2 (OvHV-2) is considered to be the cause of SA-MCF, an uncommon disease in cattle and bison in the USA. However, epizootics have been described in domestic cattle in Wyoming and Colorado. In addition, the disease has been reported in farmed deer, elk and bison. MCF in reindeer was suspected, but not confirmed, in one outbreak of SA-MCF in a petting zoo.
knowledge, this is the first report of an outbreak of SA-MCF in reindeer (*Rangifer tarandus*), in which OvHV-2 has been identified within lesions.
Results
Two female reindeer from a local petting zoo had a history of poor appetite and elevated body temperature (105.0 °F). Both reindeer were approximately 6 months pregnant. The reindeer were treated with aspirin boluses and injectable Ceftiofur sodium. The fever and anorexia persisted after treatment and the owner also noted polydipsia. One reindeer was found dead 3 days after the onset of clinical signs. The following day the other reindeer was brought to the Large Animal Clinic at Michigan State University. Clinical examination revealed an elevated heart rate, respiratory rate and elevated temperature (103.8 °F). Nasal, oral and conjunctival mucous membranes were hyperemic. Gastrointestinal sounds were not auscultated on either side of the abdomen. Urine specific gravity was 1.017, with a pH of 6, protein 1+ and moderate amounts of myoglobulin or hemoglobin. The reindeer was estimated to be 7 to 10 % dehydrated and septicemia/endotoxemia of unknown origin with possible renal involvement was suspected. A blood sample was drawn for a CBC and chemistry panel. The reindeer was placed in a stall and administered broad spectrum antibiotics and intravenous fluids. Flunixin meglumine was administered once partial rehydration was complete. The reindeer’s condition continued to deteriorate and it was euthanized.

At necropsy there were multifocal ulcers covered with fibrin along the mucocutaneous junction of the vulva that extended into the vagina. Similar mucosal ulceration was present throughout the upper portion of the respiratory (fig. 1) and digestive tracts (fig. 2). Tan-yellow viscous exudate protruded from the nares. A thick fibrinous pseudomembrane overlying multifocal ulcers covered the mucosal surface of the trachea (fig. 1) affecting especially the upper trachea and the larynx. In the oral cavity, there was loss of buccal papillae and multifocal erosions of the hard palate. The esophagus and abomasum had multifocal linear ulcers approximately 0.5 to 1.5 cm in length (fig.2). In some areas the ruminal papillae had moderately congested tips that were rarely eroded. There were multifocal serosal hemorrhages in the small intestines. Multifocal to coalescing petechia and ecchymosis were found in the subcutis and cutaneous muscles of the thorax and abdomen, in the pericardial sac and in the epicardium along the base of the heart. The lungs were diffusely congested and edematous with multifocal randomly distributed areas of atelectasis and hemorrhages. The uterus contained an approximately 15 cm long fetus that had no gross lesions.

The principal microscopic lesion was multifocal lymphoplasmacytic and necrotizing vasculitis (figs. 3 and 4), affecting primarily small to medium sized muscular arteries in the respiratory, digestive and urogenital tracts. Lesions were most prominent in the kidneys (fig. 5), esophagus (figs. 6, 6a and 6b), trachea, conjunctiva, abomasum, and vagina. Affected vessels were lined by swollen endothelial cells and the tunica media was commonly disrupted by myocyte necrosis and replaced by pale, homogenous, eosinophilic material (fibrinoid degeneration). Moderate numbers of lymphocytes and plasma cells had infiltrated the tunica media and the adventitia.
Additional lesions included multifocal lymphoplasmacytic interstitial nephritis and ulcerative lesions in the above listed organs. Sections of esophagus, spleen, lung, kidney and placenta were negative for bovine viral diarrhea virus, infectious bovine rhinotracheitis virus and adenovirus by fluorescent antibody staining and virus isolation. All samples were positive for sheep-associated malignant catarrhal fever virus (MCFV, ovine herpes virus type 2) by PCR and sequencing of the PCR product confirmed 99% homology with SA-MCFV.\textsuperscript{1, 3, 10} Additional testing for OvHV-2 was performed, using \textit{in-situ} hybridization (ISH) and immunohistochemistry (IHC). ISH was performed using the following digoxygenin-labeled oligoprobes: 5\textsuperscript{'} TACCATTGTAGACCGTAGAGGTGG 3\textsuperscript{'} and 5\textsuperscript{'} CCACCTCTACGGTCTACAATGGTA 3\textsuperscript{'} IHC was done using multiple commercially available monoclonal antibodies (15-A, 15-A-AC, N10-A, 36-A, VRMD, Pullman, WA, USA). Nuclear staining was observed with ISH in lymphocytes present in perivascular inflammatory infiltrates, and rarely focally in degenerate epithelial cells in the esophagus (fig. 7), as well as focally in cells (fig. 8) lining inflamed vessels (morphologically consistent with endothelial cells). IHC confirmed positive staining in the cytoplasm and nucleus of lymphocytes and degenerate epithelial cells (fig. 9 and 10). No staining was observed with IHC in endothelial cells.

Figure 7: Esophagus, reindeer. Positive nuclear staining of degenerate epithelial cells for SA-MCFV. \textit{In-situ} hybridization for SA-MCFV, hematoxylin staining.
Figure 8: Esophagus, reindeer. Positive nuclear staining of endothelial cells in inflamed submucosal vessel for SA-MCFV. \textit{In-situ} hybridization for SA-MCFV, hematoxylin staining.
Figure 9: Esophagus, reindeer. Positive nuclear and cytoplasmic staining of degenerate epithelial cells for SA-MCFV. Immunohistochemistry for SA-MCFV, hematoxylin staining.
Figure 10: Esophagus, reindeer. Higher magnification to illustrate strong positive nuclear and cytoplasmic staining of degenerate epithelial cells for SA-MCFV. Immunohistochemistry for SA-MCFV, hematoxylin staining.

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Discussion

Malignant catarrhal fever is a viral disease that occurs in a variety of ruminant species and can be caused by a number of closely related viruses. The alcelaphine herpesvirus 1 (AIHV-1) is the only virus in this group that has been isolated, and causes the classical African form of MCF.\textsuperscript{6, 7, 18} The virus is endemic in wildebeest and does not occur currently in Europe or North America. SA-MCF is caused by OvHV-2.\textsuperscript{3, 11, 15, 16} The virus is endemic in sheep and responsible for malignant catarrhal fever in a wide variety of ruminant species.\textsuperscript{2, 5, 6, 7, 11, 15, 16, 18} Recently, a number of closely related gammaherpesviruses associated with disease in other ruminant species have been identified.\textsuperscript{4, 9, 12, 14} The caprine herpesvirus 2 (CpHV-2) has been shown to be endemic in goats and causes disease in Sika and white-tailed deer.\textsuperscript{4, 9, 14} In the case presented here, the virus was identified as OvHV-2 causing SA-MCF, using multiple PCR assays and sequencing of PCR amplicons.

