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Prevalence of bovine tuberculosis in cattle of lower and middle ranges of north-western Himalayas

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Bovine tuberculosis, caused by *Mycobacterium bovis*, is a chronic wasting disease of cattle. *M. bovis* is principal agent of zoonotic tuberculosis and can be transmitted from infected bovines to humans and other animals (Olea-Popelka *et al.* 2017). *M. bovis* is the causal agent of human tuberculosis in about 10–15% of the cases in developing countries, and in about 1–2% of the cases in developed countries (Bolaños *et al.* 2017). Infections in humans are primarily food borne, and are transmitted mainly through consumption of unpasteurized milk and dairy products (Bolaños *et al.* 2017). *M. bovis* causes extra pulmonary disease in humans (Dürr *et al.* 2013). Studies suggest that pulmonary tuberculosis can occur in man by *M. bovis* through airborne transmission among people (Sunder *et al.* 2009, Buss *et al.* 2016). Occupationally exposed personnel such as farmers, veterinarians, slaughterhouse workers, animal product (unpasteurised milk and untreated animal products) handlers are at higher risk of acquiring *M. bovis* infections (Olea-Popelka *et al.* 2017). Bovine tuberculosis is prevalent in India but exact burden of the disease is not known (Veerasami *et al.* 2012). A study from Central India had shown a prevalence of 12.6% of *M. bovis* in man (Bapat *et al.* 2017). In Himachal Pradesh, bovine tuberculosis prevalence had been reported to be 14.3% in organized dairy herds (Thakur *et al.* 2010).

The present study was undertaken to ascertain the prevalence of bovine tuberculosis in animals reared under different farming systems in Himachal Pradesh, India. The study was carried out in 33 panchayats of 5 districts (Kangra, Kullu, Mandi, Solan and Una) of Himachal Pradesh. This region represents 3 agro-climatic zones (Zone-I, Zone-II and Zone-III, altitude varying from 200 m to 2500 m above sea level) of lower and middle ranges of Northwestern Himalayas. Animals (997) comprising 143 Jersey/Holstein Frisian (HF, all females), 611 Jersey/HF crosses (23 males and 588 females), 110 Red Sindhi crosses (all females) and 133 local pahari (46 males and 87 females) cattle, were tested for tuberculosis. One hundred seventy seven (17.8%) tested animals (7 Jersey/HF, 113 Jersey/HF crosses, 9 Red Sindhi crosses and 48 non-descriptive animals) were owned by families of 100 registered tuberculosis patients. These animals were included in the study to establish the transmission of tuberculosis between man and animals. The tested 997 animals were reared under different farming systems, organized government dairy farms (50), organized private dairy farms (95) and private unorganized farms (852).

Single intradermal (SID) tuberculin test was performed as described previously (Cousins 2010). Bovine tuberculin purified protein derivative (PPD, Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh) (0.1 ml, 2000 I.U.) was aseptically injected intradermally into the skin fold in the middle of the neck of the animal. The thickness of the skin fold at the site of inoculation was measured with a Hauptner (Hauptner-Herberrholz, Soligen, Germany) at the time of PPD injection and after 72 h, to measure the delayed hypersensitivity reaction. The results were interpreted as negative (<2 mm), inconclusive (2–4 mm) and positive (>4 mm) (Cousins 2010).

Lymph node biopsies and raw milk samples were collected from tuberculin positive reactors to detect *M. bovis* by real time PCR (RT-PCR) at Regional Disease Diagnostic Laboratory-North Zone, Jalandhar, Punjab. Lymph node biopsies were collected from pre-scapular lymph nodes. The glands were aseptically punctured and injected with 2 ml of sterilized normal saline solution. Approximately 2 ml of the fluid from lymph nodes and 50 ml (after rejecting first few strippings) of milk samples were collected from each SID positive animal and were stored and transported at –4°C for RT-PCR analysis (Mishra *et al.* 2005).

