Malignant catarrhal fever

W. PLOWRIGHT*

Summary: Whilst malignant catarrhal fever (MCF) is generally a sporadic disease, its incidence is probably increasing in farmed deer; the sheep-associated infection (SA-MCF) of cattle has, possibly, diminished recently. Wildebeest-derived disease (WD-MCF) is a problem in Africa where cattle and wildlife share pasturelands; it is also now reported more often in zoological collections and ranches with exotic ruminants.

A discussion of the clinical signs and pathogenesis of MCF is followed by an account of the epidemiology of both forms. An increasing number of species in the family Bovidae, especially in the subfamilies Caprinae, Alcelaphinae and Hippotraginae, exhibit widespread inapparent infection with herpesviruses, related to the Alcelaphinae prototypes AHV-1 and AHV-2. The circumstances in which these viruses spread from “reservoir” to “indicator” hosts are discussed. The range of the latter is still widening.

The alcelaphine viruses are gammaherpesviruses with resemblances to H. saimiri and H. aethes. Lymphoblastoid cell lines with NK cell characteristics, sometimes capable of reproducing MCF, have been established from tissues of SA-MCF infected animals; virions or viral antigens have not yet been unequivocally identified in them.

Methods for laboratory confirmation of a diagnosis of MCF are outlined. Control still depends on separation of reservoir and indicator hosts.

KEYWORDS: Antelope - Bovine herpesvirus - Cattle diseases - Diagnosis - Disease control - Epidemiology - Lesions - Malignant catarrhal fever virus - Pathogenesis - Symptoms - Wild animal diseases.

INTRODUCTION

Of the herpesvirus infections of animals which are covered by this issue, malignant catarrhal fever (MCF) is undoubtedly the least important economically. It is sufficiently infrequent that some observers publish papers which record large clusters of cases on a property as an “epizootic”. The incidence of MCF has probably declined in some European countries, as traditional practices of housing sheep and cattle together in winter have become less common, but has probably increased in importance in zoological collections and on ranches which include exotic ruminants or farmed deer. New Zealand, in particular, has seen dramatic growth of its farmed red deer population, to about 250,000 in 1983 and estimated to reach 750,000 by 1990 (86); there are now estimated to be about 300,000 exotic ruminants on ranches in Texas alone (27). In Africa the threat of wildlife reservoirs of MCF to the cattle of nomadic pastoralists or ranchers can still disrupt grazing patterns and increase pressure on “game” animals which are major tourist or sporting attractions.

Scientifically, on the other hand, MCF offers a serious challenge to the new technologies — particularly to identify the agent of the sheep-associated disease and

* Whitehill Lodge, Goring-on-Thames, Reading, RG8 0LL, United Kingdom.
to explain its epidemiology, pathogenesis and immunology. Furthermore, the evolution of a distinctive group of herpesviruses in African antelopes and wild or domesticated caprines should provide a fascinating research topic for years to come.

There have been several reviews of various aspects of MCF in recent years and it is not proposed to repeat here many details found in these papers (24, 43, 60, 61, 66, 69, 75).

GENERAL REMARKS

Malignant catarrhal fever is a clinicopathological entity which has been reported from virtually every country in the world capable of a reliable diagnosis; it should be emphasized that it is not yet proved to have a single aetiological agent, although papers are still published which imply that this is the case (30, 39). Outside Africa it can usually be associated with more or less close contact between presumed carrier sheep and susceptible indicator species. This “sheep-associated” (SA) disease has also been reported by many workers in Africa (12, 53, 54, 56), where the majority of cases are, however, known to be derived from contact with two species of alcelaphine antelopes, the blue or “white-bearded” and the black or “white-tailed” wildebeest (Connochaetes taurinus and C. gnu, respectively). The use of the term “African MCF” could, therefore, be misleading, although it has the virtue of brevity; since the isolation of the causal herpesvirus from wildebeest (62), the description “wildebeest-derived” (WD) is accurate and more informative; “wildebeest-associated” seems unnecessarily cautious.

CLINICAL SIGNS OF MCF

The classical signs of MCF have been recognised in Central Europe for well over a century, often under the name “Kopfkrankheit” which was a sporadic, non-contagious disease to be differentiated from rinderpest or “bovine typhus” (10, 87). The use of the term “head-and-eye form” for the majority of cases in cattle seems to date from a paper by Götze (15) who also described peracute, intestinal and mild forms, the occurrence of which has been frequently substantiated, especially in recent observations on the disease in deer, as well as in cattle and exotic ruminants.

The “mild” category is the one about which the greatest doubt exists, as a natural phenomenon; a few such cases have been proved to follow experimental infection with African (wildebeest) virus (see 58, 63, for example). Incidentally, the first ox in the transmission series described by Mettam (40) developed fever on one day only, following inoculation of wildebeest blood, but its blood was virulent for another ox at that time and, surprisingly, it succumbed to challenge less than four months later. There was no serological evidence, in a limited survey, that subclinical or mild infection occurred in cattle in Kenya Masailand, an area of heavy wildebeest exposure (76).

It is difficult to obtain significant figures for the mortality in naturally-occurring outbreaks of the disease, since some cases are often slaughtered, but Pierson et al. (50) described a remarkable incident in which 87/231 cattle were affected and died in Colorado, USA, whilst Maré (37) reported a 16.6% mortality
in a 1,000 cow herd over a 3 month period in California. James et al. (31) reported that all 28 cattle clinically affected on one property died in New Zealand; it is advisable to assume that any appreciable rate of recovery is an indication of concurrent infection or mistaken diagnosis. During serial transmission of MCF in cattle inoculated with wildebeest herpesvirus the mortality rate has varied from 94-100 per cent, the lower figure (292/311) being associated with concurrent anaplasmosis in some cases, which can reduce fatalities (56, 58). Essentially similar though incomplete figures were provided for N. American cattle (32).

The most important clinical manifestations of MCF are listed in Table I and additional comments follow. Good colour plates of the visible lesions are to be found in several publications (29, 80, 81).

