Chronic and recovered cases of sheep-associated malignant catarhal fever in cattle

D. O'Toole, H. Li, D. Miller, W. R. Williams, T. B. Crawford

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Malignant catarhal fever (MCF) is traditionally regarded as a disease with a short clinical course, low morbidity and high case fatality rate. Owing to the limitations of the assays used for laboratory diagnosis, it was difficult to characterise the clinical spectrum of sheep-associated MCF, particularly when the cattle recovered from an MCF-like clinical syndrome. Over a period of three years, 11 cattle that survived MCF for up to two-and-a-half years were identified on four premises. A clinical diagnosis of MCF was confirmed by the detection of ovine herpesvirus-2 DNA in peripheral blood leukocytes using a polymerase chain reaction (PCR) assay that detects a specific 238 base-pair fragment of viral genomic DNA. Of the 11 cattle examined, six recovered clinically with the exception of bilateral corneal oedema with stromal keratitis (four animals) and unilateral perforating keratitis (one animal). The 10 animals available for postmortem examination had disseminated subacute to chronic arteriopathy. Recovery was associated with the resolution of the acute lymphoid panarteritis that characterises the acute phase of MCF, and with the development of generalised chronic obliterator arteriosclerosis. Bilateral leukomata were due in part to the focal destruction of corneal endothelium secondary to acute endothelialitis. Formalin-fixed tissues and/or unfixed lymphoid cells from all 11 cattle were positive for sheep-associated MCF by PCR. These observations indicate that recovery and chronic disease are a significant part of the clinical spectrum of MCF and that such cases occur with some frequency in the area studied. The affected cattle remain persistently infected by the putative sheep-associated MCF gammaherpesvirus.

MALIGNANT catarhal fever (MCF) is traditionally regarded as a disease with a case fatality rate of 95 to 100 per cent (Campbell 1988, Plowright 1990, Heuschele and Seal 1992, Barker and others 1993, Smith 1996). The clinical disease is characterised by fever, a mucopurulent nasal discharge, severe keratoconjunctivitis, oral erosions and lymphadenopathy. There is growing evidence that most cattle with sheep-associated MCF are infected by ovine herpesvirus-2 (OHV-2) (Bridgen and others 1989, 1992, Bridgen and Reid 1991, Barker and others 1993), or a closely related gammaherpesvirus. OHV-2 is rarely detected in healthy cattle (Baxter and others 1993, Wiyono and others 1994, Crawford and others 1995, Li and others 1995b). The possibility that OHV-2 is the cause of sheep-associated MCF is strengthened by its close genomic relationship to alcelaphine herpesvirus 1 (AHV-1), the cause of wildebeest-associated MCF. The collective term 'MCF viruses' has been used for closely-related gammaherpesviruses, including AHV-1 and OHV-2, that are associated with the MCF syndrome (Li and others 1994). The precise aetiopathological association of OHV-2 with sheep-associated MCF needs to be established conclusively by transmission trials using cell-free virus. Such trials are not possible at present because OHV-2 has not been isolated in cell culture. Furthermore, the need for such trials is doubted because MCF is considered to be of limited economic importance owing to its purportedly low morbidity.

Current dogma suggests that cattle with MCF rarely survive more than two weeks after the onset of clinical signs. The statement is commonly made that cases 'never' recover (Selman 1987). There are, however, reports of mild disease in domestic cattle followed by complete recovery, recovery with recrudescence disease, and chronic MCF (Götte 1930, Daubney and Hudson 1936, Berkman and Barner 1958, Daniels and others 1988b, Hamilton 1990, Milne and Reid 1990, Baxter and others 1993, Michel and Asperling 1994, O'Toole and others 1995). Chronic and/or recrudescence MCF also occurs in sika deer (Cervus nippon) (Wilson and others 1983, Heuschele and others 1985) and bison (Bison bison) (P. Schultheiss, personal communication). Current estimates of MCF recovery rates are based on field outbreaks and on previous experimental studies with AHV-1 in Africa (Plowright 1990). These estimates may not be valid for all natural outbreaks of sheep-associated MCF because of differences in routes of infection and in virulence levels.

