Malignant Catarrhal Fever in Asian Livestock

Extreme cases of malignant catarrhal fever (MCF) in Bali cattle

Top left: early spontaneous MCF, showing salivation and apprehension. Top right: marked corneal opacity in experimental MCF. Centre left: fibrinous tags on the heart, especially along the coronary groove, in experimental MCF. Centre right: necrosis of the oral mucous membrane in experimental MCF. Bottom left: marked yellow mucoid nasal discharge and sloughing of the muzzle in experimental MCF. Bottom right: marked prefemoral lymph node enlargement in experimental MCF.

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Editors: **P.W. Daniels, Sudarisman and Purnomo Ronohardjo**

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Contents

Section 2. Pathology and Differential Diagnosis

Section 3. Aetiology

Foreword

THIS monograph represents a major study of Malignant Catarrhal Fever in Indonesia and is based on a collaborative research project between Balitvet and James Cook University, supported by ACIAR. Many of the papers were presented at a workshop on MCF held in Indonesia, being contributions from the district investigation centres and provincial veterinary services, as well as from Balitvet scientists.

Malignant Catarrhal Fever has been identified by the National Reference Coordinating Committee as a priority problem in the transmigration areas of Indonesia. Although the aetiological agent of MCF has not been identified, a great deal of knowledge has been collected and presented in this monograph, with a strong emphasis on the epidemiological aspects of MCF.

ACIAR is pleased to publish this important material in its Monograph Series, and is grateful to Peter Daniels, Sudarisman, and Purnomo Ronohardjo for their efforts in editing the text, and to Reg MacIntyre for his guidance in producing the publication.

J.W. Copland
Research Program Coordinator
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ACIAR

Preface

ON 11–12 August 1986, Balai Penelitian Veteriner (Balitvet), the Research Institute for Veterinary Science in Bogor, Indonesia, hosted a seminar on bovine malignant catarrhal fever (MCF) as part of a research project on that topic. The project was substantially funded by the Australian Centre for International Agricultural Research (ACIAR) and the Indonesian Agency for Agricultural Research and Development (AARD). Balitvet was linked with collaborating institutes in Australia—James Cook University of North Queensland and the Queensland Department of Primary Industries. This monograph presents the seminar proceedings and other contributions by the research project.

The invited speakers were drawn from the regional laboratories and provincial veterinary services in Indonesia where MCF is recognised as a problem, and from the AARD/ACIAR project both in Indonesia and in Australia. Appreciation must be expressed to Dr Hugh Reid of the Moredun Institute, Edinburgh, Scotland, for his participation, his helpful contributions to discussions, and for his allowing an edited transcript of his presentation to the seminar to be included. His contribution gave participants an important reference point against which the emerging international relevance of their own contributions could be assessed.

This monograph represents the first major study of MCF in Indonesia since the late Professor Mansjoer presented his thesis on the topic in 1954. Project personnel have given expanded contributions that include a more complete discussion of their results to date than was possible within the time constraints of the seminar. In this way it is hoped that the book will be a useful reference on which to base future research and investigations. Although Dr Mary Young was not able to attend the seminar, she has kindly contributed a paper on the pathology of MCF in Bali cattle as seen by the project, a welcome and useful addition.

In the first section, the Directorate of Animal Health gives a concise overview of MCF in Indonesia. In the subsequent epidemiology section, the situation in certain areas is elaborated to illustrate various aspects of the disease, including epidemiological patterns that have been recognised. Each paper highlights some particular aspect or anomaly of the problem. It is hoped that these contributions will be a further stimulus to more veterinarians in Indonesia to analyse and make readily accessible the details of how disease is occurring in their districts. Together they form an invaluable body of knowledge for researchers trying to define the mechanisms of spread of MCF and its pathogenesis. Any hypotheses developed must take account of the full range of situations described in these papers.

Each contributor was asked to describe the livestock industry in the area of his jurisdiction, the observations made on MCF and the consequent financial impact on the farmers. The responses to this request reflect the varying perceptions individual authors have of the problem. It has been editorial policy that apart from translating papers presented in Indonesian language, the format of submissions has been left substantially unchanged to allow any diversity of opinion to be appreciated.

The contributions therefore not only represent a definitive statement of the MCF problem in Indonesia, but equally importantly, show how the problem is

perceived by the veterinarians in the provincial livestock services and regional laboratories. It is against this background that the strategies for controlling MCF must be planned.

Since the aetiological agent of MCF in the region is unknown, the disease exists solely as a clinicopathological entity. Section 2 discusses the consequent problems in differential diagnosis of the condition, and provides the framework on which to build a nationwide consensus among Indonesian veterinary diagnosticians on what should and should not be called MCF.

Overviews of the current research of Indonesian and Australian scientists to define the aetiology constitute Section 3. The monograph benefits from being able to present research undertaken both in conjunction with the AARD/ACIAR project and independently of it. Considerable progress and interesting new developments have been achieved in the 2 years covered by the reports, including the demonstration of the extreme susceptibility of the Bali cattle to MCF, the exhaustive report on the refractoriness of the MCF agent to isolation by conventional cell culture techniques, and the isolation of new viruses in Indonesia by two independent group of researchers. The challenge is before the Indonesian scientists to firmly categorise the isolates and to collaborate in demonstrating whether they represent the same or different viruses.

The workshop concluded with a discussion session when the knowledge of the day was distilled into recommendations on the tasks for the future. The summary will, it is hoped, help make this book a useful tool for project planners and a valuable reference work for colleagues involved in MCF research.

In closing, the editors would like to thank ACIAR, and particularly Dr John Copland, the Research Program Coordinator for Animal Sciences, for their vision in supporting MCF research, a most difficult subject area. It is hoped that this book will confirm the importance of the problem in Indonesia and the continuing need for research to capitalise on recent progress. Although much of this volume deals with the epidemiology of MCF in Indonesia and research conducted in that country, the work has significance beyond the boundaries of one nation or one region. The high prevalence of disease in susceptible animals could make Indonesia one of the keys to the solution of this international mystery. The further support of ACIAR for the seminar and for the publishing of this book is gratefully acknowledged, as is the assistance of Mr Reg MacIntyre and the ACIAR publications staff.

The editors would also like to thank the many other people who have helped in many ways. In particular, the contributions of Professor R.H. Johnson and Professor R.S.F. Campbell, project supervisors at different times, and of Dr P.L. Young, project leader at its inception, are acknowledged. Dr A.J. Wilson, project manager of the AIDAB-supported project in institutional development at Balitvet, gave his complete support and also that of his administrative staff, in particular Murni Thayib, Liesye Anwar and Ambar Liano whose help with project documentation, the organisation of the seminar and the preparation of this manuscript were invaluable. The project depended on the interest and collaboration of veterinarians throughout Indonesia and from elsewhere, and many have become contributors to this monograph. We hope that their efforts will be rewarded with further discoveries in the near future.

P.W. Daniels
Sudarisman
Purnomo Ronohardjo

Summary of Discussion, and Recommendations

General

1. The workshop brought together people interested in MCF from varying perspectives: researchers with access to sophisticated technology and those relying on more basic techniques, laboratory diagnosticians and veterinarians with responsibilities for disease control at the national and field levels. Attention must be paid to good communications between the various parties and the exchange of information so that misconceptions will not lead to disappointments.

2. All discussions of MCF must be prefaced by the reminder that throughout the world MCF is known in two epidemiological forms, in which the affected animals have had contact with wildebeest, or where they have not. A herpesvirus, known by convention among MCF researchers as alcelaphine herpesvirus I (AHV-1), but also referred to previously as Bovine herpesvirus 3, has been isolated from cases of MCF in Africa and in zoos, and also from wildebeest, and will cause MCF upon inoculation into animals of susceptible species. AHV-1 is accepted as the causal agent of wildebeest-associated MCF (WA-MCF).

In many countries where MCF occurs without any possible contact with wildebeest, epidemiological observations have led to the suggestion that sheep may be a possible reservoir host for an infectious agent. Hence, MCF in the absence of wildebeest is called sheep-associated MCF (SA-MCF). No infectious agent has ever been identified as the cause of SA-MCF. Much research has been conducted towards demonstrating that the sheep harbour a virus related to AHV-1, and that cases of SA-MCF are infected by such a virus, but no definitive proof has been published.

3. MCF is of considerable economic importance in countries such as Indonesia where large and small ruminants are commonly kept together in traditional farming units and where large ruminants have a vital economic role in providing draught power. There is a need for further research funding to capitalise on the advances reported at this meeting.

4. Although there is still much to learn from field studies and transmission experiments, there is a need for research scientists in countries where MCF is a problem to have access to the techniques of modern biotechnology.

Epidemiology

5. The workshop accepted that in Indonesia and Australia the epidemiological evidence indicates that MCF is the sheep-associated form. In most cases discussed, the affected animals had had some demonstrable contact with sheep.

6. Because the agent of SA-MCF is unknown at present there can be no information about the disease based on serological or other tests. Hence, epidemiological observations play an important part in understanding the disease and the development of strategies for control and prevention.

7. The reservoir host. The role of goats as a potential reservoir host is unresolved. It is clear that in some locations goats do not spread MCF to susceptible cattle. In areas where goats are suspected as reservoir hosts, properly controlled transmission experiments involving contact exposure of susceptible Bali cattle should be conducted.

Confirmation or otherwise of the the carrier status of goats is a priority research area, so that livestock distribution programs could be safely based on the simultaneous distribution of these animals and Bali cattle to establish traditional farming units including both large and small ruminants.

8. Susceptible species. The field and transmission experimental evidence presented confirms that Bali cattle *(Bos javanicus)* are a highly susceptible species. Swamp buffalo *(Bubalus bubalis)* have a higher susceptibility than *Bos indicus* and *Bos taurus*. Note should be taken of these susceptibilities in planning control and prevention strategies.

9. Incubation period. The incubation period of the disease cannot be stated. Where Bali cattle were experimentally exposed to a large number of lambing small ruminants, disease developed in 2 to 3 months. In the field, Bali cattle that were believed to have been separated from sheep for up to 2 years were reported with MCF.

Because the incubation period is not known, it cannot be judged at what time sheep are infective. As a guide, it is noted that wildebeest calves and dams during pregnancy excrete AHV-1. Lambing sheep are often involved in Indonesia, but sheep there lamb 2 to 3 times per year. Hence, a normal Indonesian sheep population would be expected to be continuously infective. It is recommended that control based on total separation of susceptible species from sheep may be more effective than attempting separation from certain classes of sheep.

10. Prevalence. Because prevalence data may understate the true position, the economic cost cannot be fully appreciated. Until such time as the routine submission of samples for laboratory examination from field cases and from animals sent to emergency slaughter can be achieved, there is a need for well-designed prospective investigations of the turnover of large ruminants in selected study areas. Such study areas should be representative of the farming systems in various parts of the country concerned.

Ideally, such prospective studies should be coordinated by a planning committee comprised of representatives of the central animal health authorities, regional laboratories, the field services, and groups specifically involved in MCF research.

Pathology and Differential Diagnosis

11. In Indonesia there are problems in the differential diagnosis of MCF. Other diseases that must be considered are Jembrana disease in Bali cattle and trypanosomiasis in buffalo. Bovine virus diarrhoea-mucosal disease and suspected arboviral encephalitis may cause problems in special circumstances.

12. Until the results of further investigations on these diseases are available, basic principles should be followed in the diagnosis of MCF. It should be remembered that fatal MCF is usually described as being one of three overlapping clinical syndromes—peracute, gastrointestinal, and head-and-eye form. Histologically, MCF is a lymphoproliferative disease with a segmental

necrotising vasculitis with a mononuclear cell inflammatory reaction. Cases consistent with this clinicopathological syndrome can be diagnosed as MCF. Cases with some similarities but falling outside these guidelines in some aspect can be noted as suspected MCF in the differential diagnosis, and appropriate records made to keep attention focused on this diagnostic problem.

13. Mild and chronic forms. MCF is also described in mild and chronic forms. Mild febrile responses that may or may not be accompanied by other clinical signs, and a chronic wasting condition, have been seen in MCF transmission experiments in Indonesia. Such results are difficult to interpret but have epidemiological implications. Further effort in diagnosing the cause of such incidents is required, including necropsies of experimental animals early in the course of fever to develop an understanding of the pathogenesis and sequential pathology.

The capacity to investigate cases of suspect BVD-MD and arboviral disease on a routine basis must be developed by both the research and the regional diagnostic laboratories in Indonesia.

14. Differential diagnosis in buffalo. Although the case fatality rate is equally high, the clinical signs of MCF in buffaloes are not as pronounced as in cattle, and marked head and eye changes can take longer to develop, if they do so at all. Hence, an affected buffalo may not present as a classical MCF case.

Complete information on the histopathology associated with *T. evansi* infection in buffaloes in various circumstances is not available. Histopathological studies of experimental infections are required. As an adjunct to the above, full clinicopathological, serological, parasitological and virological investigations of cases of wasting diseases of buffalo in the field should be collected.

15. Differential diagnosis in Bali cattle. Although Bali cattle are a highly susceptible species, cases may not progress to show corneal opacity or severe muco-purulent nasal discharges before death intervenes. This must be remembered when making presumptive diagnoses based on clinical signs.

Experienced observers in Indonesia differentiate the Jembrana disease syndrome from MCF on the basis of clinical signs and pathology. Head and eye changes are not a feature of the former, whereas diarrhoea, focal cutaneous haemorrhages, lymphoid and splenic hyperplasia, peritoneal haemorrhages and haemorrhagic enteritis are usually marked. CNS lesions are not a feature of Jembrana disease, but the lymphoproliferative changes are prominent in all other organs and comprise predominantly large mononuclear cells of reticular cell and lymphoblastic appearance.

Although Jembrana disease as diagnosed can be recognised as a distinct syndrome, the similarities with MCF in the basic pathological changes are marked. Fatal cases of Jembrana disease may fall within the peracute or gastrointestinal spectra of MCF clinical signs. The possibility that the two diseases may have a similar pathogenesis in some respects should not be excluded.

16. Case registers. In countries where MCF is economically important or where differential diagnosis may constitute a problem, it is recommended that a case register be established. In Indonesia, the National Reference Coordinating Committee should be approached to sanction and promote a system of referrals and exchanges of case material, particularly histopathology slides accompanied by adequate case histories. The continuing MCF project at RIVS should act as a coordinating and referral centre.

Aetiology

17. Defining the aetiology of sheep-associated MCF is the primary international objective of MCF research. Only then will definitive studies of the epidemiology and pathogenesis be possible and the development of techniques on which to base accurate diagnosis and control.

18. Research in some countries, for example the United Kingdom, with the indirect immunofluorescence (IIF) test has shown some reactions between the antigens of AHV-1 and sera from cases of sheep-associated MCF. These have not been seen in Indonesia. Sera from confirmed and suspected cases should be actively collected for further testing, for holding in a national serum bank, and recorded in a case register.

19. Similarly, cross-reactions between sheep sera and AHV-1 have been reported in the IIF test. In the current work, only one clear reaction was observed, in which a 'paranuclear body' fluoresced. This serum was from an Australian sheep associated with cases of MCF in buffaloes. There is a need to develop more specific serological tests and apply them to unequivocal definition of this phenomenon.

