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OUTBREAK OF YERSINIA PSEUDOTUBERCULOSIS INFECTION IN CALLITRICHID MONKEYS CAUSING FATALITIES AND SUBSEQUENT INTERVENTION WITH AN EMERGENCY VACCINE

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Abstract
A zoo experienced several fatalities following an outbreak of a Y. pseudotuberculosis infection in Goeldi’s Marmosets (Callimico goeldii) and Cotton Top Tamarins (Saguinus oedipus). The zoo had previously abandoned its vaccination programme due to no outbreaks over the past few years. The animals underwent a post mortem examination at Greendale Veterinary Laboratories and an infection with Y. pseudotuberculosis was confirmed as the causative agent. The strains were frozen and submitted to the Ridgeway Biologicals, for the production of a formalin-inactivated whole cell emergency vaccine. The remaining colony was vaccinated. No adverse reactions to vaccination resulted and no further outbreaks have occurred.

Zusammenfassung

Résumé
Un zoo a subi plusieurs morts à la suite d’une épidémie de Y. pseudotuberculosis chez les tamarins de Goeldi (Callimico goeldii) et les tamarins à toupet blanc (Saguinus oedipus). Le zoo avait auparavant abandonné le programme de vaccination en d’épidémies les dernières années. Les animaux ont été autopsiés aux Laboratoires Vétérinaires Greendale et l’infection à Y. pseudotuberculosis a été confirmée comme agent étiologique. Les souches ont été congelées et confiées à Ridgeway Biologicals, afin de produire en urgence un vaccin à cellules entières inactif par le formol. Le reste du groupe a été vacciné. Aucune réaction indésirable à la vaccination n’a pu être observée et aucun nouveau cas ne s’est présenté par la suite.

Key words: cotton top tamarin, emergency vaccine, Goeldi’s marmoset, vaccination programme, Yersinia pseudotuberculosis

Introduction
In 1991 various monkeys in a zoo in the UK suffered from an outbreak of Yersinia pseudotuberculosis followed by sudden death. At the time an emergency vaccine was ordered from Mr. A.S. Wallis of Poulton Le Fylde, UK. When the vaccine arrived the remaining 29 monkeys were vaccinated and no adverse reaction was noted. All individuals were inoculated with 2 single doses 6 weeks apart and an annual booster thereafter until, in September 1996, the zoo ran out of
stock of the vaccine. During this time only one fatality due to *Yersinia pseudotuberculosis* had occurred in a squirrel monkey. On the basis of this, the decision was made to discontinue the vaccination programme.

Between April and June 2001 several Goeldi’s marmosets (*Callimico goeldii*) and cotton top tamarins (*Saguinus Oedipus*) died suddenly. Post-mortem examinations of the dead monkeys were carried out at Greendale Veterinary Laboratories *Yersinia pseudotuberculosis* was confirmed as the cause of death in two of the cases and implicated in the others.

The Zoo decided to initiate another vaccination programme. The protocol used by Mr. A.S. Wallis in the production of the original vaccine was used by his son, Mr. Timothy S. Wallis, in the production of this new batch of vaccine. Test vaccinations of 0.5ml vaccine were given intramuscularly to two squirrel monkeys and when no adverse reactions occurred, all monkeys (22 various Lemurs, 2 squirrel monkeys, 2 Goeldi’s monkeys, 1 cotton top tamarin and 2 white-faced sakis) were vaccinated with the same dose. No further fatalities have occurred since then.

**Review**

*Yersinia pseudotuberculosis*

*Yersinia pseudotuberculosis* infection may lead to or high mortalities in callitrichidae (2). The bacterium is zoonotic and has an extremely broad host range. It can be detected throughout much of the world but seems to enzootic in Europe (1). One important reservoir for the bacterium is rodents (3). The animals often die suddenly and post-mortem examination reveals severe suppurative enteric and hepatic lesions. From these lesions the organisms can be isolated in great numbers. It seems as if the virulence of *Yersinia pseudotuberculosis* may be related to certain plasmids in the callitrichidae (2).

Certain strains of *Yersinia pseudotuberculosis* produce an endotoxin, which leads to the formations of antitoxic antibodies (3).

**Clinical signs and pathology**

Some animals show signs of diarrhoea, depression, anorexia, weakness and dehydration (4). However often the animals die suddenly and post-mortem examination reveals severe suppurative enteric and hepatic lesions (2). Also, focal necrosis of the liver, spleen and mesenteric lymph nodes, as well as ulcerative enteritis, have been described (4).

**Transmission**

Transmission occurs by ingestion of food, which has previously come in contact with faeces of infected vermin or through contaminated material (woodchips, branches, soil, etc).

**Detection**

Culturing *Yersinia pseudotuberculosis* from rectal and faecal swabs or faecal samples very often proves difficult. The organism can also be detected by PCR and antibodies can be found with serology (1).

