An outbreak of tuberculosis in a captive herd of Arabian oryx (Oryx leucoryx): diagnosis and monitoring

J. R. B. Flamand, A. Greth, J. Haagsma, F. Griffin

An outbreak of tuberculosis induced a mortality of 25 per cent in a captive herd of Arabian oryx (Oryx leucoryx). The diagnostic screening tests used on live animals included the comparative skin test, indirect and comparative ELISA tests and lymphocyte transformation tests. Difficulties in the interpretation of these tests stemmed principally from the facts that false negatives and false positives were encountered and that the threshold of positivity was difficult to establish with the ELISA test. The presence of other mycobacterial infections in the environment was almost certainly a complicating factor.

TUBERCULOSIS in animals is a chronic infectious disease caused predominantly by Mycobacterium bovis. The disease is characterised by the development of tubercles, which are avascular nodules of chronic inflammatory tissue (Boever 1986). Almost all species are susceptible to tuberculosis, and it is distributed worldwide (Blood and others 1983). Tuberculosis has been recognised as a serious disease problem in captive and wild artiodactyls, including species of the genus Oryx (Lomme and others 1976). Control measures for both domestic stock and wild animals in zoos usually involve the destruction of affected animals, because treatment is difficult and prolonged and may induce drug-resistant strains of mycobacteria, and because the risk of relapse and the consequent danger of further transmission after the end of treatment is always present (Toen and Himes 1981, Blood and others 1983). It is also a zoonotic disease with human health implications (Blood and others 1983). The management protocols used for domestic stock for public health reasons may therefore be radically different from those used for rare animals of great genetic value.

The Arabian oryx (Oryx leucoryx), a medium sized antelope, became extinct in the wild in 1972 (Henderson 1974). The species was saved by a collaborative captive breeding programme in American zoos (Dolan 1976), but the world population remains low. The National Wildlife Research Center (NWRC) was commissioned to breed Arabian oryx and form a herd for their release into the wild in designated protected areas (Abu-Zinada and others 1988). The source herd originated from the late King Khalid’s private collection in Thumamah near Riyadh, which represented a unique gene pool, having not been mixed with other captive herds (Greth and others 1992). This paper describes the diagnosis and monitoring of an outbreak of tuberculosis in this herd, with special emphasis on the use and interpretation of different diagnostic tests. Once the decision had been made that the herd was worth keeping despite the presence of the disease, the problem became one of disease diagnosis in individual live oryx.

The herd in Thumamah, containing about 70 animals and including only four or five young (Seitre 1989), had been kept in a 600 ha enclosure, sharing the area with at least 15 other ungulate species of various origin. On April 29, 1986, 57 of these oryx were transported by air from Thumamah to the NWRC at Taif. The oryx were captured by driving the animals into four large communal crates on the day they were to be transported. The day was hot, and the animals had to wait in the crates before they were loaded into the aircraft. As a result they spent 17 hours in the transport crates. Upon arrival, the oryx were released into a 15 ha fenced paddock, and then into a 350 ha paddock where they were free to range and graze and had supplementary food provided.

Within the first two months it became clear that there was a significant mortality rate in the Taif herd (Haagsma and Poilane 1989). On June 19, a two-year-old female in good condition died suddenly. It had extensive space-occupying lesions in the abdomen suggesting neoplasia, with what appeared to be a large tumour close to the ileo-caecocolic junction and many small metastatic tumours in the lungs with pleurisy. Histopathological investigations established that the tumours were granulomatous lesions consisting of numerous epithelioid follicles, sometimes with necrotic centres. In places, the lymph node was the site of a diffuse necrotising disintegration with calcification. Acid-fast bacilli were demonstrated throughout the node by Zielh-Neelsen staining, and culture demonstrated the organism to be M bovis. In the last week of July 1986, two females died with gross post mortem lesions suggestive of acute miliary tuberculosis, and which were subsequently confirmed by histology and microbial culture as tuberculosis. Other animals showed clinical signs suggesting tuberculosis, including emaciation, coughing, rales, congestivitis and general weakness, though these signs were not always present. Some animals were observed to have an acute onset illness of short duration. Tuberculosis was confirmed by histopathology and isolation of M bovis from six other animals.