Newly developed assays for antibody and DNA show promise as diagnostic tools for MCF (Hsu and others 1990, Katz and others 1991, Baxter and others 1993, Michel 1993, Lahijani and others 1994, Murphy and others 1994, Tham and others 1994, Li and others 1995b, 1996a). The authors currently use two assays for MCF to investigate the epidemiology of the disease, particularly the role of inapparently or latently-infected ruminant species in its transmission (Li and others 1996a, b). These two assays are a competitive-inhibition enzyme-linked immunosorbent assay (CI-ELISA) based on a monoclonal antibody (mAb) to an epitope widely conserved among MCF viral strains of alcelaphine and ovine origin (Li and others 1994, 1995a, 1996b), and a polymerase chain reaction (PCR) assay based on previously reported primers (Bridgen and Reid 1991, Baxter and others 1993). These tests are used in natural outbreaks of MCF and in populations of clinically healthy ruminants (Li and others 1994, 1995b, 1996a, b, Crawford and others 1995).

Over the course of several years, while using the PCR and CI-
TABLE 1: Clinical status, PCR and c-ELISA results of 11 adult beef cattle surviving malignant catarrhal fever (MCF) for between 39 and 800 days

<table>
<thead>
<tr>
<th>Animal</th>
<th>Age/sex/breed</th>
<th>Premises</th>
<th>Clinical Status</th>
<th>Survival (days)</th>
<th>PCR1</th>
<th>ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yearling Charolais heifer</td>
<td>A</td>
<td>Subacute MCF</td>
<td>39</td>
<td>+</td>
<td>-2</td>
</tr>
<tr>
<td>2</td>
<td>Yearling Angus steer</td>
<td>B</td>
<td>Subacute MCF</td>
<td>39</td>
<td>+</td>
<td>+3</td>
</tr>
<tr>
<td>3</td>
<td>Yearling Charolais heifer</td>
<td>A</td>
<td>Chronic MCF; recrudescence</td>
<td>90</td>
<td>+</td>
<td>+4</td>
</tr>
<tr>
<td>4</td>
<td>1·5 year Angus cow</td>
<td>A</td>
<td>Mild acute MCF; recrudescence</td>
<td>150</td>
<td>+</td>
<td>+5</td>
</tr>
<tr>
<td>5</td>
<td>2 year Angus cow</td>
<td>A</td>
<td>Chronic MCF; recrudescence</td>
<td>-200</td>
<td>+</td>
<td>+6</td>
</tr>
<tr>
<td>6</td>
<td>Yearling Hereford heifer</td>
<td>D</td>
<td>Acute MCF; recovery</td>
<td>56</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Yearling Angus bull</td>
<td>C</td>
<td>Acute MCF; recovery</td>
<td>76</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Yearling Charolais heifer</td>
<td>A</td>
<td>Acute MCF; recovery</td>
<td>105</td>
<td>+</td>
<td>+6</td>
</tr>
<tr>
<td>9</td>
<td>Yearling Angus heifer</td>
<td>B</td>
<td>Acute MCF; recovery</td>
<td>150</td>
<td>+</td>
<td>+7</td>
</tr>
<tr>
<td>10</td>
<td>Yearling Angus steer</td>
<td>D</td>
<td>Acute MCF; recovery</td>
<td>205</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>2 year Charolais cow8</td>
<td>A</td>
<td>Acute MCF; recovery</td>
<td>&gt;800</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

1 OHV-2-positive by PCR on fresh or deparaffinised formalin-fixed, paraffin wax-embedded lymphoid tissues (1, 3, 4 and 7), ocular tissue (6 and 9) or on one or more occasions on peripheral blood leucocytes (2, 5, 6 and 8 to 11)
2 Seronegative after 17 and 20 days of clinical signs
3 Seropositive after 38 days of clinical signs
4 Seronegative after 52 days of clinical signs and seropositive on three occasions after 73 to 89 days of clinical signs
5 Seronegative when blood drawn 90 days before death
6 Seropositive on four occasions after 42 to 100 days of clinical signs
7 Seropositive on 12 occasions after 11 to 131 days; thereafter, negative, inconclusive or weakly positive
8 OHV-2-positive 2-5 years after recovery from MCF

ELISA to examine suspect cases of MCF in areas of Wyoming with a high prevalence, some premises were recognised that had a surprisingly high proportion of cattle with MCF which survived for long periods after the clinical onset. Some animals appeared to recover. This paper describes the clinical and pathological features of these cases and offers observations on variables relating to the diagnosis of 'atypical' forms of sheep-associated MCF.