**Prophylaxis**

Unfortunately *Yersinia pseudotuberculosis* is able to survive to long periods of time in the soil and under harsh environmental conditions (1) and thus any contaminated material, such as wood chips, branches or soil, can be a potential source of infection. Rodent and Avian pest management proves successful when combating yersiniosis. Autogenous vaccines have anecdotally proved to be successful in some institutions (2). Seasonal changes in weather correlate with the recurrence of the disease in unvaccinated animals due to increased contact, stress and hygiene problems. Preventive actions such as adequate quarantine and hygiene measurements should be implemented. The immunological status of the animal determines its competence to control or fight an infection (1). Other susceptible animals in the
vicinity of the Callitrichidae should also be included in the vaccination programme in order to minimize the spread of the pathogenic organism.

**Vaccine production**

**Emergency vaccine production**
The bacterial strain, which had been put on beads and frozen, was subcultered by Ridgeway Biologicals on blood agar and incubated overnight at 37°C. Three colonies of each strain were also cultured in nutrient broth overnight at 25°C. This broth was subsequently used to inoculate horse blood agar plates. Bacteria were harvested, inactivated with formaldehyde and blended with alhydrogel.

**Vaccination programme**
The vaccination programme comprises of 2 single doses of 0.5ml vaccine given intramuscularly two weeks apart. These are followed by a booster of a single dose one a year.

**Progress notes**
Since the vaccination programme has been put in place no further outbreaks of fatalities due to *Yersinia pseudotuberculosis* have occurred in this zoo. No local or systemic reactions to the vaccine were noted.

**Discussion**
Infections with *Yersinia pseudotuberculosis* cause a significant number of fatalities in zoo collections, especially among nonhuman primates, which may be detrimental to breeding and rehabilitation programme. In addition, the organism is zoonotic. Culture proves to be difficult because the organism is shed sporadically and pathognomic signs are lacking (1). Routine screening for *Yersinia pseudotuberculosis* should be initiated possibly with advanced techniques such as PCR and antibody detection by serology.

Since a live attenuated vaccine might cause an increase in virulence and possibly produce animals, which might shed the organism, preference was given to a killed vaccine. It is important to use the particular strain, which has been isolated from samples submitted to the laboratory. A possible danger however is that more than one strain is present and only one has been isolated from the sample. Moreover, further strains can be introduced through wild birds and rodents.

Many collections and vets are cautious about using a vaccine in callitrichid monkeys as the animals are very easily stressed and it is feared that this might trigger an outbreak of *Yersinia pseudotuberculosis* infection or other possible infections.

Currently work is under way at the University of Bristol to develop a PCR technique for the detection of this disease in mammals and birds, so that the infection might be picked up at a very early stage when it is treatable with antibiotics (personal communication with Linda Moore).

**Acknowledgement**
We are very grateful to Steven W. Cooke and Prof. John E. Cooper for reviewing the manuscript.

**References**

4. Taffs L.F., Dunn G. An outbreak of Yersinia pseudotuberculosis infection in small indoor breeding colony of red-bellied (*Sanurinus labiatu*) tamarins. Laboratory Animals 1983; 311-320
RELATION BETWEEN BOVINE TUBERCULOSIS PREVALENCE AND HERD-LEVEL INDICATORS OF CONDITION IN THE AFRICAN BUFFALO POPULATION IN THE KRUGER NATIONAL PARK

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Keywords: body condition, endoparasite load, wildlife disease.

Abstract

Bovine tuberculosis (BTB) has been found in herds of African buffalo (Syncerus caffer) in the Krüger National Park (KNP) since the early 1990s. The infection appears to have originated in the south where the prevalence is now high, and is progressively extending into the central region of the KNP. Buffalo herds in the far north of the park still appear to be free from BTB. Various other species have been found to support the infection, such as chacma baboons (Papio ursinus), greater kudus (Tragelaphus strepsiceros), cheetahs (Acinonyx jubatus) and lions (Panthero leo). The African buffalo seems to be the maintenance host for the disease and while KNP management needs to decide on how to react to the BTB epizootic, the impact of the disease on buffalo is not well known. This project explores possible associations between BTB and indicators of buffalo condition at the herd level. We use differences in BTB prevalence between three zones in the KNP, as determined by recent surveys; high prevalence south of the Sabie River; medium prevalence between Sabie River Camp and Oliphants River; low prevalence north of Oliphants River. Under the assumption that BTB causes morbidity and infects hosts and that this morbidity reduces survivorship, the objectives are to test the impact of BTB on herd structure, sex ratio, calf/cow ratio, body condition and endoparasite load.
RESULTS OF A SURVEY FOR JOHNE’S DISEASE IN THE ROYAL ZOOLOGICAL SOCIETY OF ANTWERP

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Abstract

In this study, the presence of Mycobacterium avium subspecies paratuberculosis (MAP) in the animal collection of the Royal Zoological Society of Antwerp was investigated. Faecal and post mortem samples of 38 ruminants were tested. Cultures were performed on Lowenstein-Jensen (+ mycobactine J) solid medium and with BACTEC 12B radiometric medium. PCR with primers for IS900 was performed after DNA-extraction. For post mortem samples a modified boom-extraction was used. For faecal DNA-extraction a new protocol was developed. Using both culture and PCR, no evidence of paratuberculosis was found in the animal collection.