Localization of viral DNA or protein in tissues has been extremely difficult. Whereas PCR often gives unequivocal positive results on a variety of tissue extracts, ISH and IHC have previously not been successful.\textsuperscript{3, 10} Only recently, OvHV-2 was demonstrated in lymphocytes of infected cattle and bison using \textit{in-situ} PCR.\textsuperscript{17} The use of frozen sections made morphological evaluation difficult. The amounts of viral DNA and protein detected in this case were also extremely low. Confirming previous findings, only a few lymphocytic cells in infiltrates around inflamed vessels were identified as positive for OvHV-2, supporting the hypothesis of an immune mediated pathogenesis.\textsuperscript{6, 15, 17} However, in addition small amounts of virus were also detected in degenerate epithelial cells in areas of mucosal ulceration, suggesting a possible cytopathic effect of the virus.
The epidemiology of SA-MCF is unclear, but disease has been reported most frequently after direct contact of susceptible animals with suspected carrier sheep. In this case, the reindeer had been housed with other sheep and a goat for more than a year with no problems. About 2 weeks prior to the onset of clinical signs, six newly purchased lambs during the day had access to the pasture on which the reindeer were kept during the night. Despite the lambs being clinically unremarkable, we speculate that they carried the virus and infected the reindeer on this farm.

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References

COWPOX OUTBREAK IN NON-HUMAN PRIMATES

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Abstract
This report describes an outbreak of cowpox in macaques in a sanctuary for exotic animals in the Netherlands and the possible role of wild rats in the introduction of the infection. A total of 5 barbary macaques (Macaca sylvanus) and 1 pig-tail macaque (Macaca nemestrina) were affected and proven positive for cowpox. The main lesions were some small warts on the gingiva of the jaws. In the epithelium cells of the mucosa, large eosinophylic intracytoplasmatic inclusion bodies were seen, which were suggestive for poxvirus inclusions. EM, virusisolation and PCR confirmed the diagnosis. During the outbreak there was a seroconversion in the affected macaques and their cage mates. All sick animals or animals with symptoms either died (Djerid, Macaca sylvanus and Govak, M. nemestrina) or recovered completely

Key words: cowpox virus, primates, Macaca sylvanus, Macaca nemestrina, rodents, diagnosis

Introduction
Cowpox is an orthopoxvirus infection, endemic in European wild rodents, but with a wide host range including humans and cats. However, direct evidence for the rodents as carriers is lacking for much of the virus’s geographic range (Chantrey, 1999, Hazel SM, 2000 Sandvik T, 1998). That wild rodents may be a natural reservoir of cowpox virus has been known for a long time (Marennikova, 1978, 1984). Through a combination of serology and PCR, cowpox virus was detected in bank voles (Clethrionomys glareolus), field voles (Microtus agrestis), wood mice (Apodemus sylvaticus) and one house mouse (Mus musculus), but in no other wild animal species including some birds that were tested (Chantrey, 1999, Crouch, et al, 1995). In another serological survey, samples were tested from foxes and several other wild species. Antibodies were only detected in two rodent species: bank voles and wood mice (Boulanger, 1996). In Finnish wild rodents, orthopoxvirus seroprevalence ranged from 0% to 92%. In humans seroprevalence was 100% in the over 50-age group, decreasing with each younger age group (Pelkonen, 2003). This report describes an outbreak of cowpox in macaques in a sanctuary for exotic animals in the Netherlands and the possible role of wild rats in the introduction of the infection.

Material and Methods
On 11 July 2003 Djerid, a 3-year-old male barbary macaque (Macaca sylvanus) showed some small warts on the gingiva of the upper jaw (Fig. 1). Presuming it to be a benign wart, the swellings were cauterised and meloxicam 0.2 mg/kg was given. During the annual general health check of all barbary macaques (n=15) on 18-19 August, blood was taken from all animals and Djerid’s treatment was repeated.
On 27 August the animal showed a mumps-like swelling on the right side of the face that later developed into a massive, necrotising inflammation in the maxillary gingiva. Treatment was started immediately with amoxicillin-clavulanic acid, 12.5 mg/kg orally BID (Synulox®, 40 mg amoxicillin and 10.5 mg clavulanic acid per tablet (Pfizer Animal Health, Capelle a/d IJssel, the Netherlands) and meloxicam 0.2 mg/kg SID (Metacam®, oral suspension,1.5 mg/ml Boehringer Ingelheim bv, Alkmaar, the Netherlands).

Tissue samples were taken from the lesions on 1 September. The bacterial culture showed Klebsiella sp. and anaerobic rods sensitive to enrofloxacin. The histological examination revealed a granulomatous inflammation with oedema, polymorphonuclear leukocytes and bacteria. In the epithelium cells of the mucosa, large eosinophilic intracytoplasmatic inclusion bodies were seen, which are suggestive for poxvirus inclusions (9 September). The diagnosis was confirmed by EM (23 September).

On 25 September Djerid died unexpectedly. An autopsy did not show any obvious lesions explaining the animal’s death.

In the meantime 2 other barbary macaques (Bebe, M, 2 years old and Rosa, F, 1 year old) housed on the other side of the sanctuary’s hall and in separate but adjoining cages, had both developed a swollen upper lip and gingivitis. From 23 to 25 September, blood and biopsies were taken from these animals and blood from Rosa’s cage mate Joan and Djerid’s cage mate Tchita. Nose swabs were taken from 22 wild rats (Rattus norvegicus) as well as 6 blood samples from freshly caught specimens.

Rosa was housed in a cage with 5 other barbary macaques. Rosa and 2 other animals were teething, and showed gingivitis. Biopsies and blood were taken on 29 September from both Joan (F, 2 years old) and Gizmo (M, 1.5 years old). Tchita (M, 5.5 years and Djerid’s cage mate) showed some signs of general ill health but never developed skin symptoms.

The last victim amongst the barbary macaques was Elsie (F, 3 years old). On 19 November, she developed a massive subcutaneous swelling on the right side of the body that stretched from her shoulder to the inguinal region. Blood and biopsies were taken.
The sick animals were treated with amoxycillin-clavulanic acid and meloxicam. All barbary macaques that showed some signs of illness were kept in isolation until full recovery.

In the other macaque groups only two individuals developed clinical symptoms comparable to cowpox.

An old female (Lisa) in the rhesus monkey group (*Macaca mulatta*, *n* = 8), developed a severe swelling on the face. As she had no other symptoms and was doing well generally no blood or tissue samples were taken.

In a mixed group of 1 pig-tailed macaque (*Macaca nemestrina*) and 4 Japanese macaques (*Macaca fuscata*), the leading male Govak (*M. nemestrina*, 12 years old) suddenly got ill on 15 October. He showed blisters on the facial skin, the tongue and in the mouth. He died within 2 days with haemorrhagic enteritis and bacterial septicaemia.

The skin lesions showed necrosis with polymorphonuclear leucocytes and bacteria. In the epidermis cells, many large eosinophic intracytoplasmatic inclusion bodies were seen, which are suggestive for poxvirus inclusions.

In October and November blood and/or nose swabs were taken from Japanese macaques (*Macaca fuscata*), long-tailed macaques (*Macaca fascicularis*), hamadryas baboons (*Papio hamadryas*), prairie dogs (*Cynomys ludovicianus*), and wild house mice (*Mus musculus*).

