A RABBIT MODEL OF CERVINE MALIGNANT CATARRHAL FEVER

R.E. Oliver

Central Animal Health Laboratory, Wallaceville Animal Research Centre,
Private Bag, Upper Hutt, New Zealand

ABSTRACT

Malignant catarrhal fever (MCF) of deer was adapted to rabbits and transmitted through 31 passages over a period of 12 months, using heparinised blood, pooled spleen and thymus or buffy coat suspensions for inoculation. Affected rabbits developed fever, anorexia and diarrhoea.

A pool inoculum (titre $>10^2$ rabbit lethal dose 50 per ml) was prepared from spleens and mesenteric lymph nodes of experimentally infected rabbits. The agent was labile to treatment with ether, heat (56 °C for 60 mins) or filtration (0.22 µ).

Two rabbits immunised with formalin inactivated pool inoculum were resistant to challenge with pool inoculum. Spleen cell cultures derived from experimentally infected rabbits were infectious when inoculated into rabbits. Infectivity of cultured spleen cells was maintained in cell culture for up to passage level 11.

INTRODUCTION

Malignant catarrhal fever (MCF) has been recognised as the most important disease of farmed deer in New Zealand (Beatson & Hutton, 1981; Hunter, 1981; McCallum et al, 1982). In a South Canterbury survey MCF was considered responsible for 36% of diagnosed deaths of farmed deer (Beatson & Hutton, 1981).

Under New Zealand conditions, the disease occurs more often in late winter and spring. It is most common in intensively farmed deer. Characteristic clinical features include fever, depression, severe diarrhoea, dysentery and high mortality (Beatson & Hutton, 1981).

Conditions resembling MCF have been reported in several species of deer including red deer (Cervus elaphus) (Beatson & Hutton, 1981; Hunter, 1981; McCallum et al, 1981; Reid et al, 1978), axis deer (Axis axis) (Clark et al, 1970), whitetailed deer (Odocoileus virginianus) (Clark et al, 1970; Whitenack et al, 1982; Wobeser et al, 1973; Wyand et al, 1971), Pere Davids deer (Elaphurus davidianus) (Huck et al, 1961), rusa deer (Cervus timorensis) (Denholm & Westbury, 1982) and sika deer (Cervus nippon) (Hunter, 1981). MCF has been experimentally transmitted from deer to rabbits (Buxton & Reid, 1980; Huck et al, 1961; Oliver et al, 1981 and Westbury & Denholm, 1982). The rabbit has been the only laboratory animal in which it has been possible to adapt the agent causing cervine MCF.

This report describes the development of a rabbit model of cervine MCF and reports the propagation of the MCF agent in cultured rabbit spleen cells up to the 11th passage level.

MATERIALS AND METHODS

Rabbits

Two to 3 month old New Zealand White rabbits were obtained from a specific pathogen free laboratory animal breeding colony. Inoculated rabbits were housed in isolation cages. Rabbits were clinically examined and rectal temperatures recorded daily.

Source of Initial Inoculum

Twenty mls of heparinised blood from a female deer, experimentally infected with MCF, was inoculated into each of 2 recipient rabbits by intraperitoneal injection.

Transmission

Rectal temperatures of inoculated rabbits were recorded daily. When one of a pair of inoculated rabbits became febrile (>40 °C), transmission was attempted. Blood was collected into sterile heparinised vacutainer tubes by intracardiac puncture of the anaethetised rabbit. On occasions, the spleen and thymus were collected aseptically immediately after the rabbit had been euthanised by intravenous overdosage of sodium pentobarbitone. Tissues were finely minced, pooled, mechanically disrupted using a Stomacher* and diluted to a 10% suspension in minimal essential media. Pairs of recipient rabbits were usually inoculated with 20 ml of heparinised blood or occasionally with 7.5 ml of pooled spleen and thymus suspension from the febrile rabbit. Tissues were inoculated within 2 hours of collection.

