Short Paper

Prevalence of bovine tuberculosis in zoo animals in Pakistan

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Summary

The study was carried out in zoo animals at Islamabad Zoo, Pakistan to know the prevalence of bovine tuberculosis. An overall prevalence of 3.3% was recorded in zoo animals with 3.6% in Bovidae, 3.2% in Cervidae and 0% in Equidae families. The positive animals included spotted deer (1/3; 95% CI = 0.84, 90.57), Chinkara gazella (1/5; 95% CI = 0.51, 71.64) and Blackbuck gazelle (1/30; 95% CI = 0.08, 17.22), while the negative animals were barking deer (0/4), hog deer (0/24), grey gorals (0/2), urial (0/9), mouflon (0/4), nilgai (0/5) and zebra (0/4). The results revealed significant association of live weight and number of calving with the positive tuberculin test, with 32% higher chances for females to show a positive test. Results also showed that odds of a positive test were 1.19 times higher when animal number was less than 10. The results of haematological parameters showed significant differences in total erythrocyte counts, hemoglobin, total leukocyte counts, eosinophil and basophil percentages between positive and negative animals.

Key words: Zoo animals, Tuberculosis, Tuberculin test

Introduction

Tuberculosis is one of the most important zoonotic diseases, as the *Mycobacterium tuberculosis* complex organisms infect a wide range of animals and human (Griffith, 1939; Francis, 1958). In England, relatively high prevalence of bovine tuberculosis in deer has been reported, which acts as one of the sources of infection to cattle (Ward *et al*., 2009). Understanding of the prevalence in deer in the UK is an important step in understanding the epidemiological role in transmission/ maintenance of bovine tuberculosis (Gowtage *et al*., 2009). In the USA, the role of deer in spreading bTB to cattle has been identified (Schmitt *et al*., 2002; Wilkins *et al*., 2003), and is also suspected in Spain (Aranaz *et al*., 2004). In zoos, tuberculosis has been reported in monkeys and in several other animal species including elephants (Une and Mori, 2007). During the period 2001 to 2003 in a Swedish zoo, an outbreak of bovine tuberculosis involved elephants, giraffes, rhinoceroses and buffaloes (Lewerin *et al*., 2005). Bovine tuberculosis is one of the most important zoonoses and is a disease that is prevalent in livestock in Pakistan (Javed *et al*., 2009, 2010a, b). Further, the prevalence of disease in zoo animals is a direct threat to people working in the zoo and the visitors. It has been emphasized that the TB testing in zoos may be carried out regularly, though no standard protocols are currently in place. Skin testing with tuberculin is still regarded as an efficient test for various animal species and is in use in wild and zoo animals. In Pakistan, no work could be traced indicating

prevalence of the disease in wild or zoo animals. Therefore, the present study was carried out to document the situation of bovine tuberculosis in animals at Islamabad zoo in Pakistan on the basis of positive SCCIT test.

Materials and Methods

The study was carried out in zoo animals at Islamabad zoo, Pakistan. The animals were tested by single comparative cervical intradermal tuberculin (SCCIT) test by using avian and bovine PPDs kindly supplied by a reference laboratory in Italy (Istituto Zooprofilattico Umbria e Marche, Italy) following the OIE criteria (OIE, 2004). The animals tested through SCCIT test belonged to Cervidae (n=31), Bovidae (n=55), and Equidae (n=4) families. The animals included in Cervidae family were Barking deer, Hog deer and Spotted deer, of Bovidae family were Chinkara gazella, Blackbuck gazelle, Grey goral, Nilgai, Urial and Mouflon. The animals in Equidae family were zebra. Hematological examinations of positive and negative animals were also carried out.

Data obtained were analysed by Chi-square test (Fisher exact test and Mental-Haenszel). The 95% confidence intervals and odd ratios were also worked out. For epidemiological data analysis, all the animals of equine family were excluded. The data on hematological parameters were analysed by t-test by using SAS software (SAS, 2003).

Results

The animals reacting positively to SCCIT test were spotted deer, Chinkara gazelle and Blackbuck gazelle, while the negative animals were barking deer, hog deer, grey gorals, urial, mouflon, nilgai and zebra (Table 1).