Zusammenfassung


Résumé

Cette étude recherchait la présence de Mycobacterium avium subspecies paratuberculosis (MAP) chez les animaux de la Société Royale de Zoologie d’Anvers. Des échantillons fécaux et post mortem de 38 ruminants étaient examinés. Des cultures étaient effectuées sur un milieu solide de Lowenstein-Jensen (+ mycobactine J) et sur un milieu liquide selon la méthode radiométrique BACTEC 12B. La PCR était effectuée avec des amorces pour IS900 après l’extraction de l’ADN. Pour les échantillons de l’autopsie une extraction Boom modifiée était utilisée. Pour extraire l’ADN des matières fécales une nouvelle méthode était mise au point. Aucune preuve de paratuberculose était démontrée chez les animaux ni avec la culture ni avec la PCR.

Key words: Mycobacterium, paratuberculosis, culture, PCR

Introduction

Johne’s disease, caused by Mycobacterium avium subspecies paratuberculosis (MAP), is a chronic progressive enteritis affecting domestic and wild animals (in particular ruminants). The disease is characterised by chronic diarrhoea and the symptoms of a general infectious process. Emaciation, decreased milk production, oedema, anaemia, infertility and eventually death are the dominant late signs. In the end stage of the disease, large numbers of MAP organisms may be shed in the faeces, thereby contaminating the environment (1,3).
Young animals are at the highest risk for acquiring an infection with MAP. They are most commonly infected through the faecal-oral route. This occurs either by ingesting the organism through contaminated milk or by ingestion of the mycobacterium from contaminated surfaces. The specific situation in zoo facilities (limited space for different animals) gives a cause of worry. Up to one third of zoos accredited by American Zoo and Aquarium Association reported a minimum of one infected case since 1995 (5).

Culture is considered to be the gold standard but this is a very slow and labour intensive procedure. Cultures may become positive only several weeks or months after inoculation. A rapid, sensitive and accurate identification of organisms and discrimination from other *M. avium* subspecies is possible with PCR. But faecal samples remain the most difficult specimens for DNA extraction and amplification. It has been well documented that inhibitory substances of PCR are present in faecal samples (8,11). With the sequence-capture method the target sequences can be separated from inhibitors and background DNA (10).

The objective of this study was to investigate the presence of *Mycobacterium avium subspecies paratuberculosis* in the animal collection of the Royal Zoological Society of Antwerp. Therefore, different diagnostic methods were used. In addition a new DNA-faeces extraction method was developed.

### Materials and Methods

#### Sample collection

During post mortem examination faeces, mesenteric lymph node and intestinal tissue (ileum and rectum) were collected from 38 ruminants. Faeces and tissue were stored at –20°C (table 1).

#### Culture

The double incubation method of Whitlock and Rosenberger (14) was used for decontamination. Briefly, 2-5 g faeces was mixed with 10 ml of sterile normal saline. The tube was allowed to stand 30 min at room temperature. Five ml of the surface fluid was transferred to a fresh tube containing 25 ml 0.9% hexadecylpyridinium chloride (Sigma C-5460) in half-strength brainheart infusion broth (LabM, Lancaster, UK). The sample was standing for 24 hours at 37°C. After centrifugation at 900 x g for 30 min, the pellet was resuspended in 1 ml of an antibiotic mixture (100 µg vancomycin + 100 µg nalidixic acid and 50 µg amphotericin B in 1 ml aqua dest, all Sigma reagents). The samples were incubated for 72 hours at room temperature, homogenised and used for culture. The decontamination for tissue was slightly different. Five gram of tissue was homogenised for 30 sec with sterile physiologic water in a blender. Twenty-five ml of 0.75% hexadecylpyridinium chloride was added and the sample was left standing at room temperature for 48 hours. After identical centrifugation and adding of the same antibiotic mixture to the pellet, the samples were incubated for 48 hours at room temperature, homogenised and used for culture.

For radiometric culture 0.1 ml of the sediment was inoculated in the BACTEC12B-vials (Becton Dickinson). The BACTEC12B-vials were supplemented with 200 µl of PANTA-PLUS (Becton Dickinson), 1 ml Egg Yolk, 5 µg of mycobactine J (Synbiotics) and 0.7 ml of water. The vials were incubated at 37°C. The Growth Index (GI) was determined each week with an automatic ion chamber (BACTEC 460).

Smears were made from all vials with a GI > 20. These smears were stained with Ziehl-Neelsen stain. If acid-fast bacilli were detected, the samples were processed for PCR-testing.