**Table I**

The clinical signs of malignant catarrhal fever

| (a) Sudden, persistent pyrexia |
| (b) Severe congestion, necrosis and erosion of nasal and oral mucosae |
| (c) Serous, later mucopurulent, discharges |
| (d) Scleral and conjunctival congestion; centripetal corneal opacity; hypopyon |
| (e) Generalized enlargement of lymph nodes, etc. |
| (f) Muscular tremors (meningo-encephalomyelitis) |
| (g) Diarrhoea or dysentery (especially deer) |
| (h) Dermatitis and laminitis |

(a) (e) The fever and lymphadenopathy in MCF

In typical "head-and-eye" cases caused by WD virus the pyrexia is of sudden onset, reaching a peak of about 105° to 106°F (mean 105.5°) by the 3rd or 4th days of the disease (56). Its onset is frequently used as an indicator of the end of the incubation period, but a few observers also use local or general enlargement of lymph or haemolymph nodes, which occasionally may be palpated 1-4 days prior to fever in the WD form (11, 53, 56). However, lymph node enlargement was regularly noted by others (52) as much as 5-10 days before pyrexia and the prefebrile clinical signs, including lymphadenopathy, could last 2-7 days in N. American cattle (32).

A minority of experimental cattle infected with WD virus showed a biphasic response, not attributable apparently to intercurrent infection; the first reaction was usually at 4-7 days, lasted 2-3 days and reached a lower peak, not exceeding 104°F (Plowright, unpublished). Such reactions were possibly related to an as yet undescribed generalization phase in the pathogenesis of the disease and are reminiscent of the bimodal distribution of reaction times (peaks at 3 and 11 days, respectively) noted in rabbits used for an SA transmission series (71).

In the experimental SA form a frequent delay of 2-3 days in the onset of lymphadenopathy was recorded (51), whereas Selman et al. (81) noted enlargement of nodes in 6/10 calves at 7 days post-infection, although the "incubation period" was at least 20 days.
(b) (c) Congestion, necrosis and erosion of superficial mucosae and muzzle

These signs were recorded in the oral and nasal mucosae in the majority of cases caused by WD virus (32, 52, 56), the time of first detection of oral necrosis being given in Table II which shows a peak prevalence (100%) delayed to the 6th day of pyrexia. In the naturally-occurring SA form, mucosal necrosis is noted less frequently or is less severe in some outbreaks in cattle (31) or deer (13, 67), though constant in others (80). In the experimental SA form the speed of development of mouth lesions varies considerably in cattle, except for the early diffuse congestion (81). Generally speaking, the development of marked lesions of the muzzle or oral and nasal cavities is dependent on longer survival times, which are more characteristic of WD cases and less prominent in the hyperacute or acute disease. Similarly, whilst mucopurulent nasal and ocular discharges, together with excessive salivation, are almost invariable in “head-and-eye” cases, they tend to develop less frequently in those which run a shorter course.

Table II

The frequency and time of appearance of clinical signs of WD-MCF in experimental cattle*

<table>
<thead>
<tr>
<th>Clinical sign</th>
<th>No. of animals</th>
<th>Cumulative % with lesion on given day of disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Corneal opacity</td>
<td>234</td>
<td>4</td>
</tr>
<tr>
<td>Oral necrosis and erosion</td>
<td>116</td>
<td>58</td>
</tr>
</tbody>
</table>

* From Plowright, 1964.

(d) The eye lesions in MCF

 Conjunctival and scleral congestion are recorded in the great majority of SA and WD cases, accompanied by a progressive centripetal opacity of the cornea, the keratitis being accompanied by cellular and fibrinous exudates into the anterior chamber — an iridocyclitis with hypopyon. Table II shows the time of appearance of corneal opacity in over 200 cases of the WD form which were observed daily; the frequency did not exceed 50% until the 3rd day of pyrexia.

(f) The nervous signs of MCF

 Signs of CNS involvement, such as muscular tremors, incoordination, head pressing, nystagmus, twitching of the ears, torticollis and even aggressive behaviour have been reported at various times in the majority of species infected by the WD or SA agents. The underlying meningo-encephalomyelitis is one of the most consistent histopathological changes. During serial transmission of the WD viruses, nervous signs apparently became less frequent (56).

(g) Diarrhoea or dysentery.

These manifestations have more recently come to the fore in cattle, deer and rabbits infected with the SA agent and exhibiting a rapid course to death (13, 37, 38, 46, 67). Thus, for example, 8/16 experimental cases in a Colorado (USA) expe-
rimental series were classed as predominantly "intestinal" with a further 3 developing diarrhoea (52). However, in experimentally-infected red deer, diarrhoea did not develop until the second or third days of fever, and dysentery about 24 hours later (46). In the experimental WD disease the frequency is very low, less than 1% in uncomplicated cases (56).

(h) Dermatitis and laminitis in MCF

The relative frequency of skin, hoof and horn lesions in MCF in Europe was one of the early reasons for regarding it as different from S. African "snotsiekte" (40). Severe congestion, with exudation and sloughing of the skin of the udder, was observed in the USA in an SA outbreak (37). Dermatitis is certainly infrequent in the WD form, only 2 cases being observed among several hundred experimental cases in E. Africa; both were young calves, and only one case of laminitis was detected (56). Exudation into the horn cores with loss of these appendages as well as laminitis was recorded not infrequently in the older European literature. Arthritis and synovitis have been recognized histopathologically in a majority of cases of experimental and natural SA-MCF (36) but associated clinical signs are not usually reported.

THE PATHOGENESIS OF MCF

The three essential components in the pathogenesis of MCF (55, 61) are:

1. A destruction of smaller lymphocytes, particularly those in the germinal follicles of lymph and haemolymph nodes and in the thymus (14). Diffuse areas of necrosis appear in some cases and karyorrhexis is marked, together with macrophage activation. It may be that the necrosis is a late event, coincident with the onset of fever, as in rabbits infected with SA-MCF (65, 66).

2. A proliferation and infiltration in many tissues by large lymphoblastoid cells, particularly around blood vessels and in T-cell dominated areas such as the interfollicular and paracortical areas of lymph nodes or periarterial sheaths in the spleen. This lymphoproliferation is progressive through the incubation period but may not be an essential component, as its prevention in rabbits by cyclosporin-A treatment did not change the fatal course of infection (65).

