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Evidence-based practice

Risk-based testing programme for *Mycobacterium bovis* following a clinical case in a zoological garden

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Abstract

Mycobacterium bovis is a strictly controlled disease. Outbreaks in zoos result in animal movement bans, disease investigation and euthanasia of infected animals. Both specific tuberculosis legislation and European Directive 92/65, often known as the "Balai" directive, require zoos to be free from tuberculosis in order to import and export animals. This paper describes the use of a risk based targeted testing programme for tuberculosis following a confirmed case of disease. This regime ensured a comprehensive but proportionate disease investigation developed through close co-operation with government veterinary officials, therefore limiting the impact of anaesthetic procedures and animal handling required to complete the necessary testing.

Introduction

Tuberculosis is a zoonotic disease caused by bacteria of the *Mycobacterium tuberculosis* complex, and tuberculosis infections caused by several species have been reported in zoological collections.

Mycobacterium tuberculosis is suspected to have been transmitted from infected human visitors on multiple occasions to a range of primates, antelope and tapirs (Michel et al.. 2003). Several reports of outbreaks of the newly identified *Mycobacterium pinnipedi* describe cross-species transmission, including to a human, originating from infected sealion reservoirs (Moser et al. 2008; Jurczynski et al. 2011).

Mycobacterium bovis is the cause of tuberculosis in cattle and has been reported in zoo felids, where it is thought to have been transmitted from infected meat (Helman et al. 1998; Thorel et al. 1998). Primates have also been infected on several occasions (Wilson et al. 1984; Thorel et al. 1998). There are surprisingly few accounts of this disease in antelope, deer and camelids in zoos, although it is known that these species are susceptible as it is seen in wild, ranched and farmed animals (Bengis 1999).

M. bovis is widespread in the United Kingdom and is subject to official disease control measures. Holders of susceptible animals are required to test stock for the disease and infected animals are culled. In the UK the badger (*Meles meles*) is an important reservoir of the disease (Corner et al. 2011).

Tuberculosis is of very high concern to zoological collections due to the potential public health risks, the potential loss of rare or endangered animals and the impacts of national disease control measures enforced on infected premises, which include a ban on imports or exports to the site and thus preventing participation in conservation breeding programmes.

In Europe, Directive 92/65 EC, known as the "Balai Directive", provides a basis for institutes such as zoos to become approved for use of a less stringent animal import procedure following implementation of a comprehensive programme of veterinary intervention, including disease surveillance, preventative medicine, post-mortem investigation and isolation procedures. This affords the zoo the benefits of revised animal health certification and reduced pre- and post-import disease testing, and avoids the need for officially supervised quarantine. In order to gain and retain "Balai-approved" status the zoo's animals must be free from mycobacterial infection.

This paper describes the implications of a case of *Mycobacterium bovis* infection in an animal held in a zoo approved under Directive EC 92/65, and how risk-based investigations and disease testing were utilised to allow disease control measures to be lifted and-approval under this legislation to be reinstated.

Background

A pair of llama *(Lama glama)* were tested using a comparative intra-dermal skin test in the axillary region, for routine disease surveillance purposes. The animals appeared healthy. They had been imported into the zoological collection nine months previously from a local private holder who had not had cases of

tuberculosis on their property. When re-examined 72 hours later, no reactions were seen and the animals were accepted as being free from *Mycobacterium bovis* infection.

One week after this test, the male animal was reported as being disorientated, weak and anorexic. On examination it was pyrexic and had an increased respiratory rate. Over the next 10 days the animal's condition developed, with ophisthotonus, paddling and other neurological signs. The animal was euthanased and the carcass submitted for pathological investigation. This demonstrated both gross and microscopic signs consistent with mycobacteriosis and samples were submitted for bacteriological culture. Infection with *Mycobacterium bovis* was confirmed.

Due to this finding, the zoo's Balai approved status was revoked and the zoo was placed under a Movement Prohibition Notice under the Tuberculosis (England) Order 2007. This resulted in the zoo being unable to move any animals susceptible to bovine tuberculosis until the premises were officially declared tuberculosis free by the official government veterinarians. A disease investigation was initiated to identify the source of infection, any transmission to other animals in the zoo and to facilitate eradication of the disease on the zoo site. This disease investigation was conducted under the direct supervision of government veterinary officials, who reviewed and approved the criteria used to determine the targeted testing programme. The diagnostic test regimes used in this official investigation are set out in UK law under the Tuberculosis (England Order) 2007. Any animals found positive for tuberculosis would be destroyed in order to return the zoo to its disease-free status.