The presence of lesions in various organs is shown in Table 1;
TABLE 1: Anatomical sites of tuberculosis lesions in 16 Arabian oryx

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>7</td>
<td>9</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Pharynx</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Trachea</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>Pleura</td>
<td>5</td>
<td>9</td>
<td>14</td>
<td>88</td>
</tr>
<tr>
<td>Lungs</td>
<td>7</td>
<td>9</td>
<td>16</td>
<td>100</td>
</tr>
<tr>
<td>Heart</td>
<td>6</td>
<td>4</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Thoracic lymph nodes</td>
<td>6</td>
<td>7</td>
<td>13</td>
<td>81</td>
</tr>
<tr>
<td>Peritoneum</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>38</td>
</tr>
<tr>
<td>Mesenteric lymph nodes</td>
<td>5</td>
<td>5</td>
<td>10</td>
<td>63</td>
</tr>
<tr>
<td>Ileum</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>38</td>
</tr>
<tr>
<td>Liver</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>Spleen</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>31</td>
</tr>
<tr>
<td>Kidneys</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>Uterus</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>Subcutaneous abscesses</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>19</td>
</tr>
</tbody>
</table>

* Females only considered

miliary bronchopneumonia with associated lesions in the bronchial and mediastinal lymph nodes and pleura were the most characteristic lesions. Abscesses on the trachea were also occasionally encountered, as were pericarditis, peritonitis and localised perforating lesions of the small intestine, and lesions in the spleen, liver, uterus and kidneys.

Sixteen animals had died of tuberculosis by September 1987, a mortality rate of 25 per cent, but since then no further deaths due to the disease have occurred. There was no difference in mortality between the sexes, and of the 16 oryx with extensive tuberculous lesions, 13 died naturally of the disease, and three males out of eight which were euthanased for control, had lesions. Of the three euthanased males, two showed evidence of chronic disease with calcified lesions of the thoracic lymph nodes and lungs, and one had evidence of open tuberculosis; they were therefore potential excretors, as were all the animals which died of the disease, via the lungs, uterus, digestive tract or kidneys.

In order to limit the further spread of the disease, it was necessary to identify the animals which were infected with *M. bovis*, so that they could be separated from the disease-free stock. Four diagnostic tests were used for this purpose, and for the purposes of the management of the herd, the original Thumamah animals and their offspring born up to November 1987 were termed the A generation, their offspring the B generation, and the calves of the B generation, the C generation.

Materials and methods

Comparative skin test

A comparative intradermal skin test was used to measure the allergic reaction of the animal to tuberculin antigens by the following protocol. After capture with a neuroleptanalgesic combination of etorphine and xylazine (Petit and Poilane 1989), 2000 IU of avian and 5000 IU of bovine purified protein derivative tuberculin (Central Veterinary Institute, the Netherlands) were injected intradermally in the upper third of the neck on the right and left sides respectively. This site was used after the failure of the test when it was applied to the eyelid skin and the tail fold in the first few animals. Each injection site was identified with a coloured marker pen, applied to the skin. The oryx were then confined to small cages for a period of four days so that the skin reaction could be monitored daily, either visually or by measurement of the skin fold. A positive reaction was recorded if the swelling at the site of injection of the bovine tuberculosis was equal to or larger than the swelling at the site of injection of the avian tuberculin. In order to test the validity of the skin test in Arabian oryx, eight of the positive males which were considered less valuable for breeding purposes were euthanased. Over the next five months all the oryx were captured and identified individually and allotted into small groups of five or six females to one male. These animals were then tested again at three month intervals during 1987 and 1988 with the comparative skin test, with the aim of identifying new reactors early on in the course of the disease. These tuberculin tests were read by observing the oryx carefully at a distance and examining the injection sites with binoculars. The difficulties in the use of the comparative skin test for diagnosing tuberculosis in individual animals led to a search for a more refined test. The ELISA test, which measures humoral immunity, seemed to be satisfactory when it was used in conjunction with the comparative skin test, and repeated at three-month intervals.

Indirect ELISA test

An indirect ELISA test (Central Veterinary Institute, The Netherlands) was used from August 1986 onwards. Blood was collected for this test before the skin test was applied. The ELISA test used a selected *M. bovis* preparation as antigen (Haagsma and Eger 1990). The titres were expressed as the highest dilution giving 50 per cent of the maximum obtainable value. Initially, because the herd was infected with *M. bovis*, a severe interpretation was used and titres of 1:5 and higher were considered as positive. After two years a less severe interpretation was used because the *M. bovis* infection was under control and because a problem of non-specific reactions became apparent. New thresholds were therefore used retrospectively: oryx with titres of 1:5 or less were considered negative, 1:10 and 1:20 as doubtful, and 1:40 or more as positive.

