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SHORT COMMUNICATION

Mycobacterium orygis associated generalised tuberculosis in Indian Cattle

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ABSTRACT

The present report describes two cases of generalised tuberculosis in cattle caused by *Mycobacterium orygis*. The affected cattle, both adult female cows from an organized dairy farm in Bareilly, Uttar Pradesh exhibited severe clinical signs including weakness, lethargy and respiratory distress and succumbed to the disease despite treatment efforts. Necropsy examination revealed marked emaciation, a rough body coat and pale mucous membranes. Gross lesions consisted of variably sized caseo-calcified nodules in multiple organs, including the lungs, lymph nodes (prescapular, mediastinal, mesenteric), kidneys, udder, uterus, meninges and brain. Histopathological examination revealed diffuse caseo-calcified granulomas in above mentioned organs. Duplicate sections of various organs were positive for acid-fast bacilli on Zeihl-Neelsen staining. Generalized tuberculosis caused by *M. orygis* was diagnosed based on pathomorphology, Ziehl-Neelsen staining, culture isolation and multiplex PCR. This report highlights the broader implications of mycobacterial infections in livestock and the potential risks to public health.

Keywords: Cattle, *Mycobacterium orygis*, region of difference, tuberculosis

Tuberculosis (TB), a deadly airborne disease affecting livestock, wildlife and humans worldwide is caused by the Mycobacterium tuberculosis complex (MTBC), which includes species such as *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. caprae*, *M. canettii*, *M. tuberculosis*, *M. microti* and *M. orygis*[1]. In India, Mycobacterium bovis has long been considered the primary cause of tuberculosis in approximately 21.8 million cattle affected. However, recent reports of *Mycobacterium orygis* induced TB in humans, cattle and occasionally wild animals challenge this assumption[2,3]. In this case, the infection was initially attributed to *M. bovis* due to its historical association with zoonotic TB. However, subsequent molecular characterization confirmed *M. orygis* as the actual causative agent. This misdiagnosis highlights the need to reconsider *M. bovis* as the sole proxy for zoonotic TB, particularly in South Asia, where recent molecular epidemiological studies including one from southern India found no evidence of wildtype *M. bovis*[1-3]. These findings emphasize the need to update diagnostic criteria and expand the definition of zoonotic tuberculosis to include *M. orygis*. To break the transmission linkage between humans, livestock and wild animals, timely diagnosis and appropriate preventive measures are important[3,4]. However, diagnosing bovine tuberculosis and differentiating species within the *M. tuberculosis* complex remains challenging, as traditional methods such as history, radiology, smear microscopy, physical examination and histomorphology rely on acid-fast staining but cannot identify specific Mycobacterium species[1,5]. PCR (Polymerase Chain Reaction) has emerged as a rapid and reliable tool for detecting Mycobacterium genomic DNA, offering superior accuracy compared to traditional methods and specifically identifying and differentiating specific bacteria from other *M. tuberculosis* complex members[6,7]. This report describes the pathological features of tuberculosis in cattle caused by *M. orygis* and confirms the etiological agent through molecular identification techniques.

Two adult Tharparkar cattle from an organized farm in Bareilly presented with clinical signs including weakness, anorexia, progressive emaciation and respiratory distress. Despite treatment, both animals ultimately succumbed to the disease. A detailed postmortem examination was performed on both carcasses and gross lesions were recorded. Peripheral lymph nodes were inspected and impression smears were prepared for acid-fast staining. The representative tissues pieces of visceral organs like lungs, lymph nodes, liver, intestine, spleen, kidney, heart, ovaries, uterus, adrenal glands and brain were collected and fixed in 10% neutral buffered formalin (NBF) for routine histopathological examination. Tissue samples were also collected and stored on ice for bacterial isolation and molecular studies. Smears

