

Project Title: Molecular Epidemiological study on Paratuberculosis along with Pathology of mesenteric lymph nodes and intestine in buffalo and cattle

Principal Investigator: Prof. Dr. M. Tariq Javed,
Department of Pathology, FVS, UAF

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The dairy sector is one of the vibrant sectors of Pakistan. This sector has its major share in development of socioeconomic status of the people. It has become a main player in Pakistan economy for poverty alleviation as about 35-40 million rural population of this country is dependent on Livestock. Bovine Johne's disease (BJD) or paratuberculosis (PT) is a chronic enteric disease of livestock primarily seen in ruminants. The etiological agent of BJD is *Mycobacterium avium* subsp. paratuberculosis (MAP), which is an acid fast bacillus and slow-growing organism. The disease is responsible for heavy economic losses worldwide in terms of medication, premature culling, and mortality. The disease is also of zoonotic importance because the Crohn's disease patients in humans share the same organism. The study was proposed to investigate the prevalence of paratuberculosis in cattle and buffaloes at twelve public livestock farms and two cattle colonies of Faisalabad. Another objective of the study was to compare the different diagnostic methods for the identification of *Mycobacterium avium* subsp. paratuberculosis. Animals were screened on the basis of an intradermal tuberculin test (ITT). The animals were injected 0.1ml of avium tuberculin intradermally. The tuberculin reaction was recorded after 72 hours. Faecal and blood samples were collected from tuberculin positive animals for diagnostic purposes. At twelve farms total 2181 animals were included in this study. Out of them 939 were buffaloes while, 1242 were cattle. A total of 83 faecal and blood samples with similar number of samples from negative reactors were collected from Govt. Livestock farms Khushab, Khizrabad, RakhGhulaman, Kalorkot, Dera Chahl, Jogait Peer, Jahangirabad, Chak Katora, Haroonabad, Qadirabad, Bhadurnagar and Buffalo Research Institute Patoki (BRI). Similarly, 236 animals were included at two cattle colonies of Faisalabad. A total of 24 faecal and blood samples along with same number of negative reactors samples were collected from the two cattle colonies, i.e., Aminpur road colony (Chakera), Satiana road colony (Malkhanwala) Faisalabad. To evaluate different epidemiological risk factors a complete data about the animals at these farms were recorded. For this purpose age of animal, body weight, status, lactation number, lactation length, milk yield, housing, and frequency of removal of dung, total number of animals, and total number of small ruminants at the farm and colonies were noted. Indirect ELISA was performed on skin positive samples and control samples. Faecal samples were processed for ZN staining. On the basis of tuberculin testing the overall prevalence of paratuberculosis at these farms was 3.8 %. While, ELISA showed 86 animals positive at these 12 farms. A total of 71 (3.25 %) animals were positive by ZN microscopy. In case of colonies the overall prevalence rate was (10.16 %), While, ELISA and ZN showed 6.4% and 5% animals positive. The study concluded that the disease was more prevalent in older animals. The study also revealed that the prevalence of paratuberculosis was more in relatively high producing animals. The study showed that the paratuberculosis was more in those animals which had more lactation number. The study concluded that tuberculin testing, ELISA, and acid fast staining are the prime tools for screening of dairy farms to investigate the prevalence of paratuberculosis (Johne's disease). The intestinal samples collected from slaughterhouse showed that 95% were

normal, 4% were hemorrhagic and only 1% had thickening. The suspected prevalence of paratuberculosis on the basis of ZN-Staining was 0.75%. The histopathological examination of four cases of intestine revealed that there was severe chronic inflammatory reaction in intestine involving lymphocytes and macrophages. The reaction was diffuse with micro-abscess formation at some places. The intestinal epithelium also showed sloughing.