For culture on solid medium 0.1 ml of the sediment was inoculated onto 4 tubes with Löwenstein-Jensen medium supplemented with 1 mg mycobactine J, 40 ml Panta-Plus, 4 g sodium pyruvate per litre. The tubes were incubated at 37°C. Growth was determined weekly. Ziehl-Neelsen stain was performed on positive tubes.
Table 1: Animals used in the study

<table>
<thead>
<tr>
<th></th>
<th>Animal</th>
<th>Scientific Name</th>
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<td><em>Gazella leptoceros</em></td>
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<td>Wapiti/Elk</td>
<td><em>Cervus elaphus</em></td>
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<td>6</td>
<td>Wapiti/Elk</td>
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<td>8</td>
<td>African buffalo</td>
<td><em>Synercus caffer</em></td>
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<td>Arabian Oryx</td>
<td><em>Oryx leucoryx</em></td>
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<td>Slender-horned gazelle</td>
<td><em>Gazella leptoceros</em></td>
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<tr>
<td>11</td>
<td>Sitatunga</td>
<td><em>Tragelaphus spekii</em></td>
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<tr>
<td>12</td>
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<td><em>Oryx leucoryx</em></td>
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<td><em>Cervus nippon dybowskii</em></td>
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<td>16</td>
<td>Fallow deer</td>
<td><em>Dama dama</em></td>
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<td>Dwarf forest buffalo</td>
<td><em>Syncerus caffer nanus</em></td>
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<td>Fallow deer</td>
<td><em>Dama Dama</em></td>
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<td>25</td>
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<td>37</td>
<td>Rock goat</td>
<td><em>Capra ibex</em></td>
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<tr>
<td>38</td>
<td>Sitatunga</td>
<td><em>Tragelaphus spekii</em></td>
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</table>

**Tissue DNA extraction**

The tissue samples were cut in small pieces of 1-3 mm² and transferred to a 1.5 ml microcentrifuge tube. Two hundred and fifty µl of lysis buffer (60 mm Tris pH 7.4, 60mM EDTA, 10% Tween 20, 1% Triton-100, 1.6 M Guanidine-HCl), 50 µl of proteinase K (20mg/ml, Boehringer Mannheim, Germany) and 500 µl of 0.1 mm-diameter glass microspheres were added. The tubes were incubated for 1 hour at 60°C. The samples were sonicated for 5 min at room temperature. Forty µl of diatomaceous earth solution (0.2 g/ml) was added. The tubes were incubated for 60 min at 37°C by shaking.

The tube was centrifuged for 20 sec and the supernatant was discarded. The pellet was washed twice with 900 µl of ethanol (70 %) and once with acetone. The pellet was dried in a theromoblock at 50°C for 20 min. Ninety µl of TE-buffer (10 mM Tris and 1 mM EDTA, pH 8 ) was added to the pellet and incubated for 20 min at 60°C by shaking (1000 rpm). The tube was centrifuged for 40 sec and 60 µl of the supernatant was transferred to a new tube and used for PCR.
**Faecal DNA-extraction**

One gram faeces was suspended in 10 ml of a 2% Tween-solution; glass beads were added. The samples were mixed vigorously on a vortex mixer for 1 min. The sample was left standing for 30 min. One ml of the supernatant was transferred to a new microcentrifuge tube. The tube was centrifuged at 6000 x g for 1 min. Supernatant was transferred to a new microcentrifuge tube. The tube was centrifuged at 14 000 x g for 10 min. The pellet was suspended in 500 µl of 100 mM Tris-HCl containing 150 mM NaCl and 50 mM EDTA. Five hundred µl of 0.1 mm-diameter glass microspheres and 50 ml of 20 mg proteinase K (Boehringer Mannheim, Germany) per ml were added. The samples were agitated for 50 sec, allowed to digest overnight and agitated again for 50 sec. The supernatant was recovered by centrifugation and transferred to a new microcentrifuge tube. The supernatant was heated at 100°C for 10 min and cooled to 0°C on ice. Two hundred µl of 3.75 M NaCl-2.5 pmol of biotinylated capture oligonucleotides IS900SB and IS900RB (table 2) was added. Tubes were incubated with agitation at 60°C for 3 hours to allow hybridisation. Ten µl of streptavidin dynabeads washed according to the manufacturer's instructions was added and the incubation was continued for 2 hours at room temperature. The magnetic beads were captured, washed twice with 10 mM Tris-HCl-0.1 mM EDTA (pH = 8) and resuspended in 20 µl water. Ten µl was used for amplification.

### Table 2: Biotin labelled primers used in faecal extraction

<table>
<thead>
<tr>
<th>Probe</th>
<th>DNA sequence</th>
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<tr>
<td>IS900SB</td>
<td>5 'biotine-gtt cgg ggc cgt cgc tta ggc t 3'</td>
</tr>
<tr>
<td>IS900RB</td>
<td>5' biotine-gag gat cga tcg ccc acg tga 3'</td>
</tr>
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**Bactec DNA extraction**

The ethanol extraction method described by Whittington et al. (15) was used. Briefly, the rubber stopper lid of the vial was wetted with 100% isopropanol. Two hundred µl of medium was removed with a sterile syringe and needle and transferred to a 1.5 ml microcentrifuge tube. Five hundred µl of absolute ethanol was added and the tube was left to stand for 2 min before mixing vigorously on a vortex mixer for 5 sec and centrifuging at 8 x g for 10 min at room temperature. The supernatant was transferred to a new microcentrifuge tube and the tube was centrifuged at 18000 x g for 5 min. The pellet was washed twice with 200 ml sterile phosphate buffered saline. The pellet was resuspended in 50 µl of sterile water. The tube was heated to 100°C for 20 min to lyse the mycobacteria. Five µl of the lysate was used for PCR.

