

# **The anamnestic boost effect of the skin test on antibody responses to *Mycobacterium bovis* in camelids – summary of evidence**

## **Issue**

Defra and the Welsh Government require that statutory government-funded serologic (antibody) TB testing of camelids must be performed 10-30 days after the tuberculin skin test injections. In the case of *private* antibody testing, the tuberculin injection is strongly recommended, but is ultimately the animal owner's choice.

## **The evidence**

The fundamental reason for the use of serum antibody testing in conjunction with the tuberculin skin test is to improve the overall sensitivity (i.e. the proportion of infected animals correctly identified as test-positive) of the combined testing system. In TB breakdown situations our primary aim is to increase the relatively low sensitivity of the tuberculin skin test in South American camelids so that the probability of detecting and removing all infected animals are maximised before the herd restrictions are lifted.

An increase in the sensitivity of antibody tests associated with a rise in serum antibody responses after intradermal administration of tuberculin has been identified in tuberculous camelids and other species. In scientific papers this is referred to as the anamnestic boost effect, commonly known as skin test 'priming'. The first description of this effect in camelids was in a small Canadian study using six llamas experimentally infected with *Mycobacterium bovis* and two non-infected controls (Stevens et al. 1998). The animals were skin tested with bovine tuberculin only at 80 and 143 days post-infection and their antibody responses were monitored regularly by ELISA up to the point of euthanasia. The authors concluded that without a prior skin test, the infected llamas responded poorly to the antigens used in the serological test. One of the two negative controls gave a weak anamnestic antibody response.

In a study reported by Dean et al. (2009), Chembio StatPak test results were available for serum 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) in a mixed 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.

Clearly, as with most studies in South American camelids the numbers of animals studied were small. However, they represent the best data currently available for camelids and the various studies show a consistent pattern. The antibody boosting effect is a well-recognised phenomenon that has been described in tuberculous cattle and in other species susceptible to infection with *Mycobacterium bovis* (Waters et al. 2006, Casal et al. 2014, Waters et al. 2014a, Waters et al. 2014b, Waters et al. 2015, O'Brien et al. 2017). In light of this evidence, it would be unwise to ignore the benefits of 'priming' serologic tests with the tuberculin skin test in situations where we are trying to maximise diagnostic sensitivity, such as when testing camelid herds with confirmed *M. bovis* infection or at risk of infection following the introduction of animals from known TB-infected herds (tracings).

The anamnestic boost effect can be observed from 1-2 weeks to several months after tuberculin injection, depending on the type and dose of tuberculin, the host species, stage of infection, format of the antibody test used and other factors (Waters et al. 2014a, Waters et al. 2014b). In cattle, the boosted antibody responses wane beginning ~1-2 months after the injection of tuberculin, although they can be further increased by subsequent injections (Waters et al., 2015). Thus, in contrast to skin test reactivity, repeated administration of tuberculin enhances rather than dampens subsequent antibody responses. The window for antibody testing recommended by APHA (10 to 30 days after tuberculin injection) 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 the booster effect starts to wane. It is a practical guideline designed to optimise the performance of the antibody tests in the field, but like most biological processes it is not an absolute range and there will be individual random variation.

The negative impact of the administration of tuberculin on the specificity of antibody tests in TB-free animals (i.e. the likelihood of false positive results) is a potential concern when antibody tests are used outside TB breakdown situations, such as private routine surveillance or pre-/post- movement testing. It has not been possible to assess this effect directly in camelids yet, due to lack of samples from skin-tested animals in unrestricted, TB-free herds. None of the TB-free alpacas tested during the BAS-funded study carried by APHA 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) did not suggest that the specificity of the StatPak antibody test was 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 BAS-funded study, was 97.4 % (95% CI: 95.6% - 99.2%). We have also consulted with Chembio, who developed and marketed the StatPak test, and they do not have

any evidence that the test specificity is negatively affected by prior tuberculin skin testing of TB-free animals.

Interestingly, in a study conducted in the USA no boost in antibody responses could be detected in TB-free cattle after intradermal administration of tuberculin in the caudal fold and comparative cervical tests (Waters et al. 2015). This result cannot be directly extrapolated to camelids, but it constitutes a useful precedent.

## **Conclusion**

In herds with confirmed or with a strong suspicion of *M. bovis* infection, priming of the antibody TB tests with a tuberculin skin test conducted 10-30 days before blood sampling is essential for optimal performance of those tests, i.e. to maximise the overall sensitivity (probability that an infected animal is classified as positive by the test). For private routine screening or pre-movement testing of presumed TB-free herds, skin test priming of the antibody tests is recommended. Although this increases the cost and complexity of testing, it helps identify any undetected infected animals present in those herds without impacting negatively on the test specificity.

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