20. Current hypotheses suggest that the infectious agent of SA-MCF, although related to AHV-1, is not present in affected animals in the form of complete virions, but rather is present in lymphoid cells as viral nucleic acid, and exerts its pathological effects in this form. Under these conditions, structural proteins will be produced incompletely or not at all. Serological techniques will therefore be of limited value in detecting the presence of the agent. Appropriate DNA probes for the presence of viral DNA must be developed, and detection methods then based on DNA hybridisation.

21. To serve the needs of Indonesia quickly, its scientists should collaborate internationally with laboratories that have the necessary expertise, or that are involved in research to develop the necessary specific reagents. Through collaborative research, the benefits of biotechnology can be applied to solving Indonesian animal health problems.

22. Two separate groups reported isolation of apparently similar viruses from cases of MCF, but at the present time none has been convincingly identified. Characterisation of the isolates and demonstration of their range of effects in experimental animals and large ruminants is needed.

23. Attempts to isolate the agent in culture should be directed away from traditional cell culture techniques based on the infection of established monolayers, and concentrate on the establishment of lymphoid cells in suspension cultures, supplemented by interleukin 2 or similar growth facilitators.

National Overview

Malignant Catarrhal Fever in Indonesia

Masduki Partadiredja, I. Gde Sudana and Soesilo*

Abstract

A study of malignant catarrhal fever based on results of surveillance and investigations in Indonesia showed that it has been reported from most provinces. The distribution of the disease was correlated with the transmigration of cattle. Sheep have been implicated as the reservoir of the disease in most areas. Goats are now also being taken into consideration.

Abstrak

Dari hasilo penyidikan dan pengamatan lapangan di Indonesia, telah menunjukkan bahwakasus MCF telah meluas ke beberapa Propinsi. Perluasan penyakit mengikuti penyebaran ternak sapi. Peranan domba sebagai penular penyakit ditemukan hampir di seluruh wilayah. Kambing kini mulai diperhatikan peranannya sebagai carrier.

Introduction

Malignant catarrhal fever (MCF) has been recognised for centuries. In Indonesia, MCF has been recognised for many decades, and the early reports have been reviewed (Mansjoer 1954). In the past, there have been many reports of MCF from areas like Bali, West and East Nusa Tenggara, South Sulawesi and Java. In the period 1981 to 1985, reports covered all of Indonesia except Maluku, Irian Jaya, West, Central and South Kalimantan and Aceh.

Clinical signs of advanced MCF are classic, while in the early stages signs occur which can be mistaken for foot-and-mouth disease or bovine virus diarrhoea. In the histopathological diagnosis of MCF, perivascular cuffing of the blood vessels of the brain is reported as a consistent feature in Indonesia.

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The morbidity of MCF is low but the case fatality rate is high (100%). However, epidemics can occur in certain groups of susceptible livestock. For farmers that own 3 or 4 head of cattle, the loss of one is a considerable burden, especially for the farmer with a low capability to raise livestock for repaying the Government for previous distributions of animals.

With this in mind, data were collected and reported so ways can be sought to overcome this disease.

Materials and Methods

Data collected by the Directorate of Animal Health were derived from the annual reports of each of the provincial Dinas Peternakan offices throughout Indonesia, the investigations and reports of the Disease Investigation Centres, and also the map of animal diseases in Indonesia from the Directorate of Animal Health. Also included in this paper are the results of observations of MCF cases written in conjunction with field staff.

Results and Discussion

The ruminant populations of Indonesia as a whole and by province are presented in Tables 1 and 2. These data represent the background against which the importance of the disease must be assessed. Cases of MCF have been reported since 1917 or even earlier, and the disease was researched by Mansjoer (1954). From 1954 until 1981, few cases of MCF were reported by field officers. This is perhaps because MCF occurs sporadically and is not contagious. The numbers affected were relatively low and the disease ended in death. Cases reported by farmers would be given some treatment, but after a few treatments without result would be slaughtered. Field officers

Table 1. Livestock populations ('000) in Indonesia (1981–85).

Year	beef cattle	Dairy cattle	Buffalo	Goats	Sheep
1981	6516	113	2488	7790	4177
1982	6594	140	2513	7891	4231
1983	6660	142	2538	8049	4316
1984	6867	173	2620	8141	4365
1985	7081	186	2706	8235	4416

Source: Buku Statistik Peternakan, 1986 Dit. Bina Program. Ditjennak

recommended to farmers that early cases be slaughtered, since the meat is a source of protein. The suggestion of compulsory slaughter is still discussed by colleagues in East Kalimantan because slaughter may affect the diagnosis and epidemiological pattern of disease. In addition, there would be many animals killed without diagnosis by field staff.

Endemic Areas

Before 1980, Nusa Tenggara Barat (NTB), Nusa Tenggara Timur (NTT), Bali and Java were considered areas in which the incidence of MCF was sporadic. North and South Sulawesi were later added to this list. In 1981, some MCF cases were reported from other areas. An overview of the present distribution of MCF is presented in Table 3.

In the region of the Disease Investigation Centre (DIC), Bukittinggi, an unrecorded number of cases were reported from West Sumatra, Riau and Jambi from 1981. In North Sumatra, an increasing number of cases occurred until 1984, after first reports in 1978; 182 cases occurred in Deli Serdang district(Paranginangin 1986). Aceh province has not reported any cases of MCF. The whole of Java and Madura have been considered endemic areas (Sudarto et al. 1986). Some MCF cases in cattle in the transmigration areas were reported in Bengkulu, South Sumatra and Lampung provinces (Husin et al. 1985; Prabowo and Soesilo 1986). In June 1986, in Kalimantan, cases of MCF were reported in Bali cattle 2–3 weeks after distribution to the farmers (Sudana unpublished). Sheep and goats were not found in that village, and the cattle came from South Sulawesi.

Based on observations in West Sumatra, Lampung and East Kalimantan, it appears that MCF cases follow the Government distribution of livestock, the MCF virus probably being carried by cattle from the original area. As sheep and goats were not found in East Kalimantan to act as MCF carriers or transmitters, the MCF cases in June 1986 were the last in that area. MCF cases have not been reported from Aceh, Central Kalimantan, West Kalimantan and South Kalimantan, so care must be taken in these areas to avoid the introduction of the disease.

Table 2. Livestock population ('000) by province (1982).

Province	Beef cattle	Dairy cattle	Buffalo	Sheep and Goats
Aceh	176.9	0.2	203.9	179.0
N. Sumatra	150.3	6.6	161.5	213.7
Riau	12.8	-	10.7	66.6
W. Sumatra	165.4	1.7	124.1	73.8
Jambi	18.7	-	27.5	66.9
Bengkulu	16.0	-	31.5	39.5
S. Sumatra	95.2	0.4	52.2	156.7
Lampung	78.4	0.2	30.7	181.2
DKI Jakarta	0.6	5.6	2.3	25.6
W. Java	124.1	37.3	493.	3181.1
C. Java	1021.7	32.9	319.5	3448.9
DI Yogyakarta	190.7	2.8	14.2	415.2
E. Java	2504.9	50.3	214.5	2986.4
Bali	384.7	0.3	7.2	37.6
NTB	150.0	0.1	200.	153.2
NTT	426.2	0.3	144.4	295.7
W.Kalimantan	60.0	0.1	0.5	24.7
C.Kalimantan	14.4	-	1.6	7.2
S.Kalimantan	26.3	0.1	12.4	24.7
E.Kalimantan	7.3	-	5.8	9.3
S.Sulawesi	621.3	0.9	417.6	195.2
S.E. Sulawesi	20.3	-	7.1	48.8
C.Sulawesi	119.0	-	11.0	89.5
N.Sulawesi	172.8	0.2	0.8	57.9
Maluku	16.3	-	18.8	91.6
Irian Jaya	19.7	-	-	18.4
E.Timor	-	-	-	33.6
Totals	6594.0	140.0	2513.0	12122.0

Source: Dit. Bina Program Ditjen Peternakan.

Table 3. Reported cases of MCF in Indonesia (1982–86).

Province	Source of Report	Year 1982	1983	1984	1985	1986*
N. Sumatra	Disnak*	-	-	-	-	-
	BPPH I†	22	42	62	-	-
Jambi	Disnak	-	-	-	7	4
	BPPH II	-	-	-	-	-
Bengkulu	Disnak	-	3	8	2	-
	BPPH III	7	10	32	7	-
S. Sumatra	Disnak	-	1	10	5	-
	BPPH III	-	3	19	6	-
Lampung	Disnak	-	-	-	-	-
	BPPH III	-	2	-	7	-
E. Java	Disnak	-	-	98	252	42
	BPPH IV	-	-	-	-	-
Bali	Disnak	-	-	3	19	4
	BPPH VI	-	-	-	-	-
NTB	Disnak	-	-	278	-	77
	BPPH VI	-	-	-	-	-
NTT	BPPH VI	-	-	-	-	-
E. Kalimantan	Disnak	-	-	-	-	3
	BPPH V	-	-	-	-	-
S.E. Sulawesi	Disnak	-	-	1	-	-
	BPPH VII	-	-	-	-	-

*Reports received until June
† Balai Penyidikan Penyakit Hewan (DIC)

Transmission

Some researchers have reported that transmission of MCF is associated with sheep, especially after lambing. During the period 1981 to 1985, there were MCF cases in Bengkulu, Lampung and South Sumatra in which sheep were attributed as a reservoir of the disease (Husin et al. 1985; Prabowo and Soesilo 1986).

The greatest probability of transmission occurs when sheep and cattle are housed in the same pen and grazed together. This frequently occurs because the area for grazing is limited.

In Bali, it is suspected that MCF is transmitted from goats, but further research has not yet been reported. However, the relationship between goats and Jembrana disease in cattle owned by P3B Palukan Project (Jembrana) is being studied.

In West Timor, NTT, 15% of a herd of 400 Bali cattle in Kecamatan Kupang Barat developed MCF. These cases occurred 3-4 months after the cattle were kept with sheep from Roti Island (Bale, unpublished data). Other MCF cases in cattle and deer in Kupang, NTT, occurred after the animals were kept in the same pen as sheep (Darmadi et al. 1985).

Peranginangin (1986) suggested that sheep are the most probable reservoir of the disease, but this must be proven by virological tests or virus isolation from sheep tissue. Some scientists have reported that transmission occurs via vaginal secretions after lambing. The period of time for which the infective virus is present in the excretions has not yet been reported. Ramachandran et al. (1982) successfully transmitted the disease to Bali cattle by using 250 ml of whole blood from an infected animal. Natural transmission, transmission by infection and transmission by blood dosage as described above are all difficult to achieve.

Affected Animals

Cattle and buffalo are the most commonly affected animals. Darmadi et al. (1985) diagnosed MCF from rusa deer *(Cervus timorensis)* specimens sent from Kupang. Differences in

susceptibility among animal species has not yet been reported in detail. A team of investigators from the DIC, Denpasar (Anon. 1982) reported that in East Java, of 24 MCF cases examined, 71% were in sapi Rambon (Bali cattle–*Bos indicus* crosses), 25% were in sapi PO (local *Bos indicus)* and 4% were in Brahman. However, these data did not consider distribution of population and the possibility of exposure to infection.

Sex and Ages of Affected Animals

Peranginangin (1986) and Anon. (1982) have both reported that 93% of affected animals were 24 months or older. It has been difficult to use data relating to the sex of affected animals as comparisons between male and female rates of infection because the chances of infection are not uniform in the observation areas. The economic losses caused by MCF must be considered not only in terms of deaths of animals, but also lost reproductive opportunities, decrease in draught animal power available, and the possibilities of inferior quality offspring.

MCF Complex

Because of observed similarities in clinical signs and histopathology, Ishitani (pers. comm.) suggested in 1982 that Ramadewa disease in Lampung should be included in an MCF complex of diseases. Similarly, it has been suggested that Jembrana disease is caused by an oncogenic herpesvirus. This hypothesis was based on the similarity to MCF with respect to clinical signs, lymphadenopathy and histologic changes (Dharma et al. 1985).

Conclusions

MCF is distributed throughout most of Indonesia. Regions that have not yet reported cases cannot be considered free of MCF, but further observation and research should be undertaken. MCF is a sporadic disease the incidence of which can increase. Economic loss entails not only animal death, but loss or decrease in draught animal power and the possibility of inferior offspring. The role of sheep and goats in the distribution of MCF is not fully understood and further research in this area should be undertaken. So far as is known, the animals affected are Bali cattle, buffalo and deer. Susceptibility of other kinds of livestock should be further investigated.

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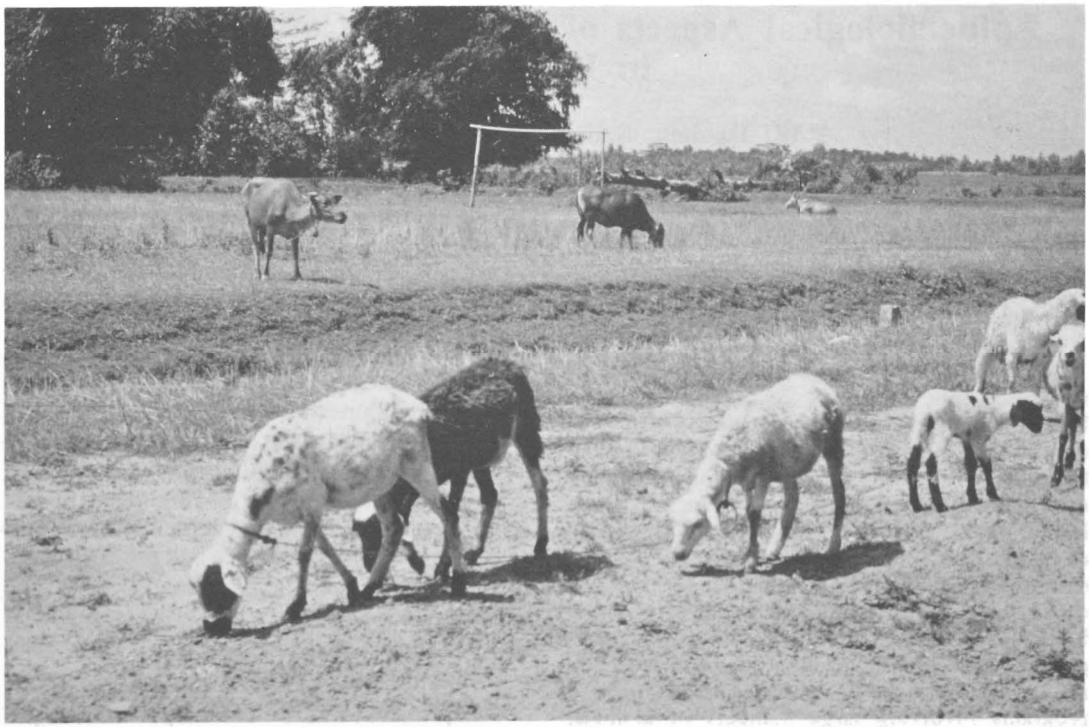

Epidemiology

Epidemiological Aspects of Malignant Catarrhal Fever in Indonesia

P.W. Daniels, Sudarisman, Agus Wiyono and Purnomo Ronohardjo*

Abstract

Indonesia has a huge rural economy, with over 90 million people depending on agriculture for a living. The large ruminant population has been estimated at 6.5 million cattle and 2.5 million buffalo. Current government planning promotes the use of indigenous breeds such as the Bali cattle. Field observations indicate that malignant catarrhal fever (MCF) is a major disease problem endemic in many areas. The buffalo and the Bali cattle seem to be more susceptible than other breeds, and sheep have been identified as a probable source of infection. Where Bali cattle are introduced into contact with sheep through programs involving large numbers of animals, epidemics of MCF have occurred. Experimental transmissions have confirmed the susceptibility of the Bali cattle.