**Preliminary results**

All sick animals or animals with symptoms either died (Djerid and Govak) or recovered completely, except for Elsie who has not responded to any treatment so far.

Cowpox virus and antibodies were demonstrated in several of the collected samples and an epidemiological study is ongoing (Martina, et al., 2004).

**Discussion**

The cowpox infection in this sanctuary did not develop into an enormous epidemic, which makes it likely that the virus was only spread by direct contact. Airbone transmission is not likely because there is a large distance between the first victim (Djerid) and the other cage where four out of six cage mates became clinically ill and tested positive in the antibody test. Most of the affected animals with symptoms were young animals showing gingivitis related to teething.

In humans in general, the lesions heal without complications; in immunosuppressed or immunodeficient individuals, however, the infection may become generalised and take a dramatic course (Nowotny, 1996). The lesions are described as dermatosis with hard black eschars covering granulating ulcers. The ulcers heal within 6 weeks and leave small scars (Stewart, 2000).

In humans, as in this case of non-human primates, diagnosis of cowpox is often rather late, even though fast and reliable diagnostic tools such as histopathological examination and polymerase chain reaction are available (Pfeffer, 1999).

Although barbary macaque Elsie shows seroconversion for cowpox, her lesions are not specific for cowpox and the non-recovery makes another underlying cause very likely. The mass that developed on the right side of her body was eventually diagnosed as myxoma or myxoid liposarcoma.

In the summer of 2003 there was a real rat plague at the sanctuary. Many of the rats tested during this outbreak were carriers of the cowpox virus. Although wild rodents are considered to be the natural reservoir (Chantrey, 1999, Hazel, 2000 Sandvik, 1998), so far no reports are available of infected wild rat populations. However, there are at least two reports in the Netherlands of a relation between cowpox in humans and rat bites (Postma, 1991, Wolfs, 2002).

During the last decade there has been much discussion on the role of the cat in the distribution of cowpox. Because improvements in hygiene have reduced direct
human contact with rodents, domestic cats could be acting as active transmitters of these viruses from rodents to humans (Nowotny, 1996, Tryland, 1998, Pfeffer, 1999, Amer, 2001). There is still very little known about the epidemiology of the cowpox virus.

In this sanctuary, house cats are not considered to be the primary source of the infection. These cats have lived on the premises for many years already. They were not infected and only one animal showed seroconversion. Transmission between host species, however, is found to be negligible despite their close cohabitation (Begon, 1999). This means that with a high infection pressure as in this sanctuary, the rat-to-macaque transmissions could be considered as isolated incidents. From the moment the infection was inside the cage, direct contact is considered to be the most likely transmission route.

Infection in wild rodents varies seasonally, with peaks in autumn. This variation probably underlies the marked seasonal incidence of infection in accidental hosts such as humans and domestic cats (Chantrey, 1999, Hazel, 2000). In Egypt, outbreaks of a dermatosis due to cowpox infection are described as occurring during the months of late summer and early autumn (Amer, 2001).

In our case, the problems also started in the autumn (September) and continued until the end of October.

There are only a few reports of cowpox infections in zoos. The first report of a “new virus”, apparently identical to other poxvirus isolates, was published in 1978 and the virus was isolated from white rats and Felidae in the Moscow Zoo (Marennikova, 1978). Another report is from Berlin Zoo where a cowpox virus infection caused stillbirth in an Asian elephant (Elephas maximus) (Wisser, 2001). This is the first report of cowpox infection in primates and is intended to make zoo veterinarians aware of the possibility of infection.

References

ISOLATION AND IDENTIFICATION OF ALLERGENIC AND PATHOGENIC FUNGI FROM THE HAIR COAT OF PERSIAN SQUIRREL (*Sciurus anomalus*)

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**Key words:** Persian squirrel, Sciurus anomalus, zoonosis, dermatophytosis, allergenic fungi

**Extended abstract**
Persian Squirrel (*Sciurus anomalus*) is a small rodent which lives in oak forest of the North West and West provinces of Iran. Although in the recent years the population of Persian squirrel as pet has increased in Iran, little is known about the zoonotic hazards of this animal.

In this study, 60 pet Persian squirrels, had been referred to the Small Animal Teaching Hospital of Veterinary Faculty of University of Tehran, were examined during a period of 12 months. Dermatologic clinical examinations were performed on all cases and the results were recorded in the standard dermatology forms.

Samples were obtained from all normal and abnormal skins using hair brushing and scrapping. The samples were cultured on sabouraud glucose agar with chloramphenicol and sabouraud glucose agar containing chloramphenicol and cycloheximide.

The number of isolates and the results of their differentiation to the genus level are demonstrated in Table1.

Among the isolated fungi, dermatophytes include Microsporum gypseum, M. canis, M. prsicolor, Trichophyton mentagrophytes and T. mentagrophytes var.

<table>
<thead>
<tr>
<th>Organism</th>
<th>All squirrels (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>1- Alternaria spp.</td>
<td>14</td>
</tr>
<tr>
<td>2- Aspergillus spp.</td>
<td>16</td>
</tr>
<tr>
<td>3- Candida spp.</td>
<td>11</td>
</tr>
<tr>
<td>4- Chrysosporium spp.</td>
<td>1</td>
</tr>
<tr>
<td>5- Cladosporium spp.</td>
<td>6</td>
</tr>
<tr>
<td>6- Fusarium spp.</td>
<td>12</td>
</tr>
<tr>
<td>7- Geotrichum spp.</td>
<td>6</td>
</tr>
<tr>
<td>8- Malasizia spp.</td>
<td>7</td>
</tr>
<tr>
<td>9- M. Canis</td>
<td>2</td>
</tr>
<tr>
<td>10- M. gypseum</td>
<td>2</td>
</tr>
<tr>
<td>11- M. Persicolo</td>
<td>2</td>
</tr>
<tr>
<td>12- Mucor spp.</td>
<td>23</td>
</tr>
<tr>
<td>13- Penicillium spp.</td>
<td>17</td>
</tr>
<tr>
<td>14- Psedoallercia spp.</td>
<td>1</td>
</tr>
<tr>
<td>15- Rhizopus spp.</td>
<td>5</td>
</tr>
<tr>
<td>16- Rhodotorula spp.</td>
<td>4</td>
</tr>
<tr>
<td>17- Scopoliops spp.</td>
<td>2</td>
</tr>
<tr>
<td>18- T. Mentagrophytes</td>
<td>1</td>
</tr>
<tr>
<td>19- T. Erinaceii</td>
<td>1</td>
</tr>
<tr>
<td>20- Trichothecium spp.</td>
<td>1</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>132</strong></td>
</tr>
</tbody>
</table>

† Percentage of all squirrels from which organisms were isolated
*Percentage of total fungal isolates
erinaceii were identified in %13.3 (8/60) of the cases. This is the first report of isolation of dermatophyte from Persian squirrel in Iran. Interestingly Malasezia pachydermatitis was isolated from %11.7 (7/60) of the cases. Aspergillus [fumigatus, flavus, niger, clavatus] %26.7 (16/60), Penicillium spp. %28.3 (17/60), Alternaria spp. %23.3 (14/60), Fusaruium spp. %20 (12/60), Mucor spp. %38.3 (23/60), Cladosporium spp. %10 (6/60) and Rhizopus spp. %8.3 (5/60) were the most important allergenic fungi has been isolated in this study. It is concluded that pet Persian squirrel may be acts as a carrier for dermatophytes, and dermatophyte infection can transfer from the animal to the owners.

