An Outbreak of Avian Tuberculosis in Red Deer

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Abstract

An outbreak of avian tuberculosis (Tb) occurred in 80 eight month old red weaner stags in a feeding trial at Invermay. Five were clinically affected and necropsy examination showed gross lesions affecting the terminal ileum and caecum which were typical of Johne's disease. When the rest of the group were slaughtered at 9 months of age, 35 had mesenteric lymph node abscessation ranging from mild to severe. Growth rates were reduced in subclinically affected animals with visceral lymph node lesions. Histopathological examinations revealed lesions "typical of Tb". Cultures after 6 to 8 weeks revealed *Mycobacterium avium* and the carcasses were released for local consumption. The infections were probably predisposed to by high challenge in a deer shed with food and water contamination by bird faeces, and stress associated with weaning, transport and adaptation to new diets. Avian Tb is unusual in domestic animals and infection in deer has implications for Tb control, Johne's disease investigation and for differentiating acid-fast lesions at slaughter. Subclinical avian Tb may also result in suboptimal growth rates in weaners.

Introduction

During a silage feeding trial at the AgResearch Invermay Deer Farm a number of red male weaner deer started to scour and lose condition during the winter and an investigation was undertaken.

History

A group of 80 recently weaned male red deer were purchased from Banks Peninsula and transported to AgResearch Invermay, arriving April 22, 1996. On arrival they were put into a covered shed and fed *ad lib* on lucerne hay and water. Over the next two weeks increasing amounts of grass silage (made from Invermay sheep pastures December 1995) was made available while the amount of lucerne was reduced, and the latter was stopped altogether on May 6. On this date they were drafted into 8 groups of 10 animals, with 4 groups put into indoor light controlled pens and 4 groups moved to outdoor feedlot pens experiencing natural lighting. From this point on all groups received *ad lib* silage and some groups also received varying amounts of grain and rapeseed supplementation to achieve 4 different levels of protein composition with similar levels of metabolisable energy (see Table 1).

Table 1 Trial design with feeding treatments for deer held indoors or outdoors

n = 10	Site	Silage	Barley	Rape seed	% protein
Group 1	Inside	ad lib	xxxx	nil	12
Group 2	Inside	ad lib	xxx	x	14
Group 3	Inside	ad lib	xx	xx	16
Group 4	Inside	ad lib	x	xxx	18
Group 5	Outside	ad lib	xxxx	nil	12
Group 6	Outside	ad lib	xxx	x	14
Group 7	Outside	ad lib	xx	xx	16
Group 8	Outside	ad lib	x	xxx	18

Health Treatments

All weaners were vaccinated with Yersiniavax (AgVax) on May 1 and June 10 and received a 4 gm capsule of copper oxide needles (Bayer) and oral moxidectin (Cyanamid) on May 1.

Monitoring

As part of the trial, group feed intakes were measured every two days and all weaners were weighed every 6 days. It was observed in July that some of the deer had stopped gaining weight and in late July, early August, 5 individuals (2 indoor and 3 outdoor) were seen to develop a chronic scour and were losing weight.

Because of the restricted diet the entire group was given 1 ml Lipiodol (Rhône Mérieux), 4 gm copper needles (Bayer) and 5 ml of Multiject B injection (Bomac). A dose of moxidectin was given to eliminate any abomasal parasites that may still be present. The 5 most severely affected animals continued to scour and lose weight and blood samples were submitted to the Invermay Animal Health Laboratory for a "Sick Ruminant" screen and faeces submitted for occult blood which might indicate abomasal or duodenal ulceration.