Materials and methods

Case histories

Eleven adult beef cattle of both sexes, aged one to two years, developed clinical signs suggestive of MCF over a period of three years (1993 to 1995) (Table 1). All 11 cattle were from four premises that also ran sheep and were within a 20-mile radius in central Wyoming; A was a cow-calf ranch and B, C and D were feedlots. Ten of the 11 cattle had clinical signs typical of acute MCF for periods ranging from one to four weeks. Many of the animals were so ill that they were considered unlikely that they would survive. One animal did not develop typical acute MCF and instead had a history of mild acute bilateral conjunctivitis that, in retrospect, may have been mild MCF (Table 1, Animal 4). Six of the 11 cattle were transferred to the Wyoming State Veterinary Laboratory where they were observed and sampled for up to six months before being euthanased and examined postmortem. The MCF outbreak at premises A has been described by O'Toole and others (1995).

Pathology

Ten cattle that had clinical signs for up to nine months were examined postmortem (Table 1, Animals 1 to 10). The remaining cow was alive two-and-a-half years after an episode of an acute MCF-like disease (Table 1, Animal 11). Selected tissues were fixed in 10 per cent neutral buffered formalin, the brain and spinal cord in 20 per cent neutral buffered formalin, and the trimmed eyes in Zenker's solution (Slatter 1981). Samples were embedded in paraffin wax and stained with haematoxylin and eosin (Gordon 1982). Pieces of carotid rete, kidney and/or cornea from six cattle (1, 3 and 5 to 8) were processed for transmission electron microscopy (TEM) and tissues from three of them were processed for scanning electron microscopy (SEM). Selected tissues were stained immunohistochemically to detect α-actin in intimal plaques, as described by O'Toole and others (1995).

Microbiological and serological assays

Samples of DNA from peripheral blood leucocytes, ocular tissue and/or formalin-fixed, paraffin wax-embedded lymphoid tissues from all 11 cattle were examined by a nested, semi-coterminal two-stage PCR assay (Li and others 1995b). The presence of an amplified 238 bp fragment of OHV-2 genomic DNA (Baxter and others 1993) was taken as a positive signal. This technique is specific for OHV-2 DNA, does not amplify DNA from alcelaphine strains of MCF virus, and does not yield false-positive signals on unfixed or fixed embedded tissues from normal cattle (Wiyono and others 1994) or from cattle infected with bovine herpesvirus-1 (BHV-1), bovine viral diarrhea virus (BVDV), or paramyxovirus-3 virus (PI-3) (Crawford and others 1995). The DNA from peripheral blood leucocytes from healthy cattle that had no previous clinical history of MCF-like illness and from cattle with acute MCF served as negative and positive control samples. Serum was collected from 10 of the 11 cattle on one- to 22-weekly occasions and examined by c-ELISA. This assay is specific for a glycoprotein complex that is conserved among all known MCF viral strains identified to date (Li and others 1994). It was not possible to collect serum from the remaining animal when terminal illness developed, but serum had been sampled 90 days before its death (Table 1, Animal 4). A range of fresh tissues from nine cattle was examined by standard fluorescent antibody techniques, and attempts were made to isolate viruses for evidence of common viral pathogens of cattle, including BVDV and BHV-1.

