



## A Modified Comparative Cervical Test: A Preliminary Evaluation S Hutchings, C Mackintosh, P Johnstone

### Introduction

As defined in the Deer Tuberculosis Veterinary Testing Officer Manual section 3TS 2.2 the standard comparative cervical test (CCT) involves the injection of 0.1ml of avian PPD tuberculin (25,000 IU/ml, normally equivalent to 0.5mg/ml) intradermally in the middle of the anterior/dorsal site on the side of the neck, and injection of 0.1ml bovine PPD (50,000 IU/ml, normally equivalent to 1mg/ml) intradermally in the posterior/ventral site. The two sites in the middle of the side of the neck shall be carefully prepared so that the hair is evenly clipped over the entire area to a mean length of  $\leq 2$ mm. Each site should be free of visible lumps and scars that could interfere with interpretation of the test. The middle of one site must be a minimum of 12cm from the middle of the second site. Prior to injection the skin fold thickness is measured in the middle of each site to the nearest 0.5mm using either electronic or vernier type caliper.

The test is read  $72 \pm 6$  hours later by remeasuring both the avian and bovine sites and subtracting the original skin fold thickness from the read day measurement, to give a net gain in skin fold thickness resulting from reaction to the tuberculin.

A deer is considered test positive if the *difference in skin fold thickness* at the bovine site is  $\geq 2$ mm **and** is  $\geq$  the *difference in skin fold thickness* at the avian site.

In the field this test is time-consuming and expensive when performed strictly to the above specifications. An alternative may be to measure the skin fold thickness between the two sites (i.e. a mid site measurement) on read day to substitute for the original skin fold thickness measurements at both the avian and bovine sites. For the purposes of this paper this proposed alternative test is defined as the modified CCT.

A trial was designed to examine whether the mid site between avian and bovine sites, or alternatively sites above or on either side of the avian and bovine sites measured on read day (72 hours post injection) could be used to represent the original skin thickness measurements.

### Aims

1. To find if skin fold thickness measured at the avian and bovine sites (for the CCT) on test day can be substituted by measurements taken around these sites on read day ( $72 \pm 6$  hours later).

2. Assuming these measurements give good approximation of original avian and bovine sites to find which individual or combination (average) of these measurements gives the best substitution of each site skin thickness.
3. To find if these measurements work in other age groups and sexes.

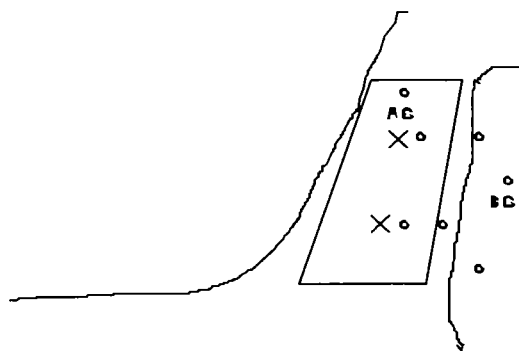
### Method

Thirty mixed aged hinds and thirty weaner deer were shaved on the side of their necks with Oster No. 40 blades. An area of approximately 30 cm long by 18 cm wide, from just below the jaw down to the shoulder and from the caudal ridge to the jugular groove was used.

Nine skin fold thickness measurements were taken prior to the injection of CSL avian and bovine tuberculin intradermally at the avian site (AS) and bovine site (BS) (Figure 1). Each of the sites was marked with permanent ear tag. They were approximately 5 cm apart and run on the horizontal and vertical axes of the neck. Measurements were recorded as;

- AA = Above Avian site
- AL = Left of Avian site
- AS = Avian site (marked x)
- AR = Right of Avian site
- AB = Below Avian site = BA Above Bovine site (i.e. mid site, between the two)
- BL = Left of Bovine site
- BS = Bovine site (marked x)
- BR = Right of Bovine site
- BB = Below Bovine site

**Figure 1. Diagram of site placements**



A metal vernier type caliper was used to measure the skin thickness. This was done by gently tenting a double skin thickness between the caliper teeth and sliding the caliper of the skin. The measurement was read to the nearest 0.1 mm. Each of these measurements was redone 72  $\pm$  6 hours later and recorded in the same way as on test day.

## Results

The data was analysed and means were calculated for each measurement over both age groups. The differences between test day and read day measurements were also calculated. The figures in brackets represent the difference between read day bovine and avian site means and the read day AB or mid site means. Table 1 shows how these results are laid out.

