Question:

Why do AHVLA, Defra and the Welsh Government recommend that the serological (antibody) tests for TB in camelids should be performed 10-30 days after the tuberculin injections?

Response:

The fundamental reason for the use of a serological (antibody) test in conjunction with the tuberculin skin test is to yield an improvement in the overall performance of the combined testing system. In TB breakdown situations the focus is necessarily on seeking an improvement in the relatively low sensitivity of the tuberculin skin test when used alone in South American camelids.

The boosting of serum antibody responses and the consequent increase in the *sensitivity* of antibody tests that occurs following the administration of tuberculin to cattle has also been identified in camelids. This is known as the anamnestic effect. In a study reported by Dean et al. (2009), Stat Pak (Rapid) test results were available for samples taken both prior and three weeks after tuberculin testing in six llamas. Of the five llamas in this cohort that were *Mycobacterium bovis* culture positive, two animals yielded negative results to testing of the pre-tuberculin test samples, but were seropositive when the StatPak test was performed on the post-tuberculin test samples.

In another study by Bezos et al. (2013) of a herd of Suri & Huacaya alpacas (age range 1 to 10 years) naturally infected with *M. bovis* in central Spain, blood sampling at 15, 30 or 42 days after tuberculin injection consistently improved the sensitivity of an antibody assay for TB, relative to blood samples taken on the day (0) of the injection. This positive effect was observed during three separate skin testing events of the same herd (January, March & June 2012). The number of culture-positive animals tested at each event varied from 7 to 39.

These numbers are clearly small, as with most studies in South American camelids. However, they represent the best data currently available for camelids. Given the fact that this is a well-recognised phenomenon that applies to bovine TB serology in general and across TB-susceptible species, it would be foolish for this anamnestic effect to be ignored in situations where we are trying to optimise test sensitivity (i.e. the proportion of infected animals detected by the test).

This 'priming' or anamnestic effect associated with the prior administration of tuberculin will not be instantaneous and can be expected to wane over time. The recommended antibody testing window of 10 to 30 days post-tuberculin testing is intended to both allow time for this effect to become established and to ensure that samples are taken before the maximum benefit associated with this effect is lost. It is a guideline designed to optimise the performance of the antibody tests, but like most biological processes it is not an absolute range.

The potential impact of tuberculin test 'priming' on the *specificity* of serological tests in TB-free animals (i.e. induction of false positive results) is also of interest outside TB breakdown

situations, such as private routine surveillance or pre-movement TB testing. We have not been able to assess this directly in camelids, because samples from skin-tested animals from unrestricted TB-free herds have not been available. None of the TB-free alpacas tested during the BAS-funded study carried by AHVLA in 2011-12 had received a skin test (Rhodes et al. 2012). Even so, analysis of data generated with sera from alpacas on premises with confirmed *M. bovis* infection in GB (under the conservative assumption [worst-case scenario] that all the non-visible-lesion seropositive animals in those herds were false positives) does not suggest that the specificity of the StatPak antibody test is substantially different between animals that undergo prior skin testing and those that do not. For information the specificity of the Statpak test on its own, as determined in the recent BAS-funded study was 97.4 % (95% CI: 95.6% - 99.2%). We have also consulted with Chembio, who developed and market the StatPak test, and they do not have any evidence that the test specificity is negatively affected by tuberculin skin testing of TB-free animals.

In summary, in herds with confirmed or suspected *M. bovis* infection, priming of the antibody TB tests with a tuberculin skin test conducted 10-30 days prior to blood sampling is essential for optimal performance, i.e. to maximise sensitivity (detection of infected camelids). For routine screening of presumed TB-free herds, priming of the antibody tests is recommended. Although this will add to the cost and complexity of TB testing, it should help identify any undisclosed infected animals in those herds without impacting negatively on the test specificity.

References:

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