

The anamnestic boost 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, 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, 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 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, Roupie et al. 2018). 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 random individual 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 fully assess this effect in camelids yet, due to lack of samples from skin-tested animals in unrestricted, presumed 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. I

If the injection of tuberculin triggered so many non-specific antibody responses in TB-free camelids as some claim, then the serum samples from camelid herds contiguous to infected cattle herds that are tested by APHA with the 4-spot Enferplex or the combined serial DPP and IDEXX tests, 10-30 days after priming with tuberculin, would yield a much higher test positivity rate than what we actually see in the lab (only 2.3% of such samples in 2017).

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 (base of the tail) and comparative cervical tests (Waters et al. 2015). In an experiment performed in Belgium with six bulls naturally exposed to *M. avium* subsp. *paratuberculosis*, a dramatic increase in MPB70/MPB83 specific antibody titres measured by IDEXX ELISA was observed in all four *M. bovis* experimentally-infected bulls 10-15 days after injection of bovine tuberculin. By contrast, in the two uninfected (control) bulls the increases in antibodies after tuberculin skin testing were much more modest. These results cannot be directly extrapolated to camelids, but constitute useful precedents.

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.

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November 2013
(last update: March 2018)

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References

Bezoz J, Casal C, Álvarez J, et al. (2013). Evaluation of the performance of cellular and serological diagnostic tests for the diagnosis of tuberculosis in an alpaca (*Vicugna pacos*) herd naturally infected with *Mycobacterium bovis*. *Preventive Veterinary Medicine* 111: 304-313.

Casal C, Diez-Guerrier A, Alvarez J, Rodriguez-Campos S, Mateos A, Linscott R, Martel E, Lawrence JC, Whelan C, Clarke J, O'Brien A, Dominguez L, Aranaz A (2014). Strategic use of serology for the diagnosis of bovine tuberculosis after intradermal skin testing. *Veterinary Microbiology* 170 (3-4): 342-351

Dean, GS, Crawshaw TR, de la Rua-Domenech R, Farrant L, Greenwald R, Higgins, RJ, Lyaschenko K, Vordermeier HM & Twomey F (2009). Use of serological techniques for diagnosis of *Mycobacterium bovis* infection in a llama herd. *Veterinary Record* 165: 323-324.

O'Brien A, Whelan C, Clarke JB, Hayton A, Watt NJ, Harkiss GD. (2017). Serological analysis of tuberculosis in goats by use of the Enferplex Caprine TB multiplex test. *Clinical and Vaccine Immunology* 24(2). e00518-16.

Rhodes S, Holder T, Clifford D, et al. (2012). Evaluation of gamma Interferon and antibody tuberculosis tests in alpacas. *Clinical and Vaccine Immunology* 19(10): 1677-1683.

Roupie V, Alonso-Velasco E, Van Der Heyden S, et al. (2018). Evaluation of mycobacteria-specific gamma interferon and antibody responses before and after a single intradermal skin test in cattle naturally exposed to *M. avium* subsp. *paratuberculosis* and experimentally infected with *M. bovis*. *Veterinary Immunology and Immunopathology* 196: 35-47.

Stevens JB, Thoen CO, Rohonczy EB, Tessaro S, Kelly HA, Duncan JR (1998). The immunological response of llamas (*Llama glama*) following experimental infection with *Mycobacterium bovis*. *Canadian Journal of Veterinary Research* 62: 102-109.

Waters WR, Palmer MV, Thacker TC, et al. (2006). Early antibody responses to experimental *Mycobacterium bovis* infection in cattle. *Clinical and Vaccine Immunology* 13(6): 643-654.

Waters WR, Maggioli MF, McGill J, Lyashchenko KP, Palmer MV (2014a). Relevance of bovine tuberculosis research to the understanding of human disease: Historical perspectives, approaches and immunologic mechanisms. *Veterinary Immunology and Immunopathology* 159: 113-132.

Waters WR, Thacker TC, Nelson JT, et al. (2014b). Virulence of two strains of *Mycobacterium bovis* in cattle following aerosol infection. *Journal of Comparative Pathology* 151: 410-419.

Waters WR, Palmer MV, Stafne MR, et al. (2015). Effects of serial skin testing with purified protein derivative on the level and quality of antibodies to complex and defined antigens in *Mycobacterium bovis*-infected cattle. *Clinical and Vaccine Immunology* 22(6): 641-649.