The affected animals all had low Total Protein levels, very low Serum Albumin levels, low serum calcium while GGT and fibrinogen values were raised compared with two clinically "normal" animals and the AHL normal reference range for deer (see Table 2). The occult blood tests were negative. It appeared the animals had a severe protein-losing enteropathy of unknown origin, and it was decided to sacrifice one to assist in making a diagnosis. On August 29, R57 (Group 5) was euthanased with intravenous barburate and the necropsy revealed severe thickening of the terminal 30 cm of ileum and the caecum, as well as enlarged abscessed mesenteric and ileo-caecal lymph nodes. Grossly the lesions appeared typical of severe Johne's disease. Serum samples taken a week earlier were tested for Johne's complement fixation (CFT) and agar gel immunodiffusion (GD). The following day, August 30, the other 4 deer had blood samples taken for lymphocyte transformation tests (LT) and were then euthanased and similar lesions were found. These

ranged from small yellow foci (3 x 3 mm) on the terminal ileum and a 5 x 5 x 10 mm abscess in the ileo-caecal lymph node to severe extensive abscessation of the entire mesenteric lymph node chain, oedematous mesenteries and severe thickening of ileum and caecum.

Table 2. Biochemistry results for five clinically effected and two "normal" deer

	GGT	Magnesium	Total Protein	Serum Albumin	A/G Ratio	Calcium	Fibrinogen	Protein/ Fibrinogen
	IU/L	*mmol/L*	*g/L*	*g/L*		*mmol/L*	*g/L*	*Ratio*
R57	22	0 56	38	9	0 31	1 81	3	12 3
G93	20	0 78	44	14	0 47	2 18	5	9
G94	23	0 64	53	17	0 47	2 23	6	9 2
Y492	37	0 74	54	17	0 46	2 1	4	13 3
R596	34	0 60	53	16	0 43	2 04	8	6 9
Mean	27 2	0 66	48 4	14 6	0 43	2 07	5 2	10 14
Y497	16	0 84	62	31	1	2 69	2	29
R512	20	0 99	69	33	0 92	2 58	3	21 7
Mean	18	0 92	65 5	32	0 96	2 64	2 5	25 35
Normal	14-21	0 64-0 8	55-71	34-50		2 26-3 62	0 0-5 0	

Histopathological examination showed the following typical range of features : a) the mesenteric lymph nodes showed large foci of caseation and calcification surrounded by epithelioid cells, neutrophils, mononuclear and very occasionally giant cells. Large numbers of acid fast organisms (AFOs) were usually seen at the edge or thoughout the necrotic areas. b) Ileum : three of the five deer were examined and two (R57, R596) had severe changes with obliteration of the mucosa and serosa with marked granulomatous reaction. Submucosal follicles appeared depleted and in many areas the submucosa was packed with epithelioid cells, mononuclear cells and neutrophils, and large numbers of AFOs were present. The caecum from one deer (Y492) had diffuse colitis but no AFOs were seen. The AHL diagnosis stated : "The pyogranulomatous reaction in the nodes and ileum .....is most likely caused by *M. paratuberculosis*. There is quite a variation in the degree of severity."

Subsequent actions and investigations

On the basis that Johne's disease was the most likely cause of the problem, an immediate ban on movement onto or off the "Flat" deer farm was put in place to prevent further spread of the disease. The local MAF office was notified of the presumptive diagnosis. Samples of fresh material were sent to Wallaceville for culture and polymerase chain reaction (PCR) tests in order to identify the causative agent. All the deer in the trial were blood-sampled. The supplier of the deer was contacted, but he had not had any animals affected with Johne's previously and herd mates that remained at his farm were apparently

healthy. We assumed at this stage that the only possible source of John's infection was the silage which had been made on the Invermay sheep farm where Johne's is endemic.

In an effort to learn as much as possible about Johne's disease in deer it was decided to conduct Johne's CFT, GD and ELISA tests in parallel on serum samples and to perform faecal smear examination for clumped acid fast organisms (AFOs) typical of Johne's organisms. A routine mid cervical Tb (MCT) skin test using bovine PPD and a lymphocyte transformation test (on heparinised blood sample) was performed on each animal to investigate non-specific reactivity. All these tests were carried out on 10/9/96. Further CFT, GD and ELISA tests were carried out on 23/9/96 to investigate the possible antibody boosting effects of skin testing

On 21/9/96 an animal (G896) showed signs of acute MCF and was euthanased. At necropsy there were typical gross lesions of *Mycobacterial* infection.