**Table 1. Layout of Skin Thickness Measurement**

	BL		AL		
BB	BS	AB	AS	AA	Head
	(Read BS - Read AB)		(Read AS - Read AB)		
	BR		AR		

**Table 2. Weaner Deer Data: Summary of skin thickness means**

**Test**

	3 003		3 190	
2.780	2 913	3.073	3.167	3 400
	2.930		3.233	
SED 0 062				

**Read**

	3 467		3 673	
3 213	3.640	3 480	4 373	3 813
	3 443		3 740	
SED 0 101				

**Difference**

	0 453		0 483	
0 433	0 727 (0 16)	0 407	1 207 (0 8933)	0 413
	0 513		0 507	
SED 0 110				

**Table 3. Mixed Age Hind Data: Summary of skin thickness means**

**Test**

	3 531		3 679	
3 417	3 672	3 841	3 766	3 659
	3 562		3 717	
SED 0 081				

**Read**

	3 576		3 738	
3 517	4 179	3 852	5 979	3 766
	3 659		3 866	
SED 0 141				

**Difference**

	0 045		0 059	
0 100	0 507	0 010	2 214	0 128
	(0 328)		(2 128)	
	0 097			
SED 0 149				

**Discussion**

1. For the weaner deer there was a highly significant thickening of the skin on test day as the measurement progressed towards the head. For the mixed age hinds the trend was for the skin to be thicker at the mid site (AB) than both the bovine and avian ends of the neck (see table 2 and 3). However, the evidence is that both these trends were largely canceled when the difference between the test and the read result is taken. (0.433 vs 0.413, SED 0.110 in the weaners and 0.100 vs 0.128, SED 0.149 in the mixed age hinds.)
2. There were no significant differences between left side and right side measurements although the average right side differences (test minus read values 0.51, 0.123) were higher than left side differences (0.468, 0.052). This could either be the effect of gravity on the oedema created in reaction to injection of tuberculin, or that the skin is tighter and more difficult to measure near the jugular groove, creating a bias.
3. Average differences between the AB (mid site) on read day and the avian and bovine sites on test day are:

	AB site read-AS site	AB site read-BS site	SED
WEANERS	0 313	0 567	0 101
M A HINDS	0 086	0.180	0 141

In the weaners the overall skin got significantly thicker on read day at all the sites, possibly as a reaction to clipping or to the tuberculin. This effect is not as pronounced in the mixed age hinds.

4. Average differences between AB (mid site) and avian and bovine sites (i.e. modified CCT) at reading were:

	Avian site - AB site	Bovine site - AB site	SED
WEANERS	0 893	0 160	0 101
M A HINDS	2 127	0 327	0 141

5. The average differences between the read and test at avian and bovine sites are higher than in No. 4 so the standard CCT results in larger differences between sites than using the mid site as in the modified CCT. This effect is more pronounced in the weaners.

	Avian site	Bovine site	SED
WEANERS	1 207	0 727	0 110
M A HINDS	2 214	0 507	0 149

In both these methods a large difference is seen at the avian site especially in the mixed age hinds. This property had a significant non specific reaction problem.

6. The number of times the difference between the read and the test sites for avian and bovine (i.e. standard CCT) is larger than the difference between the read sites and the read AB site (modified CCT) is given below.

	Number of cases	Read avian site(AS) minus test avian site is larger than read avian site minus read AB site	Read bovine site (BS) minus test bovine site is larger than read bovine site minus AB site
WEANERS	30	26	26
M A HINDS	29	18	16

7. In both sets of data a number of read minus test differences which were positive (i.e.  $\geq 2$  mm) with the standard CCT became negative ( $< 2$  mm) using the mid site difference (i.e. modified CCT) at reading.

WEANERS	2 avian sites became negative
M A HINDS	1 avian site became negative and 1 became positive 1 bovine site became negative

8. There are significant differences between the two age groups in these results. This is without considering between-sex and indeed between-farm differences.

### Comments

The skin is significantly thicker at the head end (i.e. towards avian and AA site) in the weaners and thicker at the mid site in the mixed age hinds. This difference is cancelled out in the standard CCT with the difference taken between read and test. However, if at the time of reading another 'read' site is taken to subtract from the avian or bovine site it will bias the results. Similarly, if there is any difference between left and right sites (not proven here) the effect would also bias the results. This effect (i.e. the measuring of sites to the left or right or above avian and below bovine) is smallest when the mid site (AB) is

used. The use of read mid site results to substitute test day avian and bovine sites was shown to have larger differences in the weaners than in the mixed age hinds.

The two sets of data both showed that the average mid site differences from avian and bovine sites at reading were significantly lower than the average differences between read and test at avian and bovine sites, and a disturbing number of measurements changed from positive to negative using  $\geq 2$  mm as positive and  $< 2$  mm as negative.

This is effectively reducing the sensitivity of the test.

The other disturbing feature is the between-age variation in results. This highlights the possibility that even greater variation could be found if deer of different gender, state of pregnancy, time of year (season) etc. as well as different farms were included in the trial.

### **Conclusion**

The use of other sites at reading to estimate skin thickness differences at bovine and avian sites at test and reading is variable in its results and therefore unreliable and may reduce the sensitivity of the CCT.

Of the different sites analysed the mid site was found to be the most useful. However, this effect was only seen in the mixed age hinds and not the weaners, because of the overall thickening of the skin on read day in this younger group of animals.

This study was undertaken on a limited number of animals only, and only on one property. It is therefore only a preliminary study. Future work needs to be done using mid site only in comparison to avian and bovine sites as this will be more relevant and make the design of the study much simpler. Larger groups of deer of different ages, gender and times of the year need to be used on a number farms around the country.

At present the use of the modified CCT does not comply with the specifications described in the deer Tb Testing Quality Assurance Scheme. This test has not been approved by the Chief Veterinary Officer (CVO) under the Pest Management Strategy of the Biosecurity Act and so should not be used by Deer Tb Testing Officers in the field.

### **Acknowledgments**

I would like to acknowledge the Deer Branch of the NZVA for awarding their study grant to fund this study and also the staff at Wairakei Deer Park for their assistance in the data collection.