Comparison of diagnostic tests to detect Johne's disease positive animals in western farm goats and range flock sheep

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Johne's disease is caused by *Mycobacterium avium* subspecies *paratuberculosis* (MAP), which infects the mesenteric lymph nodes and intestines of ruminant species. Adults transmit these bacteria to their fetus in utero or young via colostrum, milk and fecal contamination. This disease does not respond to treatment, so control depends on eradication. Since many large western sheep flocks graze on open range, there is further concern about the livestock-wildlife-human interface.

This diagnostic testing was initiated to accurately identify positive animals and their offspring for elimination from the flocks/herds. Because an individual sheep or goat has little economic value, we are searching for the most accurate but cost-effective means to identify a Johne's-positive animal. Range sheep are rarely confined, thus there are few opportunities for sampling during the year. Therefore, we attempted to set up our sampling protocol to find the best samples, the most convenient time, and the most cost effective method for Johne's disease flock/herd-level prevalence testing.

Three antemortem tests for detecting MAP-positive animals are being compared:

- the bovine serology **ELISA** (IDEXX Herdchek) using 0.250 S/P cutoff on sheep and goat serum, plasma and milk samples;
- **culture** of feces, tissues, and buffy coats from EDTA blood tubes, milk and colostrum; and
- the **johnin intradermal skin test** for status of cell-mediated immune response to MAP infection.

An increased sediment inoculum is being used on two culture media: BACTEC™ MGIT™ para TB liquid medium with the fluorometric manual read method; and Herrold's egg yolk agar (HEYA) with or without mycobactin J (to determine if the isolate is MAP or the MAP bovine strain).

Our samples are coming from two cooperator producers. One is a 4000 ewe range flock, and one is a 20 doe farm herd. We have ELISA tested 170 samples from adults and young. At this stage of testing, we have found that the sera/plasma (130) or milk (40) from adults with S/P results greater than 0.300 for milk and 0.400 for sera are all culture positive. Also, the serum sample S/P results from positive dams' lambs or kids range from 0.400 to 1.500 until colostral antibodies wane at 4 months of age. We have cultured 100 samples from adults from tissues and feces of both the farm herd and range flock animals. We see growth in 2 weeks from positive tissues (11) and multibacillary feces (2); and within 10 months from fecals (10) from very low shedders. None of the MAP isolates grow on the initial HEYA medium. The isolates are confirmed as MAP by subculture from MGIT acid-fast positive tubes to HEYA. Two paucibacillary does with serum S/Ps of 2.0 to 3.0 have been identified. These clinical animals were fecal culture positive but with few acid-fast bacteria in their tissues. The johnin intradermal skin test agrees (12/13) with the serology results early in infection, but this test becomes negative as the animal becomes clinical (5/11). The johnin test is more easily used on a farm goat herd than a large range sheep flock. At certain times, milk is a more easily collected sample than serum, plus the milk pellet can be cultured. **The ELISA is the most expedient test for both the serum and milk samples from adults and for serum from offspring less than three months of age.**

As we identify individual positive animals, owners donate these animals. The animal is eventually euthanized, necropsied, and sampled for culture and histopathology. Johne's disease is only one cause of wasting in small ruminants; we also test for other diseases. The sheep flock is positive for OPP virus; the goat herd is negative for CAE virus. Fecal samples are also tested for intestinal parasites. Generally, MAP positive animals tend to have high parasite loads that never clear after repeated anthelmintic treatments.