Abstrak

Peranan ekonomi pedesaan bagi Indonesia sangat penting, mengingat 90 juta penduduk menggantungkan hidupnya dalam bidang pertanian dan mereka tinggal di pedesaan. Ruminansia besar, yang di perkirakan terdiri atas 6,5 juta sapi dan 2,5 juta kerbau mepunyai andil yang sangat berarti bagi ekonoi pedesaan dan pemerintah sedang barusaha untuk mengembangkan ternak asli Indonesia, misalnya sapi Bali. Berdasarkan penelitian lapangan MCF merupakan penyakit endemik yang banyak menimbulkan masalah di beberapa daerah. Sapi Bali dan kerbau rupanya merupakan ternak yang lebih peka terhadap MCF dibanding ternak lain. Apabila pada lokasi sapi Bali ditempatkan juga domba, biasanya kejadian MCF secara endemic muncul. Percobaan transmisi telah memperkuat dugaan kepekaan pada sapi Bali tersebut.

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Introduction

Malignant catarrhal fever (MCF) is distributed widely in Indonesia. It is recognised by the animal health authorities as an important disease and by farmers as a familiar clinical syndrome resulting in deaths of large ruminants. It has been suggested (Anon. 1986) that accurate estimates of disease incidence and prevalence are difficult to obtain. The situation could not be expected to be otherwise in this country, considering the low ratio of veterinary field staff to the rural-based population.

Nonetheless, when allocating animal health research priorities, it is essential to ensure that funds are allocated to areas of genuine need. One of the aims of the AARD/ACIAR Collaborative Project on MCF has been to collect and analyse such data as are available as a prelude to further research. There is a pressing need to clarify aspects of epidemiology that could influence any control measures the veterinary services may be able to take, for it is certain that such control measures will affect the financial opportunities of rural smallholders in the field of livestock raising. This paper describes briefly the role of ruminants in the national economy and their value to farmers. Indonesian literature on MCF and official reports are reviewed, and new field and experimental observations are discussed.

The Rural Economy

An overview of the rural economy potentially affected by ruminant diseases can be extracted from data assembled in a recent major review of the Indonesian livestock sector (Anon. 1986). Of a population of 162 million people, 90.4 million (55.7%) in 17.6 million households

depend directly on agriculture for a livelihood, and their numbers and the total area farmed are increasing. Although only 25 to 30% of these households keep ruminants, and the numbers of animals per household are small, there are still more than 5 million family units containing over 28 million people involved with ruminants in some way for their financial well-being. The primary objectives of their animal husbandry are to have a store and a source of wealth, and a source of draught power.

At the national level, the major animal product is meat, with an annual production from large ruminants of more than 180 000 t and, from small ruminants, of more than 62 000 tons. The average annual per capita meat consumption of 4.33 kg is low. It is considered that the demand for meat will grow, that supply will not keep pace with demand, and that there will be an upward movement of prices for both meat and livestock.

As most households make little financial input into ruminant maintenance beyond the purchase price, ruminants offer a viable means of converting labour to wealth. The increasing demand for meat and draught power should ensure that such wealth will grow in the short and medium terms. At present the approximate value of mature cattle is US$400–450, of buffalo US$400, and of sheep and goats US$40.

To individual farmers the need for draught power is important, and must be recognised in estimates of the economic value of livestock. A pair of animals can earn US$2.50/day ploughing. It is estimated that 3.3 million cattle and 1.4 million buffalo are available for draught purposes and that in 1982 the value of their draught power was US$237 million. Animal manure as a by-product has been estimated to contribute US$40 per year per large ruminant to farm income.

An important new development in livestock usage patterns is the distributions in transmigration programs. Bali cattle is the animal preferred by the recipients and the planners because of its perceived superior productive capacity. The health and welfare of these animals is a particularly significant development problem.

The planning for the development of Indonesia is through a series of 5-year plans. Repelita IV covers the period 1984–1988. The main objectives for the livestock sector are to increase farm income and employment opportunities, to meet domestic demand for animal services and products, to increase export earnings and reduce imports of livestock products, to meet the demand for draught animals and manure for crop production, to conserve indigenous breeds like the Bali cattle, and to increase the carrying capacity of grasslands through the establishment of better quality forages.

Research to help reduce losses due to MCF can be seen as an important contribution to achieving these aims, particularly as the indigenous breeds are more susceptible.

Literature Review

Historical Aspects

A major contribution to the knowledge regarding MCF in Indonesia has been the research of Mansjoer (1954). A summary of his review of the literature to that time gives a useful background for current research. Many of the citations were not formally referenced, but the sources of information were acknowledged in the text. Readers are referred to Mansjoer (1954) for further information.

MCF was first diagnosed near Kediri in East Java (Paszotta 1894). Three buffalo with MCF were seen and four others were reported. Passage was attempted by subcutaneous inoculation of blood and ascitic fluid to one buffalo, and by subcutaneous inoculation of blood and 'ventricle' fluid to two goats. Failure to reproduce MCF within 14 days was attributed to either too small a dose or too short an observation period.

The disease rapidly became recognised throughout Indonesia (Penning 1910, 1911—cited by Mansjoer 1954). Cattle were affected sporadically. Madura was frequently mentioned as a focus of infection (Penning 1915; Van der Poel 1916—both cited by Mansjoer 1954), with affected cattle suffering 100% mortality. It should be noted that the traditional cattle in Madura are Bali-cross animals. Cases from Central Java, Madura and Sumatra continued to be reported for a number of years.

The Research Institute for Veterinary Science (RIVS) in Bogor reported MCF in 1920, 1921 and 1926. In 1920, passage by intravenous and subcutaneous inoculation of blood from a cow

showing clinical signs was successful. Nijverheid and Handel (1920) (cited by Mansjoer 1954) reported the investigation of a disease of buffalo characterised by emaciation, a pale and fish-like skin, sunken eyes, pustules on the head and shoulders, and occasionally diarrhoea and dysentery. Transmission studies with blood from such animals produced disease with clinical signs of high fever, purulent oculo-nasal discharge, wasting, and diarrhoea. Examinations for piroplasms and trypanosomes were negative. Mansjoer (1954) arrived at the conclusion that these diseases were MCF, although in some cases atypical. It seems that a problem of differential diagnosis in the buffalo has existed for some time.

Schroots (1922) (cited by Mansjoer 1954) identified 10 cases in cattle in Lombok and attempted passage to cattle, rabbits, marmots and turtle doves without success. Blood submitted to RIVS also failed to transmit the disease to cattle. In the previous year inoculations to a dog, turtle dove, rabbit and sheep had not transmitted disease. In 1922 and 1924, the Livestock Services Office identified further cases in West Java, including Bogor and Sukabumi, as well as in Central and East Java. From 1925 to 1938, MCF was mentioned by various authors in annual reports as occurring in both cattle and buffalo. Cases were widely distributed: from Medan, Madura, northern areas of Java, Central Java, East Java, West Java, Sulawesi and Kupang.

In 1935, passage by intravenous inoculation of cattle with 10 mL of blood was attempted without success (Munnik 1935—cited by Mansjoer 1954). It is clear from the writings of Mansjoer that he regarded Lombok as an important focus of infection of MCF, and that over the years from 1918 to the time of his own research there was considerable interest in the problem by local staff in collaboration with RIVS.

In 1946, the Faculty of Veterinary Science in Bogor reported cases in buffalo. This may have been the stimulus for Mansjoer's own research, which involved the examination of 27 spontaneous cases with prominent meningoencephalitis. Successful passage of MCF to cattle, buffaloes, rabbits and chicken embryos was reported. Humans, monkeys, sheep, goats, pigs, rats, mice, ducks, fowl, carrier pigeons, turtle doves and zebra doves were reported to be resistant.

It is unclear when MCF in Indonesia was first associated with sheep. Soeparman (1940) (cited by Mansjoer 1954) attributed the low prevalence in central Lombok compared with other parts of the island to a small sheep population. In Bali, it was noted that MCF occurred in villages of ethnic communities raising sheep, but not in districts where pigs were preferred (Made Bagi Asna 1950—cited by Mansjoer 1954). Farmers believed that the disease was spread by sheep urine on the grass. Mansjoer (1954) concurred that sheep were the probable carrier, and recommended that note be taken of this observation throughout Indonesia.

A Disease with a Similar Pathomorphology

In considering the epidemiology of MCF in Indonesia, similar diseases unknown elsewhere must be noted. In 1964 and 1965, an epidemic involving Bali cattle and buffalo was reported in Bali. Fever, anorexia, depression, laboured respiration, rapid emaciation, markedly swollen lymph nodes, constipation or diarrhoea and dysentery, serous ocular and nasal discharges and erosions in the mouth were observed. Death usually occurred in 5 to 12 days. The spleen was enlarged and haemorrhages were present in the gastro-intestinal tract, with the abomasum being particularly affected and thickened. The disease was easily passaged to Bali cattle, but not to *Bos indicus*. It was tentatively called Jembrana disease (Adiwinata 1967).

Thousands of animals were reported to have died. No bacteria were isolated from cases, and treatment against trypanosomes was ineffective. The possible differential diagnoses were thought to be MCF, rinderpest or bovine virus diarrhoea–mucosal disease. MCF in Bali was previously known to occur sporadically, was characterised by severely diseased mucous membranes of the head and corneal opacity, and was not as easily passaged as was the disease of the outbreak. Hence, MCF was excluded (Pranoto and Pudjiastono 1967). Rinderpest was considered the most likely possibility, and the disease abated after a rinderpest vaccination campaign (Adiwinata 1967).

However, the diagnosis was not supported by

laboratory tests (Anon. 1977). Rickettsia-like organisms were noted in tissue sections and blood smears, and their isolation was reported (Hardjosworo and Budiarso 1973), but such organisms have not yet been firmly implicated in the aetiology.

Of epidemiological interest was that the disease changed character. While still presenting in outbreaks in which the morbidity was high, the mortality became much reduced, a completely different situation from the sporadic occurrence and 100% mortality normally associated with MCF. In other aspects the diseases remained similar, with no sex predilection and with young adults being the most susceptible age group. Only Bali cattle were affected (Anon. 1977).

It is beyond the scope of this paper to review Jembrana disease in great detail. The salient aspects of the disease as currently perceived in Indonesia are provided elsewhere in this volume. The similarities and differences of the two conditions will be debated until the aetiology of each is known.

Recent Publications

Ginting (1979) examined cases with clinical signs, postmortem findings and histology consistent with MCF in Bali cattle from two commercial operations in West Java, and from Balai Penelitian Ternak (BPT), the Research Institute for Animal Production at Ciawi. The recommendation was made that the husbandry of Bali cattle not be attempted in West Java, which has a large sheep population. Cases of Jembrana disease were also reported from BPT, Ciawi, at that time (Hardjosworo et al. 1978).

MCF has been a continuing problem at BPT (Hoffmann et al. 1984a, b). From 1979 to 1982, 50 of 177 buffalo died of a syndrome described as being MCF-like. This syndrome as described was very similar to that studied previously as MCF (Mansjoer 1954). The experimental facilities for large ruminants at BPT are close to those for small ruminants which reproduced all year round. Penning buffalo 400 m from the facilities stopped the development of new cases.

Bos indicus cattle (71) held under the same conditions as affected buffalo did not contract disease, indicating a difference in susceptibility between the two populations. Further Bali cattle (4 of 7) introduced to BPT died of MCF. The disease was transmitted by blood transfusions from buffalo to *Bos indicus* and Bali cattle (Hoffmann et al. 1984b).

MCF continued to be reported in Bali and successful transmission with blood and lymph node suspensions from Bali cattle to Bali cattle was reported (Ramachandran et al. 1982).

Banyuwangi is the portion of East Java immediately adjacent to Bali. It was reported (Anon. 1982) that 400 cases of MCF were diagnosed there in 1978 and 1979. Again in 1982 approximately 150 cases were recorded. Seventy-four of these above cases were diagnosed histologically at the DIC, Denpasar, between 1979 and 1982, and most cases were submitted in the months of November and December, which is the wet season and the ploughing season. Ages of cases ranged from 1 to 9 years. Cattle and buffalo were affected. The disease of Bali cattle and their crosses in Banyuwangi had also been thought in 1979 to be Jembrana disease (Anon. 1984).

Reports of cases and outbreaks in the southern provinces of Sumatra have been published. Between 1982 and 1985, cases were reported from Lampung, South Sumatra and Bengkulu (Prabowo and Soesilo 1986), with the outbreak in Bengkulu having been reported in greater detail (Husin et al. 1985). In Bengkulu, the incidence between June 1983 and December 1984 was over 4% in imported Bali cattle. Sheep were present in five of the affected villages, but not in three others.

A disease very similar to MCF has also been reported in southern Sumatra, in the province of Lampung. Called Rama Dewa, its similarities with Jembrana Disease have been emphasised (Soeharsono and Darmadi 1976). Some descriptions of Rama Dewa (Prabowo and Ishitani 1984) also indicated considerable similarity with MCF. The characteristic feature was marked lymphoproliferation and lymphoid vasculitis. Encephalitic changes were present in fewer than 50% of cases, but necrotic changes of the oral mucosae were said to be a feature. In addition, purulent nasal discharges and corneal opacity were described, indicating that at least some of the cases showed a resemblance to the head-and-eye form of MCF. The disease was transmitted with 50 and 100 mL volumes of infected blood by Prabowo and Ishitani (1984),

and with 5 and 15 mL by Soeharsono and Darmadi (1976), volumes which seem small for MCF. Ramachandran et al. (1982) passaged the disease in Bali cattle with 50 mL of blood plus 25 ml of 20% lymph node suspension. The minimum volume necessary to transmit MCF in Bali cattle has not yet been titrated. Prabowo and Ishitani (1984) proposed that the two diseases—Rama Dewa and MCF—be considered as part of the MCF complex of diseases. The high prevalence of these similar diseases in the transmigration areas of southern Sumatra makes the MCF complex a problem of considerable magnitude.

Hence, in three locations outside Bali—at BPT, Ciawi, in Banyuwangi in East Java, and in Lampung in the south of Sumatra—Jembrana-like diseases and MCF have been reported at similar times. From the time of the earliest reports of Jembrana, MCF has been considered in its differential diagnosis (Pranoto and Pudjiastono 1967), but from then until now the two clinicopathological syndromes have been considered distinct (Soeharsono 1988). The occurrence of the two diseases in such populations indicates the susceptibility of the Bali cattle to diseases of this type. Conversely, this susceptibility is a further point of similarity between the two syndromes. As noted, Prabowo and Ishitani (1984) considered that in fact there was only one disease in Lampung, an MCF complex. Dharma et al. (1985) discussed the pathology of Rama Dewa, Banyuwangi disease, Jembrana and MCF and came to the conclusion that there were no important differences between the syndromes, that were each characterised by generalised lymphadenopathy and lyphoproliferation with mitotic figures.