**PCR**

A nested PCR was performed. Primary PCR after tissue and Bactec extraction was performed in a final volume of 25 µl containing 10 mM Tris-HCl pH 8.3; 50 mM KCl; 1.6 mM MgCl$_2$; 200 µm of each dNTP, 20 pM of each primer, 0.5 U Taq polymerase and 5 µl sample. The primary PCR after faecal extraction was performed in 50 µl containing 10 mM Tris-HCl pH 8.3; 50 mM KCl; 1.6 mM MgCl$_2$; 200 µm of each dNTP, 20 pM of each primer, 0.5 U Taq polymerase and 10 µl of sample. The primers used in the primary PCR’s are IS900S1 and IS900R3 (2) (table 3). The tubes were incubated in a thermocycler with the following conditions: one cycle of denaturation at 94°C for 4 min followed by 40 cycles of denaturation at 94°C for 45 sec, annealing at 68°C for 45 sec and extension at 72°C for 45 sec and a final extension at 72°C for 10 min.
Table 3: Primers used for PCR amplification of IS900

<table>
<thead>
<tr>
<th>Primer</th>
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<th>Product size</th>
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<td>ptb S1</td>
<td>5’ ggg ttg atc tgg acg acggtt a 3’</td>
<td>575 bp</td>
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<tr>
<td>ptb R3 (2)</td>
<td>5’ agc gcg gca cgg ctc tt 3’</td>
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<tr>
<td>ptb S2</td>
<td>5’ gga ggt tgt ggc acc tgc 3’</td>
<td>453 bp</td>
</tr>
<tr>
<td>ptb R1</td>
<td>5’ cga tca gcc acc aga tcg gaa 3’</td>
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</tbody>
</table>

The secondary PCR was performed in a final volume of 25 µl containing 10 mM Tris- HCl pH 8.3; 50 mM KCl; 1.6 mM MgCl₂; 200 µm of each dNTP, 20 µM of each primer, 0.5 U Taq polymerase and 1 µl of the first PCR solution. The primers used in the secondary PCR were IS900S2 and IS900R1. The tubes were incubated in a thermocycler with the following conditions: one cycle of denaturation at 94°C for 4 min followed by 25 cycles of denaturation at 94°C for 45 sec, annealing at 68°C for 45 sec and extension at 72°C for 45 sec and a final extension at 72°C for 10 min. PCR results were assessed by electrophoresis in 2% (w/v) agarose gels stained with ethidium bromide.

Results

Culture:
A GI of > 20 was recorded from 9 of 152 Bactec 12B vials. A Ziehl-Neelsen stain was performed on these positive vials. Only one vial (mesenteric lymph node of a wapiti) showed acid-fast bacilli. A Bactec-DNA extraction was performed. The PCR with IS900-primers was negative. To identify the mycobacterium, a panel of mycobacterium specific primers were used. A positive PCR was shown with primers (table 4) for IS901 (an insertion sequence of Mycobacterium avium subspecies avium).

Ninety-four out of 608 Löwenstein-Jensen solid media were contaminated (fungal and bacterial). Only the 4 tubes of the mesenteric lymph node of the wapiti were Ziehl-Neelsen positive. The PCR for IS900 was negative and for IS901 was positive. The acid-fast bacilli that we could culture were Mycobacterium avium subspecies avium.

The two culture methods were tested with a spiked sample (positive control) and a positive result was obtained.

Table 4: Primers used for PCR amplification of IS901

<table>
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<td>IS901S</td>
<td>5’ gca acg gtt gtt tgt gta aag gaa t 3’</td>
<td>215 bp</td>
</tr>
<tr>
<td>IS901R1</td>
<td>5’ gcg cac gca tga tgt gag cac tta c 3’</td>
<td></td>
</tr>
<tr>
<td>IS901R2</td>
<td>5’ cca ccc cgc caa cag gtc ctt aga g 3’</td>
<td>118 bp</td>
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</tbody>
</table>

DNA-extraction and PCR

The tissue extraction was performed 3 times on each sample. All the PCR’s were negative for IS900-primers. The extract of the mesenteric lymph node from the wapiti was tested with IS901-primers. The PCR was positive.

Faecal extraction was performed on all samples. All the PCR results were negative. To test and evaluate the faecal DNA-extraction method, spiked samples were used. We could detect up to 1000 bacilli per gram of faeces.
Discussion

This study was undertaken to investigate the presence of *Mycobacterium avium subspecies paratuberculosis* (MAP) in the animal collection of the Royal Zoological Society of Antwerp (RZSA). Different tests were used. None of these tests could detect MAP positive animals.

Accurate diagnosis of MAP in animals is very difficult by current tests (7). The success of culture from faeces is clearly related to the likelihood that sufficient numbers of MAP are being shed from the intestinal lesions. No isolation of MAP from the animals may have been due to the limited distributions of focal lesions with small numbers of acid-fast organisms, sporadic excretion of organisms, freezing of faeces causing loss of viability or the detection limit of the faecal culture methods employed (7,12).

