MALIGNANT CATARRHAL FEVER: WORKSHOP REPORT

P.F. Fennessy

The workshop, held in Dunedin in May, in conjunction with the New Zealand Microbiological Society and New Zealand Society for Immunology Combined Scientific Meeting, was attended by about 25 people.

The objectives of the workshop were:

a) to review the current state of knowledge of MCF,

b) to develop an awareness of current research efforts,

c) to identify research priorities, and

d) to discuss possible areas of co-operation between research groups.

A number of specific issues were also targeted including:

a) Is the New Zealand MCF the same as the UK MCF?

b) Is the sheep the real host?

c) Is there deer to deer transmission?

d) How is the virus transmitted/what triggers the disease?

e) Why are young deer not usually affected?

f) Is the development of a vaccine a realistic goal?

g) What avenues of research should be pursued?

INTRODUCTION AND OVERVIEW

Colin Mackintosh, MAFTech, Invermay

Surveys by Noel Beatson in the Canterbury area indicate that around 40% of all deer deaths are due to MCF and the majority of cases occur in adults (ie > 1 year olds) (Beatson 1984). It is thought that throughout New Zealand approximately 1% of deer die per annum of MCF, although the incidence is probably higher in southern regions. For example, Invermay loses around 5 or 6 deer from a herd of 500 adults. However, some farmers claim to lose no deer to MCF, whereas others lose considerably more than 1%. An overall annual loss rate of 1% would mean a disease cost of about $2.5 m annually (ie 3,200 hinds and 1,000 adult stags).

Although the incidence of reported cases of MCF appears a little higher in females (Orr 1986) the actual attack rate is probably higher in males because there are 3 times as many hinds as there are stags. Similarly in

cattle the attack rate of BMC is higher in males than females (Harris et al 1978). The peak of MCF cases in deer occurs in July with 70% of cases in the four months of June-September (1980-1985; Orr, 1986). This is 3-4 months earlier than the peak of BMC cases in cattle which occurs from September to November. Following fat mobilisation during the rut, stags have very low energy reserves which probably leaves them more susceptible to MCF during the winter.

There is some suggestion that the incidence rate of MCF is declining although non-reporting of the disease and fewer post-mortems may be important. However, the other possibilities include:

a) reduction in stress since the days when most deer were live-captured

b) better feeding especially in winter, and

c) an increase in genetic resistance, with natural selection of more resistant stock on farms.

While there are likely to be genetic differences in susceptibility/ resistance within the New Zealand red deer population, their impact with such a low overall death rate is unlikely to be very great. However within the Pere David's deer, which are highly susceptible to MCF, natural selection may be very important, especially in the long term. Pere David's deer in both New Zealand and Scotland have suffered a high mortality rate from MCF. At Invermay, nearly half of our herd of 24 Pere David's died in the first year after importation, although since that time, the mortality rate has declined to an annual rate of around 25%. The possible reasons for the decline include:

a) the survivors are naturally more resistant than those that have succumbed already,

b) the degree of stress is declining as the animals get used to a farming environment,

c) the amount of exposure to sheep associated virus is less now, compared with their initial site which had sheep much closer,

d) the active development of resistance by the survivors, and

e) some other unknown factors.

The difference in susceptibility to MCF between red deer and Pere David's deer highlight the genetic aspects. It also appears that fallow deer are completely resistant to the disease, while wapiti and red deer are fairly resistant, though wapiti more so. However, at the other end of the scale sika, whitetail deer and Pere David's deer are very susceptible.

Clearly we need to know more about the infectious agent, the epidemiology and pathogenesis of the disease and the response of the animal to infection.

MCF - THE DISEASE AND RESEARCH AT WALLACEVILLE

Rod Oliver, MAFQual, Wallaceville

The interesting epidemiological features of MCF in deer in New Zealand include (Beatson 1984):

a) winter-spring incidence peak with about 70% of cases occurring in the July to September period,

b) higher prevalence in the southern (colder?) parts of the country, namely Canterbury, Otago and Southland,

c) higher prevalence on intensive compared with extensive farms, and

d) lower prevalence in calves, compared with adults.