References

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ISOLATION OF CRYPTOCOCCUS NEOFORMANS (var. neoformans) FROM THE FAECAL MATTER OF PSITTACINES BIRDS ON PAL’S MEDIUM

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Abstract
A preliminary investigation was conducted to study the prevalence of Cryptococcus neoformans var. neoformans in the faecal matter of psittacine birds kept in a zoo aviary at Jamnagar, Gujarat. A total of 18 avian droppings collected from 7 macaws, 6 parakeets and 5 parrots, were cultured onto the plates of brain heart infusion agar, malt agar, Sabouraud agar and Pal’s medium (sunflower seed agar). The pathogen could be easily demonstrated only in the 5 faecal matter of parrots on Pal’s medium. The number of colonies of Cr. neoformans on Pal’s agar varied from 80 to over 300. The development of brown coloured colonies on Pal’s medium helped in early identification of pathogen. The plates of the remaining three media were badly contaminated with rapidly growing moulds and hence masked the growth of Cr. neoformans. The in-vitro genetic crossing on Pal’s medium revealed that all the isolates of Cr. neoformans obtained from the parrot droppings were ‘alpha’ mating type of Filobasidiella neoformans var. neoformans. The high prevalence and concentration of Cr. neoformans in the aviary of the zoological garden should be viewed from public health point of view. The wider application of Pal’s medium would enable the microbiologists to study the ecology, diagnosis and genetic mating of Cryptococcus neoformans.

Key words: Cryptococcus neoformans, Pal’s medium, Parrot droppings, Zoo aviary.

Introduction
Cryptococcus neoformans, an encapsulated, basidiomycetous yeast, is the chief causative agent of human and animal cryptococcosis. The pathogen occurs as a saprobe in nature and is frequently isolated from the pigeon droppings throughout the world (Ruiz et al., 1989; Pal, 1997a; Cabral Passoni, 1999; Sasaki et al., 1999). Occasionally, Cr. neoformans has been recovered from the bat guano, fruits and vegetables, decaying woods, budgerigar excreta, canary cages and droppings, faeces of munia bird besides the droppings of other avians (Pal et al., 1984; Pal and Mehrrotra, 1985; Bauwens et al., 1986, Pal, 1989; Pal, 1995 and Lazera et al., 1996). The perusal of available literature reveals paucity of information on the ecological association of Cr. neoformans with the droppings of psittacine birds kept in the aviaries of zoos. The present study deals with the screening of fresh and dried faeces of the macaws, parakeets and parrots for the presence of Cr. neoformans and also to compare the efficacy of various cultural media for the isolation of this pathogen. In addition, sexual

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compatibility of the environmental isolates of \textit{Cr. neoformans} was studied on Pal's medium by \textit{in-vitro} technique.

\textbf{Materials and Methods}

A total of 18 avian droppings which originated from 7 macows, 6 parakeets and 5 parrots, were collected in the clean polythene bags with the help of sterilized wooden spatula. In order to avoid environmental exposure of \textit{Cr. neoformans}, face mask was used while taking the excreta of the psittacine birds. The samples were brought and processed in the laboratory of Veterinary Public Health. About two gram of each specimen was suspended in 10 ml of sterile physiological saline in a sterilized test tube and shaken manually for 5 minutes. After allowing the specimen to stand for half an hour, 2 ml of supernatant fluid was mixed with 1 ml solution of chloramphenicol (0.5 mg/ml). This mixture was incubated at 37 \degree C for 1 hr. and then serial dilution of 1:10 and 1:100 were made in sterile distilled water. From this fluid, aliquots of 0.1 ml and 0.2 ml were inoculated by surface streaking method onto the plates of brain heart infusion agar (B.H.I.), malt agar, Sabouraud dextrose agar with chloramphenicol (0.1 mg/ml) and Pal's medium (1997b). The later medium contained pulverised sunflower seed 45 g, agar 20 g, chloramphenicol 500 mg and distilled water 1000 ml. The above three media were incubated at 37 \degree C while Pal's agar was kept at 30\degree C. The inoculated plates were observed daily for at least 15 days before discarding them as negative. The number of brown coloured colonies appearing on Pal's medium were enume rated. Each isolate was further subjected to urease test, KNO\textsubscript{3} utilization, growth at 37\degree C, phenolexidase activity, fermentation and assimilation test for detailed characteristics of the organism.

The morphology of the isolates were studied in a newly developed Narayan stain (Pal, 2001). This new stain contained 4.0 ml of glycerine, 0.5 ml of methylene blue and 7.0 ml of dimethyl sulphoxide.

Genetic mating experiment was carried on Pal's medium (pulverised sunflower seed 22.5 g, KH\textsubscript{2}PO\textsubscript{4} 1.0 g, MgSO\textsubscript{4} 0.5 g, agar 20.0 g and distilled water 1500 ml). The test isolates were crossed with Kwon-Chung standard tester stains by \textit{in-vitro} technique on Pal's medium (Pal, 1997a); and plates were incubated at 20\degree C. The variety of the isolates were confirmed by D-proline assimilation test (Dufait \textit{et al.}, 1987).

\textbf{Results}

\textit{Cr. neoformans} was recovered from 5 of the 18 avian excreta of the psittacine birds giving a prevalence of 27.7 per cent (Table-1).

\begin{table}[h]
\centering
\begin{tabular}{llll}
\hline
Sr. No. & Type of media used & Recovery of \textit{Cr. neoformans} from the excreta of &
& Macow (7)* & Parakeet (6) & Parrot (5) \\
\hline
1 & Brain Heart Infusion agar & 0/7 & 0/6 & 0/5 \\
2 & Malt agar & 0/7 & 0/6 & 0/5 \\
3 & Pal's medium & 0/7 & 0/6 & 5/5** \\
4 & Sabouraud medium & 0/7 & 0/6 & 0/5 \\
\hline
\end{tabular}
\caption{Isolation of \textit{Cr. neoformans} from the droppings of psittacine birds kept in an Indian zoo aviary}
\end{table}

* Number of samples examined.  
** Number of specimens positive for \textit{Cr. neoformans}.

The positive isolations came only from the parrot droppings. The pathogen could not be demonstrated in the faeces of 7 macows and 6 parakeet. All the isolations were made only on Pal's medium by observing light to dark brown pigmented colonies of \textit{Cr. neoformans} within 3-5 days at 30\degree C. On the contrary, organism could not be
recovered on BHI, Malt agar and Sabouraud medium as the fast growing filamentous moulds contaminated the plates and masked the growth of *Cr. neoformans*.