On 25/9/96, all faecal smears were negative for AFOs. PCR tests were negative for both *M. bovis* and *M paratuberculosis* (*M. ptb*). This gave some evidence that we may be dealing with an *M. avium - intracellulare* complex (*Maic*) infection although *M. ptb* could not be ruled out as the PCR is not 100 % sensitive. Therefore we went ahead with the plan to slaughter all the animals in the trial, firstly, to eliminate the risk of keeping subclinical Johne's infected animals on Invermay, secondly because the silage feeding trial had been compromised by 5 clinical and an unknown number of subclinical cases of suspected Johne's disease and thirdly in order to study the disease and assess the sensitivity and specificity of the serological tests.

On 26/9/96 all the remaining 70 deer were slaughtered through the Otago Venison Plant and in addition to the normal meat inspection procedures the mesenteric chain and ileo caecal lymph nodes were incised, lesions noted, fixed and fresh samples taken and the ileocaecal valve collected for further study.

Lesions

At slaughter, 35/70 animals had no visible lesions (NVL). Four other animals killed on 5/9 and 13/9 as part of another study plus the case of MCF were also all NVL. The other animals had a range of lesions which were scored on a rising scale of severity as follows:

NVL	0
small single abscess in mesenteric LN chain	1
small single abscess in mesenteric and ileocaecal LN	2
multiple tiny abscesses in mesenteric and ileocaecal LN	3
multiple small abscesses in mesenteric and ileocaecal LN	4
multiple large abscesses in mesenteric and ileocaecal LN	5

On this scale the 5 clinically affected animals had scores of 2, 4, 5, 5, 5. The clinically normal animals had 10 animals scoring 1, 4 scoring 2, 2 scoring 3, 14 scoring 4 and 5 scoring 5 (see Table 3). Six animals also had an abscess in a retropharyngeal or parotid lymph node. Each of the 8 groups had a similar range of NVL animals (n = 4, 5 or 6) and

lesion severity , although Group 3 had the highest mean lesion severity of 2.3, compared with the others which ranged from 1.0 to 2.0.

Table 3 CFT, GD, ELISA and lesion severity scores for the 80 deer

	Tag	Gp		CFT 22/08	GD 22/08	CFT 10/09	GD 10/09	ELISA 10/09	lesion* severity		Tag	Gp		CFT 22/08	GD 22/08	CFT 10/09	GD 10/09	ELISA 10/09	lesion* severity
G	90	1				n	n	pos	0	R	46	5				n	n	pos	0
G	91					n	n	n	0	R	47					n	n	n	0
G	92					n	pend	pos	0	R	48					n	n	pos	1
G	93		Clinical	3/8 pos	pos	*	*	*	4	R	49					n	n	n	2
G	94		Clinical	4/8 pos	pos	*	*	*	2	R	51					n	n	n	0
G	95					n	n	sus	0	R	57		Clinical	1/8 pos	pos	*	*	*	5
G	96					n	n	n	0	R	58					n	n	pos	0
G	97					4/4 sus	pos	pos	2	R	59					4/4 sus	neg	pos	4
G	98		killed13/9			n	n	n	0	R	61					n	n	n	1
G	99					n	n	sus	2	R	62					n	n	pos	0
G	185	2				3/8 sus	pos	pos	4	R	226	6				n	n	pos	3
G	186					n	n	pos	0	R	227					n	n	n	0
G	187					n	n	pos	0	R	228					n	n	pos	0
G	188					2/4 neg	n	n	0	R	229					n	n	pos	0
G	189					3/4 neg	n	pos	1	R	233					1/8 sus	pos	pos	4
G	190					1/4 neg	pos	pos	4	R	238					1/8 sus	pos	n	4
G	191					4/4 sus	pos	pos	1	R	249					n	n	n	0
G	192					n	n	pos	1	R	252					n	n	n	0
G	193					n	n	n	0	R	254					1/8 sus	n	pos	4
G	194					n	n	pos	0	R	255					1/16 pos	pos	pos	4
G	871	3				4/4 sus	neg	pos	0	R	510	7				n	n	pos	1
G	872					1/8 sus	pos	pos	5	R	512			4/8 pos	pos	3/16 pos	pos	pos	5
G	873					3/16 pos	pos	pos	4	R	513					n	n	n	0
G	874					n	n	pos	4	R	515					n	n	n	0
G	894					2/8 sus	pos	pos	4	R	517					4/4 sus	neg	n	4
G	895					n	n	n	0	R	595					4/8 pos	neg	pos	4
G	896		MCF21/9			n	n	n	0	R	596		Clinical	1/16 pos	pos	*	*	*	5
G	897					3/8 sus	pos	pos	5	R	597					n	n	pos	0
G	898					n	n	n	1	R	598					n	n	pos	0
G	899					n	n	n	0	R	599					n	sus	pos	1
PW	326	4	killed 5/9			*	*	*	0	Y	490	8				n	n	n	0
PW	327					4/16 pos	pos	pos	5	Y	491					1/8 sus	pos	pos	4
PW	328		killed 5/9			*	*	*	0	Y	492		Clinical	2/16 pos	pos	*	*	*	5
PW	329					2/16 pos	pos	n	5	Y	493					n	sus	n	4
PW	330					n	n	n	0	Y	494					n	n	pos	0
PW	331					n	n	pos	3	Y	495					n	n	n	0
PW	332					n	n	n	0	Y	496					n	pos	n	1
PW	333					2/4 neg	pos	n	2	Y	497			neg	neg	n	n	n	0
PW	334		killed 5/9			*	*	*	0	Y	498					n	n	n	1
PW	335					n	n	n	0	Y	499					n	n	n	0