More recently, an active centre of research into MCF has arisen at the DIC, Medan in Northern Sumatra. Buffalo deaths previously attributed to infectious bovine rhinotracheitis (IBR) (Noor et al. 1983) were rediagnosed as probable MCF on the basis of the clinical signs consistent with the head-and-eye form of MCF (Peranginangin et al. 1986a). Virus isolations were attempted from new cases. A virus of similar description to that isolated by the project at RIVS (Sudarisman et al. 1985) was isolated by co-cultivation of buffy coat cells from cases in buffalo. Transmissions with blood to buffalo, sheep and goats were reported in all cases to result in mild disease but not death. In each case, a virus was isolated from buffy coat cells and lymphoid tissues when the animals were killed for postmortem examination (Peranginangin et al. 1986a). No pathology was described in the animals in the transmission experiments or in the field cases. Infected cells were reported to fluoresce in an indirect immunofluorescence test with an antiserum to WA-MCF virus supplied by Plum Island (Perangingangin 1986b), but perhaps further information is needed on this point.

Apart from the obvious virological interest of these reports, they show that MCF is a frequent cause of death in buffalo in North Sumatra. Peranginangin (1986) reported that 180 cases had been observed in buffalo in the seven years 1978 to 1984, equivalent to 4.86 cases per 1000. Only two cases were observed in a similar number of *Bos indicus* in the same period. Both males and females were affected, and mature animals more than young animals. No cases were reported in calves. There was a close association with sheep in each case.

Official Reports

The Government Veterinary Services each year prepare figures on the diagnosis of disease in the provinces. These data are, of necessity, based mostly on clinical examinations, which affect their accuracy, and also represent only those cases seen by the veterinary services field staff, which means it is possible that the figures are an underestimate of the true situation.

Data derived from such sources for the last 5 years were given by Partadiredja et al. (1988). During this time, cases have been reported in 15 of the 27 provinces, confirming the widespread distribution of the disease. In terms of number of cases, some inconsistencies can be noted. Southeast Sulawesi, which claims to have a significant problem, is unrepresented in the figures. Central Java reported only two cases in 5 years, but the experience of the authors and of the DIC at Jogyakarta (Wisynu and Unruh, pers. comm.) is that sporadic MCF is endemic there.

These comments are not intended to be criticism of the reportings from the provinces named, but rather attempt to illustrate the difficulties that veterinary authorities still have in formulating an accurate estimate of the epidemiology of animal diseases. Such problems

are being overcome, but at present still exist. In summary, it seems certain that official records of MCF in Indonesia underestimate the true situation.

Field Observations

As is widely appreciated, MCF occurs in two epidemiologically distinct forms throughout the world (Plowright 1984). Susceptible species develop MCF either after contact with wildebeest (WA-MCF), or in situations where there are no wildebeest. Field observations in many countries have led to the hypothesis that sheep are the silent carriers of the MCF agent in these circumstances, and hence this latter form of the disease is called sheep associated MCF (SA-MCF). The question of the reservoir host is one of the aspects that epidemiological studies should attempt to address. As there are no wildebeest in Indonesia, note has been made where possible of any association with sheep.

Through field work in some of the districts where MCF has been reported, such as in West Timor, Banyuwangi and Central Java, and through interpretation of other government reports and personal communications, the authors have developed an appreciation of the pattern of occurrence of MCF in Indonesia.

It seems reasonable to identify two categories of disease occurrence; endemic, where the reports of disease are of a true sporadic nature, and epidemic, where there is a grouping of cases in particular herds at an incidence level above the expected norm.

Endemic MCF

In the endemic situation a low incidence is normal, although certain geographical locations experience a higher incidence of MCF. The investigation of a field case in Boyolali, Central Java, identified features of the normal Indonesia situation. A farmer who owned three buffalo reported one sick and showing classical clinical signs. MCF was confirmed by histopathology. This animal was the only animal in the immediate district known to be affected at that time and was housed adjacent to sheep including lambs. The farmer reported the loss of other buffalo from the same disease syndrome in previous years, and apparently similar cases occurred from time to time in the district. Paraveterinary staff had thought the disease to be trypanosomiasis, and had so diagnosed previous cases with similar clinical signs. Because of logistical problems histopathological confirmation of diagnoses of sporadic cases of disease was not normally sought. In this district, MCF is therefore underdiagnosed.

Similarly, a field case close to Bogor was investigated when a laboratory worker reported disease in one of his family's buffaloes. The case was clinically consistent with MCF. The buffalo was sold for emergency slaughter without the researchers being advised, and in spite of their known interest. Hence, no postmortem confirmation of diagnosis was possible.

This farmer was also familiar with this disease, and had sent animals to emergency slaughter, or had had them die, on previous occasions. He reported that others in the district shared his experience. It seems probable that again MCF is underdiagnosed and underreported. A farmer may be most reluctant to jeopardise the salvage of some of his capital by involving the authorities in the disposal of an affected animal.

In Banyuwangi, East Java, MCF is also endemic, occurring sporadically but with an apparently higher prevalence (Anon. 1982). Subsequent field work by the present authors found the paraveterinary staff in this area to be well-organised and active.

Cases, although clustered a little in certain districts, occurred thoughout the whole kabupaten. It was not the pattern that one particular village was more affected than others, or that many cases occurred in one place at the one time.

The factors leading to the reported high prevalence could not be determined. Histological confirmations of diagnosis (Anon. 1982; staff, DIC Jogyakarta, pers. comm.) suggest that MCF is not overdiagnosed to any great degree. Sheep and goats are widely spread and are owned in traditional smallholder operations. Cases can occur all year but a higher incidence has been observed in November and December which is normally the wet season and which is also the ploughing season. The less susceptible ongole *(Bos indicus)* breed has a higher-than-normal case rate, suggesting that the disease agent may be spread particularly actively or be particularly virulent in this region.

Banyuwangi is a crossroads for livestock movements from East Java and other islands towards the market in Jakarta. The livestock markets in the various districts are very active, with much local trading. It is not known whether these factors can contribute to the prevalence of MCF.

Epidemic MCF

A different epidemiological picture was seen in some situations where there were movements of identifiable groups of livestock. The authors have assisted in the investigation of two outbreaks of MCF in West Timor. MCF confirmed by histopathology (Darmadi et al. 11985) occurred when deer and sheep were brought together, and when sheep from Roti Island were moved into contact with a herd of Bali cattle in West Kupang previously unexposed to sheep. In the first case, 65% of 55 deer died, and in the second case 20% of 300 Bali cattle were affected.

It seems that similar situations have occurred in transmigration areas with the movement of Bali cattle from the breeding areas where sheep are rare, such as West Timor, into new areas with established sheep populations. Cases of MCF reported from the provinces in central and southern Sumatra have mostly been in imported Bali cattle supplied by the government to new settlers (Prabowo and Soesilo 1986).

Transmission Experiments

In Indonesia, transmission experiments have long been used to study MCF (Paszotta 1894; Mansjoer 1954; Hoffmann et al. 1984b) and the similar diseases Jembrana (Adiwanata 1967, Hardjosworo and Budiarso 1973) and Rama Dewa (Soeharsono and Darmadi 1976; Prabowo and Ishitani 1984). Thus, the infectious nature of the disease in the affected animals can be confirmed, the infectivity and associated pathology of agents associated with various incidents compared, and fresh specimens obtained for laboratory investigations. Transmission experiments have continued to give valuable information on MCF in Indonesia (Sudarisman et al. 1985). These preliminary results of the authors and others will be discussed below together with more recently derived information. Experiments have been designed to provide epidemiological information.

Contact Transmissions

(a) A young male buffalo was housed with three lambing sheep. No MCF was observed after a 20-month observation period.

(b) Two adult males each of buffalo, *Bos indicus* and Bali cattle from South Sulawesi and two young Bali cattle from West Timor, were penned in close proximity to lambing sheep and goats at BPT, Ciawi. All Bali cattle died of MCF within 15 weeks, while the other species remained unaffected after 1 year of observation, except for one buffalo that showed a 2-week episode of mild nasal discharge and fever of 39–39.5°C approximately 7 months post-exposure. The second buffalo died of misadventure after 3 weeks of exposure, and therefore was not effectively involved in the experiment.

(c) Two young Bali cattle in Kupang, West Timor, were placed in contact with sheep that had been implicated in the outbreak of MCF in deer. No deaths occurred in the Bali cattle, but they underwent two periods of illness. Ten weeks after first exposure to the sheep the cattle showed nasal discharge and diarrhoea, but recovered. After 11 months of contact there was again obvious disease with fevers of up to 41.2°C and nasal discharge. Eighteen days after the fever subsided and the animals were clinically normal one was necropsied. There was ulceration of the abomasum and mild erosions and haemorrhages in the small and large intestines.

Histopathological changes in the central nervous system, lymphoid tissue, eye, lung, heart and kidney, while not severe, were similar in some respects to those seen in MCF. However they were not sufficiently advanced to support a diagnosis of MCF.

(d) A second pair of Bali cattle in West Timor was placed in contact with the sheep from Roti Island that had been implicated in the outbreak of MCF in the Bali cattle herd

in West Kupang district. No MCF occurred in the experimental cattle, but one died of other causes.

Experimental Transmissions: Blood Inoculation, Large Ruminants

(a) Blood from diseased Bali cattle in the contact transmission experiment at BPT, Ciawi, was inoculated into other Bali cattle. One to two litres maintained at 37°C were given on two occasions 2 days apart. Large volumes were used to maximise the chance of successful transmissions, and inoculations were performed within an hour of blood collection.

Five Bali cattle previously unexposed to MCF that received whole blood from naturally induced MCF cases in Bali cattle contracted MCF and died. Four other Bali cattle that had been exposed to the MCF agent by blood transfusion from MCF-affected buffalo two years previously and that had survived (Young, pers. comm.) were also available. Second passages were attempted to these long-term survivors of the previous experiment, but only one developed MCF. Other second passages to a previously unexposed young Bali bull and a young male Bali cross were successful, but a young buffalo similarly infected at the same time remained disease-free 1 year later.

(b) Two young male buffalo were given blood transfusions from buffalo with clinical MCF at BPT Ciawi. No clinical MCF was observed, although each had a febrile period with some depression and anorexia several weeks post-inoculation. One subsequently failed to thrive, and died 12 months after inoculation. It showed no clinical signs other than anorexia and emaciation. At necropsy, the abomasum was found to be markedly ulcerated, with many erosions also present on the folds. In the rumen there was extensive loss of papillae. Individual papillae were haemorrhagic and sloughing off. Histologically, there was a necrotising vasculitis in the arteries of the abomasum.

(c) Other blood transmissions which demonstrate the infectious nature of the disease in buffalo have been conducted, primarily to further clarify the question of species susceptibility. Blood from a spontaneous case in a buffalo at BPT Ciawi was inoculated into a young Bali male, a local *Bos indicus* type (sapi Jawa), a Brahman type *Bos indicus* (ongole) and a buffalo. The buffalo, Bali yearling and ongole all developed MCF. The sapi Jawa died of non-MCF-related causes. Blood from the ongole was inoculated into four more animals of a similar range of species. None showed disease after 16 weeks of incubation.

Discussion

The primary epidemiological question regarding MCF in Indonesia is that of prevalence. There is no accurate estimate of the economic significance of the disease at the present time, nor can there be until prevalence data are obtained. The reviewed literature and field experience of the authors indicates that sporadic losses occur, and it is reasonable to assume that most of these go undiagnosed and unreported to central authorities. Compounding this problem is that of differential diagnosis, which has presented difficulties in both buffalo and Bali cattle, but for different reasons. This problem is the topic of other contributions.

Minor epidemics of MCF-like diseases occur in Bali cattle from time to time. Many are diagnosed as MCF, and there has been support for considering other named diseases such as Rama Dewa and Banyuwangi diseases as part of the MCF syndrome. Whether Jembrana is part of the same complex remains to be determined. Taken together these diseases represent one of the most visible and reported causes of death in cattle in Indonesia.

If adult animals are valued at approximately US$500, then the cash value of animals lost in Banyuwangi alone (Table 1) in each of the last two years has been US$0.25 million. Such a figure does not take into account the economic costs of lost production from loss of draught power and byproducts and lost reproductive capacity, as well as the financial hardship caused to the owners and the disincentive to others to raise livestock. Although Banyuwangi has reported a high prevalence of MCF, it is only a small fraction of one province, and there are at least 15 provinces in which the disease has been recognised. It can be concluded that the cost of MCF to Indonesia may be at least several million dollars per year.

Consideration of the field epidemiological data shows that MCF in Indonesia is SA-MCF. In all cases investigated by the authors, sheep could be implicated. The possible role of goats as a reservoir host remains unresolved. It is useful to consider the results of the experimental work in more detail, starting with the contact transmissions. In only one was contact with lambing sheep successful in reproducing MCF. Three factors in that experiment—at BPT, Ciawi—were different from the other three. Firstly, goats were involved. However, in Timor where goats are frequently kept with Bali cattle, no MCF occurs, except when sheep are imported. If goats can act as a reservoir, they do not do so in that province. A second factor was the number of reproducing small ruminants. Eleven sheep and twelve goats reproduced during the period of the experiment, whereas only two or three animals reproduced during the periods of exposure in the other experiments. The third factor was the spatial relationship of the experimental animals at Ciawi, where the cattle were penned close to the small rumimants. In this location they were maximally exposed to all fomites produced.

The second and third factors would have combined to give a large total exposure, or dose, to each animal. Alternatively, they could have resulted in a sufficiently contaminated environment for infection to occur. Consideration should be given to whether the former concept may be important. In the blood transmissions, success was achieved by using large volumes of blood. The dose of blood necessary to produce disease has not been titrated because of the cost of the animals, but it is reported widely in the literature that large volumes are necessary. Hence, one of the determinants of natural disease may be not only exposure to the reservoir host, but a quantitative effect of the dose of agent received. The sporadic pattern of natural disease could be governed either by the chances of contacting the agent in variably contaminated environments, or by the hypothesised necessity to be infected by a critical mass of agent sufficient to produce disease in a given observation period.

The transmission experiments have supported the evidence of the field observations of an ascending hierarchy of susceptibility from *Bos indicus* and *Bos taurus* breeds, through water buffalo, to Bali cattle and their crosses. All Bali cattle in the contact experiment at Ciawi died, while neither the buffalo nor the *Bos indicus* developed MCF. In the blood transmissions, the Bali cattle were the first to succumb, followed by the Bali-cross animal, and then the buffalo and *Bos indicus*, in that order. The fact that *Bos indicus* can be infected experimentally if the dose is large is of interest, for this species is not usually affected in the field, and then only in areas where MCF is particularly active (Anon. 1982).

Table 1. MCF in Banyuwangi, 1982–83 to 1985–86.

	Cases in		Emergency slaughter		Annual totals
	Cattle	Buffalo	Cattle	Buffalo	
1982–83	11	3	43	27	84
1983–84	62	40	7	6	115
1984–85	92	45	61	45	243
1985–86	148	110	25	1	284

Source: AARD/ACIAR Collaborative Project on MCF, Field Trip Report No. 11.

The species susceptibility is the essence of the MCF problem in Indonesia. The buffalo are preferred as draught animals in many areas, and the Bali cattle are preferred for livestock distribution schemes. Traditional forms of agriculture place these susceptible species in contact with the frequently implicated suspect carrier, the sheep. Confirming experimentally the impression gained in the field provides useful information for veterinary authorities for their efforts in disease control. The information is also useful for researchers, for the susceptible Bali cattle may confidently be used as a marker of infection. Further contact studies utilising this principle may be able to clarify the relative importance of goats as opposed to sheep as a reservoir host.

The development of the head and eye form of MCF in the Bali cattle placed at BPT, Ciawi, indicates that this institute is still a focus of infection under the circumstances in which large and small ruminants are housed, and that the disease in buffalo that regularly occurs there (Hoffmann et al. 1984a) is MCF. The passaging of MCF with blood from an affected buffalo to buffalo, Bali cattle and *Bos indicus* confirmed the diagnosis.