Comparative ELISA tests

Three ELISA tests (Deer Research Laboratory; University of Otago, New Zealand) compared the test serum reactivity to purified protein derivative antigens of *M. avium* (PPDA), *M. bovis* (PPDB) and purified *M. paratuberculosis* protein of *M. bovis* (Griffith and others 1991). The ELISA tests are useful for detecting seriously infected animals, but may miss animals with a low grade infection. The optical density readings for the different antigens were measured on serum diluted 1:40. Values with PPDB or MPBP which were equal to or greater than 0.2 units, by comparison with PPDA, were regarded as positive.

Lymphocyte transformation test

The lymphocyte transformation test (Deer Research Laboratory) measures in vitro lymphocytic immune T cell reactivity and was developed as a sensitive marker for tuberculosis in deer (Griffith and Cross 1986, 1989). The relative reactivities in bovine or avian stimulated cultures are compared to establish the specificity of the lymphocyte transformation. Minimal reactivity in the positive (concanaflavin A) and negative (unstimulated) control cultures is necessary to establish a valid culture set. Its special value is that it can detect disease early in its course and with a high level of sensitivity.

Rules of interpretation

Each of the tests monitors a different aspect of the host’s response to tuberculosis, dependent on the stage of pathogenesis of the disease. Testing with the New Zealand methods started in 1988, and from February 1989 all the tests were used regularly to attempt to maximise the chance of detecting infection. The best understanding of the results obtained would have been by their comparison with the results of post mortem findings and bacteriological examination, but this possibility became very limited after September 1987, when the last death occurred. For interpretation, the tests were ranked in order of value: the lymphocyte transformation test, which is a sensitive indicator of early infections, the ELISA tests and the comparative skin test. On this basis, it was possible to categorise the animals into high, moderate (also called doubtful animals) and low risk, and to follow the progression of the outbreak of tuberculosis in the herd from 1986 to 1992.
TABLE 2: Reliability of the comparative skin test in Arabian oryx

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Negative skin test</th>
<th>Doubtful skin test</th>
<th>Positive skin test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed tuberculosis*</td>
<td>8</td>
<td>1 (12.5%)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>No evidence of tuberculosis</td>
<td>15</td>
<td>11 (73%)</td>
<td>1 (7%)</td>
</tr>
</tbody>
</table>

* Post mortem and M bovis isolation

TABLE 3: Reliability of the indirect ELISA test in Arabian oryx

<table>
<thead>
<tr>
<th>Sample size</th>
<th>Negative</th>
<th>Doubtful</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed tuberculosis*</td>
<td>8</td>
<td>3 (37.5%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>No evidence of tuberculosis</td>
<td>15</td>
<td>10 (67%)</td>
<td>5 (33%)</td>
</tr>
</tbody>
</table>

* Post mortem and M bovis isolation

Results

Comparative skin test

Reactors showed an optimum reaction (determined by measurement of the skin fold) 72 hours after the tuberculin injection. Only intradermal tuberculin injections in the neck proved useful. Table 2 summarises the skin test findings in relation to post mortem findings, and shows that one of eight oryx had false negative reactions and three of 15 had false positive reactions.

Indirect ELISA test

Table 3 shows the indirect ELISA results related to post mortem findings and demonstrates that three of eight oryx, by present threshold interpretation standards, showed false negative reactions, but none had a false positive reaction.

New Zealand tests

The results for generations A and B are presented in Table 4 and compared with the results obtained at the Central Veterinary Institute. In 1989, all 17 oryx in the B generation were negative according to these tests, whereas the indirect ELISA test had shown one animal as doubtful.