All the five isolates of *Cr. neoformans* grew well on Sabouraud medium at 37°C, and failed to utilize KNO₃. The urea was hydrolysed but no chlamydospore formation occurred on corn meal agar. None of the isolates showed fermentation of glucose, lactose, maltose and sucrose. However, assimilation was observed with glucose, insoitol, maltose and sucrose. On microscopic examination, spherical, yeast-like cells with and without budding and surrounded by a thin capsule were seen in wet mount preparation of Narayan stain. The isolates were identified as variety *neoformans*, as they failed to assimilate D-proline.

Sexual crossing of all the isolates of *Cr. neoformans* with variety *neoformans* mating type “a” on Pal’s medium showed white, cottony luxurient growth around the entire margin of the paired colony after 7-15 days at 20°C. The growth from the margin of the paired colony showed long hyphae with clamp connections, basidia and basidiospores in Narayan stain. The results showed that all the strains of *Cr. neoformans* from the parrot droppings belonged to the ‘alpha’ mating type of *Filobasidiella neoformans* var. *neoformans*.

**Discussion**

The present study was attempted to demonstrate the presence of *Cr. neoformans* in the environment of 3 species of psittacine birds kept in captivity in a zoological garden. The pathogen could only be isolated from the droppings of 5 parrots on Pal’s agar, which is proved as an excellent selective medium. The development of brown colour on Pal’s medium helped us in the rapid differentiation of *Cr. neoformans* from simultaneously growing other fungi. No explanation can be given why the excreta of the macaws and parakeets failed to show the presence of the pathogen. It is advisable to undertake a comprehensive study on the natural occurrence of *Cr. neoformans* in the faecal matter of all the species of avians except pigeons to establish the ecology of the pathogen. Nevertheless, the credit goes to Emmons (1955) who first time reported *Cr. neoformans* in the droppings of the pigeon in USA.

The high rate of recovery of *Cr. neoformans* on Pal’s medium (5/18) clearly indicated the overwhelming superiority of Pal’s sunflower seed medium to B.H.I. (0/18), Malt agar (0.18) and Sabouraud medium (0/18) for the selective isolation of this pathogen. The observations of our study are in agreement with the findings of other investigators who were successful in isolating *Cr. neoformans* from a variety of environmental materials (Pal and Baxter, 1985; Pal *et al.*, 1990 and Sasaki *et al.*, 1999). Furthermore, Pal’s medium has shown excellent results for studying the genetic crossing experiment. This has helped us to know that “alpha” mating type of variety *neoformans* exist in the avian droppings. We, therefore, emphasise that Pal’s medium should be routinely employed in clinical microbiology laboratories to confirm an early diagnosis besides studying sexual compatability and epidemiology of cryptococcosis which is a global, highly infectious, enigmatic mycosis of man and a wide variety of animals.

The public health significance of environmental exposure to avian excreta particularly the pigeon is documented in the literature (Swinne, 1979). In this content, Wegner and Staib (1983) reported fatal cryptococcal infection of the central nervous system in a 69-year-old female patient who had kept the budgerigar (*Melopsittacus undulatus*) as a pet bird. The epidemiological investigation revealed the presence of *Cr. neoformans* in the dry faecal matter of the caged budgerigar. The high concentration of *Cr. neoformans* in parrot droppings is alarming and should be viewed from public health point of view, as this organism is a known human pathogen. We therefore, suggest that the workers should use face mask to prevent the inhalation of infectious cells of *Cr. neoformans* while cleaning the excreta from the bird cages. The decontamination of infected aviary with formalin is highly imperative to prevent the spread of infection in susceptible
person. Moreover, the zoo attendant with respiratory or nervous syndromes/disorders should be thoroughly investigated for cryptococcosis by using standard mycological tests.

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The authors are highly grateful to Prof. Dr. Danielle Swinne, Institute of Tropical Medicine, Antwerp, Belgium for sending the standard tester strain for genetic mating experiment. The thanks are also due to the staff of the Zoological Garden, Jamnagar, Gujarat for helping in the collection of droppings from psittacine birds. The technical assistance of the staff of Veterinary Public Health, Veterinary College, Anand is also acknowledged.

References

MANAGEMENT OF TOXOPLASMOsis IN CAPTIVE RAised PALLAS’ CATS
(Felis manul, pallas 1776)

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Abstract
Toxoplasmosis is responsible for a high neonatal mortality rate in Pallas’ cats (Felis manul, syn. Otocolobus manul) held in captivity. Therefore, Vienna Zoo evaluated 3 different methods of prophylactic treatments in young Pallas’ cats. The first method, hand-raising the kittens, was successful in preventing neonatal toxoplasmosis, but left the animals unprotected to later infections. The second method comprised oral application of clindamycin for 15 weeks. The treatment started at the 87th day of age and resulted in a survival rate of 83%. The third method consisted of the oral vaccination with Toxoplasma-infected mice and subsequent oral treatment with toltrazuril. However, no conclusion can be drawn from this method as all 3 kittens died due to a suspected viral infection. We conclude that prophylactic administration of clindamycin is effective in preventing neonatal toxoplasmosis in Pallas’ cats.

Key words: Pallas’ cat, Felis manul, toxoplasmosis, clindamycin, toltrazuril, immune deficiency, virus infections

Introduction
Pallas’ cats Felis manul, syn. Otocolobus manul, as other members of the family Felidae, are the only definitive hosts of the tissue cyst-forming coccidium Toxoplasma gondii. Felids can become infected with T. gondii by ingesting tissue cysts from intermediate hosts (mammals and birds), by ingesting sporulated oocysts from the environment (8), or by transplacental or lactational means (4). In these hosts, the parasite undergoes both an intestinal phase resulting in shedding of oocysts in the feces, and an extraintestinal phase resulting in multiplication of tachyzoites in most body tissues and eventual development of tissue cysts (7). Tissue cysts persist for an extended period, probably throughout the life of the host (4,7). The extraintestinal cycle of T. gondii can sometimes be associated with clinical signs in felids. While clinical toxoplasmosis in domestic cats is probably infrequent (4,5), Pallas’ cats held in captivity appear to be highly susceptible to toxoplasmosis (5, 14, 16, 17). The losses in captivity are unacceptably high (~ 60%) (1). The reason for this is not well understood; an immune deficiency was discussed (12). In a recent study, Brown et al. (1) found no evidence of Toxoplasma gondii-DNA in wild Pallas’ cats in Mongolia. They conclude that Pallas cats may not have evolved with this specific parasite, creating a degree of susceptibility to toxoplasmosis that is most similar to that in several island species.

A breeding pair of Pallas’ cats had been held in captivity in Vienna Zoo, Austria, since 1997 after being purchased from the Novosibirsk Zoo, Novosibirsk, Russia. Both animals were born in captivity at the Russian zoo (1996, 1995 resp.). The female gave birth after its arrival to 5 successive litters which each had a high neonatal mortality rate: 1998: 80 % (4/5), 1999:
75% (3/4), 2000: 40% (2/5), 2001: 20% (1/5), 2002: 100% (4/4), 2003: 100% (3/3). In 6 cases out of 17 (35%) toxoplasmosis was confirmed at necropsy, 2 more animals died at a later stage with the same diagnosis, 3 kittens were never found. Altogether the Zoo lost at least 47% of the manuls due to Toxoplasmosis. As previous attempts to treat clinical disease in Pallas’ cats had been unsuccessful (17), we set up 3 different methods of prophylactic treatments in young Pallas’ cats.