The patterns of disease observed in the transmission experiments where classical MCF was not produced may be important. Two Bali cattle in contact with sheep associated with the outbreak in deer at Kupang were reported to have nasal discharge and diarrhoea. About 1 year later, there was a second incident with fever and nasal discharge. In the absence of an agent on which to base a serological test, there is no way to link these disease occurrences to MCF, particularly as the histopathology in the convalescent period was equivocal. However, the incidents should be noted for comparative purposes. Recent transmission studies of Jembrana disease have also yielded mild disease (Ressang 1984). It is necessary to develop strategies to examine all such incidents rigorously so that incidental phenomena will not be mistaken for the disease under study.

The inconclusive pattern of disease after other blood inoculations should also be noted. Blood from buffalo inoculated into buffalo on the first two occasions produced fever, depression and anorexia. Mild forms of SA-MCF in experimental situations have been known for some time (Blood et al. 1961). One of the buffalo developed a chronic failure to thrive, and subsequently died. Chronic MCF has also been described in *Bos taurus* (Snowdon 1985).

Whether these two disease responses were MCF is of considerable epidemiological importance. If some of the buffalo that suffer chronic wasting conditions in the field have MCF, then the economic impact of the disease is even greater than supposed. However, one of the most intriguing epidemiological questions regarding MCF is whether animals in the field are regularly infected but show only a mild response. MCF is usually described as being sporadic in nature. Where cattle and buffalo are exposed to the reservoir host, usually only a few develop clinical disease. Are these the only animals infected, or are all infected? What are the determinants of infection and of disease? In the absence of a known aetiological agent, more intensive epidemiological studies and transmission experiments are necessary.

Acknowledgments

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Epidemiology

Endemic Areas

Malignant Catarrhal Fever in Bali

Dewa M.N. Dharma*

Abstract

Although malignant catarrhal fever (MCF) occurs in Bali, it is less prevalent there than in the neighbouring province of Nusa Tenggara Barat in the east and Kabupaten Banyuwangi in the west, where there are larger sheep populations. Bali is one of the provinces of Indonesia where the association of MCF with sheep is thought to be not clearly definable. On Bali, the majority of MCF cases occur in the kabupaten (districts) with very few sheep. There is also a problem of differential diagnosis of MCF as perceived by the Disease Investigation Centre in Bali. Jembrana disease is one complicating factor, and submission of inadequate samples and reports another. For the diagnosis of MCF a full history with clinical signs and postmortem report and also sections of the central nervous system are required.

Abstrak

Malignant catarrhal fever (MCF) walaupun terjadi di Bali akan tetapi kejadiannya tidak merata seperti yang terjadi dikedua daerah tetangganya, Nusa Tenggara Barat di sebelah timur dan Banyuwangi di sebelah barat dimana di kedua daerah tersebut domba terdapat dalam jumlah cukup besar. Keterkaitan domba pada kejadian MCF di Bali tidak jelas, sebab MCF di Bali banyak terjadi di Kabupaten dimana jumlah domba sangat sedikit. masalah lain yang juga dihadapi adalah adanya penyakit dengan diagnosis pembanding yang mirip MCF, Penyakit Jembrana dan juga pengiriman spesimen yang tidak memadai, seperti yang dilaporkan Balai Penyidikan Penyakit Hewan—Bali. Diagnosis MCF dapat dikerjakan bila diketahui sejarah penyakit, gejala klinik, laporan bedah bangkai yang leFgkap serta dibutuhkan juga potongan sistem syaraf pusat.

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Background

Geography, Topography and Climate

The island of Bali lies between 7°54' and 8°3' south and between 115°26' and 115°13' east, covering 5621 sq km. The topography is varied with a coastal belt of flat land up to 200 m above sea level, then undulating and hilly country up to 500 m. Mountains in the centre physically divide Bali into northern and southern regions. Land up to 500 m, 56% of the island, contains a large proportion of rice fields, and female cattle are plentiful. In contrast the land between 500 and 1000 m, 26% of the island, is mainly dry fields. Here there are mainly male cattle because the work is harder. These animals are also a beef cattle resource.

The distribution of livestock in Bali is affected either directly or indirectly by the climatic factors of rainfall, temperature and relative humidity. Based on the characteristics of the dry season, the island can be considered as three regions. The first is based on the north coast, Kabupaten Buleleng, and the north east, Kabupaten Karangasem, and has a 5- to 7-month dry season. The second, with a 3- to 4-month dry season, comprises much of the upland regions from Jembrana in the west through to the forest areas of east Bali around Mt Abang, and the hilly regions of Kabupaten Badung in the south. The lower land on the south of Bali, with a rainfall of 2500–2700 mm/year and a very limited dry season, constitutes the third area.

The average temperature in Bali varies with altitude, from 27.3°C at sea level to 21.6°C at Besakih (900 m above sea level) to 18.7°C at Candi Kuring (1247 m). As no part of the island of Bali is very far from the sea the relative humidity is usually high, varying from 77 to 89%.

Land Use Patterns

Bali is an agricultural province with 66% of land being utilised for this purpose. The sources of livestock feeds are agricultural waste, and natural grasses from forest areas and from beside watercourses, irrigation channels and the walls of rice fields. The proportion of land devoted to various agricultural activities is presented in Table 1.

Table 1. Land use patterns in Bali.

Type of land use	Proportion of land
Rice fields	18%
Dry fields and estate crops	48%
Forest	22%
Mixed gardens	3%
Other uses	9%

Livestock Population

The population of Bali cattle, buffalo and goats is increasing. The distribution of livestock throughout Bali is presented in Table 2. It should be noted that in Kabupaten Klungkung and Bangli there are no records of sheep, while in Kabupaten Gianyar and Tabanan only very few sheep are recorded.

Malignant Catarrhal Fever in Bali

The area covered by the Disease Investigation Centre (DIC) Region VI in Denpasar includes not only Bali but also the provinces of Nusa Tenggara Barat (NTB) and Nusa Tenggara Timur (NTT). In the period 1982–85, of 422 cattle and 11 buffalo submissions examined from Bali, 4% were diagnosed as MCF. No buffalo had MCF. In submissions from NTB, of 212 cattle and 35 buffalo cases, 95 cattle (45%) and 10 buffalo (29%) were diagnosed as MCF. Of 27 cattle submissions from NTT, 5 were MCF.

Diagnosis

All diagnoses of MCF are based on histopathological findings supported by clinical signs, epidemiological data, and postmortem changes. Such supporting data are, however, often not accurate. Specimens may even be submitted without an accompanying necropsy report. Sometimes no brain is submitted, and this is regarded as a most important organ in the diagnosis of MCF. The necrotic and fibrinoid vasculitis and perivascular cuffing are pathognomonic.

In the diagnoses from DIC Denpasar, a disease category 'virus infection' is recognised. This may be a problem in the differential diagnosis of MCF. Animals in this category show mononuclear cell infiltrations in the heart, liver, kidney, lung, and intestine, but not in the brain. This category accounts for 8.5% of total bovine submissions from Bali, and 17.5 and 12.5% of submissions from NTB and NTT, respectively.

Treatment and Control

According to reports from the Veterinary Services section and the experiences of the DIC staff, treatment of cases with antibiotics or B vitamins is unsuccessful. Hence, farmers always slaughter affected animals to reduce the financial loss. Although there are no accurate data, information from the farmers indicates that a sick animal can be sold for only 25% of its value when healthy.

To control MCF, cattle, buffalo and deer must be separated from sheep. However, this advice receives no support from the farmers as their animals are their store of wealth. To meet a large financial commitment such as a ceremony they will sell large ruminants, but for smaller obligations they keep sheep and goats to sell. For this reason, the joint husbandry of large and small ruminants is frequently practiced.

Discussion

MCF is described in two forms depending on the nature of the carrier: a wildebeest-associated form and a sheep-associated form. The former is not found in Indonesia as there are no wildebeest, nor are there any cattle from Africa. Field evidence indicates that MCF in this country is sheep-associated, particularly in districts such as Banyuwangi in East Java and Bima in NTB, where sheep and cattle are grazed together. The proportions of submissions to the laboratory that are diagnosed as MCF are higher for the provinces of NTB and NTT, where there are more sheep than in Bali. Most of the cases of MCF diagnosed by the DIC Denpasar come from Bima.

Table 2. The livestock population of Bali (1985).

Kabupaten	Bali cattle	Dairy cattle	Buffalo	Goats	Sheep
Badung	40 171	35	77	3903	95
Gianyar	46 358	-	16	1053	3
Klungkung	34 816	-	53	205	-
Karangasem	88 603	-	35	13100	18
Bangli	62 077	-	3	657	-
Buleleng	73 406	88	1190	16842	406
Jembrana	23 506	-	6531	29534	67
Tabanan	55 160	8	516	4572	2
Totals	424 097	131	8421	71 715	591

If the correlation between MCF cases and the populations of cattle and buffalo and sheep are considered further, some interesting aspects emerge. In Bali, there are only 400–500 sheep, with the highest population being in Kabupaten Buleleng (Table 2). However, there are no cases of MCF reported from this district (Table 3). In contrast, there is a relatively high prevalence of MCF in Kabupaten Tabanan where there are almost no sheep.

It seems that sheep may not be the only carrier. Perhaps goats, pigs or dogs are also involved as these animals have a high population on Bali. Further research is required. The cattle population is 400 000 and the goat population 60 000–70 000, so the ability of the goat to transmit this disease must be considered.

Complaints arise from field veterinarians when no definitive diagnosis is reached on specimens submitted. If certain diagnosis is not possible the case may be classified 'Z disease'. It has been noted that on histopathological observations viral infections have been suspected.

Table 3. Histopathological diagnoses of MCF in Bali.

Kabupaten	1982	1983	1984	1985	Totals
Jembrana	0/1*	0/2	0/6	4/37	4/46
Tabanan	1/67	0/26	6/68	3/22	10/183
Badung	1/26	0/32	2/76	0/23	3/157
Gianyar	0/0	0/0	0/0	0/0	0/0
Bangli	0/2	0/1	0/0	0/1	0/4
Kelungkung	0/1	0/7	0/7	0/10	0/30
Karangasem	0/1	0/1	0/1	0/3	0/0
Buleleng	0/1	0/1	0/0	0/1	0/3
Total	2/105	0/75	8/158	7/81	17/433

*Cases of MCF—cattle and buffalo submissions

Further problems lie in the difficulty in differentiating between MCF and Jembrana disease. If the diseases present classically, there should be no problem, but for MCF there are five recognised forms: the peracute, the gastrointestinal, the head and eye, the chronic and the mild. The first two, if they occur in Bali, are similar to Jembrana disease. A similar problem of differential diagnosis occurs with samples from NTB and NTT if cases are submitted without the brain.

It is therefore suggested that further research is needed to differentiate between MCF and Jembrana. The prevalence of infectious bovine rhinotracheitis (IBR) and bovine virus diarrhoea-mucosal disease complex (BVD-MD) in Indonesia should also be investigated, as all these diseases may present with similarities in some of their clinical signs and pathology.

By considering the field and laboratory data, some preliminary conclusions may be reached. In some regions, sheep are strongly suspected as the carrier, and so cattle and buffalo populations should be kept separately from the sheep. In Bali, further research is needed, in order to identify a possible alternative carrier. In Bali, there is a further problem of the differentiation of MCF and Jembrana disease in some cases. Research on both these diseases must be supported.

Malignant Catarrhal Fever in West Java

Endang Suharya*

Abstract

Malignant catarrhal fever is endemic in West Java. Cases have been seen in dairy and beef cattle and in buffalo. Sheep have been implicated as the source of infections.

Abstrak

Malignant catarrhal fever merupakan penyakit yang endemis di Jawa Barat. Beberapa kasus terlihat pada sapi perah, sapi potong serta pada kerbau. Domba sering dikaitkan sebagai sumber infeksi.

Introduction

The purpose of this paper is not only to give information about malignant catarrhal fever (MCF) in the province of West Java but also to discuss the methods for prevention and control. MCF is considered to be endemic in West Java. The livestock population is shown in Table 1 and features a large sheep population.

Reports of Malignant Catarrhal Fever

Between 1980 and 1985, 21 dairy cattle died at Lembang in the Bandung district, and several beef cattle and buffalo died at Karawang, Tasikmalaya, Kuningan, Majalengka and Garut as shown in Table 2. Laboratory confirmation of diagnosis implicated MCF. The cases occurred in villages where there were sheep. Most commonly, the disease was sporadic in occurrence, with only individual animals being affected. It was seen in association with the mixing of cattle and lambing ewes, which act as carriers of the infection.

The clinical signs found were:

- fever (40–41°C)
- depression
- dyspnea
- profuse mucopurulent nasal discharge
- kerato-conjunctivitis with purulent ocular discharge and partial cloudiness to complete opacity of the cornea.

Treatment and Control

MCF treatment has little value, although some animals survive. Antibiotic administration is to control secondary bacterial infection.

To prevent the widespread dissemination of MCF disease, the Livestock Services of West Java instituted a program as follows:

- rectal temperatures of dairy cattle surrounding signal cases were measured for 1 month;

Table 1. Livestock population of West Java.

	1982	1983	1984	1985
Beef cattle	131 852	133 609	135 963	139 860
Dairy cattle	30 796	43 243	47 643	49 352
Buffalo	471 283	425 896	429 869	438 681
Goat	1 010 048	1 064 137	1 067 272	1 128 591
Sheep	1 795 238	1 845 069	1 926 370	1 998 334

*Department of Livestock Services, Bandung, West Java.

Table 2. Malignant catarrhal fever in West Java (1980–85).

Year	Subdistrict area	Affected animals	Histological diagnosis
1980	Bandung	dairy cow	No
1984	Karawang	buffalo	Yes
1985	Tasikmalaya	buffalo	Yes
	Kuningan	buffalo	Yes
	Majalengka	buffalo	Yes
	Garut	beef cattle	No

- if the body temperature reached more than 39°C, the animal was treated with antibiotic; and
- the affected area was isolated from livestock traffic.

Conclusions

- MCF has attacked dairy cattle, beef cattle and buffalo in West Java.
- Cases have been sporadic.
- Sheep act as carriers of the infection, so dairy cattle should be kept isolated from those animals.
- To prevent the wide dissemination of MCF, a program should be implemented as outlined above.

Malignant Catarrhal Fever in South Sulawesi

I. Sulaiman, H.M.G. Siregar, and Isbandi*

Abstract

During the period 1977–78, at the Disease Investigation Centre (DIC) Maros, seven Bali cattle died in two outbreaks of an acute disease with clinical signs and macroscopic changes suggestive of a diagnosis of MCF. Histological examination revealed that the most pronounced lesion was vasculitis of most organs with perivascular cuffing being predominant in the brain of all affected cattle.

Abstrak

Pada Balai Penyidikan Penyakit Hewan, Maros, antara tahun 1977 dan 1978 terdapat kematian tujuh sapi Bali dari dua kali wabah penyakit yang bersifat akut. Berdasarkan gejala klinik dan bedah bangkai, maka diagnosis mengarah pada malignant catarrhal fever (MCF). Sementara dari pemeriksaan histopatologik, terlihat adanya vaskulitis pada sebagai besar organ dengan 'perivascular cuffing' menonjol pada otak hewan terserang MCF.