In deer, MCF is predominately an acute disease; fever, diarrhoea, dysentery, dehydration, shock and death within 48 hours of the onset of diarrhoea are typical clinical signs. Severe diarrhoea and massive intestinal haemorrhage are the principal causes of death in infected deer (Oliver et al 1986).

The pathogenesis of acute MCF in deer has been investigated. Changes in blood coagulation parameters were monitored during the course of experimental MCF. Following the onset of fever, blood platelet counts diminished and prothrombin and activated partial thromboplastin times increased. Increased fibrinogen levels, decreased anthrombin III levels and increased fibrinogen degradation products were detected. These coagulation abnormalities, together with haemorrhage and thrombosis indicate disseminated intravascular coagulation is an important mechanism in acute MCF in deer.

Transmission of MCF from deer to deer is readily achievable by inoculation of blood or lymphoid tissue suspension from affected deer. However, contact transmission between deer appears to be an infrequent event. Adaption of MCF from deer to rabbits was achieved and a rabbit model of MCF in deer developed. Transmission in rabbits was maintained for over 12 months by inoculating susceptible rabbits with blood from febrile rabbits. A consistent pattern of experimental disease was obtained with fever, diarrhoea, acute mesenteric lymphadenitis, typhlitis and colitis being the predominant features. The mean incubation period was 13 days, irrespective of inoculum dose. Backpassage from experimentally infected rabbits to deer was not achieved. Horizontal transmission of MCF by contact between rabbits was not demonstrated experimentally (Oliver 1984).

The virus causing MCF was propagated in spleen explant cell cultures derived from spleens of febrile rabbits experimentally infected with MCF. Spleen cells, in culture for up to 11 passages in vitro, were shown to transmit MCF when inoculated into rabbits. However the virus was not visualised in infected cells.

HISTOPATHOLOGY OF MCF

Marjorie Orr, MAFQual, Invermay

The clinical signs of MCF in deer vary depending on the rate of progress of the disease. The disease may be peracute with animals simply found dead. They may appear to be ill for only a few hours showing dullness and fever. Those cases which last for a few days often develop diarrhoea (and dysentery). In longer lasting cases, which linger for several weeks, the animal becomes emaciated, while there may be nasal and ocular discharges, buccal erosions, conjunctivitis or corneal opacities.

Post mortem changes usually feature haemorrhage which is often into the intestinal tract. The terminal ileum and the ileocaecal part of the intestine contain blood; there may be tiger striping in the colon. There may be erosion in the oesophagus and abomasum as well as the mouth.

None of these clinical signs or post mortem changes are pathognomonic of MCF although they are characteristic, with the diagnostic lesions being histological. There is general agreement that there are 3 types of histological lesions:

a) vasculitis,

b) lymphoid proliferation and infiltration, and

c) tissue necrosis

While most workers (McAllum et al 1982, Oliver et al 1983, Wilson 1983 - red deer; also Denholm and Westbury 1982 - rusa deer in Australia) describe vasculitis as the predominant lesion, Reid and Buxton in Scotland, describe a lymphoid reaction and tissue necrosis as the predominant lesions (references 1984).

These apparently differing views on the relative predominance of the histological lesions were highlighted when Reid et al (1987) and Orr and Mackintosh (1987) published independent accounts of MCF outbreaks in Pere David's deer. The former attach more significance to the lymphoid proliferation and tissue necrosis and also have developed a convincing hypothesis to explain the pathogenesis of the lesions. In contrast, Orr and Mackintosh described vasculitis as the most consistent and conspicuous lesion of MCF.

The contrasting views are not incompatible. While the lymphoid reaction and tissue necrosis may be more significant in the pathogenesis of MCF, it is the vasculitis which is of more relevance in diagnosis of the disease. Other factors may also be involved - the UK cases may be generally less acute than those seen in New Zealand or different strains of the agent may be involved in the two countries.

MOREDUN STUDIES

Hugh Reid, Animal Diseases Research Institute, Moredun, Research Institute, Edinburgh.