Case report
In 1998 the queen gave birth to 5 kittens. 4 of them disappeared at the age of about 7 weeks. As remainings of the kittens were never found, we believe that they died and were subsequently eaten by their parents.

In 1999 3 of 4 kittens, 10 weeks of age, displayed weakness, ataxia, anorexia, dyspnoe and an absence of escape reflex. Single doses of 62.5 mg sulfadoxin, 12.5 mg trimethoprim (Borgal® 7.5%, Intervet) were administered subcutaneously. However, all kittens died a few days later. At necropsy toxoplasmosis was diagnosed.

In 2000 3 of 5 kittens were taken away at the age of 10 days in order to handraise them, 2 were left with the mother. The kittens left with the mother became ill at the age of 8 weeks and died a few days later. Toxoplasmosis was serologically and histopathologically confirmed in the kittens (14, 17). The remaining kittens did not develop any signs of disease as long they were kept isolated. One of these 3 was given to a different zoo at the age of 7 months and died 6 weeks after the arrival due to Toxoplasmosis. Another animal of this litter had to be moved to a different enclosure within Vienna Zoo, at that time 3 years old. It also died due to Toxoplasmosis 3 weeks after the removal.

In 2001, 6 kittens were born and left with the mother. Blood was taken for serological examination between day 45 and 295 after birth. The kittens were vaccinated at 68 and 87 days of age (at the time of 2nd and 3rd blood sampling) against Feline Rhinotracheitis, Calicivirus and Panleucopenia infection (Fevaxyn i-CHP®, Fort Dodge, IRL). At 84 days of age, 1 of the kittens (kitten no. 5) displayed weakness, ataxia, anorexia and an absence of escape reflex. Single doses of 62.5 mg Sulfadoxin and 12.5 mg Trimethoprim (Borgal® 7.5%, Intervet) were administered subcutaneously, however, the kitten died 2 days later. Toxoplasmosis was diagnosed at necropsy (15).

Clindamycin (Antirobe®, Pharmacia, Austria) was administered orally to the remaining kittens and the breeding pair via medicated mice for 15 weeks (15 mg/mouse; 1 mouse/animal), beginning at 87 days of age/post parturition respectively. All of them survived. Toxoplasma gondii oocysts were detected in fecal samples of at least one of the kittens from the 72nd to 86th day of age and from the 198th to 219th day of age.

In fecal samples of the queen, T. gondii oocysts were observed between 59 and 86 days post parturition and between 192 and 199 days post parturition.

The blood samples of the kittens were tested for antibodies against T. gondii by the agglutination test (AT) and the immunofluorescence antibody test (IFAT) (15). The results are shown in tables 1 and 2, respectively. In the summer 2001 the breeding pair suffered from an acute herpesvirus infection with severe clinical symptoms. They recovered both after 2 weeks of treatment with 8 mg/kg azyclovir (Zovirax®).

In 2002 4 kittens were born, all of them died on the first day of an unknown reason. Necropsy showed a well filled stomach in all 4, but not any pathological alterations. The antibody titer against T. gondii in the ingested milk was 1:40,960, in the blood of 2 kittens 1:20,480, in one 1:10,240 and in the last one 1:5,120.

In 2003 3 kittens were born. The kittens were vaccinated at 76 and 99 days of age against Feline Rhinotracheitis, Calicivirus and Panleucopenia infection (Fevaxyn i-CHP®, Fort Dodge, IRL). At day 119 after birth they were orally vaccinated with Toxoplasma-infected mice. Oral treatment with about 25 mg/kg toltrazuril (Baycox®, Bayer) started at the same time. This medication was less well accepted than clindamycin in the manul family, all of them started to vomit at day 12 of treatment. The cats improved after reducing the dose of toltrazuril. However, all 3 kittens died at the age of 158, 160 and 167 days, respectively. At necropsy all 3 kittens showed atrophy of the lymphatic tissue, but no signs of toxoplasmosis.

Virological examinations were performed for Feline Herpesvirus 1 (cell culture isolation and
Polymerase chain reaction - PCR), Feline Calicivirus (cell culture isolation), Feline Leukemia Virus antigen (MegaScreen FeLV, MegaCor, Austria), Parvovirus antigen (FASTest Parvo Strip, MegaCor), Parvovirus specific nucleic acid (PCR), Canine Distemper Virus specific nucleic acid (PCR) with necropsy material, and for antibodies against Parvovirus (haemagglutination inhibition assay), Feline Herpesvirus-1 (virus neutralisation assay) and Feline Immunodeficiency Virus (Witness FIV, MegaCor) in blood samples. From the lung of manul 3 calicivirus was isolated. The gut contents of all three kittens were positive for Parvovirus antigen using the FASTest Parvo Strip. However, immunhistochemical testing on gut material and preliminary results with Parvovirus PCR did not verify these results. The results are shown in Table 3.

Discussion
It can be stated that handraising young Pallas’ cat and keeping them isolated, as performed in 2000, was effective in preventing the disease but left the animals unprotected against toxoplasmosis: 2 of 3 died when the animals had to be moved to different places. In 2001 the oral application of clindamycin to prevent the outbreak of toxoplasmosis was highly effective if using the correct timing: medication must start at the time when the kittens begin to eat mice and before the apparently protective maternal antibodies reach the bottom line. In the present study, the detection of anti-\textit{T. gondii} antibodies at 45 days of age in all of the kittens, the progression to negative results by AT (Table 1) and the subsequent increase of the anti-\textit{T. gondii} IgG titers are suggestive of an initial colostral transfer of antibodies followed by post natal infection, varying the time of infection in each kitten. The results obtained by IFAT show the same type of progression (Table 2). Maternal antibody titers had decreased below serological detection level (Titer <1:40 by AT) between 9.5 weeks (kitten no. 5) and 11.5 weeks of age (kittens no. 1, 2, 3, 4 and 6).

The small number of kittens studied does not allow any statistically valid generalization, but it is of note that kitten no. 5, which showed the lowest maternal antibody titer and the earliest antibody depletion, suffered a \textit{T. gondii} infection before other littermates, and developed a generalized toxoplasmosis. Why kitten no. 5 had a lower anti-\textit{T. gondii} antibody titer is unknown, but this could be related to the volume of colostrum ingested, the timing of initial suckling or individual absorption capability.

The other kittens with higher maternal antibody titers became infected later and showed no signs of disease. The mild course of the infection in these kittens could have also been influenced by the clindamycin administration. During the clindamycin administration, two of the kittens showed anti-\textit{T. gondii} antibody seroconversion suggestive of \textit{T. gondii} infection (kittens no. 3 and 6). This indicates that, at least at the doses and method of treatment employed, clindamycin does not exclude the possibility of a \textit{T. gondii} infection, but may be useful in the prophylaxis of clinical disease in Pallas’ cats. Felids excrete \textit{T. gondii} oocysts in feces 3 to 10 days after ingesting bradyzoites, ≥ 18 days after ingesting sporulated oocysts and ≥ 13 days after ingesting tachyzoites (7). The bradyzoite-induced cycle in cats is considered the most efficient as nearly all cats fed tissue cysts excrete oocysts, whereas < 30% of cats fed tachyzoites or oocysts excrete oocysts (7). It is hypothesized that after oocyst ingestion, \textit{T. gondii} invades extraintestinal tissues and merozoits produced in these tissues return to the intestine to initiate the intestinal cycle (9). The assumed infrequent rupture of cysts may account for the complete coccidian cycle occurring only in a low percentage of cats (7).