3. An angiitis, affecting all components of the walls of arteries and veins. This is irregularly segmental in distribution, most readily seen in medium-sized arteries, such as those which abound in the capsule of the adrenal gland, in the carotid rete and arcuate arteries of the kidney cortex. There is often a fibrinoid degeneration of the medial elements with hypertrophy and lymphoid infiltration of the intima. Partial occlusion of the vascular lumina is common, complete thrombosis leading to infarction relatively rare. Particularly good descriptions of the vascular lesions are given by Liggitt and others (33, 34).

Similar infiltrative and degenerative changes to those in blood vessels occur in the capsule and trabeculae of lymph nodes and spleen, in perinodal connective or adipose tissues and in the smooth muscle layer of hollow viscera. Changes of types 2 and 3 occur in the lamina propria and deeper layers of many mucosal surfaces and the skin where they are associated with degeneration and necrosis of the surface epithelia (35). They are often predominant in the supporting tissues of parenchymatous organs such as liver, kidney and lungs, whose specialized cells may show degeneration and necrosis.
There is increasing evidence that these changes are primarily cell-mediated, immunopathological events and this would be consistent with an extended but variable incubation period, long prepatent viraemia associated with lymphocytes and the virtual absence of viral cytopathology, antigens or virions in any of the lesions.

Suggestions that hypersensitivity to viral antigens, autoimmune (graft-v-host) reactions or immune complexes are involved (61, 75) have not yet been substantiated but the work of Reid and his collaborators in establishing the association of MCF infectivity (SA and WD) with large, granular T lymphocytes possessing cytolytic (NK) activity does offer a novel and credible hypothesis (65, 66).

EPIDEMIOLOGY

The epidemiology of both the SA and WD forms of MCF is essentially the same world-wide. The great majority of outbreaks can be related to close contacts between the reservoir species, sheep or wildebeest and the indicator hosts; the reservoir animals show no clinicopathological evidence of infection although suspected MCF in sheep has been reported recently (79). The reservoir hosts are not capable of transmitting infection at all times, otherwise MCF would be a much more serious problem. With very few reservations it can also be said that transmission by contact between indicator hosts does not occur.

(a) Sheep-associated disease (SA-MCF)

After prolonged, sometimes acrimonious, exchanges during the 1930’s about the origin of MCF it was generally accepted that direct sheep contacts, during the previous 6 months or so, could be established on the overwhelming majority of properties where multiple cases of MCF occurred, albeit spread over long periods. In these cases removal of the sheep normally resulted in cessation of MCF within 3-4 months (Götze (16a) with whom was associated the dictum “ohne Schafe kein Katarrhhalieber”). It was also shown convincingly, as on many later occasions, that mixing known “carrier” sheep with cattle could lead to numerous cases of MCF (12, 15, 17, 54, 83, 84). Others, such as Wyssmann (87, 88), were unsuccessful in demonstrating sheep: cattle transmission. Wyssmann found that only single cases were reported from 83% of affected farms, on 20-25% of which there could not possibly have been any contact with sheep. In New Zealand, 95% of cases from 1974 to 1976 were single animals and sheep association was reported for 77%, with no information on 12% (22). The basic questions about alternative hosts to sheep have still not yet been answered and, furthermore, it has not yet been possible to produce typical MCF in cattle by inoculating them with material from suspected carrier sheep or other potential caprine reservoirs.

Several more recent publications have reiterated that infection of cattle or deer is most likely during the sheep lambing season (21, 65, 66, 69) and the seasons of highest incidence for cattle, often reported as late winter and spring to early summer in temperate zones, would be consistent with this hypothesis (e.g. 50). Others have reported more cases in autumn and winter, as for example in Yugoslavia (80) whilst, in Finland, the highest incidence amongst 335 cases, over 5 years to 1949, was in summer (84). In South Africa SA-MCF was not related to season (1), whereas in New Zealand the disease in deer is most frequent in mid-winter, i.e. June/July (38, 65) and in cattle in September/October (22).
Numerous old references to cases of MCF which could not be related to direct or indirect contacts with sheep (84, 87, 88) certainly necessitated an open mind on the possible existence of other reservoirs, an increased need since the accession of serological evidence for MCF infection in other species of the subfamily *Caprinae* (64, 27). The most frequently positive species (≥20%) appear to be in the ibex varieties (*Capra ibex* spp.), moufflon sheep (*Ovis orientalis*), Barbary sheep (*Ammotragus lervia*), markhor (*Capra falconeri heptneri*) and Himalayan tahr (*Hemitragus jemlahicus*), all in the subfamily *Caprinae*, and the chamois (*Rupicapra rupicapra*) in the subfamily *Rupicaprinae*. Until the herpesviruses present in these species have been characterized, it is too early to judge their significance.

Equally well, it is necessary to accept that intermediate contagion could play a role in transmission from sheep to cattle or deer (67) and there is also some evidence that the infection may, unusually, spread amongst highly susceptible deer by contact (38, 71), but the evidence for this in cattle is absent or unconvincing (37).

(b) Wildebeest-derived (WD) MCF

WD-MCF has long been known as a menace to cattle owners in Africa (60, 61). The strains of agent studied by important earlier workers (11, 40, 53) were all probably derived from wildebeest and had the advantage over SA “viruses” that they were easily transmissible, both to cattle and rabbits. Except in one case (40), however, proof of the species of origin was lacking. The recovery of a herpesvirus from 5/35 blue wildebeest in Kenya in 1959/60 proved the role of this species as a reservoir, confirmed the ready transmissibility of the agent and showed its limited cytopathogenicity and strictly cell-associated infectivity in cell cultures and intact hosts (62).

Similar viruses are apparently universal in populations of blue and black wildebeest, whether in Africa (1, 19, 60, 61) or in zoological collections (9, 27). Small groups of animals (<10) can probably maintain the viruses indefinitely, since there is long-term persistence in at least some individuals and transplacental transmission has been demonstrated (9, 28, 62). Young wildebeest, if not congenitally infected, acquire the virus by horizontal spread in the annual calf crop, in spite of the presence of maternal neutralizing antibody. They develop a leucocyte-associated viraemia which is most frequent (40% positive) in the second month of life, declining to 2% by the end of the first year and which is very infrequent thereafter (61). There is no good evidence for clinical signs or a virus-associated pathology in viraemic wildebeest, though it was suggested that a vasculitis found in a yearling or adult animals was similar to that in MCF (8).