The decontamination method and culture methods are widespread used (2,9,15,16). Bactec12B + egg yolk, mycobactine J and Panta -Plus appeared to be satisfactory for culturing MAP (15,16). But the development of a GI in BACTEC12B does not guarantee the presence of MAP. Detection of IS900 by PCR on a aliquot taken from the primary culture is a rapid and effective test but is quite costly.

A higher percentage of contamination was recorded (15% on solid medium and 5,2 % in BACTEC12B vials). This was also reported in other studies (7).

When using a stronger decontamination method, the chance of affecting the MAP is great and it would be more difficult to grow or culture the mycobacterium. Alternative procedures have been used to detect the organisms without need for culture and therefore speed up the process. PCR provides a rapid, specific test for detecting MAP in clinical samples. Results can be obtained in 24 – 48 hours. Sample preparation poses the greatest obstacle to adoption of PCR.

Faeces contains a lot of PCR-inhibitors (8,11) and difficulties are experienced in recovering DNA from a small number of organisms in such complex matrix (13). Sequence capture-PCR eliminates essentially all cellular DNA and eliminates inhibitory substances present in crude samples (4).

Although magnetic-particle technology has been reported as a mechanism for isolating specific DNA targets from complex mixtures, the result is rarely a complete absence of PCR inhibition (10). Generally, PCR based tests have been found to be less sensitive than faecal culture (13). When applied to tissue samples, PCR is more sensitive and reliable. Although the use of biopsy samples is not practical in the case of field studies, the technique is useful for confirming diagnosis at necropsy.

Faeces are a lot easier to sample in a zoo than blood or biopsy material. For these practical reasons, a faeces DNA-extraction was developed and used in this study.

Conclusion:

Although up to one third of zoos accredited by American Zoo and Aquarium Association reports at least one case of the infection with MAP since 1995 (5), we couldn’t detect a positive animal in the RZSA with the specific diagnostic tests used. Further investigation is planned.

Acknowledgements

We thank the Flemish Government for structural support to the RZSA. E. D’Haese is a postdoctoral research fellow of the Fund for Scientific Research (FWO) - Flanders (Brussels, Belgium).

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Corynebacterium pseudotuberculosis IN THE ZOOLOGICAL GARDEN BASEL, SWITZERLAND: DETECTION AND ERADICATION STRATEGIES

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Abstract

Caseous lymphadenitis was diagnosed in a group of dwarf goats from the pet zoo of the Zoological Garden Basel in Switzerland using clinical examination, serology, bacteriology and pathology. The results revealed a correlation between serology and pathology as well as between serology and bacteriology. There was no correlation between clinical signs and the results of the other tests. Therefore serological screening and culling of animals with a positive result is the method of choice for eradication programs as they are needed in environments with zoonotic potential or risk for valuable exotic ruminants. The clinical examination is not sufficient to eliminate the disease successfully.

Zusammenfassung


Résumé

Le diagnostic de pseudo-tuberculose des petits ruminants a été établi dans un groupe de chèvres naines du Jardin Zoologique de Bâle en utilisant, comme méthodes d’analyse, des examens clinique, sérologique, bacteriologique et pathologique. Les analyses ont montré une corrélation significative entre les résultats sérologiques et bactériologiques d’une part et les résultats sérologiques et pathologiques d’autre part. Les résultats de l’examen clinique n’étaient par contre corrélés à aucun des résultats des autres tests. L’examen sérologique suivi de l’élmination des animaux positifs est donc la démarche à adopter pour combatte la pseudo-tuberculose dans un environnement où l’homme et des animaux exotiques de valeur sont menacés. L’examen clinique seul n’est pas suffisant pour eradiquer la maladie dans un cheptel.

Key words: small ruminants - Corynebacterium pseudotuberculosis - caseous lymphadenitis

Extended Abstract

In a group of 35 dwarf goats from the pet zoo of the Zoological Garden Basel in Switzerland several animals showed recurrent lymphadenitis in the least years, mainly in a retropharyngeal location. Corynebacterium pseudotuberculosis was identified in the lesions after repeated bacterial culture. An eradication program was started because of to the relevant zoonotic risk for visitors (especially children) but also for the zoo animal collection. Caseous lymphadenitis is a contagious bacterial disease that affects predominantly sheep and goats but may also infect a large number of exotic species (1, 2, 3). Corynebacterium
*pseudotuberculosis* is a gram-positive short rod with two distinct serotypes. Type 1 is usually isolated in goats and sheep. It produces an exotoxin with both phospholipase D and sphingomyelinase activity, (4, 5) which is essential for abscess formation, and is also a major immunodominant antigen (4).

The lesions are characterised by the development of pyogranulomas mainly in the skin, retropharyngeal lymph nodes, lung tissue, mediastinal and mesenteric lymph nodes (5). Histological lesions consist of macrophage and lymphocyte layers distributed around a necrotic centre, which may also be surrounded by a fibrous capsule.

Immunohistochemistry studies revealed that in immature lesions, T cells of the CD4+ subset predominate, whereas in mature lesions the proportions of CD8+ T lymphocytes and also cells expressing the gamma/delta chains for the T cell receptor are increased (6). Numerous cells may also express interleukin-2 receptor. A large individual variability in the proportions of macrophage and T cell subsets may be observed for lesions of the same age. This heterogeneity suggests a different cellular pattern in relation to the persistence or the elimination of bacteria by the host and could be important in disease progression (6).