Action

The concepts of the OIE Risk Analysis Framework (1994) were applied to this investigation using the risk question "What is the likelihood that *M. bovis* has been transmitted to other animals in the zoo?"

The standard risk terminology for this framework was used as shown in Table 1, and a risk pathway was produced (see Fig. 1).

Transmission of tuberculosis can be by a number of routes, including direct contact, short distance aerosol, contaminated fomites or reservoir species. The risk pathway was used to identify which animals could have been a source of the disease and which could possibly have been infected by the llama. The animal at highest risk was the female llama, as the only animal the male llama had been in direct contact with since his arrival at the zoo. There were no other animals sufficiently close for aerosol transmission.

The llamas were cared for by a team of keeping staff who could have inadvertently transmitted the disease on clothing, tools or other equipment, and therefore all susceptible species cared for by the same keepers were considered at higher risk of infection. It was unlikely that susceptible species cared for by other keeping

Table 1. Standard risk terminology.

Term	Definition
Likelihood	Probability; the state or fact of being likely
Likely	Probable; such as well might happen or be true; to be reasonably expected
Negligible	So rare that it does not merit to be considered
Very low	Very rare but cannot be excluded
Low	Rare but does occur
Medium	Occurs regularly
High	Occurs very often

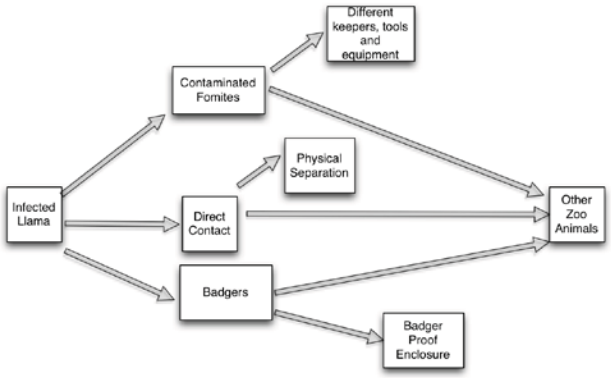

Figure 1. Risk pathway.

teams would be at risk as all equipment was kept separate and only used within a specific house or area.

The final risk factor considered was wildlife reservoirs. The zoo does have a number of setts of the European badger on its site that in theory could be the source of the infection or transmit the disease to other zoo animals. Any dead badgers found on site were examined for evidence of disease and due to the lack of previous cases of *M. bovis* in the badgers in the zoo it is likely that the resident population was free of the disease.

The badger population on the zoo site is isolated from other populations, separated from them by major roads and heavily populated urban development. The nearest cattle and badger populations are significantly beyond the foraging or dispersal range of the species. This significantly reduces the risk of badgers being the source of the infection. However the badgers move around the zoo freely and can enter some enclosures and therefore could have become infected and could be a risk for onward transmission. As the badgers were rarely seen during daylight hours, those species that were housed indoors overnight or had enclosures that prevented access to the badgers were excluded from the investigations.

So in summary the animals that were of highest risk of being infected were the animal in direct contact with the infected llama, those susceptible animals cared for by the same keeping team as the llama and those susceptible animals that may have had contact with badgers.

This risk assessment determined which animals needed targeted testing for *M. bovis* to identify spread of the disease. As the risk of the female llama being infected was high and ante-mortem testing had already proved unreliable, it was decided to euthanase this animal so that definitive investigations could be undertaken. The animals cared for by the same keepers were Bactrian camels (*Camelus bactrianus*), guanaco (*Lama guanaco*), alpaca (*Lama pacos*), domestic sheep, domestic goats and domestic pigs. The camelids could also have had contact with the badgers. The only susceptible species other that could have had contact with the badgers was the group of reindeer (*Rangifer tarandus*), which was located closest to the llama exhibit.