Introduction

Malignant catarrhal fever is well known as an acute generalised viral disease of cattle, buffalo and deer, characterised by high fever, profuse nasal discharge, severe hyperaemia, diffuse necrosis of oral and nasal mucosae, corneal opacity and enlargement of lymph nodes (Blood and Henderson 1974; Selman et al. 1974; Reid et al. 1979).

Typical clinical features help in forming a presumptive diagnosis. Confirmation depends upon the identification of typical histopathological changes in a number of tissues (Jubb and Kennedy 1970).

The disease is transmitted from natural reservoirs to susceptible cattle. Wildebeest are known as natural reservoirs in Africa (wildebeest-associated MCF) and sheep are thought to be reservoirs elsewhere (sheep-associated MCF). Transmission occurs where cattle are grazed with these animals, particularly following parturition (Blood and Henderson 1974).

Reported here are episodes of disease in 1977 and 1978 in the DIC Region VII, Maros, South Sulawesi, in which several cattle died showing clinical, macroscopic and histopathological changes typical of MCF.

*Disease Investigation Centre Region VII Ujung Pandang, South Sulawesi.

Background Information

Geography and Climate

The province of South Sulawesi is divided into 21 kabupaten and 2 kotamadya (Table 1). It has a total area of over 6 248 254 km^2. About 60% of the land area is forest and woodland, about 24% is devoted to agriculture, about 7% is pasture, about 7% is wasteland, lake, and mountains, and 2% is devoted to urban development. South Sulawesi has a tropical climate with temperatures between 20 and 35°C and an annual rainfall between 85 and 105 cm.

Livestock Population

The estimated livestock populations of ruminant species in South Sulawesi have been presented for each kabupaten as an average livestock density to give an appreciation of the relative risk of exposure of susceptible species to sheep (Table 1). The deer population is unknown.

Case Histories

During the period 1976–77 at the DIC in Maros, there were four Grati (local Friesian–Holstein) bulls, Five Bali cows 3–4 years old and 15 local breed sheep. The two species of ruminants were grazed together on the same pasture.

Case I: in May 1977, all the Bali cows showed signs of illness a few days following parturition of a ewe, while the bulls showed no signs. The cows died over a period of 1–6 weeks after the first symptoms.

Case II: in January 1978, four Bali cows 3 years of age were purchased from a farmer in Maros. During the next 7 months, two of the Bali cows showed the same symptoms as in the first case. This incident also occurred after a ewe lambed. The cows died over a period of 2–4 weeks, whereas the other cattle including the two remaining Bali cows showed no signs of illness. Subsequently,13 sheep were killed, while two rams were kept for laboratory purposes.

Clinical Signs

The first signs were observed when two Bali cows refused to eat, were fevered (40–41°C), and showed lacrimation, conjunctivitis and profuse nasal discharge. A few days later there developed mucopurulent nasal discharge, corneal opacity, hyperaemia and erosion of the gingiva and the dorsum of the tongue. Treatment with antibiotic and vitamins gave no response. They died 2–3 weeks after the first signs. One week later, three more Bali cows showed the same signs of illness. Death occurred 1–2 weeks after the first signs. The clinical signs in the second case were the same as in this first case.

Gross Pathology

The most prominent lesions were in the oral cavity, and included the presence of extensive erosions involving the dorsum of the tongue and erosions of gingiva and hard palate. The trachea was severely hyperaemic and haemorrhagic and covered with serofibrinous exudate. The mucosal membrane of the urinary bladder showed foci of haemorrhage. Other organs did not reveal any macroscopic lesions.

Histopathology

Histopathological examination revealed that the most consistent change was a vasculitis affecting blood vessels of most organs examined.

In the lung, the wall of the alveoli was thickened by proliferation of epitheloid cells and accumulation of serofibrinous oedema fluid. These were also found in the submucosa of bronchi and bronchioles, and in addition, the air passages were filled with serofibrinous exudate containing inflammatory cells, erythrocytes, and epithelial cell debris. Most blood vessels were infiltrated by lymphoid cells, and endothelial cells of the vascular intima were hypertrophied.

In the spleen, there was destruction and depletion of lymphocytes and replacement with proliferating immature lymphocytes, with the infiltration of such lymphocytes also being found in the trabeculae. In the kidney, dilatation and epithelial degeneration of cortical and medulary tubules and the presence of cell infiltrations of the marginal cortex and glomeruli were seen.

In the brain, perivascular cuffing by lymphoid cells was predominant. Lymphocyte infiltration was also found in the submeningeal spaces.

Table 1. Estimated densities of the livestock population in South Sulawesi (1984–85).

Kotamadya/ Kabupaten	Land area (km^2)	Number of head/km^2			
		A*	B	C	D
Pangkep	880	76	949	8	5
Pare-pare	60	117	0.3	85	4
Ujung Pandang	100	5	42	3	4
Jeneponto	880	5	28	33	4
Bantaeng	400	64	34	51	3
Gowa	1700	29	21	10	2
Sidrap	2350	39	4	5	1
Sinjai	1160	48	24	22	2
Wajo	2460	31	23	11	0.5
Selayar	1340	2	8	22	0.5
Takalar	400	12	87	23	0.25
Bulukumba	940	65	23	45	-
Bone	4190	61	12	3	-
Soppeng	1520	43	0.8	4	-
Pinrang	2010	38	4	3	-
Majene	870	37	22	161	-
Maros	1330	28	21	13	-
Enrekang	1720	18	7	17	-
Barru	930	60	-	4	-
Polmas	4280	9	2	13	-
Mamuju	11430	2	1	1	-
Luwu	18300	4	2	0.8	-
Tana Toraja	3610	0.7	10	1	-

*A, cattle; B, buffalo; C, goats; D, sheep.
Source : Annual Report of Dinas Peternakan Propinsi Dati I Sulawesi Selatan 1984–1985.

Discussion

The clinical features and macroscopic lesions of the cases reported here were similar to reports of MCF elsewhere. The pattern of lesion distribution remained constant in the oral cavity, respiratory tract and the eye. The other organs showed no significant macroscopic lesions. However, histopathological changes consistent with the diagnosis of MCF, namely vasculitis, were found in most organs examined. The presence of perivascular cuffing by lymphoid cells in the brain was predominant.

We believe that the source of infection was associated with the presence of sheep, especially the lambing ewe. Although MCF was suspected in the first case, a confident diagnosis was not reached, so no sheep were killed. When the incident occurred the second time, the diagnosis was confirmed, with the lambing ewe being the probable source of the infection.

Therefore the sheep were killed, except two rams kept for serological and bacteriological laboratory purposes. So far, no further sign of the disease has been observed at the DIC. Whether the two rams cannot transmit the infection to the cattle and whether the infection can only occur through the ewe is unknown. No Grati breed animals were affected, and it might be possible that this breed is more resistant than the Bali breed.

No reports of the disease have been received from Kabupaten areas in South Sulawesi. This may be because of lack of information from the farmers, because no particular investigations for MCF have been conducted, or because of the low population of sheep. Although the origin of the sheep at the DIC is not known precisely, it might be possible to suggest that sheep distributed in areas in South Sulawesi are a source of infection. This investigation of MCF at the DIC therefore indicates the presence of the disease in South Sulawesi.

Acknowledgment

We acknowledge Dr Sobari for his confirmation of diagnosis and for providing photographic slides to complete this paper.

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Epidemiology

Transmigration Areas

Cases of Malignant Catarrhal Fever in West Sumatra, Riau and Jambi

Samrosi Pakpahan*

Abstract

Based on clinical signs, epidemiology, anatomical pathology and histopathology cases of disease in Kabupaten Natuna, Riau Province, and Kecamatan Muara Bulian, Jambi Province, were diagnosed as malignant catarrhal fever (MCF). The cases of this disease were found in Bali cattle from South Sulawesi and Nusa Tenggara Timur Provinces. There were no reports of MCF in other breeds of cattle or in other species. The disease occurred after the introduction of Bali cattle from other areas.

Abstrak

Berdasarkan pemeriksaan klinik, bedah bangkai, histopatologis dan secara epidemiologi, maka penyakit yang terjadi di Kabupaten Natuna, Propinsi Riau, dan di Kecamatan Muara Bulian, Propinsi Jambi adalah malignant catarrhal fever (MCF). Semua hewan terserang adalah sapi Bali yang berasal dari Propinsi Sulawesi Selatan dan Nusa Tenggara Timur. Kasus tersebut tidak dilaporkan pada bangsa sapi dan hewan lainnya. Penyakit muncul setelah ada sapi Bali yang didatangkan dari daerah lain.

Introduction

Malignant catarrhal fever (MCF) is a disease that occurs sporadically, more often affecting cattle than buffalo. Clinical signs usually involve the mucous membranes of the head, sometimes with nervous system involvement and signs of septicaemia with or without haemorrhagic enteritis (Rosenberger 1970).

Dahme and Weiss (1983) stated that the morbidity is low, while fatalities are high, usually more than 90%. According to Blood et al. (1979), clinical signs are stomatitis and gastroenteritis, dysfunction of the respiratory tract, keratoconjunctivitis, encephalitis, and enlargement of lymph glands.

In West Sumatra, this disease has been thought to occur since 1978, while in Riau and Jambi it was first observed in 1984.

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Materials and Methods

The Disease Investigation Centre (DIC) at Bukittinggi services the three provinces of West Sumatra, Riau and Jambi. The data in this report are based on the observations of the DIC from 1984 until July 1986. Histopathological investigations were on organs submitted to the laboratory.

Results

Background of the Disease

In West Sumatra, MCF has been suspected since 1978. In Riau Province, reports of a disease resembling MCF have been received from Natuna Island in the Kabupaten of Riau Island. Bali cattle from South Sulawesi were introduced to Natuna in June 1984. According to the report of the Provincial Dinas Peternakan in Riau, there were 148 males and 852 females. The sheep population in this kabupaten is officially 20.

In Jambi Province, clinical signs suggestive of MCF were reported in Bali cattle imported from Nusa Tenggara Timur (NTT) Province. These were introduced into Kecamatan Muara Bulian in Kabupaten Batanghari in 1984. The sheep population in this kabupaten was 400, with more than 70 in Muara Bulian.

Prevalence

There are no prior reports of MCF in either of these provinces. Details of the total number of

specimens received and the method of diagnosis are given in Table 1.

Clinical Signs

Information was collated from the reports of veterinary officers and farmers. Signs observed included fever, hyperlachrymation followed by inflammation of the eyes progressing to opacity in severe cases, yellow mucoid nasal discharge with foul smell, low appetite but increased thirst, bloody diarrhoea and death within 5 days.

Case Report

DIC staff, together with veterinarians from the B type laboratory in Jambi, had the opportunity to necropsy an affected adult female Bali cow.

Clinical Signs

The body condition was very poor, there was conjunctivitis, yellow mucoid nasal discharge and inflamed vulval mucosae.

Anatomical Pathology

In the respiratory tract there was laryngitis, pharyngitis and inflammation of the trachea. Mucous membranes were red with a yellow mucoid exudate. The diaphragmatic lobes of the lungs were enlarged and frothy on the cut surface, and there was some cornification, and inflammation of the apical and cardiac lobes.

The pericardium was cloudy and atrial fat was atrophied. There was dilatation and petecheae on the bicuspid valves.

Mucous membranes of the stomach showed hyperaemia, and there were areas of inflammation in the intestine. The liver was swollen, red and cirrhotic. The spleen was thickened and on the cut surface the red pulp was dark and the connective tissue unusually prominent.

The kidneys were of normal size and the capsules easy to peel, but there was atrophy of the fatty tissue. The surface of the organ was rough like a tumour, and on the cut surface the pyramidal line had disappeared.

Table 1. Specimens received and the method of diagnosis.

Province/ Kabupaten	Species	Clinical diagnosis	Histo-pathology
Riau, Kep. Riau	Bali Cattle (2)	2	2
Jambi, Kep Batanghari	Bali Cattle (14)	14	5

Histopathology

The lungs showed bronchopneumonia, mononuclear cell infiltrates and vasculitis. There was extensive haemorrhage and vasculitis in the heart.

Mononuclear cell infiltrates and vasculitis were present in the liver. The spleen showed haemorrhage and inflammation. There was mononuclear cell infiltration in the kidney. There was a nonpurulent encephalitis, vasculitis of vessels in the central nervous system with necrosis of vessel walls, and gliosis.

Discussion

Signs of a disease resembling MCF in Bali cattle first occurred in Natuna, Riau Province, and in Muara Bulian, Jambi Province, in 1984 after the introduction of Bali cattle from Sulawesi Selatan and NTT, respectively. Cases in other species of livestock have not yet been reported in the two mentioned areas, which have sheep. Rosenberger (1970) stated that sheep act as the carrier of MCF.

From the macroscopic pathology seen in the intestine and in the head, MCF can be suspected. Dahme and Weiss (1983) stated that, in MCF, the changes in the intestine always happen in the head and eye form. Moreover, they also said that the changes in the mucosae of the nose, pharynx, larynx and trachea were a diffuse to acute catarrhal inflammation.

In a discussion of the diagnosis of MCF, Dahme and Weiss (1983) referred to the clinical signs, epidemiology, anatomical pathology, and especially to the histopath-ology. Characteristic histopathological changes are the nonpurulent inflammation of the blood vessels, and mononuclear cell infiltrates in the lungs, liver and kidney. Changes in the brain include a

massive meningoencephalitis and vasculitis and necrosis of the walls of the blood vessels. In the early stages, there is subendothelial infiltration of lymphocytes and granulocytes, while in the parenchyma can be found multifocal necrosis and gliosis. Pathology in the examined cases was considered consistent with these features.

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Malignant Catarrhal Fever in Lampung, South Sumatra and Bengkulu

Hadi Prabowo*

Abstract

Malignant catarrhal fever has been investigated in Lampung, South Sumatra and Bengkulu. Clinical signs have been recorded and histopathological examinations have been carried out. Until now, only buffalo and Bali cattle have been affected by this disease. From field investigations and field reports it seems the disease happens if cattle or buffalo are grazed together with sheep.

Abstrak

Malignant catarrhal fever di propinsi Lampung, Bengkulu dan Sumatra Selatan, telah didiagnosis berdasarkan pemeriksaan gejala klinik dan histopatologik. Sampai sekarang yang terserang hanya sapi Bali dan kerbau. Berdasarkan penyelidikan dan laporan dari lapangan diduga bahwa penyakit muncul apabila sapi atau kerbau digembalakan bersama domba.

Introduction

The Disease Investigation Centre (DIC) at Tanjungkarang has a responsibility for acting as the veterinary diagnostic laboratory for the provinces of Lampung, South Sumatra and Bengkulu, which together cover the southern third of the island of Sumatra (Fig. 1).

Malignant catarrhal fever (MCF) has been recognised as a serious disease of livestock in these provinces in recent years. This report outlines the nature and scope of the problem.

Materials and Methods

MCF has been diagnosed particularly on the basis of clinical signs. If veterinary staff were present when cattle or buffalo died or were slaughtered, tissues were fixed in 10% formalin and embedded in paraffin wax. Histological sections were stained with haematoxylin and eosin.

Results

Basic data on livestock numbers in the three provinces are presented in Table 1.

Based on the records of the DIC laboratory, MCF has been occurring at a low level for some years. MCF has been diagnosed from clinical symptoms such as high fever, decreased appetite or anorexia, depression, reddening of conjunctiva, lacrimation, serous or mucopurulent nasal discharge, and diarrhoea. Usually, faeces containing blood, and corneal opacity were found. Erosions and ulcers of the tongue, pharynx and palate were also observed.