Discussion

It is clear that tuberculosis was present in the Arabian oryx at Thumamah before they were moved to the NWRC in April 1986. Subsequent findings clearly demonstrated that the disease persisted in the oryx remaining there, and in other species with which they shared their enclosure, notably fallow deer (Dama dama). The small number of calves mentioned by Seitre (1989) was possibly due to a high mortality due to the disease and would have resulted in a low recruitment rate. The capture of the oryx and their prolonged confinement in communal transport crates on a hot day would have provided an ideal opportunity for the transmission of tuberculosis from one animal to another, owing to the importance of aerosol transmission and inhalation as a route of infection. In addition, the stress of their capture, confinement, movement and release into new surroundings would have been sufficient to make a latent infection manifest itself in oryx which had previously managed to contain the infection or could have made uninfected animals more susceptible to infection with the tubercle bacillus. Indeed, two months after their transportation, the disease manifested itself explosively. The high incidence of lesions in the lungs and thoracic cavity indicated a respiratory route of infection. There was no difference in morbidity between the sexes, despite the expectation that females might be more susceptible owing to their closer social contacts than males and their exposure to the stresses of pregnancy and lactation. However, the overriding means of transmission in this outbreak would have been during the animals’ confinement in the transport crates, when all the animals would have been similarly exposed.

The signs were typical of an acute, miliary tuberculosis, although many of the animals did not lose condition and some remained fat until they died. Indeed, with some of the animals there were no indications that they were about to die. The lesions observed post mortem were similar to those described for domestic ungulates (Blood and others 1983). In all cases, the most notable post mortem lesions were in the lungs, where hardly any normal lung tissue remained. After the disease had manifested itself, it was difficult to distinguish infected from non-infected oryx. It was therefore decided to attempt to eradicate the infection by the use of sanitary, therapeutic and management measures (Greth and others 1994). It became essential to distinguish between the infected and non-infected oryx, when potentially clean calves were being reared. One fear was that one infected calf might contaminate the calves with which it was being reared. It was hoped that the available tests would help to make this distinction, and that by the end of the tests in combination it was reasonable to assume that, if a suspicious result was obtained in one test while all the other tests remained negative, the animal in question was less likely to be infected. Thus, on the basis of agreement between a number of tests, the chances of missing an infected animal would be diminished.

The comparative skin test as used in cattle proved unreliable in individual oryx, at least partly owing to the difficulty in reading it. This difficulty was also recorded by Lomme and others (1976) for bactrian oryx (Oryx gazella bactra) from East Africa. They found that only one of three confirmed tuberculous oryx gave a positive tuberculin skin test. In 1986, during the acute phase of the disease and when only the skin test and limited ELISA testing were available, 58 per cent of the tuberculous oryx were skin test negative (Haagsma and Polianc 1989). This was almost certainly a reflection of the inadequacy of the skin test as a test in individual Arabian oryx, and the practical problems of reading the test adequately in wild animals.

At the end of 1986, the first ELISA test results became available, and these increased the number of doubtful and high risk animals. The interpretation of the ELISA test depends on the threshold of positivity chosen. The interpretation of the New Zealand comparative ELISA and the lymphocyte transformation data (Griffin and Cross 1989, Griffin and others 1991) was empirical because the animals were not examined post mortem to establish sensitive parameters of disease diagnosis. However, later studies from another herd involving a clinically affected tuberculous oryx, showed highly specific M bovis reactivity in lymphocyte transformation and a remarkable ELISA titre (1:64,000). It was not surprising that a significant number of the A generation (Table 4) had M bovis-specific cellular reactivity in the lymphocyte transformation test. It is likely that these animals had all been exposed to M bovis before or when they were moved so that residual sensitisation to M bovis is likely to have occurred.

The significant reduction in immunological markers of M bovis in the B generation oryx provided evidence against the transmission of the disease to the offspring. In conjunction with diagnostic testing, it was decided to treat all the oryx from late 1987. In 1988 the number of positive high risk animals increased, when the complete range of screening tests became available. A number of younger animals born that year were thought possibly to be infected, owing to caution being expressed by the respective laboratories in their attempt to establish sensitive reference values for the diag-

TABLE 4: Comparison of the results for A and B generation Arabian oryx in 1989 obtained in the New Zealand ELISA test, the lymphocyte transformation test and the indirect ELISA test

<table>
<thead>
<tr>
<th>Test Type</th>
<th>A generation</th>
<th>B generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRL* ELISA test (positive)</td>
<td>6/36 (17%)</td>
<td>0/17 (0%)</td>
</tr>
<tr>
<td>Lymphocyte transformation test (positive)</td>
<td>6/27 (22%)</td>
<td>0/17 (0%)</td>
</tr>
<tr>
<td>Indirect CVID ELISA test (doubtful)</td>
<td>10/36 (28%)</td>
<td>1/36 (3%)</td>
</tr>
</tbody>
</table>