The maximum annual incidence of WD-MCF in cattle is probably of the order of 7% in Kenya Masailand, concentrated in the months of April to July, following the wildebeest calving in February to April. The times are earlier but comparable in Tanzania but in southern Africa it is not clear whether peak cattle transmission (September to December) is associated with the wildebeest calving (1). In individual herds the incidence of WD-MCF cases in a single season may reach 13-20% (1, 56).

When viraemic wildebeest calves were housed experimentally with cattle they developed MCF after maximum incubation periods of 30-47 days in 4/5 cases, 81 days in the fifth case (61): this corresponds closely with delays of 29, 68 and 82 days in the death from MCF of 3/4 banteng (*Bos javanicus*) exposed to newborn wildebeest in the San Diego Wild Animal Park (18). The cell-free herpesvirus is excreted
as early as one to four days (28, 43), both in the ocular and nasal secretions and was recovered once from faeces (28) but not in urine or saliva. The titre, particularly in calves 6-8 weeks old, may be $\geq 10^{3.2}$ TCD$_{50}$/ml, probably derived from sites of replication in the cornea and turbinate mucosa; after 3 months of age the secretions contain neutralizing antibody, predominantly IgA, which accounts for the rapid decline in virus shedding and cessation of lateral transmission (43, 61).

Excretion and transmission of MCFV to cattle by adult wildebeest has occurred under experimental conditions — housing and exposure to heat stress (Plowright, unpublished), but attempts to reproduce the phenomenon failed. The nasal and oral secretions of infected cattle do contain MCF infectivity but this is probably in cell-associated form (32, 44) and contact transmission in this species does not occur or is very rare (11, 12, 58). Similarly, deer-to-deer transmission of WD-MCF was not observed (85).

Although "close contact", particularly on common grazing or on housing together, is generally regarded as necessary for the transmission of the WD virus from the reservoir host, there are now several accounts of apparent transfer across fences or even at distances of up to several hundred metres, i.e. direct contact is not essential (1, 37, 89). The route of entry of virus is probably by the nasopharynx, as intranasal instillation of infectious cell suspensions, or virulent cell-free virus, leads readily to infection in cattle and rabbits (61); aerosols are also successful. Hence, transfer in air currents seems more likely than intermediate contagion and oral uptake of virus.

(c) Other potential sources of MCF viruses

Work in Africa (61) established that antibodies which neutralize WD-MCFV (alcelaphine herpesvirus 1 or AHV-1) are by no means limited to wildebeest. Sera from hartebeest (Alcelaphus buselaphus), topi (Damaliscus korrugion) and fringed-eared oryx (Oryx beisa calotis) were all found to be frequently positive. In addition, strains of herpesvirus were isolated from free-living hartebeest and topi (45), which were serologically related to AHV-1. One of four of the hartebeest isolates (AHV-2) was shown to be slightly different in that wildebeest sera neutralized it to lower titre than AHV-1. The hartebeest viruses produced MCF in cattle, in three cases atypical, whereas the topi isolate, also cell-associated, grew only in topi cells and was not infectious for cattle or rabbits (see 61). Similar viruses have been isolated recently from the Cape hartebeest (Alcelaphus buselaphus camaa) and Jimela topi (Damaliscus lunatus jimela) in the USA (27).

The ultimate objective of the African studies was to obtain an MCF-related virus which would immunize cattle against AHV-1 in the same way that turkey herpesvirus is used as a vaccine against Marek's disease, but in this they have, so far, failed. The recent surge of interest in MCF in the USA, especially in zoological collections, has led inter alia to serological surveys of many species (26, 27, 64). The methods used have included indirect immunofluorescence, which gives an essentially "group-specific" reaction for MCF viruses and also cross-reactivity with some bovine herpesviruses (25, 61, 75). The most discriminating antibody is that neutralizing the virus and Tables III and IV show, predominantly, results obtained by its detection. Details of the test virus, conditions of exposure of virus-serum mixtures, and the culture system used for assay, vary considerably and may account for some anomalies. The methodology should surely now be standardized as soon as possible.
### TABLE III

*Antibody to alcelaphine herpesvirus 1 in the subfamily Alcelaphinae*

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Sera positive for antibody (titre range)</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Connochaetes taurinus</em></td>
<td>Blue wildebeest (white-bearded)</td>
<td></td>
<td>23/23*</td>
<td>111/120</td>
<td>33/54</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(4-256)</td>
<td>(2-256)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>Connochaetes gnu</em></td>
<td>Black wildebeest (white-tailed)</td>
<td></td>
<td>—</td>
<td>—</td>
<td>4/11</td>
<td>30/46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(12-256)</td>
<td>—</td>
</tr>
<tr>
<td><em>Alcelaphus buselaphus</em></td>
<td>Hartebeest</td>
<td></td>
<td>124/206</td>
<td>0/1</td>
<td>9/20</td>
<td>14/22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(4-256)</td>
<td></td>
<td>(4-256)</td>
<td></td>
</tr>
<tr>
<td><em>Damaliscus kordigum</em></td>
<td>Topi</td>
<td></td>
<td>25/62</td>
<td>0/20</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td><em>Damaliscus lunatus</em></td>
<td>Tsessebe or Tiang</td>
<td></td>
<td>—</td>
<td>26/72</td>
<td>7/14</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(4-16)</td>
<td>(2-128)</td>
<td>—</td>
</tr>
<tr>
<td><em>Damaliscus dorcas</em></td>
<td>Blesbok</td>
<td></td>
<td>—</td>
<td>0/3</td>
<td>4/16</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(2-6)</td>
<td>—</td>
</tr>
</tbody>
</table>

* No. positive/No. tested. Neutralizing antibody except for (4).

(1) Reid *et al.* (1975), *Res. vet. Sci.*, 18, 269-273. See also Plowright (57) - 172/181 and Rossiter *et al.* (77) - 131/133.
(2) Hamblin and Hedger (19).
(3) Heuschele *et al.* (27).
(4) Ramsay *et al.* (64). Results are for IIF tests only on sera diluted 1:20.