* Lesion severity

0 NVL

1 small single abscess

2 small abscess mes and IC LN

3 multiple tiny abscesses mes and IC LN

4 mutiple small abscesses mes and IC LN

5 multiple greatly enlarged abscessed mes and IC LN

Serology

A summary of results is shown in Table 3. Sensitivity is the percentage of animals with gross lesions which were positive to the test, and specificity is the percentage of animals which were negative to the test which do not have lesions, using various endpoints. Various *M. avium* or *M. paratb* strains, some of which were cross-absorbed with *M. phlei*, were used in these tests.

Overall the absorbed ELISA was the most sensitive (72.5 %) but had the poorest specificity (62.2 %). The CFT, when using the normal deer endpoint of $\geq$ 4/8, had poor sensitivity (22.5 %) but was 100 % specific. When the CFT was read at the normal cattle endpoint ($\geq$ 4/4) the sensitivity rose to 57.5 % and specificity fell to 97.2 %. The GD using the "pos" endpoint was 55 % sensitive and 100 % specific. If the GD endpoint included "pos", "weak pos" and "suspicious" the sensitivity rose to 65 % and specificity fell to 94.6 %. There was little or no boosting effect of skin testing with bovine PPD two weeks prior to the second blood sampling.

Skin test and lymphocyte transformation tests

A single intradermal skin test with bovine PPD resulted in 64 % of animals having an increase in skin thickening $\geq$ 1 mm and almost all the animals had avian reactivity in LT suggesting that the entire group had been heavily exposed to *M. avium*. It showed that avian Tb had the ability to severely compromise the normal mid cervical skin test (MCT) but that the LT should indicate the true cause of skin test reactivity.

Subclinical effects

The animals were weighed every 6 days as part of the silage-feeding trial and, analysis of the data showed that for the 3 month period, May 15 to August 19 there was a significant negative relationship between the severity of avian Tb lesions and growth rate. The analysis showed that the 5 clinically affected animals gained the least weight (and in fact lost weight just prior to euthanasia) and the rest of the animals with subclinical disease gained weight at a rate inversely proportional to the severity of the lesions found at slaughter.