The presentation covered the following aspects, with only the main points of the paper covered here.

a) Bovid gamma herpes viruses
b) Evidence for infection in sheep and the relatedness of AHV-1 and SAV and other herpes viruses
c) Experimental transmission
d) Molecular studies
e) Pathogenesis of MCF
f) Conclusions

a) Bovid gamma herpes viruses
It is well established that the cause of MCF in cattle in Africa is a gamma herpes virus carried by wildebeest known as Alcelaphine Herpes Virus 1 (AHV-1). It is now believed that MCF in cattle and deer in the UK, NZ and many other countries is caused by a closely-related virus carried by sheep, known as the Sheep Associated Virus (SAV) of MCF. Whereas AHV-1 is mainly confined to Africa (and zoos worldwide), SAV has a worldwide distribution associated with sheep. In both cases, experimental transmissions to cattle, deer, rabbits and hamsters have been achieved.

AHV-1 is endemic in wildebeest with virtually all animals infected and is present in other African bovids. For example, neutralising antibodies were detected in 23/23 wildebeest, 124/206 Coke's hartebeest, 25/62 topi, and 3/3 oryx. Although the hartebeest, topi and oryx had a high proportion of carriers, they do not appear to transmit the disease to cattle with the same ease as wildebeest.

b) Evidence for infection in sheep and the relatedness of AHV, SAV and other herpes viruses
Blood samples from sheep flocks from a number of countries were tested using neutralising antibody and indirect immunofluorescent antibody (IIF) tests. While there was no evidence of reaction in the more specific neutralising antibody (to AHV-1) test virtually all sheep were positive in the more cross-reactive IIF antibody test.

In one study, hamsters were infected with the AHV-1 strain of MCF from wildebeest, and with the SAV strain from field cases of MCF in red deer, cattle and Pere David's deer. Using IIF tests with antibodies to AHV-1, reaction was detected in hamster sera from 22/22 wildebeest (AHV-1, the control), and cross-reaction in 28/36 red deer, 24/25 cattle and 33/34 Pere David's deer.

At Moredun, conventionally derived lambs were all found to be IIF positive from birth. A group of 6 specific pathogen free (SPF) caesarian-derived lambs were negative at birth but while in isolation all seroconverted up to 40 days after birth suggesting that at least one had been infected in utero and had infected the in contact lambs.

Two other SPF lambs that were isolated remained seronegative up to the age of 1 year old until they were joined with normal sheep, after which they seroconverted around 14 days later.

Although IIF is not the test of choice for specificity in identifying related viruses, the data have shown a consistent pattern, when testing hamsters infected with a wide variety of bovine and ovine herpes viruses. Unlike the cross-reaction in the IIF system with SAV, there was no cross-reaction with these other known herpes viruses.

c) Experimental transmission
MCF infection has been transmitted from both cattle and deer to rabbits and from those rabbits to hamsters but not mice, rats or guinea pigs.

d) Molecular studies
Using cloned DNA probes from AHV-1, significant homology between AHV-1 and the SAV was apparent (this technique involves taking pieces of single stranded DNA from AHV-1, cloning it in a plasmid to produce defined material, isolating the DNA and then running it against DNA from SAV infected cells; binding of the two species of DNA indicates complementarity - ie, homology between the two). Western blotting involves antibody:protein reactions; using immunological probes from AHV-1 with this technique, showed that the SAV and the AHV-1 had many antigenic components in common. The Western blotting, in particular, provided convincing evidence of the relatedness of AHV-1 and SAV.

e) Pathogenesis of MCF
The Moredun group's working hypothesis is that MCF is a lymphotropic virus. The target cells for the virus are a particular subset of T cells. The large granular lymphocytes (LGL) are "natural killer" (NK) cells which can be isolated simply from MCF-infected deer (eg, LGL isolated from CSF in 5/6 and from lymph nodes in 7/9 deer), but not from normal deer. Normally the NK cells, present in all animals, kill only virus-infected or transformed cells. It seems likely that the viral infection deregulates the cells resulting in an increased production of interleukin-II, which stimulates the LGL further. The final death of the host occurs when the LGL turn on the animal's own epithelial and endothelial cells.