Preparation and Titration of a Pool Inoculum

The mesenteric lymph node and spleen from 4 febrile rabbits experimentally infected with the MCF agent were collected aseptically, pooled and a 10% suspension prepared in minimum essential medium (MEM) with 10% foetal calf serum (FCS) and 10% dimethyl sulphoxide (DMSO). The suspension was stored at -80 °C in 5 ml aliquots, after controlled freezing.

For titration, an aliquot of pool inoculum was thawed and pairs of rabbits were inoculated by intraperitoneal infection of tenfold dilutions of pool inoculum, adjusted to 5 ml final volume with MEM.

Physicochemical Characterisation of the MCF Agent

Aliquots of pool inoculum were thawed and treated for ether sensitivity, heat lability (56 °C for 1 hour) and ultrafiltration (0.22 μ) as described (Hsuing, 1973). Pairs of rabbits were inoculated with 1.0 ml of treated inoculum by intraperitoneal inoculation. Rabbits were observed and rectal temperatures recorded daily.

Immunisation of Rabbits

Aliquots of pool inoculum were thawed and treated with 0.33% formalin (volume/volume) overnight at 4 °C. Pairs of rabbits were inoculated with 2.5 ml of treated inoculum by intraperitoneal inoculation and boosted 4 and 6 weeks later. Immunised rabbits and susceptible controls were challenged 2 weeks later with 0.1 ml of pool inoculum and were rechallenged after a further 8 weeks with 1.0 ml of pool inoculum. Pairs of susceptible control rabbits were also challenged.

Infectivity of Spleen Cell Cultures from Experimentally Infected Rabbits

Rabbits experimentally infected with MCF agent, were euthanised after they developed fever. Spleens were removed aseptically and established in tissue culture by the explantation technique. Growth media consisted of MEM and 20% FCS.

Once confluent monolayers were established, cells were passaged at weekly intervals by trypsination, using a 1 to 2 split ratio. For transmission to rabbits, recipient rabbits were inoculated with trypsinised spleen cells from a 75 cm^2 tissue culture flask, resuspended in the old growth media. Rabbits were inoculated by the intraperitoneal route of infection and were observed clinically and rectal temperatures taken daily.

Pathology

Necropsies were performed on infected and control rabbits. Tissues were fixed in formalin and HE stained sections were prepared and examined.

*Stomacher, A.J. Seward, VAC House, Blackburn Road, London

RESULTS

Transmission

The agent causing MCF of deer was adapted to the rabbit and passaged routinely by intraperitoneal inoculation of heparinised blood or spleen and lymphoid suspension. Three of the 4 lines of transmission were maintained through 30 passages extending over a period of 12 months. The fourth line of transmission was lost after 4 passages (Figure 1). Incubation periods ranged from 5 to 31 days (x=17 days). Serial transmission of the agent in rabbits when rabbit lymph node suspensions stored at -80 °C with 10% dimethyl sulfoxide were infectious on thawing and inoculation into rabbits.

Clinical Disease

Clinical signs were not observed prior to the onset of fever (>40 °C). Febrile rabbits were depressed and anorexic. Diarrhoea developed within 24 hours of the onset of fever. Rabbits with severe diarrhoea usually died within 24 hours. Rectal temperatures dropped prior to death. Haemorrhages were noted on the conjunctiva and sclera.

In the occasional chronic cases, diarrhoea did not occur. Affected rabbits developed mucopurulent conjunctivitis and rhinitis, lost body condition and developed jaundice.

Pathology

Gross lesions - Livers were swollen with a mottled grayish brown lobular appearance. Mesenteric lymph nodes were enlarged with patchy congestion and haemorrhage, and discrete focal areas of necrosis and haemorrhage up to 0.5 cm in diameter. Petechial haemorrhages were present on the caecal serosa. The caecum was dilated with brown watery contents.

Discrete haemorrhagic lesions were found on the mucosa of the sacculus rotundus and caecal appendix. Lesions appeared to start as focal haemorrhagic areas and developed into circumscribed haemorrhagic necrotic foci, up to 1.0 cm in diameter. The mucosa over the lesion was ulcerated. On the cut surface, the necrotic area was demarcated from adjacent normal tissue.