Table III summarizes the results to date for species in the subfamily *Alcelaphinae*. Two viruses, one from blue wildebeest, the other from a hartebeest, have been provisionally designated alcelaphine herpesviruses 1 and 2 (72). At least three species in the genus *Damaliscus* (topi, tsessebe, blesbok) show evidence of infection with related viruses; many topi had neutralizing antibody, even when they had not been exposed to wildebeest.

In the subfamily *Hippotraginae* (Table IV) there is also widespread evidence of infection with MCF-related herpesviruses in the *Addax* and *Oryx* spp., including the gemsbok of southern Africa (1) and the Arabian oryx. VN antibodies were found in all of fifty fringe-eared oryx sera in Kenya, but it was not possible to isolate a virus from them (41); more recently, Heuschele *et al.* (27) reported isolation of an MCF-related agent from a healthy scimitar-horned oryx (*O. gazella dammah*) in San Diego. There is contradictory evidence for the roan and sable antelopes (Table IV) but recent confirmation of the involvement of the latter is now available (1). The waterbuck, reedbuck and kob species, sometimes regarded as a separate subfamily, the *Reduncinae*, rather than a tribe, may also be infected, but some workers have encountered difficulties with cytopathogenicity of sera from these species. The occurrence of neutralizing antibody (low-titre) in free-living members of the families such as *Antelopinae* and *Hippopotamidae* (19) as well as several others in zoological collections (27) requires further investigation.
TABLE IV
Antibody to alcelaphine herpesvirus 1 in the subfamily Hippotraginae

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Sera positive for VN antibody (titre range)</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Oryx gazella beisa</em></td>
<td></td>
<td></td>
<td>3/3*</td>
<td>1/5</td>
<td>(0-2)</td>
<td></td>
</tr>
<tr>
<td><em>O. gazella gazella</em></td>
<td>Scimitar-horned oryx</td>
<td></td>
<td></td>
<td>55/102</td>
<td>(2-256)</td>
<td></td>
</tr>
<tr>
<td><em>O. leucoryx</em></td>
<td>Arabian oryx</td>
<td></td>
<td></td>
<td>12/19</td>
<td>(2-48)</td>
<td></td>
</tr>
<tr>
<td><em>O. gazella</em></td>
<td>Gemsbok</td>
<td></td>
<td></td>
<td>30/46</td>
<td>(2-256)</td>
<td>13/16</td>
</tr>
<tr>
<td><em>Hippotragus equinus</em></td>
<td>Roan antelope</td>
<td></td>
<td></td>
<td>3/14</td>
<td>(2-6)</td>
<td>0/14</td>
</tr>
<tr>
<td><em>Hippotragus niger</em></td>
<td>Sable antelope</td>
<td></td>
<td></td>
<td>33/73</td>
<td>(2-160)</td>
<td>33/211</td>
</tr>
<tr>
<td><em>Addax nasomaculatus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4/23</td>
</tr>
<tr>
<td><em>Redunca redunca</em></td>
<td>Reedbuck</td>
<td></td>
<td>0/14</td>
<td></td>
<td>5/7</td>
<td></td>
</tr>
<tr>
<td><em>Kobus defassa</em></td>
<td>Defassa waterbuck</td>
<td></td>
<td></td>
<td>5/22</td>
<td>(8-16)</td>
<td>7/7</td>
</tr>
<tr>
<td><em>Kobus ellipsiprymnus</em></td>
<td>Ringed waterbuck</td>
<td></td>
<td></td>
<td></td>
<td>11/11</td>
<td>(45-1024)</td>
</tr>
<tr>
<td><em>Adenota kob</em></td>
<td>Uganda kob</td>
<td></td>
<td>0/12</td>
<td>6/19</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* No. positive/No. tested. Neutralizing antibody except for (4).
(2) Heuschele *et al.* (27).
(3) Hamblin and Hedger (19).
(4) Ramsay *et al.* (64). Results for IIF tests only, on sera diluted 1:20.

It must be emphasized that only wildebeest can yet be incriminated as a source of MCF for cattle or other susceptible, exotic ungulates. The additional carriers of MCF-related viruses can apparently be grazed or even housed together with indicator species with impunity. It should also be borne in mind that any disease induced by herpesviruses which are harboured by species other than wildebeest, could be atypical as well as infrequent, and might well have passed unrecognized. The most interesting epidemiological and virological question is how these viruses have evolved and diverged in the rich bovid fauna of subtropical Africa.
(d) The range of indicator species of MCFV

All breeds of cattle and domestic buffaloes are susceptible, at all ages, to MCF; some data suggest a higher incidence in yearling beef cattle in New Zealand (22). It is evident from the expanding literature that the number and diversity of species which can develop more or less typical MCF, in the clinicopathological sense, is continually increasing (24, 60, 65). In recording cases, a differentiation should always be made, where possible, between those probably or certainly derived from alcelaphine, as opposed to caprine, reservoir species, as the respective indicator host ranges of the agents may vary. It is now relatively easy to confirm the presence of several alcelaphine MCF viruses in the laboratory, even if the caprine members are still virtually impossible to identify. Secondly, if a species is a putative or proven reservoir host it seems unlikely that it would develop MCF in response to infection. For example, it has sometimes been claimed that sheep developed signs of the disease when infected with presumed SA-MCF and naturally-occurring cases have been reported (79). The evidence was by no means convincing, except perhaps in the case of four lambs inoculated in utero (4). Whilst varying accounts of the susceptibility of sheep to AHV-1 have been published, it may be that different breeds of sheep or different inocula and routes of infection account for the discrepancies reported and their susceptibility is more plausible (61). It is also surprising that an Arabian oryx was reported to have died of MCF in San Diego (24).

Judging from the frequency of cases reported in zoological collections, the species most susceptible to AHV-1 disease are probably the Asiatic wild cattle (24), Bos javanicus (the banteng) and B. gaurus (the Indian gaur), also the N. American and European bisons (Bison bison and B. bonasus). Some African antelopes, the kudu (Tragelaphus strepsiceros) and the sitatunga (T. spekei) are certainly susceptible and the Asian nilgai (Boselaphus tragocamelus). The Cervidae also appear to be much more susceptible than cattle to SA-MCFV (65, 69), or to AHV-1. Severe outbreaks of SA-MCFV affecting sika, axis, Père David and red deer have been reported whilst Rusa, Barasingha and roe deer have now been added to the list (24, 65, 71). It has even been suggested that there could be ecological advantages for sheep and some antelopes in carrying viruses so pathogenic to their competitors (65). It is particularly significant that some of these taxa, Père David deer and European bison, for example, are "threatened species", which should never be subjected to the threat of MCF from known reservoirs.