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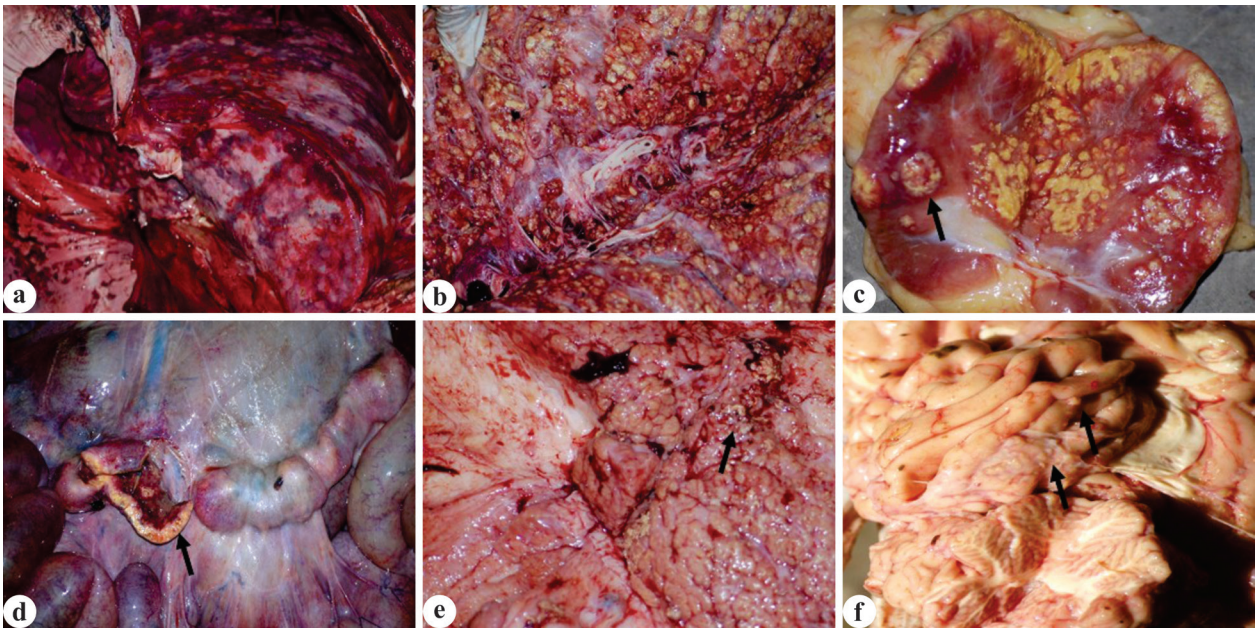

Fig. 1a. Multifocal to coalescing, poorly demarcated, variably sized, pale yellowish caseous nodules on both the surface and cut surface of the lung parenchyma, accompanied by severe consolidation. **b.** Dissected nodules in the lung revealing caseo-calcified, pale-white material. **c & d.** Prescapular and mesenteric lymph nodes exhibiting enlargement with gritty, caseous nodules visible on the cut surface (arrow). **e.** Hard, indurated udder showing tiny caseous nodules (arrow). **f.** Meninges displaying tuberculous nodules (arrow).