While the \textit{T. gondii} stage involved in the transmission and the route of infection influences the oocyst excretion and the prepatent period, it is of note that during the 15 weeks of clindamycin administration none of the kittens shed \textit{T. gondii} oocysts. The effect of clindamycin on reducing the number of \textit{T. gondii} oocysts shed by infected cats was earlier documented (3). Kitten no. 3 and no. 6 showed seroconversion during the medication but the first \textit{T. gondii} oocysts were detected in the feces 12 days after ending the therapy. Clindamycin reduces the level of replication of \textit{T. gondii} by inhibiting the protein synthesis of the parasite and impairs the ability of tachyzoites to infect host cells (11). It is stated that clindamycin may have some effect on the disseminated stages of \textit{T. gondii}, preventing bradyzoites from Close this window to return to IVIS
reaching the gut and undergoing sexual reproduction in the intestine with subsequent oocyst shedding (2). Greene et al. (10) and Lappin (13) reported occasionally gastrointestinal side effects to clindamycin. The kittens in this study showed no gastrointestinal intolerance to clindamycin.

In 2003 we tried to control the infection and subsequent duration of oral medication by vaccinating the kittens with Toxoplasma-infected mice. The subsequent treatment of the kittens with toltrazuril could apparently prevent toxoplasmosis but all 3 kittens died most likely due to a virus infection although the results of necropsy were not satisfying. Calicivirus was isolated from the lung of one kitten, which is in accordance with the respiratory symptoms observed. As the antigen test for Parvovirus was positive in all three kittens and an atrophy of the lymphatic tissue was found at necropsy, a parvovirus infection may have been responsible for the death but cannot be confirmed as no parvovirus could be detected in any body tissue immunohistochemically, neither could paroviral nucleic acid be detected by PCR. However, these examinations are not yet completed.

Nevertheless, concurrent virus infections raise the question for an existing immune deficiency in these animals. The occurrence of concurrent herpesvirus infections in animals with suspected immune deficiency was already described by Ketz-Riley et al. (2000) (12) and Zenker et al. (2000) (17).

**Conclusion 1**
Treatment of Pallas’ cats that already showed clinical signs of toxoplasmosis was unsuccessful.

**Conclusion 2**
Handraising young Pallas’ cat and keeping them isolated was effective in preventing the disease but left the animals unprotected against toxoplasmosis.

**Conclusion 3**
Prevention of Toxoplasmosis with clindamycin was effective and safe.

**Acknowledgements**
We thank Michi Salaba, who handraised the 3 kittens and cared for them. Special thanks also to my co-author, Prof. Prosl, whose knowledge saved many Pallas’ cat’s lives.

**References**

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Table 1. Longitudinal assessment of T. gondii-specific IgG antibody titer development in the serum of Pallas’ cats kittens as determined by Agglutination Test (n.d. = not done)

<table>
<thead>
<tr>
<th>Kitten no.</th>
<th>Chip no.</th>
<th>Days of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45</td>
<td>68</td>
</tr>
<tr>
<td>1</td>
<td>119766</td>
<td>1:640</td>
</tr>
<tr>
<td>2</td>
<td>045508</td>
<td>1:640</td>
</tr>
<tr>
<td>3</td>
<td>119521</td>
<td>1:640</td>
</tr>
<tr>
<td>4</td>
<td>119102</td>
<td>1:640</td>
</tr>
</tbody>
</table>

Table 2. Longitudinal assessment of T. gondii-specific IgG antibody titer development in the serum of Pallas’ cats kittens as determined by Indirect Immunofluorescence Test (n.d. = not done)

<table>
<thead>
<tr>
<th>Kitten no.</th>
<th>Chip no.</th>
<th>Days of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45</td>
<td>68</td>
</tr>
<tr>
<td>2</td>
<td>045508</td>
<td>n.d.</td>
</tr>
</tbody>
</table>
Table 3. Results of the virological and serological examination of the kittens born 2003 (n.d. = not done)

<table>
<thead>
<tr>
<th>Virus / antigen / nucleic acids</th>
<th>Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kitten 1</td>
</tr>
<tr>
<td>Parvovirus Antigen in gut contents</td>
<td>positive</td>
</tr>
<tr>
<td>Feline Herpesvirus</td>
<td>negative</td>
</tr>
<tr>
<td>Feline Calicivirus</td>
<td>negative</td>
</tr>
<tr>
<td>Canine Distemper Virus</td>
<td>negative</td>
</tr>
<tr>
<td>Feline Immunodeficiency virus</td>
<td>n.d.</td>
</tr>
</tbody>
</table>
MENINGOENCEPHALOMYELITIS CAUSED BY *NEOSPORA CANINUM* IN A JUVENIL FALLOW DEER (*Dama dama*)

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**Key words:** Fallow deer, *Dama dama*, *Neospora caninum*, Meningoencephalomyelitis

**Extended abstract**
A 3-week-old female fallow deer (*Dama dama*), with a 2-day history of hind leg paresis was euthanized and submitted for necropsy to the Institute for Animal Pathology, University Berne, Switzerland. On the day of euthanasia the calf had a body temperature of 39.2°C, a respiratory rate of 120 and a heart rate of 96. The deer was treated with an infusion of antibiotics (Tetracycline) and Vitamin B1, but it had to be euthanized, due to its rapidly deteriorating condition and apparition of opisthotonus.

The calf originated from a captive fallow deer group, composed of 4 adult females, one adult male and one yearling. One month prior to the reported case, another 2.5 week-old calf from the same group was euthanized after showing to the same clinical signs. Necropsy was performed in a private pathology laboratory and revealed similar microscopic lesions as the submitted case. Unfortunately no material from this deer was available for further investigation.

At necropsy the calf was in a good body condition, with a weight of 9.2 kg. Macroscopically the lungs were congested and oedematous. The grey and white matter throughout the length of the spinal cord showed a brownish marbled discoloration and multiple dark spots were visible in the brain stem. All other organs appeared grossly unremarkable. Microscopically lesions were characterised by multifocal areas of necrotizing and granulomatous inflammation that affected the meninges and were scattered throughout the grey and white matter of the brain and spinal cord. Foci of malacia were associated with endothelial proliferation of capillaries, micro- and astrogliosis, perivascular infiltration composed of lymphocytes, macrophages and plasma cells, and focal areas of mineralization. Numerous protozoal cysts were observed within the inflammatory areas, free or intracellular, occasionally also within neurons. Besides a severe congestion and alveolar oedema in the lungs, no pathological lesions were observed in the other organs.