CULTIVATION OF THE AGENT OF SA-MCF

Numerous attempts over many years to cultivate the agent of SA-MCF by methods successful for the alcelaphine herpesviruses, yielded several candidate viruses, none of which would, however, reproduce the disease. The most important recent discovery in respect to SA-MCF has been the cultivation by Reid and collaborators of lymphoblastoid cell lines from rabbits, deer and cattle infected with the agent. The sequence of events, as described by Reid and Buxton (66) was briefly as follows:

The first, MF120, line was propagated by co-cultivation of fetal ovine kidney cells and rabbit lymph node cells, treated with polyethylene glycol. The kidney cells degenerated, whilst refractile, rounded cells appeared in the fluid phase and had the morphological and functional characteristics of large granular lymphocytes (LGL) or
natural killer (NK) cells. They carried "T" cell antigens and Fc receptors, were of rabbit karyotype, required "feeder" cells for replication, were cytotoxic to cultured primary cells or established cell lines and, most importantly, were shown to carry MCF infectivity on inoculation into rabbits in a dose down to $10^{-2}$.

Similar lymphoblastoid cell lines were later cultivated directly from the cerebrospinal fluid of lymph nodes and cornea of cattle and deer affected by SA-MCF but these did not reproduce disease on subsequent inoculation into cattle, deer or rabbits. Similar cells were also cultivated from rats infected with AHV-1, after rabbit passage, but again failed to reproduce the disease on return to rabbits or cytopathic effects in permissive cell cultures. These rat cells induce lymphoproliferative disease in weanling rats and were remarkable in not requiring a feeder layer (70). Not only do these lines of LGL/NK cells lack infectivity for MCF-susceptible hosts, they exhibit no electron microscopical evidence of herpes or other viruses and no viral antigens can be demonstrated in them or on their cell membranes by immunofluorescence using AHV-1 antisera (66, 68). It was suggested that MCF cell lines were similar to lymphoblastoid strains cultivated from tissues of marmosets infected with *Herpesvirus ateles* and *H. saimiri*, which are also lymphoproliferative agents in unusual hosts; in these cases only a small proportion of the viral genome (27%) is essential to maintain transformation. It is proposed that the MCF lines may have a similarly small proportion of the total viral genome which is inadequate for expression of viral antigens and, usually, for infectivity in any available system.

Reid and Buxton (65, 66) summarized their hypothesis as proposing that LGL/NK cells are a prime target for MCF virus replication; NK cells appear in significant numbers only after the onset of clinical disease in rabbits and lead to the characteristic lymphoproliferative disease, probably by inducing a benign and polyclonal T-lymphocyte hyperplasia, either by production of abundant interleukin 2 and/or by suppression of T-helper-cell function. The necrosis of cells, including that of lymphoid elements and those in vessel walls would, according to this hypothesis, be due to direct cytotoxic action of the proliferating NK cells.

The proposal is a very attractive one in providing an explanation of an otherwise puzzling and controversial pathogenesis (61, 75). It appears to fit the facts better than a proposal that, as some of the features of MCF viruses are obviously similar to those of other gamma-herpesviruses, MCF should also be regarded as an infectious neoplastic disease along with Marek’s disease, for example (30). The presence of a small fraction only of the viral genome in LGL/NK cell lines may also account for the variable, but always severe, difficulty of transmitting SA-MCF from cattle or deer to other deer and rabbits (71) and would not conflict with the suggestion that the viral DNA may be integrated with the cell genome or present in episomal form (30).

There are no indications at present as to how the "virus of MCF" is maintained in or excreted by sheep; fully productive infection of superficial cells is presumably required to make possible transmission to indicator hosts, however infrequent this may be in contrast with the widespread infection of sheep.

**THE RELATIONSHIP BETWEEN SA AND WD FORMS OF MCF**

All observers are agreed that it is not possible to differentiate the SA and WD forms of MCF on clinicopathological criteria and their epidemiology is also essen-
tially similar. There is increasing evidence that SA-MCF will eventually be shown to be due to a herpesvirus related to but not identical with AHV-1 (27, 66). Significant data are as follows:

(a) Antibody (IIF) titres against AHV-1 in sheep from many countries were comparable to those in wildebeest, but there was no significant neutralizing activity. Congenital infection was suggested by the detection of antibody in gnotobiotic and colostrum-deprived lambs (73).

(b) Neutralizing antibody was found fairly frequently in species of the subfamily Caprinae, including domestic sheep and goats in the USA [see Epidemiology, (a)]. Titres were low, except in the Barbary sheep, Ammotragus lervia. It was also reported that antigens reacting with AHV-1 antisera could be demonstrated by immunofluorescence in the circulating leucocytes of some caprines (27). In the United Kingdom 86/508 (17%) sheep sera from 13 flocks showed VN antibody for AHV-1, with titres between 1:2 and 1:32 (20).

(c) Using AHV-1 antigens in Western blot tests, it was shown that all sheep sera reacted with some of the same bands that were produced by wildebeest sera (66). Unfortunately, attempts with bovine antisera to demonstrate AHV-1 related antigens, by immunofluorescence, in cultured lymphoid cells or in rabbit tissues carrying SA-MCF infectivity, have failed. However, it is also very difficult to show specific fluorescence in animal tissues infected with the homologous agent, except following their in vitro cultivation and putative derepression of viral protein synthesis (see 61, 66, 75). The results of applying radioactive probes developed from the DNA of AHV-1 are eagerly awaited.

(d) In the course of SA-MCF, cattle from Europe, the USA and Australia frequently showed rising titres of IIF antibodies specific for AHV-1, but this finding was not invariable in the United Kingdom and it was suggested that an antigenetically different agent may sometimes be involved (20, 74).

