CASE REPORT OF PARATUBERCULOSIS IN A MINIATURE DONKEY (*EQUUS ASINUS F. ASINUS*)

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INTRODUCTION

*Mycobacterium avium* subsp. *paratuberculosis* (MAP) has been isolated from different non-ruminant wildlife species by culture or detected by PCR, but no clinical cases of paratuberculosis have been recorded in these animals to date [1]. Macroscopic lesions have been observed only in naturally MAP-infected rabbits [1]. Otherwise, four cases of natural infections of domestic equids with clinical signs of paratuberculosis have been described worldwide: in a pygmy ass, a Sicilian ass, a miniature donkey [2, 3, 4], and a horse [5]. In addition to the detection of acid-fast organisms in the intestine of these animals, MAP was cultivated only from the pygmy ass [2], and, furthermore, from a healthy horse killed for human consumption [6]. The isolates were not genotyped. Such a rare case of paratuberculosis in a donkey with typical clinical and pathomorphological signs is reported here again. The infection strain was isolated, genotyped and epidemiological data were discussed.

CASE REPORT

Origin of the animal. A male miniature donkey born in France spent the first eleven months of life at a donkey breeding farm with free-ranging cattle in the neighbourhood, commingled in common pastures. The farm was localized in Burgundy, west of the Parc Naturel régional Morvan. Together with a female donkey the animal was moved to a private owner in Saxony, Germany. Both animals were kept together with their foal born in Germany, two Cameroon sheep, a cat, and a dog. In between, the male donkey had contact with two other donkeys and two sheep. It appeared healthy.

Course of disease. At the age of two years and eight months the male donkey progressively lost weight, associated with foul-smelling diarrhoea. Treatment by Equest® (Pfizer, Germany), later by Equimax® (Virbac, Germany) adduced no result. Examinations for parasitic or bacterial diseases were negative. Different blood parameters, including blood cell counts and differential haemogram, and blood chemistry were inconspicuous. Only a low anaemia was revealed. The winter coat remained up to May, later the animal grew a dull coat with areas of alopecia at the hooves and ankles. However, the donkey showed an attentive alert behaviour and appetite but was more frequently found recumbent. Further treatment with different antiparasitics as well as antibiotics and cortisone were not helpful. The emaciation proceeded continuously. Diarrhoea persisted. After eight months a repeated examination of the fluid faeces resulted in the detection of bacterial dysbiosis and intestinal mycosis caused by *Candida albicans* connected with a low pH-value of 5.5, but no parasites. After 9 months - the animal was lying down more often – a decubitus developed. Comprehensive bacteriological and parasitological examinations were again without result. Ten months after the onset of the first clinical symptoms the general condition fell off rapidly and the animal had to be euthanized.

Pathologic-anatomical, histological, and microbiological analysis. Clinical and pathomorphological signs of this equid were similar to manifestations of paratuberculosis described for ruminants. Proliferative enteritis was visible and the intestinal lymph nodes were swollen. In the area of cardia and fundus erosions and ulcerations were observed. The intestinal loops were segmentally thickened, corrugated, and signs of granulomatous lymphadenitis were noticed within the distended mesenteric lymph nodes. The intestinal content was viscous and dirty grey; no parasites or their ova were detectable, but *Candida albicans* was found in high amounts. Yeasts were also detectable within the gastric mucosa. Large quantities of acid-fast bacilli were revealed by Ziehl-Neelsen (ZN) staining in the mucosa and submucosa, mainly in the small intestine and in the intestinal lymph nodes, especially in macrophages and giant cells. In the liver moderate interstitial hepatitis and a low number of acid-fast bacilli within the macrophages were found. In the kidney, profound chronic interstitial nephritis was observed, but acid-fast bacilli were not detectable. Brain, lung, and spleen were without pathological findings. No appreciable morphological signs of immune suppression were seen.
Cultural isolation and molecular biological analyses for diagnosis and genotyping. After four weeks of culture on Herrolds Egg Yolk Medium (HEYM) with Mycobactin J, MAP was successfully isolated from intestinal content, also from the small intestine, liver, kidney, and even muscle tissue. The Mycobactin J dependence of the isolates was tested by comparative cultivation on HEYM with and without Mycobactin J. Other mycobacteria could not be detected. MAP-specific DNA sequences (IS900 pos., F57 pos., IS1245 neg.) were verified by PCR after direct isolation of MAP-DNA from faeces and from cultures of the above-mentioned tissues. The MAP-isolates were characterized by high resolution multi-target genotyping: MIRU-VNTR typing [7], MLSSR analysis [8], and IS900-RFLP using BstEII- and PstI- digestion [9]. Isolates from different tissues exhibited identical typing results. The MAP isolates belonged to the MAP-type-II group which was revealed by a specific PCR [10]. A unique genotype with the profile 52332228 (MIRU-VNTR loci 292, X3, 25, 47, 3, 7, 10, 32) and 9g-12g-4ggt-5tgc (MLSSR loci 1, 2, 8, and 9) was detected which is different from MAP strains previously studied in Germany [9, 11, 12] and France [13]. In contrast, by IS900-RFLP the strain exhibited the common pattern C1-P1.

Check-up of contact animals. Within the faeces of the female donkey MAP could be detected by nested PCR after direct isolation of DNA. But cultural isolation was negative for about 42 weeks. The other animals of the German owner, the female donkey, the foal, the two Cameroon sheep, the dog, the cat, and four other contact animals (two donkeys, two sheep) from two further animal holders in Germany were also MAP negative using faecal culture for about 42 weeks. Up to now (one year after the death of the male donkey) all these animals have remained healthy. Unfortunately, samples from the donkey breeding farm in France were not available.

DISCUSSION
In the present study a case of paratuberculosis in a miniature donkey (Equus asinus f. asinus) was described with typical clinical and pathomorphological signs, inter alia diarrhoea, emaciation, and very high numbers of MAP organisms in intestine, lymph nodes and faeces; parts of this study have recently been published in German [14]. The initial good condition of the animal, the course of disease with examinations by the veterinarian, the pathological, histological and microbiological investigation demonstrated that paratuberculosis was the basic disease of the donkey. Candida albicans may have settled in because of the damaged intestine after excessive antibiotic treatment. No evidence for an immune deficiency was traceable by the clinical and pathomorphological examinations carried out by the veterinarians; no specific tests were performed. In future cases it is indicated to use additional methods to find out if a kind of specific immune deficiency of “naturally” infected non ruminant animals exists. However, most probably the animal was infected during infancy in the breeding farm in France by contact with large amounts of MAP from shedders: infected ruminants or related donkeys on the breeding farm. After more than two years there must have been a stress situation which triggered the onset of disease. Equids such as donkeys and horses are often permitted to mingle with cattle; therefore a high danger exists to infect individual infant equids which possibly exhibit a specific unknown susceptibility to MAP. In Germany, paratuberculosis is a notifiable disease in cattle, sheep, goats and other ruminants, but not in equids. Equids are considered resistant to natural infection by MAP. No case of paratuberculosis in a donkey has been noticed in Germany before. Therefore, the veterinarian did not consider paratuberculosis as a cause of chronic diarrhoea. This can be dangerous for farms where different animal species are kept together, including precious species.

CONCLUSION
Donkeys should be considered as paratuberculosis-susceptible animals in exceptional cases and as possible reservoirs or disseminators of infection. Donkey breeding farms should be strictly separated from cattle herds, sheep or goats with an unknown paratuberculosis status.
REFERENCES


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