Results

Clinical signs

Bilateral ocular lesions were the most obvious clinical sign. Four of the five cattle with subacute-chronic MCF developed severe bilateral panophthalmitis, progressing in one animal to unilateral corneal perforation and iridal prolapse (Table 1). By contrast, the ocular lesions in the six recovering cattle that gradually

FIG 1: Carotid rete; animal 3. This heifer developed MCF 90 days earlier. There is marked intra-arteri
al intimal thickening, and associated arteritis-periarteritis. Verhoeff-Van Gieson, bar = 100 μm
regained weight consisted of chronic bilateral central stromal keratitis, either with or without corneal pigmentation (Table 1). The leucoc mata either remained essentially stationary or resolved slowly. One of the six developed unilateral perforating corneal ulceration, but in other respects the clinical condition of this animal improved markedly before it was euthanased 56 days after the onset of clinical signs (Table 1, Animal 6). Two recovered cattle were slaughtered for human consumption (Table 1, Animals 9 and 10). Two others could have gone for slaughter after fattening but were euthanased earlier for logistical reasons (Table 1, Animals 7 and 8).

**Pathology**

Gross and microscopical lesions primarily involved the eyes and arterial vessels (Figs 1, 2, 3a-d; Table 2). The extent and severity of the lymphoid arteritis that characterises the acute stage of MCF (Liggett and DeMartini 1980) waned with time after the initial disease and vasculitis was essentially absent in the two recovered cattle that survived for the longest periods after clinical onset (150 and 205 days). There was marked hyperplasia by α-actin-positive myocytes in the tunica intima, resulting in oblitative arteriopathy of medium calibre arteries (200 to 600 μm)
Vascular lesions were present in all the major organ systems and were particularly evident in carotid rete, kidney, leptomeninges, bowel, posterior ciliary arteries, enteric serosa, spermatic cord and broad ligament. The severity of arteriosclerosis diminished with longer survival times.

All nine cattle whose eyes were examined histologically had extensive bilateral lesions, including moderate to severe stromal keratitis. Leucomatous lesions corresponded to moderate or marked oedematous thickening of the substantia propria (Fig 2a). Perforating corneal ulcers with anterior synchiae developed unilaterally in two cattle (Fig 2b). The migration of limbal melanocytes into the basal layer of the corneal epithelium resulted in corneal melanosis (Henkind 1965) (Fig 3a). There was intercellular oedema of the corneal epithelium in animals with stromal oedema and keratitis (Fig 3b). Anterior uveitis was severe in some animals (Fig 3c). Light microscopic and SEM preparations demonstrated fibrin and adherent inflammatory cells overlying extensive areas of denuded Descemet’s membrane (Fig 3d), with spindle-shaped cells at the leading edge of the damaged endothelial monolayer. In TEM preparations, the endothelial monolayer was attenuated adjacent to denuded parts of Descemet’s membrane, endothelial cell junctions were lost, and lymphocytes and melanin-laden macrophages infiltrated the endothelial monolayer. In many areas, a 0.5 to 2.0 μm layer of extracellular matrix that included collagen was interposed between the corneal endothelium and Descemet’s membrane. Uveitis and retinitis became less severe with longer survival times.

**Microbiological and serological assays**

All the samples from all the 11 cattle examined were positive by PCR for OIV-2, as were known positive control samples from cattle with clinical signs typical of acute MCF (Fig 4; Table 1). The PCR amplified the target 238 bp fragment, as well as two larger fragments of DNA of approximately 340 and 420 bp (lanes 3 and 4, Fig 3: Ocular lesions in chronically affected and recovering cattle. a) Central portion of cornea of animal 7; there is marked pigmentation of corneal keratocytes, due to lateral migration of limbal melanocytes (‘limbal slide’). Haematoxylin and eosin, bar = 50 μm. b) Central portion of cornea of animal 9; there is spongiosis in the superficial part of the corneal epithelium. The substantia propria is oedematous. Haematoxylin and eosin, bar = 50 μm. c) Ciliary body of animal 3; severe lymphocytic uveitis is present. Haematoxylin and eosin, bar = 50 μm. d) Corneal endothelium of animal 3; mononuclear cells accumulate on both sides of Descemet’s membrane and are adherent to Descemet’s endothelium. Gaps in the endothelium indicate focal destruction of the partly detached monolayer (arrows). Haematoxylin and eosin, bar = 25 μm.
Fig 4). Samples from clinically healthy cattle were negative by PCR. By contrast, the presence of detectable levels of antibody by 
CH-ELISA was less reliable as a diagnosis of MCF. Of the 11 animals, eight were seropositive and three were negative (Table 2). One animal (10) tested serologically over seven months was consistently CH-ELISA-positive between 11 and 131 days after the onset of clinical signs and thereafter the results were either weakly positive, inconclusive or negative until it was euthanased 205 days after the clinical signs developed. Tissues from two animals (5 and 9) were positive for the antigen of BVDV and those from one (7) were positive for BHV-1 on fluorescent antibody tests, but attempts to isolate BVDV or BHV-1 from these cattle were unsuccessful.