All of the animals were tested using comparative intra-dermal skin tests, except for the Bactrian camels ,which were tested using lateral-flow rapid tests (RT) as described by Dean et al. (2009). These are the diagnostic tests that are required to be used by UK law in these species.

Government public health authorities were notified of the outbreak but due to the routine vaccination of humans in the UK, lack of reported clinical signs in the keepers, that this outbreak was due to *M. bovis* and that an official veterinary investigation was being undertaken the zoonotic disease risk was very low and no action other than biosecurity precautions needed to be taken.

Table 2. Risk assessment.

Source of TB	Justification	Risk Assessment before further testing	Risk Assessment after further testing
Direct contact with infected zoo animals	Only had contact with female llama	High to female llama, Very Low to all other stock.	Negligible – PME examination negative
Indirect contact through contaminated fomites	All other animals cared for using same tools and keepers etc.	Medium	Very Low – all in contact animals tested negative
Infection carried by badgers to other susceptible stock	No evidence of badgers infected with TB on site. Only reindeer and camelids have potential contact with badgers other than llamas.	Low	Very Low – all other stock tested negative

Consequences

The post-mortem examination and bacterial culture from the euthanased female llama did not demonstrate infection with *M. bovis.* The two camels had negative serological tests. All of the animals that were tested using comparative intra-dermal skin testing were negative except a single 16-week old reindeer calf and a domestic goat, which both had inconclusive tests. Therefore these animals needed to be retested at 120 days and 60 days respectively after the initial test. These animals were found to be negative on retesting using the same methodology.

This testing programme demonstrated that the risk of transmission of *M. bovis* from the original animal to others on the site was very low to negligible. The specific testing was supported by the zoo's ongoing programme of clinical examinations and routine post-mortem examination of all animals that die. On the very rare occasion a badger carcass is found, it is also examined for signs of disease.

As no other animals were found to be infected it was concluded that the source of the disease was the male llama and that it is likely he was latently infected when brought into the zoo. This is supported by the fact that the female llama was not found to be infected. This animal had been at the zoo for longer than the male and they had only been housed together.

As all the at-risk animals had tested negative and therefore the site was considered to be free *of M. bovis*, the Movement Prohibition Notice was lifted 326 days after the death of the male llama. Approval under Directive 92/65 was reinstated 331 days after the death of the male llama, allowing movements of susceptible animals into and out of the zoo to recommence.

This case study demonstrates the potential devastating impacts of infection with *M. bovis* both to the health and welfare of zoo animals but also the operation of the zoo as a conservation breeding centre. For almost a year this zoo was unable to import or export animals susceptible to *M. bovis*.

One of the many challenges of mitigating and investigating tuberculosis in zoos is the lack of validated tests. The "gold standard" of confirming an infection with organisms from the *M. tuberculosis* complex is the isolation of the bacteria. The intra-dermal skin test, which is the accepted method of detection in tuberculosis in domestic hookstock, primates and man, has not been standardised in many zoo mammals but is used widely (Kaandorp 1998; Sternberg et al. 2002). Use of the intra-dermal skin test raises several issues. It requires double handling for injection and then reading of the test, therefore requiring two anaesthetic procedures in many zoo animals. It detects only infection, not necessarily active disease, and it has a low sensitivity: animals with advanced disease can give anergic responses to tuberculin (Bengis, 1999,) The low specificity of this test can cause false-negative test results in animals other than domestic hoofstock and nonhuman primates (Bengis 1999; Kaandorp 1998). In some instances, even false-positive reactions can occur. Reindeer have been shown to regularly produce false positive reactions to the intra-dermal skin test (Palmer et al. 2006; Waters et al. 2005). These false positive results could lead to the unnecessary euthanasia of these animals if this species anomaly was not recognised. However, it has been determined that reindeer infected with *M.bovis* do reliably develop a robust response to the intra-dermal test and that false negatives are rare (Palmer et al. 2006).

As in this case, inconclusive tests can occur. This happens when there is a small reaction to the tuberculin but under the thresholds set for confirmation of a positive response (Sternberg et al. 2002). In this case the thresholds were set by the veterinary authorities, with a positive test being described as "an increase in skin thickness at the site of injection of PPD *M. bovis* by more than 2mm above the amount of increase at the site in injection with PPD *M. avium*". Inconclusive tests can be caused by incorrect injection of the antigens, incorrect reading of the test or abnormal immune reactions, for example in young animals with maternal antibodies. In these cases the animals are retested at a defined interval following the initial test.

