Intra-tonsil inoculation: an experimental model for tuberculosis in deer

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Introduction

For research into tuberculosis (TB) it is essential to develop an infection model which reproduces the disease as naturally as possible, causing the typical range of disease outcomes and immune responses seen in naturally infected animals. The model should be efficient, repeatable, relatively easy and safe to work with, and economical. We have developed an experimental TB infection model for *Mycobacterium bovis* in deer which fulfils most of these criteria.

The majority (around 60%) of single TB lesions found at slaughter in farmed deer in New Zealand occur in the head lymph nodes, primarily the medial retropharyngeal, whereas single lesions occur in the thoracic and abdominal lymph nodes in 20-30% of cases. Seriously diseased deer usually have lesions in the head, thoracic and abdominal nodes. This suggests that the natural route of infection in deer is via the oral or nasal cavities. The palatine tonsil is the most likely point of entry and it drains directly to the medial retropharyngeal lymph node.

Intra-tonsil inoculation with *M. bovis* produces lesions primarily in the medial retropharyngeal LN and a spectrum of pathological and immunological responses typical of naturally occurring TBin deer. An initial trial (Mackintosh *et al.* 1993) compared intra-tracheal, intra-nasal and intra-tonsil inoculation with either 2×10^2 or 2×10^4 colony forming units (cfu). The animals were monitored for 35 weeks using periodic lymphocyte transformation (LT) and antibody (ELISA) assays and then slaughtered. Intra-tracheal inoculation especially at the higher dose, led to severe progressive lesions in the thoracic cavity, high LT levels and the early appearance of circulating antibody. Intra-nasal inoculation gave variable results with 20% infection at the lower dose rate and severe progressive disease at the higher dose rate. Intra-tonsil inoculation resulted in a range of lesions in the medial retropharyngeal lymph nodes and typical LT and antibody responses, which mirrored patterns of reactivity found in naturally infected animals.

Subsequent trials (Table 1) have shown that infections can be established with doses as low as 8 cfu given by intratonsilar inoculation

This route results in 70-100% of animals becoming infected using dose rates of 100-500 cfu (see Table 1). This means that group sizes of 5 to 10 animals are usually sufficient for most studies. Trials are usually run for 6 to 8 months to allow for lesion development and to discriminate between cured, contained and progressive disease.

Table 1: Results from six trials in which 102 red deer have received intra-tonsil inoculation with various doses of virulent M. bovis

Trial	Animals	Infecting dose cfu	No. infected withTB	Duration (months)
1	Weaner female (1992/93)	$2x10^2$ $2x10^4$	4/5 5/5	8
2	Weaner female (1993/94)	8 80	5/8 8/8	8
3	3 y.o. stags (1993/94)	8 80 $0.8x10^4$	4/7 7/8 7/7	8
4	3 y. o. stags (1994/95)	$5x10^2$	34/39	7
5	Yearling hinds (1993/94) (Vaccine trial control group)	$2x10^2$	6/6	7
6	Yearling hinds (1994/95) (Vaccine trial control group)	$5x10^2$	10/10	6

On the practical side, intra-tonsil inoculation is relatively easy and safe to undertake. It involves instilling 0.2 ml of inoculum into the left tonsil crypt while deer are heavily sedated. After 6 to 8 weeks lesions are detectable and mostly confined to the medial retropharyngeal LN although a small proportion are in tonsils, lungs and mesenteric LNs. Under pasture and yarding conditions the rate of transmission to in-contact control deer has been low. This is in contrast to the risks associated with aerosol infection procedures and resultant productive lung lesions.

The model has been used in epidemiological and immunological studies on Tb in deer and in two *M. bovis* BCG vaccine trials, both of which demonstrated significant protection afforded by two doses of live vaccine.

Intra-tonsil inoculation with *M. bovis* in deer provides a practical, efficient, repeatable model to test new generation vaccines for domestic livestock and humans (Griffin *et al.* 1995).

Bibliography

Mackintosh, C.G. & Griffin, J.F.T. (1993). *Proc. NZ.V.A. Deer Br.* **10:** 297-304.

Mackintosh, C.G., Griffin, J.F.T. & Waldrup, K.A. (in press). *Proc. Third Int. Cong. on Biology of Deer,* Edinburgh 1994.

Griffin, J.F.T., Mackintosh, C.G. & Buchan, G.S. (1995). *Trends in Microbiology.* **3**: 218-224.