Discussion

It is generally assumed that most or all cattle with signs of MCF die after a short clinical illness (Smith 1996). Indeed, the perception that a diagnosis of MCF is incompatible with extended survival leads some veterinarians to amend a clinical diagnosis of MCF retrospectively if the animal recovers (G. Goodall, personal communication). This perception may make estimates of the high case fatality rate in MCF something of a self-fulfilling prophecy. Recovery from MCF has been difficult to document in the past, because such animals are unlikely to have been examined post-mortem, and because reliable diagnostic assays have been developed only recently.

This study indicates that several animals in Wyoming have recovered from MCF. The diagnosis of MCF in these animals was based on several criteria: typical clinical signs of acute MCF, histological lesions consistent with active or recent panvasculitis, the detection by PCR of OHV-2 DNA in peripheral blood lymphocytes and/or tissues, and the absence of other infectious agents that might have caused similar clinical signs and lesions. The absence of BVDV in these animals was corroborated by immunohistochemical staining of tissues with a mAb specific for BVDV (Haines and others 1992); the tissues of the 10 animals tested were negative (D. M. Haines, personal communication). The detection of OHV-2 DNA is a reliable method for identifying cattle that have experienced MCF and subsequently recovered or developed chronic disease. This test detects three DNA sequences that are specific for OHV-2: the target 238 bp sequence and two larger fragments of approximately 340 and 420 bp (Li and others 1995c). The CH-ELISA is less reliable for identifying persistently infected cattle.

Owing to the small number of cattle in the present study it is difficult to identify what proportion of animals with MCF subsequently recover. Nevertheless, some crude estimates are possible. Of the 10 cattle from premises A that developed MCF over a period of a year (O'Toole and others 1995), two recovered completely and another three died or were killed owing to chronic MCF after a clinical illness of up to 280 days. This suggests a recovery rate of 20 per cent, with another 30 per cent developing 'atypical' chronic MCF. In premises D, where one to three cases of acute MCF occur annually, three of six animals recovered after developing MCF during 1994 to 1995. This suggests a recovery rate approaching 50 per cent for cattle on these premises. A fourth recovered animal from premises D was recognized in 1996 (unpublished observations). This unusually high recovery rate is probably associated with the managerial practice of slaughtering only animals in extremis, clinical confidence in making a diagnosis of MCF with subsequent recovery, and the willingness to retain blind animals for fattening. It was concluded from a study of 120 cases of MCF in Finland that 35 per cent of affected cattle recovered (Stenius 1952, cited by Berkman and Barner 1958). Prospective studies on premises with endemic MCF may determine whether chronic disease and recovery are significant components of the disease spectrum and whether OHV-2 infection is synonymous with a past history of MCF-like clinical disease in individual animals.