Thymic lesions were variable with severe congestion and haemorrhage in some cases, atrophy in chronic cases and no lesions in several. Peripheral lymph node lesions were variously pale brown and hyperplastic or congested with haemorrhages throughout. Discrete foci of necrosis and haemorrhage were seen on the cut surface. Extensive oral and nasal mucosal ulceration was seen only in 1 chronic case.

Microscopic Lesions

There was widespread perivascular cuffing and infiltration of lymphoblasts in several tissues including the portal triads of the liver, the lung, the renal cortex, eye, brain, tongue, trachea, salivary gland and intestine. In the sacculus rotundus and caecal appendix there were multifocal to confluent areas of acute necrosis with thrombosis of peripheral vessels. Lymph nodes were hyperplastic with enlarged germinal centres and infiltration of paracortical areas by lymphoblasts. Focal to confluent areas of acute necrosis with thrombosis of peripheral vessels were also found in these lymph nodes.

Titration of Pool Inoculum

Results of inoculation of pairs of rabbits with tenfold dilutions of stock inoculum are presented in Table 1. The titre of the stock inoculum was estimated to be $>10^2$ rabbit lethal $dose_{50}$ (RLD_{50}) per ml.

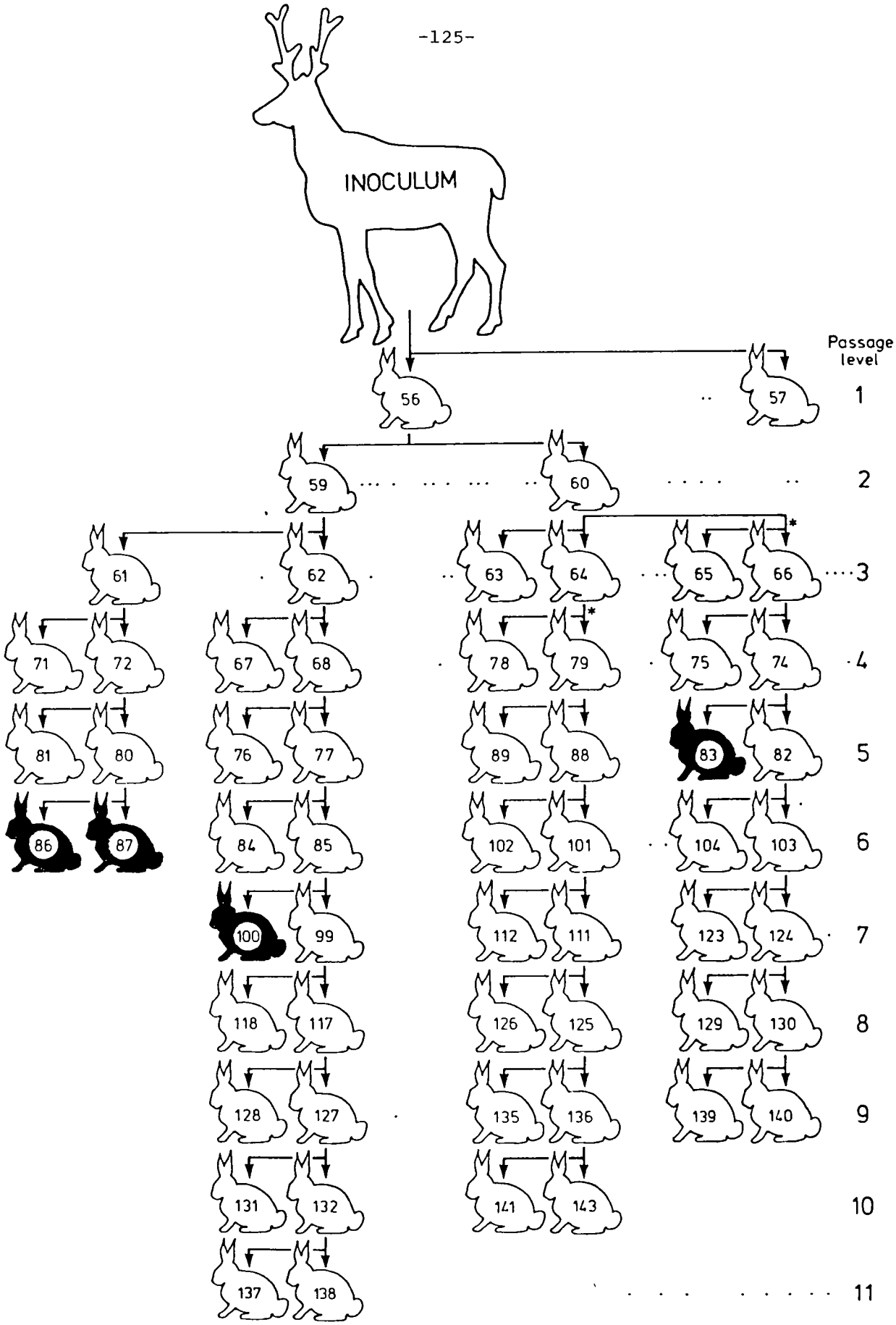

Figure 1: Diagram of serial transmission of cervine malignant catarrhal fever (MCF) in rabbits. Rabbits were inoculated with 20 mls of heparinised blood. Where indicated, rabbits were inoculated with 7.5 mls of pooled thymus and spleen suspension (*) Black coloured rabbits did not develop MCF.

Physicochemical Characterisation of the MCF Agent

Results of effect of ether, heat (56 °C for 60 mins) and ultrafiltration (0.22 μ) treatment on infectivity of MCF stock inoculum are presented in Table 2.

Table 1

Titration of pool inoculum

Volume of inoculum* (ml)	No. Rabbits Inoculated	No. Rabbits Developing MCF
5.0	2	2
1.0	2	2
100 μl	2	2
10 μl	2	2
1 μl	2	0
0.1 μl	2	0

Adjusted to final volume for inoculation of 5.0 ml.

Table 2

Effect of physicochemical treatments on infectivity of MCF agent

Inoculum treatment	No. Rabbits Inoculated	No. Rabbits Developing MCF
