'TUBERCULOSIS - TEST AND SLAUGHTER'

N. S. BEATSON

J. B. HUTTON

G. W. de LISLE

INTRODUCTION:

Tuberculosis (Tb) caused by Mycobacterium bovis (M.bovis) was diagnosed for the first time in New Zealand farmed red deer Cervus elaphus by Lincoln and Wallaceville Animal Health Division (AHD) Laboratories in 1978. One of the first properties involved was located in the Mid Canterbury area. Tuberculin testing in this herd commenced in September 1978 and continued until the remainder of the whole herd was slaughtered in 1983.

This paper gives a preliminary report on the results of the test and slaughter programme adopted in this red deer herd.

BACKGROUND:

The property farmed in partnership by a father and son was located in the foothills of Mid Canterbury and farmed in conjunction with their sheep breeding and fattening unit on 400 hectares. The area deer fenced was approximately 40 hectares divided into 9 paddocks. In 1976 they started farming deer by purchasing a small number of hinds. In 1977 an agistment agreement was entered into and a number of stags and hinds were placed on the property. The agisted deer all came from Rotomanu, West Coast.

In September 1978 one aged hind (ex West Coast) with a swelling behind the shoulder was examined by their Veterinary practitioner. This on lancing, appeared to be a simple abcess fortunately a swab was taken and on Ziehl Neelsen (Z.N.) stain proved positive, so the hind was immediately slaughtered. Necropsy revealed generalised tuberculosis throughout the abdominal and thoracic cavities. Culture confirmed M.bovis.

As a result of the discovery of Tb and in an attempt to determine the extent of spread within the deer herd 'experimental' tuberculin testing commenced on the 22 September 1978. Little was known about cervine tuberculosis in New Zealand and other parts of the world, and nothing on testing of red deer. (1, 2, and 3). Naturally the initial testing technique was somewhat 'trial and error!' However it was soon established that the mid-cervical site gave the most 'consistant' results and was the test used from 1979 onwards. Voluntary tuberculin testing was continued until March 1981 when a special project was started and this was continued for 2 years until March 1983, when the remainder of the herd was slaughtered.

OBJECTIVES OF THE PROJECT:

1. To establish if routine test and slaughter using 2 mg/ml tuberculin at the mid-cervical site can control and eradicate M.bovis from a farmed red deer herd.

2. To determine whether the bovine comparative cervical test (CCT) using 1 mg/ml CSL bovine and 0.5 mg/ml Weybridge avain tuberculin has any application in a Tb infected farmed red deer herd.

3. Monitor the efficiency of the tuberculin test in an infected farmed red deer herd.

TUBERCULIN TESTING:

Facilities - an excellent yarding system and handling pen made the task of testing easier. The handling shed was covered and darkened and the crush pen measured 1.5m x 2m. The deer were all tested in this pen 'free range' with the minimum of restraint being used.

Timing of the test - testing was aimed at a minimum interval of 60 days. At certain times of the year it was not practical to test and during these periods the interval was longer than 60 days. As a general rule the adult hinds were tested five times, the adult stags four times and the weaner stags and hinds six times in any one year. Where possible testing was done as a whole herd but at times this was not always practical (during the roar it is possible to test the hinds but not the stags). The property had excellent double fenced tree lines so where part herd testing was carried out adequate isolation was possible.

Tuberculin - In some of the earlier tests 1 mg/ml tuberculin was used from 1981 onwards 2 mg/ml tuberculin was used (CSL bovine PPD).

For the CCT 1 mg/ml CSL PPD bovine and 0.5 mg/ml Weybridge PPD avian was used.

Site - In all but the initial test on 22 September 1978 deer were injected in the mid-cervical site. At the initial test a number of deer were injected in the tail. At each subsequent test the side of the neck alternated so that no two consectutive tests were carried out on the same side.

Site Preparation - Several methods were used but the most sucessful was electric clippers. It was found that scissors tended to produce a more uneven surface and when it came to reading the test interpretation was made more difficult. The noise of the electric clippers was a disadvantage but was worthwhile putting up with for the improved injection site. The area clipped was approximately 8 cm x 8 cm with the hair clipped as close to the skin as possible.

For the CCT two 8 cm x 8 cm areas were clipped 10 cm apart and then a smaller area 3 cm x 3 cm was shaved and the injections made into the shaved areas.

Testing Procedure - Prior to injection, double skin thickness to the nearest mm using callipers was measured and recorded, then 0.1 ml of tuberculin injected intradermally using a dental syringe.