THE DIAGNOSIS OF MCF

(a) Clinicopathological diagnosis

The majority of cases of MCF, whether of the SA or WD forms in cattle, deer or exotic ruminants, can be readily recognized from the clinicopathological features (see previous sections). Hyperacute cases, which are not infrequent in some of the species of deer, may not arouse suspicion because the characteristic discharges, necrosis of mucosae, skin and eye lesions, diarrhoea or dysentery, may all be absent; nevertheless, typical histopathological changes may still be present.

The gross and histological features have been described in detail many times (for the WD form, see 11, 32, 40, 52, 55, 56; for the SA form, see 34, 35, 36, 52, 80, 81). In the case of SA-MCF, diagnosis primarily on clinicopathological grounds is still essential, as no methods for isolation or demonstration of the causal virus(es) have yet been developed. The demonstration of rising antibody (IIF or IIP) titres to AHV-1 can be helpful but this does not occur regularly (see above).

(b) Laboratory confirmation of diagnosis

As there are extremely few cells in infected animals which exhibit immunofluorescing antigens with AHV-1 antisera and SA-MCF antisera have not been developed,
such methods have not generally been found to be of diagnostic value (61, 75). The claim (26) to have demonstrated MCFV antigens by direct immunofluorescence in smears of peripheral blood leucocytes from diseased or "carrier" (caprine) animals, using serum from a chronic AHV-1 infection in a sika deer, is interesting but not yet corroborated.

The development of antibodies to AHV-1 has, however, proved useful in following experimental infections and is potentially useful in diagnosis of clinical cases. Antibodies usually appear during the incubation period or the early clinical phases and prior to the almost invariable death; the most useful techniques are indirect immunoperoxidase (IIP) or indirect immunofluorescence (IIF) using appropriate anti-IgG reagents (61, 75). Nevertheless, Heuschele (25) did not find IIF was often of value. Complement-fixation methods were also useful but immunoprecipitation and immunoelectrophoresis were not found successful in cattle (61, 75). The specificity of immunohistochemical techniques for antibody has been questioned and investigated (24, 25, 27, 78); there are undoubtedly cross-reactions with other bovid herpesviruses, especially BHV-1 (IBRV) and BHV-3 (BCMV) but titres are usually much lower with heterologous sera. As would be expected, there are similar indications of broad specificity using ELISA tests, as for IIP or IIF.

The main application of serological techniques in MCF research has been in epidemiology and VN tests, which were the first to be developed (42, 67) and which remain the most specific and economical for investigating the distribution of MCF viruses in reservoir species, whether in zoos (26) or free-living populations (19). However, increasing evidence for antigenic (VN) relationships between SA (caprine)-MCF herpesviruses and the alcelaphine agents has now blurred the divisions between the two groups and much more work is necessary on the relevant epitopes using monoclonal antibodies and molecular virological techniques.

Earlier recommendations for the diagnosis of MCF by virus recovery (58, 59, 60) stressed those characteristics of WD-MCF viruses which are important in the collection and handling of materials from suspected cases. Firstly, virtually all infectivity in blood or tissues is associated with intact, living cells and is lost immediately on cell death. Secondly, virus infectivity is associated predominantly with lymphoid cells. Washed blood leucocytes (buffy coat fractions) can be readily prepared from live animals, for inoculation into susceptible cell cultures, especially monolayers of primary and secondary bovine thyroid (BTh) cells, which exhibit foci of a characteristic cytopathology within 18-21 days. Lymphoid tissues collected at death, or within 2-3 hours of this, should be lymph nodes, possibly thymus or spleen; these and also leucocyte suspensions should be treated to retain cell viability, i.e. dispersed in a manner to avoid cell damage, stored at about −4°C (not frozen) in a nutritive, serum-containing medium and inoculated as soon as possible, certainly within 3-4 days, into animals or cell cultures. Alternatively, suspensions may be stored at or below −70°C, provided that 10% DMSO or other cryoprotectant is used but some loss of infectivity occurs in the freezing process. Oliver et al. (46) reported retention of infectivity in 10% suspensions of deer (SA-MCF) lymph node stored for 8 months at −70°C without cryopreservatives, but this was very exceptional. The stage of disease for collection of bovine, WD-MCF materials is immaterial as the titre of blood or lymph nodes is nearly always in excess of 10^2 TCID\(_{50}\)/ml or 10^3 TCID\(_{50}\)/g, respectively (56, 61).

The difficulty in producing adequate quantities of BTh cells led to attempts to use serially-passaged strains of calf (BK) or bovine embryonic kidney (BEK) cells,
especially for *in vitro* passage of virus isolates, for immunofluorescence tests and for production of cell-free virus for virus characterization and neutralization (42, 60, 61, 78). It was difficult, however, to maintain a passage series in BK cells using released virus as an inoculum. Strains of calf testis (BT) cells were later found to be highly sensitive for the detection and assay of infectivity in animal tissues and produced high titre cell-free, infectious virus (up to $10^5$ TCD$_{50}$/ml), even in early passages (48, 49, 60). Infected bovine (calf) blood and lymphoid tissues assayed in tube culture of BT cells gave infectious centre counts up to about $10^4$ /ml and $10^6$ /g, respectively, figures which are comparable to or greater than those obtained by simultaneous titration of similar materials in cattle and in calf thyroid monolayers (56). In addition, cytopathic foci are more easily detected in BT cells, as the syncytia exhibit long refractile processes and generalize rather than regress or even disappear, as happens in BTh, BK or BEK cells; the differences are presumably due to the early production of cell-free virus in BT cells (Plowright and Watt, unpublished; 48).

The ease with which BTh and BT cells, in particular, can be used for direct quantitative recovery of MCFV infectivity from animal tissues or from wildebeest secretions (43) contrasts with the experiences of N. American workers (6, 7, 25) who used BTh and BEK cells and concentrated inocula but reported syncytia with intranuclear inclusions usually after the second, or even up to six subcultures. Two procedures were advocated to overcome the difficulties. Firstly, inoculation at 32-34°C was employed, a procedure first shown in E. Africa to increase the speed of appearance and amount of cell-free virus in culture fluids and change the predominant cytopathic effects from syncytium formation to rounding and refractility of affected elements (21). Secondly, fusion of suspect cell suspensions with permissive cells by polyethylene glycol (PEG; MW 1000-4000) has been recommended (8, 9). Again, the use of a cell strain (FAK) derived from fetal aoudad (barbary sheep) kidney has been advocated (25, 26), but no data have been provided on the relative sensitivity of this cell as opposed to those previously employed for detection and assay of AHV-1 infectivity in animal tissues. BTh or BT cells give rapid results, positives being seen in unstained cultures within 3-4 days of inoculation of blood leucocytes from sick cattle.