There were 55 animals in Bovidae family and two (3.6%) of them reacted positively to skin test, while there were 31 animals in Cervidae family and only one (3.2%) animal reacted positively to skin test and none of the four animals tested in Equidae family reacted to skin test (Table 2). The overall prevalence of bovine tuberculosis was 3.3%. However, this prevalence was 3.5% when animals of Equidae family were excluded. The results revealed that among animals of Bovidae and Cervidae families, the odds of females being positive in skin test were 1.32 times. Results also revealed that in Bovidae family, there are higher chances of females to show positive skin test, while in Cervidae family there are higher chances of males to show positive skin test (Table 2). Results also showed that odds of a positive test were 1.19 times higher when animal number was less than 10 (Table 2). Results showed a significant ($P<0.05$) association between the number of calving with the positive test and the reactor animals increased with the increase in number of calving (Table 2). The live weight showed significant ($P<0.05$) association with positive skin test when the results of Cervidae and Bovidae families were combined (Table 2).

The results of hematological studies showed significant differences in total erythrocyte counts, hemoglobin concentration, total leukocyte counts, eosinophil percentage and basophil percentage between positive and negative

Table 1: Positive SCCIT test recorded in different animal species tested at Islamabad zoo, Pakistan

Animal species	Positive	Negative	Positive %	95% CI
Barking deer (*Muntiacus muntjak*)	0	4	0	0.00 to 60.24
Hog deer (*Axis porcinus*)	0	24	0	0.00 to 14.25
Spotted deer (*Axis axis*)	1	2	33.3	0.84 to 90.57
Chinkara gazella (*Gazella gazella bennettii*)	1	4	20	0.51 to 71.64
Blackbuck gazelle (*Antilop cervicapra*)	1	29	3.3	0.08 to 17.22
Grey gral (*Nemorhaedus goral*)	0	2	0	0.00 to 84.19
Urial (*Ovis orientalis vignei*)	0	9	0	0.00 to 33.63
Mouflin (*Ovis musimon*)	0	4	0	0.00 to 60.24
Neilgai (*Baselaphus tragocamelus*)	0	5	0	
Zebra (*Equus zebra*)	0	4	0	

Table 2: The SCCIT test positive zoo animals divided into different groups

	Positive	Positive %	Negative	95% CI	Chi-square or odd ratio
Families of animals					
Bovidae	2	3.6	53	0.44 to 12.53	
Cervidae	1	3.2	30	0.08 to 16.70	Fisher's P=0.9
Equidae	0	0	4	0.00 to 60.24	
Total	3	3.3	87	0.69 to 9.43	
Sex					
Male	1	2.9	33	0.07 to 15.33	ODDS RATIO = 0.76
Female	2	3.8	50	0.47 to 13.21	
Family of animal and sex					
Bovidae					
Male	0	0	25	0.00 to 13.72	Fisher's P>0.49
Female	2	6.67	28	0.82 to 22.07	
Cervidae					
Male	1	11.11	8	0.28 to 48.25	Fisher's P>0.28
Female	0	0	22	0.00 to 15.44	
Number of animals					
<10	2	3.7	52	0.45 to 12.75	ODDS RATIO =1.19
>10	1	3.1	31	0.08 to 16.22	
Number of calving					
0	0	0	26	0.00 to 13.23	
1-4	2	9.09	20	1.12 to 29.16	M-H P<0.05
>4	1	20	4	0.51 to 71.64	
Age of animal					
<5	2	4.65	41	0.57 to 15.81	
5-8	1	4.76	20	0.12 to 23.82	M-H P>0.18
>8	0	0	22	0.00 to 36.94	
Cervidae					
<5	1	6.25	15	0.16 to 30.23	
5-8	0	0	7	0.00 to 40.96	M-H P>0.30
>8	0	0	8	0.00 to 36.94	
Bovidae					
<5	1	3.7	26	0.09 to 18.97	
5-8	1	7.14	13	0.18 to 33.87	M-H P>0.32
>8	0	0	14	0.00 to 23.16	
Live weight (excluding of equine)					
<25	2	11.76	15	1.46 to 36.44	
25-50	1	1.61	61	0.04 to 8.66	M-H P<0.05
>50	0	0	7	0.00 to 40.96	
Cervidae					
<25	1	14.29	6	0.36 to 57.87	
25-50	0	0	22	0.00 to 15.44	M-H P>0.08
>50	0	0	2	0.00 to 84.19	
Bovidae					
<25	1	10	9	0.25 to 44.50	M-H P>0.12
25-50	1	2.5	39	0.06 to 13.16	
>50	0	0	5	0.00 to 52.18	

zoo animals (Table 3).