After 72 hours the injection site was observed, palpated, double skin thickness measured and results recorded.

Reactors - Any swelling or change in skin thickness which could be palpated was taken as a reactor. A detailed description of the reaction, was made and recorded. Any deer which showed clinical signs of Tb, notably loss of weight or obvious abscesses was identified and slaughtered along with the tuberculin reactors. All reactors were permanently identified by a metal ear tag. They were then held in isolation until a CCT was completed, and slaughtered. (Not all reactors were reteated with the CCT).

Comparative Cervical Testing - At a later stage of the testing programme in an effort to increase our knowledge on the test a number of tuberculin reactor deer were retested using the standard CCT for cattle. This test was initially applied immediately on the reading day of the primary test but a number were also done up to 21 days later. The test was applied on the opposite side of the neck and read at 72 hours. Following this all reactors to the primary test were slaughtered irrespective of the CCT result.

AUTOPSY METHOD:

Reactors - Where possible P.M. was carried out immediately following the reading of the test. Where large numbers of reactors were involved there was a slight delay but never more than one week.

Non reactors - All deer that died on the property were subjected to a critical necropsy examination.

All deer that were slaughtered at Deer Slaughter Premises (DSP), were subjected to the normal Meat Divison examination, followed by a detailed examination as described below.

Briefly all lymph nodes (ln's) from the deer were carefully incised and examined for gross Tb lesions. Where lesions were seen, fresh and fixed tissues were collected if no lesions were seen ln's were pooled into four groups: head, body, thorax and abdomen for later cultural examination at Wallaceville AHD Laboratory. Lungs, liver, kidney, tonsils and spleen were also examined and incised. For each necropsy examination a detailed report was completed indicating site and lesion description.

RESULTS:

Tuberculin Testing - A summary of the testing history is seen in Table I. A total of 3620 individual animal tests were done at 29 different testing episodes and resulted in 107 reactors being identified and slaughtered. 82 (76.6%) of these reactors showed gross lesions consistant with a diagnosis of Tb.

TABLE I

TUBERCULIN TESTING HISTORY

Test Date	No. Tested	No. Reactors	Necropsy	Tb	NGL
22.9.78	106	1		-	1
8.12.78	28	3		3	-
21.5.79	261	5		2	3
8.7.79	18	0		-	-
3.8.79	224	0		-	-
8.2.80	105	1		1	-
23.6.80	189	0		-	-
10.7.80	98	0		-	-
5.9.80	273	1		1	-
3.11.80	168	1		1	-
25.1.81	144	10		7	3
19.3.81	194	26		19	7
18.6.81	159	4		4	-
22.6.81	121	4		2	2
20.8.81	115	2		2	-
21.8.81	153	8		7	1
22.10.81	132	15		14	1
18.12.81	61	4		4	-
22.1.82	104	7		5	2
25.2.82	159	10		5	5
3.5.82	145	0		-	-
28.5.82	89	4		3	1
19.8.82	57	1		1	-
10.9.82	110	0		-	-
8.11.82	106	0		-	-
10.1.83	54	0		-	-
1.2.83	43	0		-	-
28.2.83	33	0		-	-
7.3.83	117	0		-	-
TOTAL	3620	107		82	25

Non reactor deer slaughtered at DSP's - a line of 47 mixed aged stags was slaughtered at the Hokitika DSP (Stag Corp) in June 1982. No gross lesions of Tb were seen but the culture of pooled ln's isolated M.bovis in 11 (23%).

In January, February and March 1983 a further 194 mixed sex and age deer (whole herd) was slaughtered at Ashburton DSP (Canterbury Venison). Gross lesions consistent with a diagnosis of Tb were seen in 6 (3%) deer with a further 12 (6%) having suspicious lesions. Histology and culture confirmed the diagnosis in 4 (2%) of these gross lesion deer. Another 7 (3.5%) were culture positive. (These were not the deer that had gross or suspicious Tb lesions). Of the total of 241 deer slaughtered M.bovis was recovered from 22 (9%). For details of these results refer to Table II.

TABLE II

Post mortem and culture findings from 241 deer slaughtered immediately after negative tuberculin test.

