

## Molecular identification of *Mycobacterium tuberculosis* transmission between cattle and man: A case report

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### Abstract :

The study explain a case of transmission of *Mycobacterium tuberculosis* (*M. tb*) infection of a man with cattle on a farm in Gombe state, northeast Nigeria. *M. tb* was isolated from the bronchial lymph nodes of a 2-year-old heifer which responded strongly positive to the intradermal caudal fold tuberculin test but showed no gross pathological lesions at slaughter. The cattle assistant reported to hospital sick and was diagnosed smear sputum positive and he died at the time when the heifer was diagnosed as *M. tb* infected. The owner's sputum and the heifer's bronchial lymph nodes were all cultured on Lowenstein Jensen media containing both pyruvate and glycerol. The isolates obtained were Ziehl Neelsen stained in order to obtained smear positives. The smear positive isolates were subjected to Bioline (SD TB Ag MPT64 Rapid) assay to differentiate them in to *Mycobacterium tuberculosis* complex (MTBC) and environmental *Mycobacterium*. The MTBC were more subjected to PCR technique with GenoType MTBC technique to differentiate them into their various species. It was found that both the isolates from the man as well as that of the heifer were *M. tuberculosis* species, it was then concluded that there was a possibility of an anthroponozoonotic transmission. Further studies using VNTR and spoligotyping is herby suggested.

### Clinical case of acute equine piroplasmiasis in a horse in Malaysia

This is the primary report on acute equine piroplasmiasis in a horse in Malaysia. A 20 years old thorough bred mare, weighing 350 kg, used for sport showed clinical signs likewise anorexia, emaciation, nervousness, fever (40.1°C), congested mucous membranes with petechial haemorrhages on 3rd eyes lid and conjunctiva, difficulty in movement with muscular rigidity, in-coordination, ataxia, oedema on the fetlock joint of the hind limbs and hemoglobinuria, increase respiratory rate, heart rate and capillary refilling time. Haematology revealed decrease in total erythrocyte count, haemoglobin concentration, packed cell volume, thrombocytes, mean corpuscular volume and mean corpuscular haemoglobin concentration reflecting to microcytic hypochromic type of anaemia. Whereas, there is increase in erythrocytes sedimentation rate and reticulocytosis. The total white blood cells count was increased due to neutrophilia and lymphocytosis. The serum biochemical analysis reflect an increase in aspartate amino transferase, alanine amino transferase, alkaline phosphates, blood urea nitrogen and total bilirubin. Moreover, there was decrease in the total protein albumins and globulins, calcium, phosphorous, glucose and creatinine. Thin blood smear stained by 10% Giemsa was positive for *Theileria equi* and *Babesia caballi* with parasitemia of 18.2%. Competitive ELISA assay on the horse sera test was analytical and confirmative to antibodies against *Theileria equi* and *Babesia caballi*. Multiplex PCR assay was positive for *T. equi* and *B. caballi*. The horse was euthanized after asking the owner's permission and post mortem was performed. The gross morphology appears general cachexia, yellowish discolouration of subcutaneous tissue, pulmonary tissue at the cardiac lobe of

left lung was congested with multiple solid discrete prominent nodules scattered on the surface of the lobe adjacent to pinkish white emphysematous focal lesions. The heart was enlarged covered by huge amount of pericardial fat, coronary and aortic mucosa were icteric. The spleen and liver were extremely inflamed splenomegalic and hepatomegalic. Both Kidneys were enlarged, pale to red-brown coloured. The urinary bladder was full with dark coloured urine (coffee like). Small and large intestine was suffering catarrhal enteritis, petechial haemorrhage and ecchymosis detected in various parts of the GI tract. Distinct microscopic tissue alterations were observed in brain, heart, lung, liver, spleen, lymph nodes and kidney

### Materials and Methods

In April, 2010, during tuberculin skin testing in cattle which was part of the National Bovine tuberculin tuberculosis epidemiological survey, a 2 year old heifer from a dairy farm reacted strongly to bovine tuberculin test. In the farm, 15 animals were considered to have positive reactions, out of 350 cows, and 3 animals, including the said heifer that had the strongest reactivity to the tuberculin test were purchased and slaughtered. The submandibular, tracheobronchial, retropharyngeal, bronchial, mediasternal, portal and mesenteric lymph nodes were collected for bacteriological investigation. No gross pathological changes were visible in the lymph nodes and tissues inspected at slaughter. However, after 8 weeks of incubation, colonies were observed on Lowenstein-Jensen slopes and were stained with Ziehl-Neelsen, and acid-resistant culture isolates were obtained. Likewise, samples of sputum from the farm attendant who came to the sick hospital were also obtained, samples of 3 sputum were pooled and stained with Z-N before being treated for culture. All Z-N positive isolates from the cow and the breeder were analyzed using the Bioline analysis and the Hain Assay test as described in their HLEA manual (GenoType MTBC analysis).

Bacteriological investigation of the collected lymph nodes as well as the sputum was performed according to the protocol described by Kent and Kubica, 1985. After homogenization, decontamination and concentration, the materials were inoculated on Lowenstein-Jensen slopes (with pyruvate and the other with supplemented glycerol), whose growth was checked once a week for eight weeks. Colonization identification - All suspected growths were subjected to Z-N staining and those which were positive were chosen and subjected to the SD Bioline® analysis, which differentiates them into the *Mycobacterium tuberculosis* complex and other environmental mycobacteria. A GenoType® MTBC for the molecular genetic assay for the differentiation of the *Mycobacterium tuberculosis* complex from the cultivated material was used (Hain Lifescience, Germany). The kit was used in accordance with the manufacturer's instructions.

### Results

All the colonies which were positive for Z-N were subjected to a

Bioline® analysis, which identified them as a Mycobacterium tuberculosis complex. The Mycobacterium tuberculosis complex was then subjected to the Hain Assay test (Hain Lifescience, Germany). It was observed that, the samples belonging to the heifer as well as the attendant were found to be Mycobacterium tuberculosis species.

#### References

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