Sections of formalin fixed, paraffin embedded brain tissue were send to the Diagnostic Center for Population and Animal Health at Michigan State University to test for the presence of an infection with *Toxoplasma gondii*, *Sarcocystes neurona* and *Neospora caninum* using immunohistochemistry (IHC) and polymerase chain reaction (PCR). The protozoal cysts in the brain and large numbers of free or intracellular zoites stained strongly positive using a antibody against *Neospora caninum*, but negative for *T. gondii* and *S. neurona*. PCR was performed on paraffin sections from the same tissue block. The identified PCR product was characteristic for *N. caninum*, based on gel electrophoresis and sequence data, confirming the diagnosis of an infection with *Neospora caninum*.

Since its first recognition in 1984 in dogs in Norway (2), neosporosis has emerged as a serious disease of cattle and dogs worldwide. *Neospora caninum* has been recognized as a frequent cause of bovine abortion (1, 3, 5, 7) and prenatal neosporosis appears to be one of the most important causes of bovine abortion in Switzerland (10, 11, 14). Furthermore, naturally occurring abortion and stillbirth associated with *N. caninum* has been occasionally reported in...
sheep, goats, horses, two lesser kudus, two black-tailed deer, an Eld's deer, and a rhinoceros. Also antibodies to *N. caninum* have been found in the sera of water buffaloes, African buffaloes, elands, Thompson gazelles, impalas, black and white tailed deer, chamois, roe deer, red deer, alpine ibex warthogs, zebras, red and grey foxes, coyotes, camels, spotted hyena, lions as well as free-ranging and captive cheetahs (4, 8, 9).

Domestic dogs are the only known definitive hosts for *N. caninum*. They excrete coccidial oocysts in their feces after ingesting tissue cysts, but can also be intermediate hosts developing clinical neosporosis (4, 12, 13). Tachyzoites and tissue cysts occur intracellularly in the intermediate hosts. Cysts are found primarily in the central nervous system and the characteristic pathologic lesion is a focal encephalitis with necrosis and non-suppurative inflammation. The parasite can be transmitted vertically. Transplacental transmission is the main route of infection in cattle, but initial introduction of the parasite into the herd occurs by ingestion of food contaminated with dog feces. *Neospora caninum* causes abortion in cattle and the foetuses may die in utero, be resorbed, mummified, stillborn, born alive with clinical signs, or born clinically normal but chronically infected. However, up to 95% of calves born congenitally infected from seropositive dams remain clinically normal. Clinical signs have only been reported in calves younger than 2 months of age (4).

The clinical signs and pathological findings in the fallow deer reported here were similar to those found in cases of classical bovine neosporosis. An infection with *N. caninum* was confirmed by PCR and immunohistochemistry. Whether the transmission was vertical or horizontal remains unclear. There are no serological data from the other deer and an infection via contaminated food or water cannot be ruled out. Although *N. caninum* is known to be a causative agent of abortion and perinatal mortality primarily in cattle, it has to be considered as differential diagnosis in abortion and neonatal death in other species, especially ruminants. Definitive diagnosis of neosporosis remains challenging. Serology from aborting cows is only indicative of exposure to *N. caninum* and histologic examination of the foetus, as well as immunohistochemistry and PCR are necessary. However, results of molecular biological investigations have to be interpreted cautiously, as the efficiency of the diagnosis by PCR depends on the laboratory, the sampling procedures and stage of the foetus, and immunohistochemistry is considered as an insensitive method (4).

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SARCOPTIC MANGE IN THE BLACK BACKED JACKAL (Canis mesomelas)
POPULATIONS OF CAPE CROSS AND LÜDERITZ, NAMIBIA

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Keywords: Black backed jackal, Canis mesomelas, mange, Sarcoptes scabiei

Abstract
Sarcoptic mange is a highly contagious skin disease resulting from the infection with the burrowing mite Sarcoptes scabiei, that affects a wide range of mammals, including humans. Mange epizootics in wildlife populations are well documented (1,4,5), although the epidemiology is still not well understood and seems to differ according to geographical area and host population. Sarcoptes scabiei has been reported to be a potential threat to some endangered species including chamois and ibex in European mountain regions (1) and cheetahs (3), wild dogs (3) and mountain gorillas (2) in Africa. This paper reports the results of an investigation on sarcoptic mange in black backed jackal (Canis mesomelas) populations of the Cape Cross Seal Reserve and Lüderitz Diamond Restricted Area, Namibia. The full external examination of 41 captured individuals included body condition score, body weight and an estimate of age. The sarcoptic mange infestation status was scored according to detailed description of extent and severity of the skin lesions, based on schematic drawings, photographs and clinical findings. The clinical investigation of sarcoptic mange included skin scraping to demonstrate the presence of mites in the field (6/41, 14.6% positive), blood smears to estimate the white cell count, particularly eosinophils, and serodiagnosis ELISA (55/72, 76.4% positive), that indicates a high exposure to Sarcoptes scabiei and confirms the sensitivity of the serological test. The seroprevalence of sarcoptic mange related statistically to the age estimate (N=72, P<0.001). The serological titre was found to correlate to the estimate of body surface affected (N=41, r=0.045). Skin biopsies were taken on 39 individuals for histopathology. Parakeratosis was recorded in 27 specimens (65.9%) and hyperkeratosis in 19 specimens (46.3%). Only two specimens showed serocellular crust. The possible role of black backed jackals in the transmission of this disease to other carnivores, particularly brown hyenas (Parahyena brunnea) and domestic dogs (Canis familiaris), is discussed.
Acknowledgements
This study was funded by the Zebra Foundation and the Zoological Society of London Veterinary Department and Institute of Zoology. We acknowledge Greendale Veterinary Laboratories for collaborating on the serology. We are very grateful to the Jackal Project volunteer team on site, Victoria Procter, Marc Higgins and Matt Grainger who provided encouragement and assistance with this project.

References
MINIMALLY-INVASIVE ENDSURGERY OF BIRDS & REPTILES

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Key words: Avian, bird, endoscopy, surgery, orchidectomy, salpingohysterectomy

Abstract
Diagnostic endoscopy has proven to be an important diagnostic tool for zoo veterinarians wishing to visualize and biopsy from internal structures; however, such procedures have concentrated on single-entry techniques (1,3,4). The evolution of multiple-entry endoscopic procedures for birds has developed from human paediatric laparoscopy. The addition of a second and third port using 2.5 or 3.5 mm cannulae has facilitated the use of 2 or 3 mm instruments within the body cavity. Triangulation of various instruments coupled with radiosurgical hemostasis has made several endoscopic procedures possible, including salpingohysterectomy and orchidectomy, in a variety of avian and reptilian species (1,2). In addition, endoscope-assisted, minimally-invasive procedures including enterotomy, enterectomy, cloacopexy, and pneumotomy may be initiated internally and completed using more established techniques. The majority of avian species are relatively small and delicate, and standard coeliotomy procedures cause permanent damage to airsacs and are not particularly well tolerated. The advent of minimally-invasive endoscopic surgery offers significant benefits including rapid and accurate diagnosis, reduced need for extensive coeliotomy, reduced surgical stress, improved pulmonary function, more stable anesthesia, and reduced surgical and hospitalization periods.

References