The camelids have proved to be especially problematic to test reliably for tuberculosis. The intra-dermal skin test has been demonstrated to have a sensitivity of only 14% in llama. Twomey et al. (2010) identified seven animals out of a cohort of 70 that tested negative for tuberculosis by intra-dermal skin test, but were confirmed to have the disease by post-mortem examination within one to two months. Therefore at least some of them will have been infected with *M. bovis* at the time of the skin test.

In this case the male llama was definitely infected at the time of the initial routine skin test as he was examined by post-mortem that confirmed infection only 19 days later. Anergy to tuberculin when an infected animal fails to give a measurable hypersensitivity cutaneous response is a potential cause of a false-negative result but has never been proven in South American camelids (Twomey et al., 2010).

The lateral-flow rapid test VetTB STAT-PAK is a serological test that has been used widely in domestic and zoo animals (Lyashchenko et al., 2008). This test has the advantage that it only requires the collection of a single blood sample. In zoo animals it has been used where the risk of two anaesthetic procedures in a short period of time is considered to be high. In this case the RT was used on the Bacterian camels, which were not handled and therefore would have required anaesthetising for effective intra-dermal skin testing. The sensitivity and specificity of the rapid test in Bactrian camels is unknown, but during investigations of an *M. bovis* outbreak in a racing herd of dromedary camels, the rapid test accurately detected all three confirmed infected camels out of the 55 tested with no false positive results (Wernery et al., 2007).

Again South American camelids are the exception, Twomey et al. (2010) found a high rate of false positives with only 10 animals out of 54 testing positive to tuberculosis by the RT being confirmed to be infected with *M. bovis* at post-mortem examination. The RT is not specific to *M. bovis* and so the results could be caused by

infections with other mycobacterial agents, but no evidence of this was found. The most appropriate testing regime, which should have been used initially for this llama, is a combination of intra-dermal skin test and serological testing as described by Bezos et al. (2013). Preventative veterinary medicine best practice indicates that the llama should have been tested for tuberculosis prior to import as this outbreak and its consequences would have been prevented by the disease being detected at this stage (BIAZA 2008). However, this is not required by either tuberculosis legislation or EC Directive 92/65. Balai-approved premises are required to have a general disease surveillance programme and post-import isolation process in place, both of which were complied with. The zoo should review its standard disease prevention measures and surveillance programme in light of this outbreak.

With the difficulties of handling, restraint and potential anaesthetic procedures combined with the challenges of reliably testing the animals for *M. bovis*, a risk-based process for undertaking the testing regime was required. This attempted to avoid testing animals where the risk of infection was considered low and there was the possibility of negative impacts on the health and welfare of the animals through undertaking the procedure. These risks could be further mitigated by the choice of test, as in the case of the Bactrian camels.

By applying the risk-based criteria the number of animals that required testing was considerably reduced and the process eliminated many zoo species where interpretation of the test results is not standardised and therefore interpretation difficult. The risk-based procedure had the unforeseen advantage that the highest-risk species were primarily domestic species from the zoo's children's farm. Diagnosis of *M. bovis* in domestic species has been standardised and specific protocols and test interpretations have been defined.

In this case the zoo worked effectively with local environmental health officers to ensure that the potential risks to humans were being addressed appropriately, through biosecurity, disinfection, protective clothing and equipment, and access restrictions. This avoided any adverse impact on the zoo's visitors and allowed the zoo to operate during the disease incident.

Close cooperation, collaboration and communication with the government veterinary authorities was essential for the successful resolution of this disease incident. The control of *M. bovis* on infected premises is defined in legislation written primarily for domestic commercial farms and therefore its application in zoos and non-domestic animals can be problematic, primarily in interpretation of diagnostic testing. The risk-based testing regime was defined by the zoo and the veterinary authorities working in collaboration, following discussion, inspections of working practice and assessments of operating procedures to define transmission risks. This resulted in an evidence-based investigation which was proportional and effective. Further, by having defined testing regimes and defined test interpretations, even more prolonged restrictions on the zoo premises were avoided.

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