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Diagnosis of Bovine TB: Gross Necropsy, Histopathology and Acid-fast Staining

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Diagnosis of bovine tuberculosis (TB) in cattle involves several steps. The result of each test determines if follow-up tests are necessary. Cattle suspected of being infected with bovine tuberculosis (TB) on the basis of results obtained from the caudal fold tuberculin (CFT) and comparative cervical tuberculin (CCT) test are submitted to an appropriate animal diagnostic laboratory for necropsy (or autopsy). During the necropsy, several techniques are used to examine cattle for evidence of infection with bovine TB. These techniques include gross and histological (microscopic) examination for lesions and organisms compatible with bovine TB.

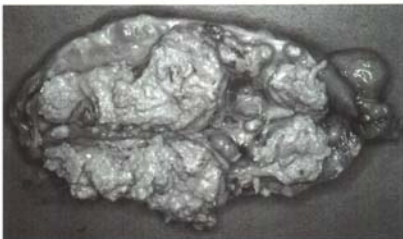
Gross Necropsy

Upon submission, cattle are humanely euthanized and then closely examined by a veterinary pathologist for gross lesions suggestive of bovine TB. The characteristic gross lesion seen in an animal infected with bovine TB is the presence of "tubercles" within the body. A tubercle is a white nodule usually 1 mm to 2 cm in diameter within a lymph node or organ. Tubercles commonly occur in the thoracic cavity (chest), though they may be found in other major organs such as the liver. In cattle, bovine TB most commonly will cause lesions in the lymph nodes of an infected animal. Therefore, during necropsy, the lymph nodes, especially those associated with the head, thorax and abdomen, are closely examined. Many factors contribute to the formation of tubercles. Consequently, an animal may be infected with bovine TB despite the absence of tubercles. Just as importantly, other diseases are capable of causing lesions that are grossly indistinguishable from tubercles. Therefore, finding gross lesions compatible with bovine

TB is not conclusive evidence that the animal is infected with the disease. Further testing — including histopathology, acid-fast staining of tissues, polymerase chain reaction (PCR) and culture — is required to make a definitive diagnosis of bovine TB.



Gross lesions in the lung of a cow with bovine TB.



Gross lesions in the lymph node of a cow with bovine TB.



Throughout the examination, multiple tissue samples are taken and saved in formalin (a preservative) for histopathological (microscopic) examination. If gross lesions suggestive of bovine tuberculosis are found in any of the tissue samples examined, the animal is identified as **lesioned**. Animals in which lesions are not found are classified as **no gross lesions (NGL)**, but are still subject to further, more sensitive testing for *Mycobacterium bovis* (the bacterium that causes bovine TB).

Histopathology and Acid-fast Staining

During the necropsy of cattle suspected of being infected with bovine TB, tissue samples are collected and examined for histopathological (microscopic) lesions that are compatible with *Mycobacterium bovis*. The histologic lesion most commonly associated with bovine TB is granuloma, a collection of inflammatory cells within the suspect tissue. When the animal's immune system recognizes the bacteria within the tissues, special inflammatory cells (macrophages) are sent to dispose of it. *Mycobacterium* is resistant to destruction, and once ingested by the macrophages, it may replicate in and kill the macrophage instead of being killed. The animal's immune system continuously sends more macrophages to help destroy the bacteria, resulting in an accumulation of living and dead macrophages at the site of the bacteria. The accumulation of the living and dead macrophages, bacteria and tissue cells in a focal area comprises the tubercle. Over time, a thick capsule may form around the tubercle, walling it off from other tissues and forming the granuloma.

In addition to looking for specific lesions under the microscope, pathologists can use a special stain to identify organisms that are compatible with *Mycobacterium bovis*, the bacterium that causes bovine TB. This is called an **acid-fast stain**. The cell walls of bacteria belonging to the *Mycobacterium* family contain structural elements for which the acid-fast stain is specific. Following acid-fast staining, bacteria that take up this stain, including *Mycobacterium bovis*, will appear as short red or pink rods when examined under a microscope. The finding of acid-fast-staining organisms in tissues is only suggestive of infection with bovine TB — other bacteria may also take up the acid-fast stain. More definitive laboratory testing is required to make a definitive diagnosis of bovine TB infection.

On the basis of the examination of the tissues for lesions and the acid-fast staining, three possible outcomes can occur:

1. ***Mycobacterium bovis* histopathology non-compatible** — No lesions or acid-fast organisms compatible with *Mycobacterium bovis* are seen.
2. ***Mycobacterium bovis* histopathology suggestive** — Lesions compatible with *Mycobacterium bovis* are seen but no acid-fast organisms are present.
3. ***Mycobacterium bovis* histopathology compatible** — Lesions and acid-fast organisms compatible with *Mycobacterium bovis* are seen.

Regardless of the outcome of the acid-fast staining and histopathological examination, tissues are submitted for further diagnostic evaluation by PCR and bacterial culture. The results of all test findings are used to make a definitive diagnosis of bovine TB.

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