The anamnestic boosting effect of the skin test on antibody responses to *Mycobacterium bovis* in camelids – summary of the 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 in England, 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 the primary aim is to increase the low sensitivity of the tuberculin skin test in South American camelids, so that the likelihood of removing all infected animals is 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 injection of tuberculin has been identified in tuberculous camelids and in other species. In scientific papers this is referred to as the anamnestic (immunological memory) 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* positive on culture, 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, Roupie et al. 2018). 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 boosting effect can be observed from one to two weeks following tuberculin injection and can persist for up to several months, 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, Roupie et al. 2018, Lyashchenko et al. 2020). In cattle, the boosted antibody levels wane beginning ~1-2 months after the injection of tuberculin, although they can be further increased by subsequent injections (Waters et al., 2015, Lyashchenko et al. 2020). 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 random individual variation.

The possibility that prior tuberculin skin testing could have a detrimental effect on the specificity of antibody tests and lead to false positive results in presumed TB-free animals has been raised as a potential concern for some camelid keepers. However, several studies carried out in ruminants (cattle and cervids) have failed to detect such an effect (Lyashchenko et al. 2020).

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 (base of the tail) and comparative cervical tests (Waters et al. 2015).

Similarly, a study carried out at the Institute for Animal Health, UK (Thom et al. 2004), stated that antibody responses were not evident as a result of repeat skin testing prior to experimental infection with *Mycobacterium bovis*, but were seen at seven weeks post-infection following a skin test.

In an experiment performed in Belgium with six bulls naturally exposed to *M. avium* subsp. *paratuberculosis*, a dramatic rise in MPB70/MPB83 specific antibody titres measured by IDEXX ELISA took place in all four of the bulls that were experimentally infected with *M. bovis*. This antibody boost was observed 10-15 days after the injection of bovine tuberculin. By contrast, in the two uninfected (control) bulls the increases in antibodies after tuberculin skin testing were much more modest.

In Spain, repeated comparative skin testing of TB-free red deer at a six-month interval confirmed that it did not affect serological responses to *M. bovis* measured by ELISA (Che-Amat et al. 2016). Results in cattle and deer cannot be directly extrapolated to camelids, but do constitute useful precedents from other artiodactyl species that are related to camelids.

It has not yet been possible to experimentally assess the effect of tuberculin injections on antibody responses in TB-free camelids, due to a lack of samples from skin-tested animals in unrestricted 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 antibody testing was substantially different between animals that underwent prior skin testing and those that do not.

Furthermore, the evidence gathered so far through mandatory field antibody testing of camelid herds in GB does not support the hypothesis that tuberculin 'priming' is a common cause of false test positive results. In 2020 the Animal and Plant Health Agency (APHA) blood tested 581 camelids in England and Wales that were co-located with or contiguous to TB-infected cattle herds. All those animals received an injection of bovine (and in some cases also avian) tuberculin 10-30 days before blood samples were collected for 4-spot Enferplex or combined serial DPP and IDEXX testing in an APHA laboratory. Eleven (i.e. 1.9%) of those animals were deemed TB seropositive and removed. And since it is quite possible that a proportion of those 11 animals were truly infected with *M. bovis*, the proportion of false positive results was even lower.

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) and thus avoid missing infected animals.

For private routine screening or pre-movement testing of presumed TB-free herds, skin test priming of the antibody tests is strongly recommended. Although this increases the cost and complexity of TB testing, it helps identify any undetected infected animals present in those herds without impacting negatively on the test specificity.

Dr Ricardo de la Rua-Domenech, APHA Veterinary Adviser to Defra's Bovine TB Programme

Dr Shelley Rhodes, TB test consultant, APHA Weybridge

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