Discussion

The prevalence of bovine tuberculosis in a zoo was investigated and it was around 3.3. The presence of infection in zoo animals is not only a potential danger to the workers and veterinarians working there, but also to the general public who visit the zoo. There were already a few unpublished reports about the presence of tuberculosis in zoo animals on the basis of postmortem findings supported by laboratory results. There are

Table 3: Hematological values in positive and control zoo animals

Variables	Positive	Control	P-value
TEC (millions/cu.mm)	5.82 ± 1.93	6.05 ± 1.03	P<0.0001
ESR (mm/h)	83.80 ± 30.86	99.86 ± 27.25	
Hb (g/dl)	11.23 ± 1.67	14.00 ± 1.15	P<0.0001
PCV (%)	30.88 ± 3.87	31.00 ± 2.47	
TLC	4581.39 ± 1883.54	7376.67 ± 1053.71	P<0.0001
Lymphocyte (%)	52.66 ± 8.71	52.80 ± 2.14	
Neutrophil (%)	40.55 ± 8.28	37.93 ± 3.47	
Eosinophil (%)	3.11 ± 1.60	6.40 ± 1.18	P<0.0001
Monocyte (%)	2.94 ± 2.31	3.20 ± 1.26	
Basophil (%)	3.8 ± 0.50	0.06 ± 0.25	P<0.0001

reports from other countries about the presence of infected animals in zoos (Sternberg *et al*., 2002; Kiers *et al*., 2008). The infection in deer has been known in the wild where it acts as a reservoir for cattle infection in many European countries (Aranaz *et al*., 2004; Surujballi *et al*., 2009; Ward *et al*., 2009; Jaroso *et al*., 2010). In another study, 21 out of 36 tuberculin positive fallow deer showed positive culture for *M. bovis* (Jaroso *et al*., 2010). These results strengthen our findings on the basis of a positive tuberculin skin test. The positive animals identified were one spotted deer, one Chinkara gazella and one Blackbuck gazelle. All other animal species including hog deer, grey gorals, urial, mouflon, nilgai and zebra were negative. These results do not mean that infection does not occur in the latter.

The prevalence recorded in animals of Bovidae (3.6%) and Cervidae (3.2%) family was almost similar, suggesting an equal level of susceptibility of these animals. Results of the combined data suggested that there is a 32% higher chance for females to show a positive tuberculin test than males. Further, in Bovidae family, there are higher chances of positive test in females, while in Cervidae family there are more chances of positive test in males. However, on the basis of these data it cannot be generalized and further studies are needed to clarify these facts. Results also suggested that there were slightly (19%) higher chances of a positive test when animal number was less than 10. Now, this is something not often seen as the larger the herd the higher are the chances of infection or spread of disease. But it may be possible in zoos where the social setup among animals might be a contributory factor, e.g., when the number of animals is small, the offering of fodder is also in small quantities that bring social dominance into play for feeding. However, when the animal number is large, then the fodder offering is also in higher quantities, giving more chances to weaker animals to get some share. This is just a presumptive explanation and there may be many other factors involved. The higher chances of a positive skin test in females given more births may be related to the stress of parturition bringing the immune system of the animal down. We were unable to find any difference in prevalence in animals of different age groups. However, another study from Spain reported a significant association of age with tuberculosis in deer (Jaroso *et al*., 2010). Further, it has been reported in a study from New Zealand that factors including age, environment, population density, exposure and genetics play a role in deer susceptibility (Mackintosh *et al*., 2004). Another study reported the prevalence of disease in deer between the age from 1.5 to 5.5 years (Schmitt *et al*., 1997). The latter fact seems quite true as we were also unable to find a positive animal of more than 8-year-old. Further studies are needed to confirm it. Results showed animals with lower weight were infected more often than the animals having good weight. As previously explained, the social setup among the deer might have played some role in causing general body weakness and lowering the immune system. Another possibility is that the animals might have become weaker with the passage of time as being infected. It is our opinion that, both factors are involved as the infection rate was higher in herds with a

lesser number of animals and the live weight further decrease in infected animals.

The hematological results showed anemia, leucopenia with proportionate decrease in leucocytes not affecting their percentages and eosinopenia. However, basophilia was also recorded. A recent study reported basopenia and slight lymphopenia in positive compared to TB-negative deer (Beechler *et al*., 2009). The number of animals in deer during the present study was small, so these results need to be looked at in that context. Further studies are required to reach a conclusion.

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