The most distinctive clinical feature in cattle that recover from MCF is the presence of persistent bilateral leukomata. This chronic corneal lesion can be regarded as strongly suggestive of MCF with recovery when it is combined with a clinical history of fever and ocular inflammation. Similar ocular lesions were described recently in a cow that recovered from sheep-associated MCF (Michel and Asperling 1994). The slow rate at which these lesions resolve is distinctive, because the bovine cornea regenerates quickly and with minimal scarring after all but the most severe injuries (Severin 1995). Partial destruction of the corneal endothelium probably accounts for the chronic nature of the MCF-induced leukomata. Severe endothelialitis and endothelial cell necrosis are common lesions in the acute stage of MCF (Whiteley and others 1985). The repair of corneal endothelium, which in most species is limited, is associated with the formation of microguttae, corneal oedema, and a loss of transparency (Cogan 1949, Van Horn and others 1977). These lesions are readily apparent in scanning and transmission electron microscopical preparations of the cornea. Factors contributing to the chronic persistence of leukomata in MCF-recovered cattle are low-grade chronic stromal keratitis and, in cattle with a heavily pigmented limbus, corneal melanosis. The corneal tissue of both animals that were examined for OHV-2 DNA by PCR was positive, suggesting a persistent intraocular infection.

Obliterative arteriopathy may be a useful hallmark lesion to identify cattle that have recovered from a previous episode of MCF. These lesions persist for an extended period, as demonstrated by their presence in one animal that was slaughtered more than six months after the clinical onset of the disease. Identical generalised obliteratorative arteriopathic lesions were observed recently in an OHV-2 DNA-positive bison that had an earlier episode of acute MCF-like clinical illness (P. Schultheiss, personal communication). These MCF-associated arteriosclerotic lesions are most readily detected in the retinal artery, the same tissue that is used to establish a morphological diagnosis of acute MCF (Daniels and others 1988a), although they may be found in any vascularised tissue. Similar healing lesions develop in polyarteritis nodosa (Schoen 1994), an idiopathic disease of people in which both its acute and chronic phases has features in common with MCF.

Cattle that recover from MCF remain persistently infected with OHV-2. Earlier studies also found that cattle that have recovered remain persistently infected (Baxter and others 1993, Michel and Asperling 1994). This condition raises the question whether such animals are a potential source of horizontal or vertical infection for other members of the herd. There is considerable evidence that cattle with acute MCF rarely transmit the infection to other cattle (Plowright 1990). The same is probably also true for recovered, persistently infected cattle. The owner of premises A retained a persistently infected animal for more than two years without the development of any further active cases of MCF. Only one of three asymptomatic calves born to this cow was OHV-2 DNA-positive on the one occasion it was tested, and this calf did not develop MCF.
(unpublished observations). Attempts to transmit MCF or to detect a serological response to MCF virus in hamsters inoculated with cell suspensions from two cattle with extended survival times were unsuccessful (unpublished observations).

The results of this study suggest that MCF can be a source of chronic non-fatal loss on some premises and that the case fatality rate may be lower than generally estimated. The rate will be defined in future studies. For the present, practitioners and diagnosticians may want to consider the possibility that the clinical spectrum of MCF is wider than currently assumed.

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Abstracts

Occult cardiomyopathy in 54 dobermanns

PROGRESSIVE left ventricular dysfunction and ventricular tachyarrhythmias were detected in 54 apparently healthy dobermanns, 14 of which died suddenly before showing any signs of congestive heart failure. Twelve of these 14 had ventricular tachycardia, and ventricular tachycardia-fibrillation was detected in the other two immediately before death. In comparisons with the 40 dogs that died after the onset of congestive heart failure, the sudden deaths of the 14 dogs were significantly associated with sustained ventricular tachycardia. The lesions in the heart consisted of multifocal interstitial fibrosis with foci of variable-sized muscle fibres which were most numerous in the left ventricular papillary muscles and the interventricular septum. The condition is relatively common in dobermanns with left ventricular dilation and a decreased ejection fraction, and echocardiography and Holter recording can help to identify dogs at risk.


Treatment of calf diarrhea with a solution containing glutamine

A HIGH-CALORIE oral rehydration solution containing glutamine was more effective in correcting the plasma, extracellular fluid and blood volume of calves with diarrhea due to Escherichia coli than other high-calorie solutions which did not contain glutamine. It was the only solution to improve the calves’ plasma volume and correct their packed cell volume within 48 hours, and to maintain these improvements throughout the treatment. The calves treated with the glutamine-containing solution were the only ones which did not suffer a significant loss of weight. The solution also had more beneficial effects on hypotonia and metabolic acidosis than a standard oral rehydration solution.