SID tuberculin test detected 1.7% (17/997) of the tested animals as positive reactors to tuberculin PPD whereas, 3.3% (33/997) and 94.9% (947/997) were identified as inconclusive and non-infected animals, respectively. All positive (17) cases were detected in organized government dairy farms and prevalence of tuberculosis was 34.0% (17/

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Table 1. Delayed type of hypersensitivity reaction to cattle from different breeds and age groups

Breed	No. of animals	Age (Years)			Delayed hypersensitivity reaction to tuberculin PPD, Skin fold thickness (mm)*		
		1–5	5–10	>10	<2 mm	2 mm to 4 mm	>4 mm
Jersey/Holstein Friesian	143	57	77	09	131	08	04
Jersey/Holstein Friesian crosses	611	153	390	68	596	15	00
Red Sindhi crosses	110	21	70	19	91	06	13
Local Pahari	133	23	58	52	129	04	00
Total	997	254	595	148	947	33	17

*Interpretation criteria as per OIE, 2016.

50) in this farming system. Animals (8%, 4150) dairy tested in organized Government farms were inconclusive reactors. Our findings were in agreement with previous study conducted in organized Government dairy farm of Himachal Pradesh, which reported a prevalence of 34.4% (Thakur *et al.* 2010). Cattle herds should be tested annually and tuberculin reactors should be removed from the herd before they start excreting tubercle bacilli and become a persistent source of infection. In private organized dairy farms (including Go-Sadan), none of the 95 tested animals reacted positively to the PPD but 7.4% (7/95) animals had inconclusive reactions. About 2.8% (22/852) of the tested animals reared under unorganized farming system showed inconclusive reaction. High stocking density and poor ventilation facilitates the spread and transmission of tuberculosis from infected animals to susceptible ones in farm houses. Highest number of tuberculin positive reactors were detected in the age group of 5–10 years (15/595, 2.5%) followed by age group of 1–5 years (2/254, 0.8%) and none in age group more than 10 years (0/148). Higher rates of tuberculin reactions in older animals can occur due to non-specific immune responses to environmental mycobacteria (Thakur *et al.* 2010, Philips *et al.* 2003). The difference in results between cattle of different ages can also be attributed to slow progression of disease to a detectable level (Thakur *et al.* 2010).

Prevalence of tuberculin reactors was highest among Red Sindhi crosses (11.8%, 13/110) followed by pure Jersey/HF animals (2.8%, 4/143) (Table 1). None of the Jersey/HF crosses or local pahari tested animals reacted to tuberculin PPD. Various workers from different parts of the world have also recorded significant differences in the incidences of bovine tuberculosis among crossbred and purebred cattle (Thakur *et al.* 2010, Ameni *et al.* 2003). None of the tested male (69) animals reacted positively to tuberculin testing. In females, out of 928 tested for tuberculosis, 17 (1.8%) were positive, 33(3.6%) inconclusive and rest 878 (94.6%) were negative. Among 177 animals owned by 100 registered tuberculosis patients, none gave a positive reaction to PPD. Only 4 (2.3%) had inconclusive reaction to tuberculin.

Milk samples (17) and 10 lymph node biopsy fluid samples from SID positive reactors were tested for the presence of *M. bovis* by RT PCR (Mishra *et al.* 2005). Around 20% (2/10) lymph node biopsy samples and 17.6% (3/17) milk samples were detected positive for *M. bovis* by RT-PCR. Excretion of *M. bovis* in milk poses a serious public health threat as milk from all the yielders is pooled in organized farms before disposal. Further, adding fresh contaminated milk to raw/fermented milk or vice-versa, leads to continued presence of mycobacteria in milk (Mariam 2014).

SUMMARY

This study was conducted to ascertain the prevalence of bovine tuberculosis in cattle of different breeds reared under different faming systems and agro-climatic zones of Himachal Pradesh. Tuberculin reactors (17) were detected only in organized dairy farms. Prevalence (15/595, 2.5%) of the disease was highest in animals aged between 5–10 years with 88.2% (15/17) of total reactors being detected in this age group. Tuberculin reactors were found among animal from Red Sindhi crosses (11.8%, 13/110) and pure Jersey/HF animals (2.8%, 4/143). RT-PCR detected *M. bovis* in milk (20%, 2/10) and lymph node biopsy samples (17.6%, 3/17). *M. bovis* is zoonotic and shedding of bacteria in milk is a serious public health hazard. Raw milk and products prepared from unpasteurized or raw milk are major vehicles of *M. bovis* transmission and causation of extra pulmonary tuberculosis in humans.

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