The disease can become endemic in a herd or flock and is difficult to eradicate by virtue of its poor response to therapeutics, the ability of the bacteria to persist in the environment, and the limitations in detecting subclinically affected animals and therefore may have significant economic impact on the small ruminant industry (7). Infection routes are wounds like shearing cuts, aerogen and per os. Additionally it must be considered that the available vaccination allows only the reduction of the incidence of lesions in the vaccinated population but not the elimination of the pathogen (8).

Although pseudotuberculosis is a zoonotic disease, human infections are rare. Suppurative lymphadenitis is described world-wide after occupational or sporadic contact with mainly farm animals (5, 9). In reported cases patients did not show underlying disease, or predisposing conditions like immunosuppression (10). Surgical excision of the affected lymph nodes is the usual treatment in humans, followed by antibiotic therapy (10).

The group of 35 dwarf goats was examined clinically for the presence of enlarged or suppurated lymph nodes. All animals were tested serologically. Animals with clinical lesions or positive serology were euthanatised, necropsied and a bacteriological examination was performed. Some animals were slaughtered without a clinical examination, but were tested serologically. Gross examination and histopathology of all animals (euthanatised or slaughtered) were focussed on target organs like lymph nodes or lung parenchyma. Statistical analyses of the results were accomplished using the Fisher’s Exact test.

<table>
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<th>Table 1. Results</th>
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<td>Clinical signs</td>
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<td>Serology</td>
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<td>Pathology</td>
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<td>Bacteriology</td>
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<th>Table 2. Statistical correlation’s (Fisher’s Exact test)</th>
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<tr>
<td>Clinical signs</td>
</tr>
<tr>
<td>p=0.17</td>
</tr>
<tr>
<td>Serology</td>
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<td>Pathology</td>
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The statistical test revealed a strong correlation between serology and pathology as well as between serology and bacteriology. There was no correlation between clinical signs and the results of the other tests. These results demonstrate that in presence of a positive serology the probability that the animal shows gross lesions or positive microbiology result is high.

With these findings we conclude that clinical examination is not helpful for the detection of all affected animals and that the total number of animals infected with *Corynebacterium*...
pseudotuberculosis is higher than suggested by the clinical appearance. The high correlation between serology and pathology indicates that serology is the appropriate method for eradication programs. As recently reported from an experimental study, the period between infection and first detectable gross lesions is about 8-9 days, whereas for a detectable seroconversion 15 to 20 days are needed (11). Therefore we suggest a serological screening of potential hosts (especially small ruminants from pet zoos) and the culling of animals with a positive or doubtful result. In addition an isolation period of at least 20-25 days and subsequent testing are considered suitable to prevent introduction of infected animals into zoo collections.

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References


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LEPTOSPIROSIS IN BAIKAL SEALS (*Phoca sibirica*)

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**Abstract**

Two male Baikal seals aged 6,5 and 3,5 died very fast after one resp 2 days of illness. By means of histological, serological examinations leptospirosis has been diagnosed. Positive serum titers of 1:400 (*L. icterohaemorhagica*) and 1:800 (*L. heptomadis*) in one of the dead animals were noticed.

**Zusammenfassung**


**Résumé**

Deux phoques du Baikal mâles âgés de 6,5 et de 3,5 ans sont morts de leptospirose. Suraigu?, respectivement aigu? chez le second, le diagnostic s’appuie sur l’examen histopathologique et sérologique. Les résultats sérologiques ont révélé un titre positif de 1:400 pour *L. icterohaemorhagica* et de 1:800 pour *L. heptomadis*

**Key words:** leptospirosis, Baikal seals

**Introduction**

Keeping results in Baikal seals in captivity are very unsatisfactory (7). Reproduction in captivity did not yet occur. Since 1959, Baikal seals have been kept with more success at Leipzig zoo than in other places. Only skin diseases were a problem over a longer period (2). Only 2 seals died already during the first year of their stay at the zoo. One death was caused by pneumonia, the other of a volvulus, after having eaten a lot of fallen leaves. The other 9 animals lived or are still living, at least 5 until 11 years.

We had no losses during the quarantine period like other premises. As we have had also reported in 1997, we believe that our prophylactic measures against an infection caused by *Pseudomonas sp.* after a transport of the seals are very effective. These measures consists of the applications of enrofloxacin and Baypamun® P, as well as high dosages of multivitamin preparations daily over a period of at least 3 days and more (concerning vitamins). We used this schedule successfully again in 2,2 arrivals in 1998. Unfortunately, we lost our two males very surprisingly on leptospirosis in 1996/97.
Clinical case history

On the 29th of November 1996, the male “Gorbi” (6.5 years old, 47 kg body mass) showed signs of anorexia. A day later, he was laying in a tucked-up position only on the land of the Bakail seal facility. Its fur was very dry, suggesting that “Gorbi” was already a longer time outside the water. Therefore, we darted the animal with 300 mg enrofloxacin and 100 mg prednisolon. Two hours later, the animal died. Leptospirosis was diagnosed at necropsy.