To understand the transmission of the disease, it is necessary to understand the various strategies of herpes viruses, which are outlined here:

i) productive infection: the host cell synthetic processes are switched over to produce whole virus; cell death results and virions are released.

ii) latent infection: the host cell functions normally; viral DNA persists but there is no viral expression.

iii) semi-productive infection: the host cell synthetic processes are deregulated; there is partial virus expression but no intact virions are released.

It appears that in SAV infections in sheep, both productive and latent infections occur while in deer and cattle semi-productive and possibly latent infections occur. Sheep with productive infections release the infective virions to the environment. The virus is picked up by deer where it is possible that it may remain latent for a time before becoming semi-productive, and precipitate fatal disease. Because no complete viral particles are produced deer and cattle are "dead end" hosts under natural conditions although transmission can be achieved artificially using cellular preparations from infected animals.

f) Conclusions
All sheep are naturally infected with SAV.

Deer are "dead-end hosts" because they experience a semi-productive infection and incomplete viral expression and therefore it is most unlikely that there is any deer to deer transmission in the field situation in red deer.

The pathogenesis of MCF is thought to be due to widespread tissue damage caused by killer T cells attacking normal cells, especially blood vessel walls, and mucosal cells.

DISCUSSION

The Discussion addressed the specific issues listed previously, with the important points being summarised here.

a) Is the NZ MCF the same as the UK MCF?
There was general agreement that the similarities between the descriptions of the NZ and UK diseases were much greater than the dissimilarities. However, the omission of the vasculitis in the description of the disease in the UK was initially a stumbling block, but it does in fact occur. Although there may still be subtle differences in the necropsy findings, it has to be noted that MCF is not a static disease but has a spectrum of presentations. The conclusion was that they are the same disease but that there may be subtle differences between the agents, which would require isolation and sequencing.

b) Is the sheep the real host? (Dr Hugh Reid comments)
The evidence strongly favours the hypothesis that the virus is a sheep-associated gamma herpes virus. Although Herpes viruses are considered quite fragile (Mareks disease surviving in chicken litter for years is an exception), there is evidence of Bali cattle (which are highly susceptible) becoming infected from sheep about 40 metres away; this suggests transmission via aerosols/wind-borne spread. In this respect, it is almost certain that sheep are the reservoir for the SAV form of MCF in Bali cattle and buffalo. However, there are two intriguing things about Indonesia. Firstly, whereas the domestication of cattle and sheep were associated in most parts of the world, the situation in Indonesia was different, in that sheep were introduced only about 100 years ago; ie, the contact between sheep and Bali cattle is recent. Secondly, there are areas of Indonesia where there is legislation against the keeping of any small ruminant just to protect the Bali cattle.

The specific pathogen free (SPF, Caesarean derived) lambs had no contact with other sheep but seroconverted 20-40 days after birth. We suggest that at least one of these lambs was infected in utero and that the virus was latent from the time of infection until some time after birth. Later the virus was triggered off into a productive infection that then infected the in contact lambs. We propose that the antibody appeared only after the productive infection and that there was no immune stimulation during the period of latency.

It is likely that immunosuppression of sheep would cause productive expression and excretion of the SAV. Although this has been tried, the SAV was not detected, possibly because an inappropriate substrate was used in the test.

One of the intriguing things about the SAV is that while all sheep are apparently infected, some flocks are very effective at transmitting the disease to cattle whereas others are much less effective.

c) Is there deer to deer transmission?
There is no evidence and it appears most unlikely that there is deer to deer transmission of MCF under field conditions with red deer. There was one case at the Moredun where a red deer, which was extraordinarily affected, infected an in contact animal although the latter could have eaten infected material.