DATE		NO. SLAUGHTERED	TYPE	POST MORTEM			HISTOLOGY	CULTURE
Tested	Slaughtered			Susp/Tb/NGL				
28.5.82	1.6.82	47	M.A. Stags	1	0	46	0	11
10.1.83	12.1.83	21	M.A. Stags	1	0	20	0	0
1.2.83	9.2.83	23	18 M.O. Stags	2	0	21	0	0
28.2.83	3.3.83	33	M.A. Stags	3	0	30	0	2
7.3.83	9.3.83	35	Weaners	4	2	29	1	2
7.3.83	14.3.83	30	1-2 Y.O. Hinds	0	0	30	0	1
7.3.83	17.3.83	52	M.A. Hinds	2	4	46	4	6*
Totals		241		13	6	222	5	22

* Culture results still being processed at 1.6.84.

Clinical condemnations - During the testing there was a total of 12 clinicals identified, and lesions consistent with Tb were seen in 6 (50%) of these. Ten of these deer were identified at the completion of a test. There were two which were identified by the farmer some time after passing a test.

Pathology - Lesions were rarely calcified to the extent seen in cattle. They usually had a gross appearance of an abscess with a soft or semi-liquid central zone and a thin outer fibrous capsule. These abscesses may spread via the blood stream to produce a large number of abscesses throughout various organs e.g. the lung - or may enlarge to 5-10 cm in diameter. Occasionally lesions were seen on the pleura adjacent to a lung lesion and some of these spread through the thoracic wall to form a subcutaneous abscess. The characteristic microscopic lesion of deer Tb is the tubercle, starting as a cluster of neutrophils surrounding the invading bacteria. These become necrotic and are replaced and surrounded by macrophages (epithelioid cells). The bacteria produce toxic substances and more necrosis occurs with more epithelioid granulation around the central necrotic area. In this outer zone macrophages coalesce to form Langhan's giant cells. These have a large central zone of acidophilic cytoplasm and an outer ring of nuclei and often engulf the Tb organisms. In the outer zone of the granulation tissue lymphocytes accumulate and fibrous granulation develops to wall off the lesion.

In a number of cases lesions seen in the lungs developed a more granular appearance with no obvious abscess formation. Microscopically these lesions tended to be more granulomatous and some had many Langhan's giant cells present.

Histological diagnosis was based on the finding of Langhan's giant cells and the presence of acid fast bacilli in Ziehl Neilsen stained sections. In some cases ZN positive organisms were plentiful but often they are sparse and only located after diligent searching.

Necropsies of Natural deaths - Throughout the trial there were 68 deer examined. In a total of 25 (37%) of these Tb was diagnosed. Of the 25, in 15 (60%) the disease had become dissemmated throughout the deer and could be described as generalised tuberculosis (GTb).

C.C.T. - The test results on reactors to 2 mg/ml bovine PPD tuberculin.

TABLE III

Tuberculin	Animals Reacting	
	Number	%
2 mg/ml bovine	79*	100
1 mg/ml bovine	21	26.5
0.5 mg/ml avian	10	12.5
Bovine increase over avian $<$1mm	12	15
Avian increase over bovine $<$1mm	1	1.2

* 72% of these were confirmed as having Tb on necropsy.

Tb Lesion Site - Tb lesions were located throughout the animal. Table IV - shows the lesion : site area association observed in this herd.

Lesion Area Association	Deer group by Gross Lesion Association.	
	Number	%
Head only	53	56.3
Head and Thorax	5	5.3
Head and Abdomen	10	10.6
Head, Thorax and Abdomen	4	4.2
Thorax only	8	8.5
Thorax and Abdomen	2	2.1
Abdomen only	6	6.5
Others	6	6.5
TOTAL	94	100

The location of lesions in 94 deer in this herd are seen in Table V

Lesion Site	Lesions found in each site	
	Number	%
Medial retropharyngeal ln	81	43.3
Parotid ln	2	1.0
Mandibular ln	2	1.0
Tonsil	5	2.5
Lung	18	9.6
Bronchial ln	13	6.9
Mediastinal ln	7	3.7
Pleura	8	5.3
Hepatic ln	2	1.0
Gastric ln	3	1.6
Mesenteric ln	27	14.4
Peritoneum	3	1.6
Prescapular ln	5	2.5
Popliteal ln	4	2.0
Precrural ln	2	1.0
Others	5	2.6
TOTAL	187	100

TABLE VI

Sire Group Tb Association - During 1981 detailed records of several mating groups were kept, a summary of one of these groups is as follows -

		Fate	Necropsy		
			GTB	Tb	NGL
Sire stag	1	1 Reactor	1	-	-
Adult hinds	18	8 Reactors	0	7	1
		6 Died	4	-	2
Stag fawns	4	1 Reactor	-	1	-
		2 Died	2	-	-
Hind fawns	8	6 Reactors	2	4	-
		2 Died	2	-	-

Of the original 31 at 31.3.81 there were only 5 left by 22.12.81.