Discussion

When initially confronted with 5 weaners which had stopped growing and had developed varying degrees of diarrhoea, the differential diagnosis included dietary upset/imbalance as a result of silage feeding plus supplements leading to some combination of rumenal ingestion, acidosis, copper, iodine or Vitamin B deficiency, and a possibility of parasitism due to animals having missed treatment. When treatment failed to improve their condition, faecal samples were taken for an occult blood test which might indicate the presence of abomasal or duodenal ulcer which can result from chronic stress. Blood samples were taken and these indicated a protein losing enterpathy. Even at this point experience suggested that Johne's disease was likely in 7 to 8 month old animals. One weaner was euthanased in order to diagnose the problem and the other 4 were killed the next day.

The first 5 deer that were clinically affected all had gross lesions characteristic of Johne's disease. Histopathological examinations suggested that the ileal and caecal involvement were the basis for the "protein-losing enterpathy" indicated by the biochemical results. Subsequent necropsies showed that none of the other subclinically affected deer appeared to have any gross involvement of the intestines, although analysis showed that growth rates of these animals were adversely affected by the disease. These affects were not large and would easily be overlooked without accurate weighing and record-keeping. The decision to kill all the animals in late September was based largely on the fact that the outbreak of disease had seriously compromised the silage feeding trial. If the disease was due to *M. ptb* then there was a high risk that many more animals were affected and their condition was likely to deteriorate and a cost-benefit analysis showed that the cost of maintaining the animals on the feed-lot would not have been compensated for by the anticipated weight gain, especially if animals were growing suboptimally. A different decision may have been made in a fully commercial situation, especially if the animals could have been turned out onto pasture in spring. However, the risk of contaminating paddocks by Johne's affected animals would have affected that decision. Never-the-less, it demonstrates the value of cost/benefit analysis and the importance of accurate animal health information in decision making.

It appears that deer are unique farm animals in that *M. avium* can cause outbreaks of disease. In this case, 6.25 % of the group were clinically affected and 50 % had subclinical lesions detected at slaughter. The infections were probably predisposed to by a number of factors:

1. Stress due to weaning, transportation, housing in a strange environment and adapting to new diets.
2. Heavy challenge with *M. avium* from faecal contamination of food, water and air in the deer shed. Deer at this unit frequently have avian sensitisation but there have been few lesions and no clinical cases previously.
3. The strain of *M. avium* is being investigated at AgResearch Wallaceville and it is possible that it is more pathogenic than other organisms in the *M. avium-intracellulare* group.

The infections appear to have taken place in the shed soon after arrival because the attack rate and range of lesion severity is similar for all the groups irrespective of their subsequent location (indoors or outdoors) or feeding treatment.

Because of the lesions which were found in the clinically affected animals it was no great surprise to find lesions in the other animals at slaughter. The MAF vet and meat inspector had been fore-warned and assisted in sample collection. Never-the-less, all carcasses with lesions were detained and samples submitted to the Invermay Animal Health Laboratory. Histopathological examinations showed lesions "typical of Tb" and it was not until 6 to 8 weeks later that cultures confirmed *M. avium* as the causative organism, thus taking the "Flat" Deer Farm off Movement Control. The downgrading of these carcasses to "local" consumption due to the prolonged detention resulted in considerable loss. Thus *M. avium* has the potential to cause considerable delays and loss of income from animals

sent for slaughter. It also highlights the necessity of culturing all lesions "typical of Tb" so that, in the case of avian Tb, *M. bovis* is ruled out and farmers are not incorrectly put on Movement Control.

The serological results demonstrate that deer with *M. avian* lesions are very likely to be test positive to the Johne's GD, CFT and ELISA. This is not surprising since *M. avium* and *M. paratb* are closely related and some of the tests actually use antigens from organisms in the *M. avium-intracellulare* group which grow more easily in the laboratory than *M. paratb*. It is characteristic of Mycobacterial infections that protection against disease is associated with the cell-mediated immune response and that the presence of antibody is usually associated with lesions, indicating a failed or suboptimal immune response.

Johne's disease is emerging as a problem in the deer industry and urgent steps should be taken to prevent it becoming more serious. However, *M. avium* infections in deer have the potential to interfere with the usual diagnostic tests for Johne's disease and it is essential that research is directed at understanding the epidemiology of these two diseases and better diagnostic tests are developed to differentiate them and assist in control and prevention.

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