One reason for the reported differences between N. American and other workers, in the recovery of alcelaphine herpesviruses, could be a preoccupation of the former with supposedly low titre materials and the real difficulty of detecting cytopathic foci before they regress in BTh or BK cells. This has led to the use of large inocula whether of buffy-coat or lymphoid tissue suspensions. Both of these latter in high concentration were found to suppress cytopathic effects, in addition to lymph node producing cytotoxic effects (56). The suppression may be partial or complete and has been investigated again recently by assays of infected bovine lymphoid tissues in a sensitive line of calf testis cells (BT11) (Plowright, unpublished). Typical results are shown in Table V, which indicates that washing the cells 3 times (in serum saline) did not remove the inhibitory factor, which was detectable down to a w/v dilution of $10^{-2.7}$; secondly, subculture of apparently negative tubes at the lower end of the titrations gave positive results in a proportion only and added very few positives at the upper end of the titration. The inhibitory factor was not reduced by the removal of plastic-adherent cells from the infected suspensions and was not demonstrable in high-speed supernatants of crude, infected lymph node suspensions. Normal calf lymph node suspensions did not show the effect, which thus appeared to be mediated by non-adherent cells in infected nodes and not by antibody or other soluble factor.
TABLE V
Titrations of calf lymphoid tissue for MCFV (C.500 isolate) in tube cultures of the BT11 strain of bovine testis cells

<table>
<thead>
<tr>
<th>Dilution w/v of lymph node</th>
<th>Crude Ten Broeck suspension. Cytopathic effects at day 19</th>
<th>Washed (× 3) cell suspension. Cytopathic effects at day 19</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(a) Primary inoculation</td>
<td>(b) First subculture</td>
</tr>
<tr>
<td>10^-1.2</td>
<td>0/5</td>
<td>ND</td>
</tr>
<tr>
<td>10^-2.0</td>
<td>0/5</td>
<td>4/5</td>
</tr>
<tr>
<td>10^-2.7</td>
<td>0/5</td>
<td>3/5</td>
</tr>
<tr>
<td>10^-3.4</td>
<td>5/5</td>
<td>ND</td>
</tr>
<tr>
<td>10^-4.1</td>
<td>5/5</td>
<td>ND</td>
</tr>
<tr>
<td>10^-4.8</td>
<td>4/5</td>
<td>5/5</td>
</tr>
<tr>
<td>10^-5.5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Titre (log_{10} TCD_{50})</td>
<td>10^{3.08}/g</td>
<td>10^{3.15}/g</td>
</tr>
</tbody>
</table>

* The cells were in their 16th in vitro passage. Cultures received 1 ml of inoculum, which was removed after 2 hours at 37°C by washing twice with serum saline. Subculture of negative tubes was carried out on the 19th day by mixing versenised cells with clean cell suspensions and dispensing into 5 tubes.

The identification of cytopathogenic isolates as WD-MCF herpesviruses is readily made by IIF or IIP using specific antisera prepared in cattle or rabbits. There is a need for standard antisera from animals not previously exposed to other bovid herpesviruses, a need certainly not met by one proposed “international reference” (2). Neutralization tests can be performed as soon as cell-free virus is available — a process accelerated by incubation at 32° – 34°C and the use of bovine testis (BT) or fetal caprine (FAK) cells (21, 25, 60). Virus neutralization may well distinguish some members if reciprocal quantitative tests are performed, but cross-neutralization of AHV-1 by some caprine sera argues for very broad antigenic relationships. Electron-microscopical examination of cultured cells can be used to supplement observations on the cytopathology in unstained or in fixed and stained preparations (5). In addition, parenteral inoculation of infected cultured cells into rabbits, cattle or deer may produce the disease, but not all alcelaphine viruses are pathogenic (61).

The differentiation of alcelaphine, hippotragine or caprine MCF viruses by serological or biochemical methods is in its infancy. Gradient centrifugation of DNA from AHV-1 gave two peaks similar to those (M and H) of *Herpesvirus saimiri* and *H. ateleis* (25). Restriction endonuclease patterns of AHV-1 viral DNA show a clear separation from the homogeneous BHV-4 isolates (bovine cytomegaloviruses) and minor differences between the high-passage, low-virulence, WC11 strain and the virulent C500 isolate or a black wildebeest isolate (23, 47). The cloning of restriction fragments of AHV-1 into four vectors opens up the prospect of probes to investigate its relationship to SA-MCF (3).

THE CONTROL OF MCF

All forms of the disease can, of course, be prevented by avoiding contacts between reservoir and indicator species, especially at times when the former are producing
offspring. In practice, this can be difficult in some parts of Africa where there are no stock-proof fences and thousands of wildebeest competing with cattle for grazing and water. The occasionally catastrophic results of allowing cattle and other susceptible species to have close contacts with sheep and possibly other caprine reservoirs, emphasize the desirability of separating the two classes of host.

In the case of zoos and ranches with mixed exotic species, especially those threatened with extinction in the wild, it has been advocated recently that routine serological testing should be employed to avoid introduction or movement of potential carriers of virus and that notification of cases of MCF should be mandatory. It is also advocated that hand-rearing of calves of reservoir species, such as wildebeest, should not be undertaken to avoid the particular risks from these high-level excretors (24, 26, 64).

In those parts of Africa where cattle share the grazing with large herds of wildebeest, particularly in the Masailand of E. Africa and in Botswana, MCF vaccination could be an attractive alternative to restriction or elimination of the game. Attempts to develop attenuated vaccines (58, 61, and Harkness J.W., personal communication) or killed virus vaccines (75) by empirical methods have, so far, been a failure or of limited success, although they were antigenic.

Much further work on the molecular immunology and virology of MCF will be necessary before a suitable product is likely to emerge and the economics are not attractive; furthermore, the possibility of superinfection with wild virulent viruses and congenital infection of the offspring has to be borne in mind (63).

* * *

**REFERENCES**