Microscopically, the most common and prominent findings were the proliferation of lymphoid cells throughout the body. Perivascular lymphoid cell infiltration and lymphoid vasculitis were found in most organs. Encephalitic changes were apparent.

In Lampung, MCF has been reported from the Seputi Mataram, Sukadana, Seputi Raman, Punggur, Sekampung, Metro and Kalirejo districts in Kabupaten Central Lampung, from Teluk Padang in Kabupaten South Lampung, and from Kedaton in Kabupaten North

Table 1. Livestock population in Lampung, South Sumatra and Bengkulu provinces.

Province	Sheep	Goats	Cattle	Buffalo
Lampung	31379	18800	97727	35081
South Sumatra	38119	101477	267238	148198
Bengkulu	23600	93000	57959	69300

Source: Annual Report BPPH Wil. III 1984/85.

*Disease Investigation Centre Region III Bandar Lampung.

Fig. 1. Location of the provinces of Lampung, South Sumatra, and Bengkulu.

Lampung. Cases have occurred in a central band from the east through to the south of the province.

In South Sumatra, cases have been reported only in the west of the province, in areas adjacent to some of the districts where cases have been reported in the neighbouring province of Bengkulu. Districts affected were Ulu Terawas, Tugumulyo, Jayaloka and Muara Kelingi in Kabupaten Musi Rawas and Kota Lahat in Kabupaten Lahat. In Bengkulu, cases have been reported from Curup and Kepahiyang in the eastern Kabupaten of Rejang Lebong, in Kercap in Kabupaten north Bengkulu and from Talo in Kabupaten South Bengkulu.

Table 2. Incidence of MCF in Lampung, South Sumatra and Bengkulu.

Year	Lampung		South Sumatra		Bengkulu	
	Cattle	Buffalo	Cattle	Buffalo	Cattle	Buffalo
1980	5	-	-	-	-	-
1982	4	2	3	-	5	-
1983	4	1	1	4	12	-
1984	-	-	18	1	33	-
1986	3	1	-	-	-	-

The incidence of MCF per province is presented in Table 2. There has been variability in the number of cases each year, with a tendency for Bengkulu to suffer the greatest losses. In the provinces of South Sumatra, both cattle and buffalo have been affected, but most cases have been seen in cattle.

Discussion

Depending on market factors and the quality of the animal, the price for bovines ranges from Rp300 000 to Rp500 000, while for buffalo the price, at Rp350 000 to Rp550 000, is slightly higher (Rp 1100 = US$1.00). If an animal is sold for emergency slaughter because of illness, then the farmer receives only about half its the nominal value. Hence MCF imposes severe hardship on the farmer whose animal is affected. For control, it is suggested that cattle and buffalo be kept separate from sheep.

Malignant Catarrhal Fever in Southeast Sulawesi

Hasa Mardijono*

Abstract

Malignant catarrhal fever (MCF) in Southeast Sulawesi is confined to two regions, Kendari and Kolaka. Only Bali cattle are affected, and the case fatality rate is 100%. The number of cases is high in areas where the disease is reported for the first time. Although sheep are implicated as the carrier, contact is not necessarily direct, but can be by flowing water. Because of the high mortality development of effective methods of prevention and cure is a high priority. MCF is causing a constraint to increased production, not only of Bali cattle, but also of sheep in Southeast Sulawesi.

Abstrak

Kejadian malignant catarrhal fever (MCF) di Sulawesi Tenggara terbatas hanya pada dua daerah, yaitu Kendari dan Kolaka. Hewan terserang hanya sapi Bali dengan angka kematian 100%. Pada daerah dimana pertama kali MCF ditemukan, jumlah kasus yang ada sangat tinggi. Domba diduga merupakan hewan pembawa penyakit, akan tetapi kasus MCF dapat muncul tidak perlu harus dengan adanya hubungan langsung antara hewan peka dengan domba, tetapi dapat juga melalui aliran sungai. Pencegahan dan pengobatan merupakan prioritas utama karena angka kematiannya sangat tinggi. Menghambat peningkatan produksi ternak, tidak hanya terhadap sapi Bali akan tetapi juga terhadap domba di Sulawesi Tenggara.

Introduction

Southeast Sulawesi is a young province on Sulawesi Island. It is located between 3° and 6°S. latitude and 120°45' and 124°E. longitude, and has an area of 36 091 km^2. The province is divided into four Kabupaten (Kendari, Kolaka, Buton and Muna) and then further divided into 45 kecamatan containing 720 desa and kelurahan (villages). In 1980, the population was 950 000, giving 25 persons/km^2. The annual rate of population growth is 3.1%.

Climatically, the province may be divided into two regions: the north where the rainfall is about 2000 mm per year; and the south where the rainfall is lower. The period of highest rainfall is between April and July, and the lowest is between August and October. Minimum temperatures are in the range 18–21°C and maximums 32–34°C. Average humidity is 87–89%.

Livestock population data are given in Table 1. In 1985, 95% of the cattle were Bali cattle, with some local and imported *Bos indicus* and some imported Sahiwals.

MCF in Southeast Sulawesi

The first clinically diagnosed MCF case occurred in 1978 in Kecamatan Panomeeto in Kendari. Throughout 1978 further fatalities were recorded. Local reports indicated that the disease had probably been occurring in the district for some years, although not at epidemic levels.

Since 1975, field officers have continued to observe MCF cases. Reports were received from two neighbouring villages in a transmigration area, Tatibali and Sindangkasih. Treatment of field cases was attempted without success.

The following points were noted about these sporadic cases of MCF. Only Bali cattle were affected, and the disease rarely affected more than one of any animals housed together.

In Tatibali there were no sheep, but cases occurred along an irrigation ditch which came from Sindangkasih where sheep were present. All cases died or were slaughtered for salvage of the meat. It is known that cases of suspected MCF in these villages have been slaughtered without being reported to field officers.

According to SEST/ADP Project data, the

*Livestock Services, Southeast Sulawesi, Kendari.

Table 1. The livestock population of Southeast Sulawesi.

Species	Population	Annual growth (%)
Cattle	106 082	22
Buffalo	12 052	11
Goats	69 966	6
Sheep	302	14

population of Bali cattle in Sindangkasih in February 1982 was 122, decreasing to 51 in February 1986, a 14.5% decrease per year. Data on the MCF cases in this village and the neighbouring Tatibali were collected. Of 45 Bali cattle owners contacted, 18 reported having had cattle affected by MCF. Of an estimated 185 cattle owned by these farmers during the period, 24 were affected.

In 1983 an outbreak of MCF was reported in Kolaka region after sheep had been introduced into an area upstream on a river running through the site at Sambilambo village. In the third week of August, four cattle died of the same clinical syndrome. Subsequently, although treatment and isolation of sick animals was practiced, a total of 26 cattle died or were sent to emergency slaughter. Diagnosis of the disease was based mainly on clinical signs. In some cases, specimens were submitted to the Disease Investigation Centre (DIC) in Ujung Pandang and MCF was confirmed histologically.

Initial precautions taken to prevent the disease included antibiotic therapy of affected animals. This was discontinued because of unsatisfactory results, and replaced by slaughter of animals and disposal of organs. Affected animals were isolated and the stall cleaned. Field officers advised farmers of the risks involved in keeping sheep with Bali cattle.

Discussion

According to the information available, MCF cases in Bali cattle in Southeast Sulawesi have been associated with sheep imported from West Java. These came with transmigrants to Sindangkasih in 1979, and with spontaneous transmigrants from the Mamuju region to Kabupaten Buton and North Kolaka, who brought a few sheep that subsequently reproduced. Most sheep distributed in Buton went to Kecamatan Poleang. Although Bali cattle were kept in the same villages here, they were kept separately from the sheep. A similar situation was found in north Kolaka, in Kecamatan Lasusua. From these two districts there have been no reports of cases of MCF, perhaps because of the separation of the sheep and cattle. According to the data on livestock population growth, cattle are increasing more rapidly (22% per year) than sheep (14% per year). It was therefore hoped that the farmers could be persuaded to concentrate their efforts on cattle only, but because of the traditional forms of agriculture practiced, this approach has not been widely accepted. Reliance has therefore been placed on educating farmers of the potential danger posed by sheep as potential carriers of MCF.

Sheep movements associated with transmigration increase the distribution of sheep as each year passes. The officers of the livestock services have tried to stop this movement, especially to areas where there are Bali cattle.

MCF causes economic losses to farmers. To solve the problem by separation of sheep and cattle is difficult, as farmers are reluctant to put a constraint on sheep production. Compounding factors are the lack of effective treatments and preventative vaccination. Collection of accurate epidemiological data is also difficult because farmers familiar with the syndrome kill affected animals without reporting the illness to veterinary field services. The susceptibility of Brahman and Sahiwal crosses with Bali cattle is not yet known in Southeast Sulawesi, an aspect which needs more attention.

Epidemiology

Special Situations

A High Prevalence of Malignant Catarrhal Fever in Banyuwangi, East Java

M. Tranggono*

Abstract

Throughout the region of Banyuwangi, cattle, buffalo, sheep and goats are raised, with sheep and goats being in approximately equal numbers. All districts have reported cases of malignant catarrhal fever (MCF). Bali cross cattle are the most frequently affected, with the age of affected animals varying from 2 to 5 years. MCF causes large financial losses for farmers, either through the death of the animals or through forced sales at low prices. The disease is most frequently found in areas with plantation crops or rice fields, and occurs mainly in the dry season between April and September. Sheep are suspected as the carrier animals, but this remains to be proven. Control at present can be attempted only by separation of large ruminants from small. MCF causes losses every year and more research is considered an urgent priority.

Abstrak

Sapi, kerbau, domba dan kambing merupakan ternak yang dipelihara di semua wilayah Banyuwangi dengan jumlah domba dan kambing kurang lebih sama. Malignant catarrhal fever (MCF) dilaporkan terjadi di semua kecamatan, dengan sapi Bali persilangan dan keturunannya yang berumur antara 2 to 5 tahun paling banyak terserang. MCF mengakibatkan kerugian besar bagi peternak, baik karena kematian ternaknya maupun karena ternak sakit dijual dengan harga sangat murah. Penyakit ini sering muncul di daerah perkebunan dan juga persawahan terutama pada saat musim kemarau antara bulan April dan September. Domba diperkirakan merupakan hewan pembawa penyakit, akan tetapi hal ini harus dibuktikan lebih lanjut. Pencegahan penyakit sejauh ini hanya dapat dilakukan dengan cara pemisahan antara ruminansia besar dan kecil. MCF menyebabkan kerugian besar bagi peternak setiap tahun, sehingga penelitian terhadap penyakit ini harus diperhatikan sebagai prioritas utama.

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Introduction

Kabupaten Banyuwangi is located at the eastern end of the province of East Java. It is the largest Kabupaten in East Java and has the potential to support increased animal husbandry development. To the north and west, it adjoins other districts of East Java, and to the east and south the Bali Strait and the Indian Ocean, respectively. As a consequence, the topography tends to slope from high land in the northwest to lower land in the southeast. Most of the land lies between 500 m and sea level, with a high proportion of the land in the southeast being rice fields. Land use patterns are given in Table 1.

The Kabupaten has a total area of 459 903 ha and a population of 1.37 million, most of whom are farmers in the rural villages.

For local government administration, Banyuwangi is divided into 19 kecamatan containing a total 175 village units. Each kecamatan has been assigned an animal husbandry technical officer with an agricultural technical high school education. For the present, these people are considered adequately trained for their tasks.

The cattle population in Banyuwangi is increasing, while there is a small decrease in the numbers of sheep and pigs. In 1985, there were an estimated 90 854 cattle, including 270 dairy cattle, 1685 Bali cattle and 7623 Bali cross cattle (sapi rambon). There were 30 200 buffalo, 43 660 goats and 37 129 sheep. However, animal production is always accompanied by the problem of losses caused by animal disease, with most deaths in cattle and buffalo being caused by malignant catarrhal fever (MCF).

Animal Disease in Banyuwangi

Kabupaten Banyuwangi, besides being the major

Table 1. Land usage patterns (%) in Banyuwangi.

Rice fields	15
Dry fields	13
Plantation crops	11
Forests/National Parks	57
Other	5

livestock-producing area in East Java, is also a reservoir of such animal diseases as MCF, trypanosomiasis, haemorrhagic septicaemia, piroplasmosis, scabies, helminthiasis, distemper, Newcastle disease, chronic respiratory disease complex and perhaps Jembrana disease, as well as problems affecting individual animals. The diseases are mostly seasonal, and tend to be found mainly in certain areas.

Not only is Banyuwangi a crossroads for animal traffic originating from Bali and other islands, but the climate and topography favour dissemination of infectious agents. Even if disease does not result in death, the farmers still feel a financial loss.

Efforts to alleviate the problem, including treatments where appropriate, have had only limited success. There are several limitations, and the situation is not helped by the action of those who seek to profit from the farmers' problem.

Malignant Catarrhal Fever

Also known as malignant head catarrh, corysa gangrenosum bovum, epitheliosis, snotsiekte, and radang tenggorokan jahat, MCF is an acute infectious disease of livestock, especially cattle and buffalo. It is caused by a filterable virus that is very difficult to isolate. Until the present time, sheep have been considered the source of the disease.

A particular characteristic of MCF is the thick, mucopurulent and foul-smelling discharge from the respiratory tract. The digestive tract, the eyes and the brain can also be involved, and there is dehydration. The number of cases is usually low but the case fatality rate is high, ranging from 20–100%. In 1985, there were an estimated 186 cases of MCF diagnosed in cattle and 113 cases in buffalo. Cases occur mainly in the dry season (Table 2).

Clinical Signs

General Signs of Disease

In the peracute situation, there is anorexia, depression and a high fever of 41–42°C. The muzzle is hot and dry and the eyes inflamed with a turbid lachrymal discharge. The skin is dry and rough, with piloerection. There is an early serous nasal discharge that becomes turbid. The breathing is laboured and the animal tends to hold its head straight to the front. Death occurs in 1–2 days.

In the acute situation, the animal shows similar signs, which develop further. There is a sudden loss of body weight, the discharges from the eyes and nose become more mucoid and darker and develop a foul odour. They may form a crust around the nostrils. The eyelids become swollen, the sclera becomes reddened and the cornea becomes progressively opaque, to the extent that the animal becomes blind.

Specific Signs

The specific signs of an animal with MCF include severe respiratory distress caused by thick mucous production throughout the whole respiratory tract and resulting in the head and neck being held extended and a foul odour accompanying the nasal discharges. There is corneal opacity and also swelling of the lymph

Table 2. Seasonal distribution of malignant catarrhal fever in Banyuwangi in 1985.

	MCF cases		Rainfall	
	Cattle	Buffalo	mm	Rainy days
January	1	5 (1)*	161	17
February	2	2	208	14
March	16 (2)	2	321	15
April	13 (1)	6	86	10
May	29 (1)	12	97	16
June	19 (3)	10 (3)	21	7
July	27 (2)	15 (2)	56	11
August	31 (6)	18 (2)	2	4
September	16 (2)	17 (2)	3	2
October	5 (1)	2 (1)	141	11
November	13 (1)	8 (1)	158	13
December	14 (2)	16 (1)	29	15

* Number of cases confirmed histologically.

glands, especially of the head and neck. There may be a foul-smelling diarrhoea, and the case fatality rate is very high.