No treatment	2	2
Ether	2	0
Heat (56 °C for 60 mins)	2	0
Ultrafiltration (0.22 μ)	2	0

Immunisation of Rabbits

Rabbits were resistant to the initial challenge but the susceptible control did not develop MCF. On repeat challenge 8 weeks later using a higher challenge dose (100-1000 RLD_{50}), both immunised rabbits were resistant on challenge, whereas the susceptible control succumbed to MCF.

Infectivity of Spleen Cell Cultures from Experimentally Infected Rabbits

Spleen cell culture suspensions derived from 18 febrile rabbits experimentally infected with MCF agent were inoculated into recipient rabbits. Eleven of 18 spleen cell cultures were infectious for inoculated rabbits. In some cases only 1 of 2 inoculated rabbits developed MCF. Lesions were indistinguishable from lesions observed in serially transmitted cases of MCF. In one case cells maintained in culture to passage level 11 were infectious when inoculated into rabbits.

DISCUSSION

The agent causing MCF of red deer has been adapted to the laboratory rabbit and serially passaged using heparinised blood from febrile rabbits. The agent causing MCF in deer has been adapted to rabbits previously (Buxton & Reid, 1980; Huck et al, 1961; Oliver et al, 1981; Westbury & Denholm, 1982).

In our study, intestinal lesions were frequently present. Profuse diarrhoea occurred together with the onset of fever or soon afterwards; rabbits with diarrhoea often died within 24 hours. The clinical and

pathological features observed in these rabbits closely resemble those seen in acute MCF of red deer (Beatson & Hutton, 1981; Oliver et al 1981).

Establishment of a pool inoculum has permitted cervine MCF to be more fully investigated. Characterisation studies indicate that the agent is enveloped (ether sensitive) and heat labile (inactivated by heating to 56 °C for 1 hour). Removal of infectivity by passage through a 0.22 μ filter may have resulted from exclusion of virus with cellular membranes. Initial immunisation studies whereby rabbits, immunised with formalin inactivated pool inoculum, were resistant to challenge with virulent virus suggests that vaccination may be a feasible option to control MCF in deer.