Colin Mackintosh then referred to the intensive grooming of the Pere David's deer by one another. It is possible under such situations that infected cellular material could be consumed. It is also proposed that a smaller challenge is required to produce the transmission in Pere David's deer. Consequently it is conceivable that there could be deer to deer transmission in Pere David's.

d) How is the virus transmitted/what triggers the disease? (Dr Hugh Reid comments)
Although there is no direct evidence it is likely that ewes in the peri-parturient period are responsible for much of the viral shedding, non-pregnant adult sheep may also infect younger sheep. The SPF lamb situation was described above. However, it may be that it is the intensity of the challenge which is important in causing an infection. For example you may require a much lesser challenge to get infection in Pere David's deer or Bali cattle than in red deer or cattle.

It is possible that the natural challenge by cell-free virus (presumably entering via the nasal mucosa) could have a much longer incubation (latent?) period than the experimental challenge which was via large volumes of infectious material. In this case the incubation period is 2-3 weeks. However, there are the Rowett cases where animals became infected by contact with someone who had also been working with sheep and where the incubation period was also about 2-3 weeks.

There is also the question as to what actually triggers the disease in deer. It is possible that the virus is latent within the deer for a time and that a triggering event may allow the virus to transfer to an appropriate type of cell. It is also possible that the different

susceptibility may be at the receptor/target cell level in the lymphoid cell series. Incidentally the Moredun group have detected antibodies (IIF) in only one case of MCF in deer, a chronic case of 3 months duration.

e) Why are young deer (ie, less than 1 year old) not usually affected?
MCF is very rare in calves less than 6 months of age and is not common in animals of 6-12 months. Although cases do certainly occur in deer of less than 12 months of age, the attack rate is much lower than for any other age group.

f) Is the development of a vaccine a realistic goal?
Several groups have tried various AHV vaccines in cattle and the animals challenged with cell-associated virus, which is of course an unnatural type of challenge. Often high antibody titres have been produced and animals have survived the challenge.

Dr Reid suggests that there would be practical problems with developing a suitable vaccine against SAV for female deer because of the danger that the hinds may become permanently infected as occurs with cattle vaccinated against AHV. Cattle have been protected by vaccinating with an attenuated form of the AHV. Although they survived challenge, they remained permanently infected with the wildebeest strain of MCF and subsequently the foetuses were aborted or the calves were infected in the first month of life. Herpes viruses are notoriously difficult to vaccinate against. The vaccine may protect against the disease but not protect from infection. Therefore an alternative possibility would be vaccination of stags to prevent wastage in this group of high value breeding and velveting animals. Another theoretical, though not practical, alternative in the NZ situation is the development of a modified virus with an appropriate deletion to vaccinate sheep.

g) What avenues of research should be pursued?
The most appropriate research in NZ would involve some co-operation with Dr Reid's group at the Moredun. The urgent need in NZ is to devise a research programme which will identify the mode/situation of transmission of the virus to deer and to define the infective process so that appropriate control measures can be devised. For example, when do sheep shed the virus - how long should paddocks be spelled - how close is too close to a sheep - even though all sheep may be infected are all sheep infective?

The Alcelaphine gamma herpes virus (AHV) causes MCF in the African wildebeest. The immunological and molecular probes developed from this virus have provided the convincing evidence that there is a related virus in sheep which is the cause of the MCF in deer and cattle. The objective of the Moredun programme is to employ these reagents to isolate, identify and characterise the sheep virus. As appropriate, aspects of this work could also be carried out in NZ.

There is also a place for studies to investigate the effect of various stressors and other factors which might influence the development of the disease in the NZ situation. This is particularly relevant when it is considered that the principal losses occur during winter. This

could involve examining the immunological response of deer to infection with SAV and attempting to determine the "trigger factors" which might precipitate infection or the transformation from latency to the semi-productive state.

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WORKSHOP REPORTS

The following four papers consists of case study presentations considered at intensive workshop session. Course delegates were required to work through and discuss each case.

Data is presented here to give readers an understanding of the content, format and approach to these sessions, and to encourage others to study them and refine their own approach to such problems:

Feed management budget	R R Fraser J Stantiall
Content for Deer Herd Health Workshop	P Wilson I Walker
Reproduction Workshops	P F Fennessy N S Beatson M J Bringans
Breeding and Genetics Workshop	C M Rapley