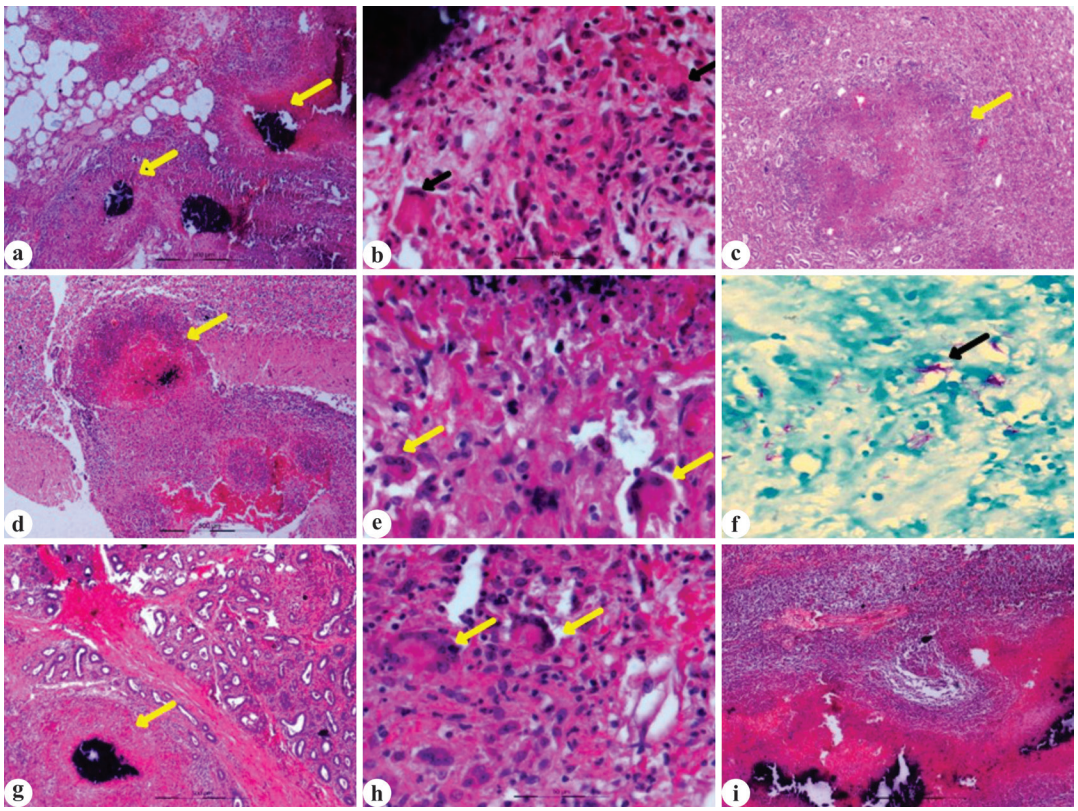

Fig. 2a & b. Lung sections showing multiple granulomatous nodules with central caseo-necrotic areas surrounded by a mixed population of inflammatory cells, including a few multinucleated giant cells (arrow) around the caseo-granulomatous lesions (H&E x100, x400). **c.** Kidney sections exhibiting caseous granulomas (arrow) encircled by a severe inflammatory reaction (H&E x100). **d & e.** Meninges around the cerebellum showing tuberculous granulomas surrounded by multinucleated giant cells (arrow) (H&E x100, x400). **f.** Cerebellum showing few acid-fast bacilli (arrow) in the caseous material (ZN x1000). **g & h.** Mammary gland sections revealing caseous granulomas with multinucleated giant cell formation (arrow) (H&E x100, x400). **i.** Lymph node sections displaying small tuberculous granulomas in the medulla (H&E x100).

from lymph nodes, lung and lesions in other organs were stained with Ziehl-Neelsen (ZN) to detect acid-fast Mycobacteria. Lowenstein-Jensen (L-J) medium containing glycerol and pyruvate were used for bacterial cultural isolation[2]. DNA was extracted from both cultures and tissues and subjected to multiplex PCR. Primers used for amplification of MTBC targeting 16S rRNA, MPB70 gene[6] and specific conventional PCR for species differentiation targeting region of difference (RD9 and RD12) were used[1].

Necropsy examination revealed similar findings in both cases, including pale mucous membranes and a debilitated body condition with a rough hair coat. Internal examination revealed multifocal to coalescing, caseous nodules, varying in size, on the surface and cut surface of the lung parenchyma, accompanied by severe consolidation (Fig. 1a). Upon dissection, these nodules contained caseo-calcified, pale-white material (Fig. 1b). The mesenteric lymph nodes exhibited enlargement with gritty, caseous nodules on the cut surface (Fig. 1c & d) and the hard, indurated udders were partially necrotic and ulcerated (Fig. 1e). The meninges exhibited tuberculous nodules (Fig. 1f), while the uterus showed granulomas in both the endometrium and myometrium. Histopathological examination of the lung sections revealed multiple granulomatous nodules with central caseo-necrotic areas that were surrounded by a mixed population of inflammatory cells, along with a very few multinucleated giant cells around the caseo-granulomatous lesions (Fig. 2a & b). The kidney sections exhibited caseous granulomas surrounded by a severe inflammatory reaction (Fig. 2c). The meninges around the cerebellum contained tuberculous granulomas encircled by multinucleated giant cells (Fig. 2d & e) and the sections of the mammary gland showed caseous granulomas with multinucleated giant cell formation (Fig. 2f & h). Similarly, lymph node sections showed tiny tuberculous granulomas in the medulla (Fig. 2i). The duplicate sections from the lungs, lymph node, brain and mammary gland showed acid-fast bacilli on ZN staining (Fig. 2f). The presence of MTBC was confirmed by culturing the sample on Lowenstein-Jensen (LJ) medium containing sodium pyruvate, which yielded moist, granular colonies. Acid-fast staining of the cultured sample revealed clusters of acid-fast bacilli. Isolated DNA samples were successfully amplified for the 16S rRNA and *mpb70* genes, yielding amplicon sizes of 1030 bp and 372 bp, respectively, which are specific to *Mycobacterium tuberculosis* complex (MTBC) organisms (Fig.3). Additionally, *M. orygis* was identified using specific PCR targeting regions of difference (RD9 and RD12) showed a positive amplification with an amplicon size of 410 bp for RD9 and 264 bp for RD12, indicating infection caused by *M. orygis*.