That spleen cell cultures derived from experimentally infected rabbits were infectious on inoculation into recipient rabbits and that infectivity was maintained for up to 11 passages in culture demonstrates that the agent can be propagated in vitro for lengthy periods and that these cells are persistently infected without overt cytopathic effect. This finding concurs with the report of Reid et al who reported transmission of MCF to rabbits with large granular lymphocytes derived from rabbit mesenteric lymph node cells fused to foetal ovine kidney cells (Reid et al, 1983).

Over the past 4 years, research MCF has progressed from a clinicopathologic problem of unknown aetiology to the level of persistently infected rabbit spleen cells. Further work will now be directed to identification and propagation of the agent in cell culture, development of antigen and specific antibody and immunisation of rabbits.

REFERENCES

1. Beatson, N.S.; Hutton, J.B. (1981): A study of the malignant catarrhal fever syndrome on Mid and South Canterbury deer farms. Proceedings of a deer seminar for veterinarians, Deer Advisory Panel of the New Zealand Veterinary Association, 117-21.
2. Buxton, D.; Reid, H.W. (1980): Transmission of malignant catarrhal fever to rabbits. Vet. Rec. 106: 243-45.
3. Clark, K.; Robinson, R.M.; Marburger, R.G.; Jones, L.P.; Orchard, J.H. (1970): Malignant catarrhal fever in Texas cervids. J. wildlife Dis. 6: 376-83.
4. Denholm, L.J.; Westbury, H.A. (1982): Malignant catarrhal fever in farmed rusa deer (Cervus timorensis). I. Clinico-pathological observations. Aust. vet. J. 58: 81-7.
5. Huck, R.A.; Shand, A.; Allsop, P.J.; Paterson, A.B. (1961): Malignant catarrh of deer. Vet. Rec. 73: 457-65.
6. Hsuing, H.G. (1973): In "Handbook of Diagnostic Virology", Yale University Press.
7. Hunter, J.W. (1981): Malignant catarrhal fever - Taupo area. Proceedings of a deer seminar for veterinarians, Deer Advisory Panel of N.Z. Veterinary Association: 121-24.
8. McCallum, H.J.; Mavor, N.M.; Hemmingsen, P. (1982): A malignant catarrhal fever-like disease in red deer (Cervus elaphus) in New Zealand. N.Z. vet. J. 30: 99-101.
9. Oliver, R.E.; Cathcart, A.; Poole, W.S.; Julian, A.F. (1981): Malignant catarrhal fever of deer - transmission studies. Proceedings of a deer seminar for veterinarians. Deer Advisory Panel of N.Z. Veterinary Association, 125-29.
10. Oliver, R.E.; McNiven, R.A.; Julian, A.F.; Poole, W.S. (1981): Isolation of caprine arthritis-encephalitis virus from a goat. N.Z. vet. J.
11. Piercy, S.E. (1955): Studies on bovine malignant catarrh. VI. Adaptation to rabbits. Br. vet. J. 115: 484-91.
12. Reid, H.W.; Buxton, D.; Corrigull, W.; Hunter, A.R.; Rushton, R. (1978): Outbreak of malignant catarrhal fever in red deer (Cervus elaphus). Vet. Rec. 104: 120-23.

13. Reid, H.W.; Buxton, D.; Pow, I.; Finlayson, J.; Berrie, E.L. (1983): Res. vet. Sci. 34: 109-113.
14. Westbury, H.A.; Denholm, L.J. (1982): Malignant catarrhal fever in farmed rusa deer (Cervus timorensis) 2. Animal transmissions and virological studies. Aust. vet. J. 58: 88-92.
15. Whitenack, D.L.; Castro, A.E.; Kocan, A.A. (1982): Experimental malignant catarrhal fever (African form) in white-tailed deer. J. Wildlife Dis. 17: 443-51.
16. Wobeser, G.; Majka, J.A.; Mills, J.H. (1973): A disease resembling malignant catarrhal fever in white-tailed deer in Saskatchewan. Can. vet. J. 14: 106-9.
17. Wyand, D.S.; Hemboldt, C.F.; Neilsen, S.W. (1971): Malignant catarrhal fever in white-tailed deer. J.A.V.M.A. 159: 605-10.