On the 30th of December, the other male “Peter” (born in 1993, 27 kg body mass) also fell sick. He was very weak and could be manipulated without any aggressiveness. Lordosis was thought as a symptom of a painful abdomen. We applied 1 Mill. units penicillin G and 2 mg dexamethasone intramuscular (i.m.) and 500 mg of the analgesic metamizole i.m. as well. In the afternoon, “Peter” ate fish very well. The 3 females were given 2 g ampicilline orally as a metaphylactic measure. Next morning, the male was a little bit more active but anorectic again. We administered 1 ml Tardomyocel® and 1 g streptomycin, both i.m., Baypamun® P i.m. and 500 mg metamizole again. On the 1st of January the behaviour of the animal had not changed. We repeated the treatment at 10 o’clock in the morning but the seal died in the afternoon, showing symptoms of a severe dyspnoea. The females did not show any signs of illness. Titre control was not possible.

Necropsy

The outer inspection of the Baikal seal “Gorbi”, showed bean-sized lesions of grey-white colour and partly concentric and ring-kind structures in the skin at the inside areas of both hind extremities. The histological findings of the skin changes showed intra-epidermal pustules, local erosions, and local ulcers that were covered with sero-cellular crusts. In the crusts and pustules fungal hyphens were detected.

At autopsy, the seal was in a good state of nutrition. The heart showed at a strong dilatation of both ventricles with subepicardially located local bean-sized foci of fibrosis. The lungs were congested and strongly oedematous. Besides local emphysema, the alveolar areas showed milary yellowish, central-necrotic lesions. Histologically, they proved to be central-necrotic granulomes with fungal hyphens.

Liver, spleen, kidneys, and adrenal glands showed an extreme congestion. The histological findings revealed single cell necroses in liver and adrenal glands. In the right kidney, a stone lay in a calyx renalis of a renculus. The affiliated renculus had hydronephrotic changes. By means of the silverplating technique, according to Warthin and Starry, the histological examination of kidneys showed leptospires in the tubuli.

At the opening of the oesophagus, a bean-sized ulcus lay in the stomach lining. The intestinal tract was macroscopically and histologically unchanged. The immune-histological examinations of the stomach lining for Helicobacter sp., the examinations of organs with regard to the existence of viruses such as dog distemper and the Aujeszky disease, as well as the parasitological examination of the gastro-intestinal tract, showed negative results. Bacteriological findings revealed alpha-haemolytic streptococci and Candida albicans in the skin. In the liver, spleen, kidneys, and intestines just a very small content of Escherichia coli could be detected.

Macroscopy of Baikal seal “Peter” revealed a dilatation of both ventricles, a minor spleen swelling, congestion and oedema in the lungs, as well as bleedings in the stomach lining of miliar up to wheatgrain size.

Duodenum, the proximal jejunum, liver, and kidney were extremely congested. A coprostasis could be detected in the colon. The macroscopic results could be confirmed by histological examinations of organs. In the kidneys, leptospires could be detected, isolated in the cups by applying the silverplating technique according to Warthin and Starry.

A histological examination of the brain and a bacteriological examination of lung, liver, spleen, kidneys, intestine, and inte stinal lymph nodes brought negative results. A serological examination of the serum gained from heart blood brought positive titers of 1:400 (L. icterohaemorrhagica) and 1:800 (L. hebdomadis).
Discussion

There are some reports about leptospirosis but only for wild ranging California sea lions (*Zalophus californianus*) (1,5,10). An epizootic along the West Coast northward to Seattle, Washington in 1984 was caused by *Leptospira* serovar *pomona* (1). A similar epizootic occurred already in 1970 and the causative agent was isolated and identified as *Leptospira interrogans pomona* (10). It is assumed that Leptospirosis is endemic in the Californian sea lion female breeding population of the southern coast of North America (5). Also in a rehabilitated pacific harbour seal from California leptospirosis was diagnosed (8). But there are no reports on leptospirosis in seals in captivity in the standard issues of Zoo animal medicine (3,4).

The clinical findings in our Baikal seals are more or less similar to those, described in sea lions. But the clinical course was much more dramatic and therapy not very effective. Symptoms like icterus, haemoglobinuria, and anaemia as seen in domestic animals with leptospirosis, were neither observed in sea lions (1) or in our Baikal seals.

The origin of the causative agents could not been found out. Rodents have never been seen in the surrounding of the Baikal seal facility. At the end of November, living fresh water fishes of our water mouts were fed to the Baikal seals. Perhaps only the males caught these fishes. It is assumed that various fish may overwinter the leptospires (6). Therefore, it might be possible that the fishes were the carrier of the Leptospires. Especially the proof of the species *L. hebdomadis* may suggest such an idea in the case of the outbreak of leptospirosis among our Baikal seals.

The question is whether we can save our seal populations in captivity by vaccinations. Vaccination of sea lions is not recommended since an adequate polyvalent bacterin has not been developed yet. Furthermore, its use could confuse serologic diagnosis as it does in domestic animals (9). Afterwards, we tested Canimed® L, Merial, in an old female South African seal without any side effect. Further investigations should be initiated.

References