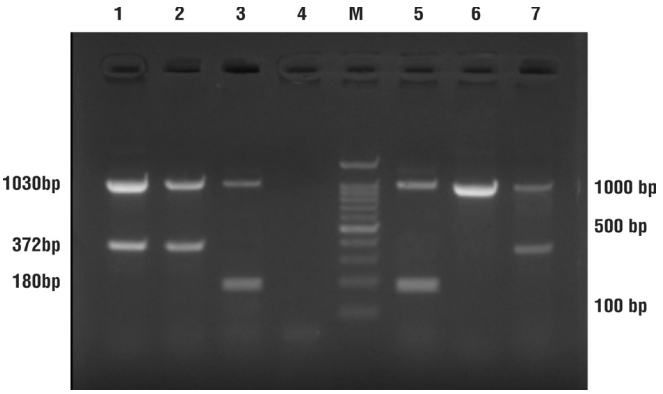

Fig. 3. Agarose gel electrophoresis: Amplification of 16S rRNA and MPB70 gene segment to differentiate MTBC organisms from NTM in tissue samples by conventional multiplex PCR. Lane M: 100 bp DNA ladder. Lane1-2: Sample of cattle lung showing both 372 bp and 1030 bp amplicon specific for MTBC organisms; Lane 3: Positive control showing both 180 bp and 1030 bp specific for *M. avium*; Lane 4:Negative control; Lane 5: Positive control of *M. avium*; Lane 6: Positive control of NTM; Lane 7:Positive control of MTBC

Tuberculosis continues to be a significant airborne disease, affecting humans, domestic and wild animals and birds and causing substantial morbidity and mortality[8-11]. This report describes two cases of generalized tuberculosis in adult Tharparkar cattle caused by *M. orygis*, a member of the *Mycobacterium tuberculosis* complex that is increasingly being recognized. However, an increasing number of cases have now been reported in humans, domestic animals and wild animals[1-3]. This study highlights the capability of *M. orygis* to cause systemic disease in cattle, marked by extensive granulomatous lesions in multiple organs, including the lungs, lymph nodes, udder, uterus, adrenal glands and brain. These findings underscore the severity and widespread pathological impact of *M. orygis* infections in bovine hosts. Histopathological evaluation revealed characteristic granulomas, while advanced molecular tools, including PCR targeting specific regions of difference, confirmed the pathogen, overcoming the limitations of traditional diagnostic methods[10,11]. The finding of this study highlight the growing importance of *M. orygis* as a pathogen in livestock health, particularly in the context of bovine tuberculosis[1,2,8,11]. Its zoonotic potential raises concerns about interspecies transmission, further complicating public health and veterinary control measures. These findings align with prior studies, reinforcing *M. orygis* role in systemic tuberculosis outbreaks in cattle and highlighting its diagnostic challenges. This case report underscores the need for robust surveillance systems, rapid and precise diagnostics and effective intervention strategies to curb the spread of *M. orygis* induced tuberculosis. Such efforts are vital to safeguard livestock, prevent zoonotic transmission and protect public health.

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