* DRL: Deer Research Laboratory, University of Otago, New Zealand
† CVID: Central Veterinary Institute, Leelay, The Netherlands
nosis of tuberculosis in oryx. In 1989 and 1990, the whole range of tests was used in screening the herd, which reduced the uncertainty of interpretation. The ability to categorise the oryx into three categories (high, doubtful and low risk) was a fundamental improvement in the understanding of the tuberculosis status of the herd and of individual oryx. It also allowed systems of management to be used to stratify the risk associated with offspring from oryx with different levels of risk for tuberculosis. The low risk assessed with B generation animals gave the authors confidence that offspring were being salvaged for future breeding without the risk of disease transmission. Even when the comparative ELISA tests were introduced, there always remained the doubt that an animal might be infected, because the interpretation of the results was sometimes equivocal, thus demonstrating the value of using several tests comparatively. The fact that treatment might affect the tests, or complicate the interpretation of the tests, was recognised.

It was clear that many of the unexplained positive ELISA and skin test reactions could be due to sensitisation with environmental mycobacteria. Infection with atypical mycobacteria can result in sensitisation to bovine tuberculin (Pritchard 1988). This possibility was confirmed when a female which had been consistently positive with the comparative ELISA tests and the bovine lymphocyte transformation test, died as a result of foot wounds in March 1990. On post mortem examination, no signs of tuberculosis were evident. A lipomatous lesion associated with, but not adherent to one kidney was the only abnormal finding. A bacteriological examination revealed the presence of Mycobacterium fortuitum from a pool of organs and from the kidney lesions. M. fortuitum is a ubiquitous mycobacterium, present in the soil, which sometimes causes abscesses but is more often considered non-pathogenic (Pilet and others 1979). The isolation of M. fortuitum from this animal demonstrated that it or other environmental organisms might have been implicated in falsifying many of the earlier test results. Indeed, it may have appeared in 1988 when there was an apparent increase in the number of doubtful and positive reactors compared with the previous year.

Tuberculosis no longer poses a threat to the NWRC herd. All the high risk oryx of 1989 and 1990 have yielded low ELISA titres. The B generation oryx seem to be clear of tuberculosis, even if some false positive reactions sometimes appear, and they have now produced their own C generation calves. During post mortem examinations of 27 oryx made between 1987 and 1992, no gross lesions indicative of tuberculosis infection were detected. Twenty-one young C generation oryx have already been reintroduced to the wild, as tuberculosis-free animals.

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References

Absracts

Repair of acetalbar fractures in dogs
THREE cranial, seven central, one caudal and five comminuted acetalbar fractures in 16 dogs were repaired with reconstruction plates. Notches cut into the plates made it possible to contour them in three planes so that they could be fitted accurately. The acetalbar fractures were accompanied in all the dogs by multiple pelvic fractures, and a repair of a non-acetalbar fracture or luxation was required in 10 cases. In 12 of the 15 dogs for which follow-up information was available the outcome was excellent or good, and the configuration of the fracture or the presence of multiple fractures did not affect the prognosis. Two of the dogs died suddenly late in their convalescence, but the cause of death was not established.


Biology of maedi-visna virus
MAEDI-VISNA virus is the prototype virus of the lentivirinae. It was isolated and characterised as a result of an epidemic of a progressive pneumonia (maedi) which was accompanied in some cases by a progressive paralysis (visna) among sheep flocks in Iceland; both signs were shown to be due to the same virus. This review provides a short history of the background to its discovery, followed by detailed descriptions of the structure and organisation of the genome of the virus and of the virion-encoded polypeptides. The life cycles of the virus in vitro and in vivo are compared and contrasted and its tropism is discussed. Its mode of transmission, the immune response to the virus and the possible mechanisms of the pathogenesis of the disease are also described.


Distribution of lesions in ovine salmonellosis
FOUR forms of salmonellosis were recognised in sheep kept in feedlots or transported at sea: septicemic, acute, subacute and chronic. The acute disease involved the abomasum and small intestine, whereas the subacute disease affected mainly the lower small intestine and upper large intestine; the chronic disease involved considerable repair of the ileum, caecum and proximal colon. The septicemic form of the disease was often accompanied by acute enteritis and occasionally by acute cholecystitis. S typhimurium was the most common serotype.


References