DISCUSSION

In line with the project objectives the following points can be made -

1. Routine test and slaughter did not eradicate Tb from this farmed red deer herd.

2. The comparative cervical test as used in the bovine Tb control programme in New Zealand had no useful application in this herd.

3. It was not possible to monitor the efficiency of the tuberculin test in this herd.

Based on the results of the last tuberculin tests (1983) it could be considered that control of the Tb situation had been established. However the slaughter of the non-reactor deer demonstrated that Tb was still present in this herd. In February 1983 at least three of the fawns were noticed to have enlarged mandibular lns. One of these, when slaughtered, was confirmed as having Tb. The mother of this fawn was identified and on slaughter had multiple Tb abcesses in the alimentary tract. This was considered similar to events in 1980 when several negative tests were followed by a major breakdown in 1981, and with a change of farming policy (farm was sold) the opportunity to slaughter the herd was taken.

The 4 non-reactor deer identified at slaughter with gross lesions of Tb were all adult hinds. 3 of these originated from the West Coast and had been resident in the herd since 1977. All 4 of these had only single-site lesions.

In the non-reactor deer slaughtered immediately following a tuberculin test it was anticipated that M.bovis would be recovered from some of these. In the 47 slaughtered in 1982, a total of 11 (23.4%) had M.bovis recovered from groups of nodes submitted for culture examination. Of the next 211 examined, M.bovis was recovered from 7 (3.3%) and it is considered that this result is more likely to reflect the true situation.

The C.C.T. gave very poor results. The low number (26%) of deer giving any reaction to the bovine 1 mg/ml, and the lower number (15%) of deer with a bovine increase greater than that of the avian indicated the limited value of this test in this herd. Also there was no correlation between deer reacting to C.C.T. and their necropsy findings. During the project, in an effort to improve the C.C.T. in deer, the interval between the primary test and the application of the C.C.T. was increased to 14 or 21 days. Increasing this interval did not entirely overcome the problem, but at least more animals showed a response to the bovine tuberculin than before.

The lesion site and site area association were similar to those reported earlier, Beatson & Hutton (pers comm.) (1,2).

The importance of head examination in determining Tb status is underlined by 56.3% of all lesions occuring in the head only and 76.4% of all lesions occuring in the head and another site. The medial retropharyngeal ln was the most common node or tissue infected; 43.3% of all sites. This node, because of its situation, is not easily located and it is therefore most important that it is examined to accurately determine the Tb status of any deer.

From an analysis of the lesion sites and the method of lymphatic drainage described by Livingstone (4) it can be concluded that in this herd the respiratory route was the most important method of infection. The confining of deer in enclosed yards for handling would seem to be providing an excellent method for M.bovis to spread. It is also important that person involved in testing Tb infected deer are aware of the potential health hazards.

It should be remembered that the results of this project may not reflect the normal pattern of spread of the disease within a deer herd (Corrin pers comm). However the final result of this project indicates that the concern within the New Zealand Deer Farmers Association and Veterinary Profession is fully justified. The work toward improving the tuberculin test must therefore be continued.

ACKNOWLEDGEMENTS

We wish to thank all the AHD staff in Timaru District, Lincoln and Wallaceville AHD Laboratories, K. Corrin, Head Office, M.A.F. and the property owners whose generous attitude and assistance made the whole project possible.

REFERENCES

1. Quinn, J.F., Towar, D. 1963: Tuberculosis problem in deer park in Michigan. Am.Vet.Med.Assoc.Sci.Proc. 100: 262-264.

2. Jones, D.M., Manton, V.J.A. 1976: Tuberculosis in a herd of Axis deer Axis axis at Whipsnade Park. Vet.Rec. 98: 525-526.

3. Kollias, G.V. 1978: Clinical and pathological features of mycobacterial infections in Sika and Fallow deer. Smithsonian Institute Press: 173-177.

4. Livingstone, P.G. 1980: The evaluation of Tuberculin tests in a tuberculous farmed, red deer Cervus elaphus herd in N.Z. -

Thesis for Degree of Master of Preventative Veterinary Medicine, University of California U.S.A.