Diagnosis

For a field diagnosis, apart from observing the clinical signs, an accurate history from the farmer is required. A description of the early clinical signs is helpful. However, in the early stages MCF may be difficult to differentiate from haemorrhagic septicaemia, ephemeral fever or pneumonia.

Treatment

At the present time there is no effective treatment or prevention. Antibiotics, vitamins and antihistamines prevent only secondary infection and provide additional support for the animal. To prevent further spread, separation from other livestock is recommended. The housing should be clean, dry and protected from strong winds.

Prevention

In the season when the disease is active it is recommended that the livestock do not graze in the fields, and that their work should not be excessive. Housing should be clean and dry, and there should be adequate food. Attempts should be made to keep cattle and buffalo housing away from that of sheep.

Discussion

Because of the large livestock population, animal disease is particularly important in Banyuwangi. MCF is the disease causing the greatest losses to the farmer either through death or forced sales. A sick animal returns only 25–40% of its former value when sold for emergency slaughter. A compounding problem is that farmers sell sick animals early in the course of the disease to reduce their losses, before the differential diagnosis is clear.

Although MCF is found in every district, it is more prevalent in areas of rice fields and estate crops. More investigation is needed on possible correlations with the system of agriculture, in order to identify possible predisposing factors. Communal grazing, including grazing the rice fields after harvest, may be a factor. Because of work commitments in the rice fields, the body condition of animals here is often poor, particularly in the drier harvest season. MCF is more prevalent in this season than in the wet season. The strong winds that blow at that time may also facilitate spread.

Careful observation will show that in most grazing groups several types of livestock can be found, including cattle, buffalo, goats and sheep. In most cases of MCF there has always been previous direct contact with sheep. However, there are also farmers who raise cattle and sheep in adjacent barns who never encounter the disease. More study is needed in order to define the role of sheep in the spread of MCF.

Malignant Catarrhal Fever in West Timor, East Nusa Tenggara (NTT)

Agus R. Bale*

Abstract

Cases of malignant catarrhal fever in West Timor in cattle and deer have been associated directly with sheep. The attack rate in four investigations varied from 6.5 to 88%, while the case fatality rate was 100%. Bali cattle were involved more than other breeds. No cases were recorded in animals under 1 year old.

Abstrak

Kasus malignant catarrhal fever di Timor Kupang pada sapi dan rusa berkaitan erat dengan adanya domba. Attack rate pada empat kali pengamatan menunjukkan angka antara 6.5 - 88%, sedangkan case fatality rate mencapai 100%. Sapi bali lebih sering terserang dibanding jenis lainnya. Tidak ada kasus yang tercatat pada hewan dibawah umur satu tahun.

Introduction

Malignant catarrhal fever (MCF) is an acute, infectious and usually fatal disease of cattle characterised by encephalitis, symptoms in the upper respiratory tract and a wide range of other symptoms (Hungerford 1975; Smith et al.1974). The disease usually appears in cattle which are in contact with sheep or wildebeest (Smith et al. 1974).

Both sexes and all breeds of cattle are susceptible to MCF. All ages are attacked but the incidence is highest in ages 6 months to 4 years (Jensen and Mackey 1974).

Besides affecting cattle, MCF has been observed in wild deer (Jensen and Mackey 1974). The disease has a worldwide distribution. The first report in Australia was in 1953 (Belschner 1974), but the disease has been known in Indonesia for almost a century (Mansjoer 1954).

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Background

Geography and Climate

The province of NTT lies at the eastern end of the chain of islands that starts at Bali and also includes the province of NTB. The major islands comprising NTT are West Timor, Flores and Sumba, but there are also many smaller islands. This makes animal disease surveillance a difficult task, particularly as the local government districts must straddle many different islands. An example of this is Kabupaten Kupang, which includes the western end of West Timor and the smaller islands of Rote and Savu, each of which has a livestock population quite different from that on the main island.

NTT lies to the east of the Wallace line and, by definition, has a climate markedly different from that of the western parts of Indonesia. It is a monsoonal climate with seasonal rains that start in November and are mostly finished by March or April. A relatively long dry season follows. As a consequence of the climate, farming is not as widely practiced as on Java and Baii, whereas the raising of livestock by grazing is more important. The estimated livestock populations are presented in Table 1.

The kabupatens in West Timor—namely Kupang, South Central Timor, North Central Timor and Belu—are the main cattle-producing areas in NTT. Although West Timor also has the most sheep, these are not usually kept on the main island because of prohibiting legislation but are kept in large numbers on the

Table 1. Livestock population in East Nusa Tenggara (NTT) Province.

District	Cattle	Buffalo	Sheep	Goats	Deer
Kupang*	135 495	29 011	63 767	100 894	70
South Central Timor (TTS)	161 274	2 820	-	39 636	-
North Central Timor (TTU)	97 237	2 122	-	15 049	14
Belu	86 652	16 980	-	38 475	14
Alor	3 565	-	91	14 829	22
East Flores	783	17	4 984	41 056	-
Sikka	3 144	182	-	27 992	20
Ende	5 318	3 187	79	10 621	27
Ngada	15 572	13 558	?	13 207	-
Manggarai	4 074	29 022	49	12 849	-
East Sumba	31 402	28 246	1 351	8 185	8
West Sumba	9 833	45 095	-	11 660	-
Total	554 349	170 240	70 321	334 503	175

Source: Annual Report of Provincial Livestock Services 1984/85
*Kupang District includes the islands of Savu and Roti.

islands of Roti and Savu. For historical reasons, the species of cattle kept on the main islands differ somewhat. West Timor has mainly Bali cattle, although artificial insemination has led to the production of cross breeds of different kinds. Some *Bos indicus* have also been imported to locations along the north coast.

MCF in West Timor

In West Timor, MCF was first recorded at the end of 1975 in Lili village in the Kupang district. The Provincial Livestock Services had imported fat tail sheep from Savu Island and they had subsequently been allowed to graze with cattle. MCF was recorded in adult animals but not in animals under 1 year old.

Altogether four outbreaks of MCF have been recorded in NTT in recent years, and the case histories are presented below. Buffalo and goats have not been affected, although they are reared with sheep on some of the islands in the province.

Outbreak at Lili, Kupang District

The outbreak occurred at a holding ground of the Livestock Services section. In March 1974, six head of fat tail sheep from Savu Island were introduced to the area holding 30 head of Bali cattle. The sheep and cattle were housed separately but grazed together. The first case occurred in a Bali bull in September 1975. Two ewes had lambed 2 months previously.

In October 1975, 80 more sheep were imported from Savu to this location, which by this time also held 101 head of cattle. A few days after arrival, five ewes lambed, and 4 weeks later several cattle showed clinical signs including diarrhoea or dysentery, yellow mucopurulent nasal discharge, serous or purulent ocular discharge, corneal opacity to varying degrees, and hypersalivation. A week after the onset of symptoms in the herd, approximately 20 head of cattle had died. In November 1975, the 80 newly imported sheep were moved to Konotuef village in Central North Timor, but the six original sheep remained behind. In the period to March 1976, 89 cattle died, a mortality rate of 88% (Table 2).

Specimens for laboratory examination were sent to the Disease Investigation Centre (DIC) at Denpasar, where MCF was diagnosed histopathologically.

Outbreak at Konotuef, Central North Timor

In November 1975, the 80 sheep from Lili were placed in a holding yard containing

Table 2. Malignant catarrhal fever in cattle in West Timor.

Location	Time of incident	No. of sheep	Cattle at risk	No. affected	Attack rate (%)	Case fatality (%)
Lili, Kupang	Oct 75 –Mar 76	6–86	101	89	88%	100%
Konotuef, TTU	Dec 75 –Jan 76	80	100	70	70%	100%
Lifuleo, Kupang	Feb 84	11	400	26	6.5%	100%
Lifuleo, Kupang	May–Jun 84	16	400	60	15%	100%

approximately 200 head of goats and 100 Bali cattle. The first case of MCF in the cattle was observed in December 1975, and in a 1 month period approximately 10 sheep had lambed. Between December 1975 and February 1976, about 70 cattle died, giving an attack rate of 90% (Table 2). No specimens were submitted for laboratory examination.

Outbreak at Lifuleo Village, West Kupang Subdistrict

In March 1983, nine head of sheep were illegally imported from Roti Island. The sheep were yarded separately about 300 m from cattle, but the two groups were allowed to graze together. The cattle were 400 head of Bali cattle owned by one person. In early 1984, two ewes lambed and 1 month later the first clinical signs of MCF occurred. Twenty-six head of cattle are reported to have died.

In April, five more ewes lambed, and again a month later new cases occurred. From May to June 1984, approximately 60 head were affected. Half died and the rest were slaughtered by the owner or bartered for rice, corn or brown sugar.

Specimens were submitted to the DIC Denpasar and MCF was diagnosed histopathologically.

An outbreak in deer at Kupang

At the Governor's residence in Kupang, 55 head of Timor deer *(Cervus timorensis)* and 16 head of fat tail sheep were held. The sheep came from Savu Island in 1981 and the deer from various places in NTT province. At first they were kept in different paddocks, but were grazed together from the beginning of 1984. In the period to May 1984, four sheep had lambs.

In May, cases of MCF started to occur. Diarrhoea with or without blood, mucopurulent nasal discharge, lacrimation, salivation and depression were observed. In 3 days, seven deer died, although 10 were reported sick. At postmortem, haemorrhages on the abomasum and small intestine were seen, as well as swelling of the liver and congestion of the brain. The deer were shifted from this location, but a further 29 died. Overall this represents an attack rate of 65.5%.

Specimens were submitted to the DIC in Denpasar, and to Balitvet in Bogor. MCF was diagnosed histologically.

Discussion

As a result of the field investigations of MCF outbreaks in NTT province, there is no doubt that sheep have an important role as the source of the disease. Clinical signs of MCF were observed if cattle and sheep were kept together, particularly after parturition in the sheep.

The field experience in NTT is that Bali cattle are more susceptible than other breeds. There are no reports of MCF from Sumba and Rote Islands where sheep and ongole *(Bos indicus)* cattle are present, and no reports have been received from Flores where Madura cattle are kept as well as sheep. However, these are remote areas and sporadic cases may be unreported. The recent increase in the veterinary field and

laboratory staff in NTT province can be expected to help in the confirming of this situation.

Treatment of cases with broad spectrum antibiotics has been unsuccessful. This has resulted in owners tending to slaughter or barter sick animals. However, the price of such an animal is usually 25% or less of the value of the animal when healthy. In NTT, the normal price of a 300-kg liveweight bull is about Rp300 000, but when the animal is sick the price drops to Rp60 000–75 000.

Disease Control

1. In districts where sheep and Bali cattle are kept, it is suggested they be kept separately. This applies to certain districts on the island of Flores, and in Alor district.

2. In areas where the Bali cattle population is concentrated, sheep should be removed. This has been done in all of West Timor.

3. The importation of sheep from Savu and Roti Islands to West Timor is prohibited.

Acknowledgments

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Malignant Catarrhal Fever Disease Situation in West Nusa Tenggara (NTB)

Abdul Muthalib*

Abstract

Malignant catarrhal fever (MCF) has a high incidence in West Nusa Tenggara (NTB), with an average 420 cases occurring annually. Cases are normally associated with contact with sheep. Cattle are more often affected than buffalo because they are more frequently raised with sheep. The price obtained for sick animals is approximately one-quarter the value when healthy, so the disease has serious financial consequences. Prevention is being attempted by working towards movement of cattle into sheep-free areas.

Abstrak

MCF di Nusa Tenggara Barat (NTB) sangat tinggi angka kejadiannya, dengan perkiraan 420 kasus terjadi setiap tahunnya. Kejadian umumnya dihubungkan dengan adanya kontak dengan domba. Sapi lebih sering terkena dibanding kerbau, sebab ia lebih sering dekat dengan domba. Harga dari hewan yang sakit akan mefjadi seperempat dari harga normal, yang berarti penyakit ini sangat penting artinya dari segi ekonomi. Pencegahan sedang diterapkan dengan memisahkan sapi pada area yang bebas domba.

Introduction

West Nusa Tenggara (NTB) Province is one of the provinces in Indonesia with a large agricultural potential, and serves as a grain and a beef cattle resource for the other provinces. There are some obstacles to the further development of farming in NTB, and one of these is animal disease. Among the animal diseases the one most difficult to handle is MCF.

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The clinical signs of MCF in cattle are high fever, erosions of the mouth and nose, corneal opacity and mucopurulent discharges from the eye and nose. MCF occurs both sporadically and enzootically throughout NTB. Bali cattle and buffalo are the susceptible animals. Field reports show MCF occurs with a high annual incidence. To date there is no effective treatment. Sheep, the suspected carrier, are kept with susceptible animals in several regions, particularly on Sumbawa Island. Separation of sheep and cattle is the only method of prevention.

Background Information

Geography, Topography and Climate

NTB is included in the Nusa Tenggara group of islands lying at 115°46'E. and 8°5'S. There are two main islands—Lombok and Sumbawa—surrounded by the Java Sea to the north and the Indian Ocean to the south. To the west is the island of Bali.

The climate of this province is tropical, with minimum temperatures between 21.1° and 23.5°C and maximum temperatures between 29.8° and 31.3°C. Average annual rainfall in Lombok is 1653 mm and in Sumbawa 1290 mm.

Lombok is 4739 km^2 in area and Sumbawa 15 414 km^2, giving a total area of 20 155 km^2 for the province. The land usage patterns are outlined in Table 1.

Livestock Population

Livestock population figures are presented in Table 2. It is of particular interest that no sheep are recorded in Dompu.

Table 1. Land usage patterns (%) in NTB.

Rice fields (sawah)	10.0
Dry-field farms and mixed cropping	7.5
Estate crops	1.5
Forest	67.0
Other, mostly unproductive	14.0

The MCF Disease Situation

MCF is distributed throughout NTB. Clinical diagnoses of MCF by field staff for the period 1981–85 are given in Table 3. These figures indicate an average of 420 cases per year in the province. It can be seen that the districts of Dompu and Bima had the highest incidence, and that cattle were more affected than buffalo. For confirmation of diagnosis, specimens from some cases were submitted to the Disease Investigation Centre (DIC) in Denpasar. Bima was the most active district in this respect, with 196 of 212 submissions from NTB in the period 1982–85 being from there. In 40% of submissions the diagnosis was confirmed as MCF. There were no significant differences in percentages of confirmations between districts, or between cattle and buffalo submissions.

The sale price of sick animals drops considerably in NTB, with such animals realising only a quarter to a third of their previous value. Hence, for a 1-year-old, the price may drop from US$100 to US$25, and for an adult male from US$300 to US$80.

Prevention, Control and Treatment

Sheep are suspected as the carrier of MCF. Because of the lack of a vaccine, the only way to achieve control is to keep sheep and susceptible species separate. This criterion is strictly enforced in development programs in NTB in which livestock are distributed to farmers. Enforcing this rule is seen not only as a necessary protection for the cattle distributed, but also as contributing to an education process.

Once disease has occurred the only control is to slaughter the affected animals. There is no treatment yet for MCF cases. The use of antibiotics and vitamins has proved unsuccessful.

Table 2. Population of livestock in NTB (1984).

Kabupaten	Cattle	Buffalo	Goats	Sheep
West Lombok	74 470	7 118	38 728	5 094
Central Lombok	78 846	26 847	42 922	14 307
East Lombok	78 373	11 287	81 413	13 293
Sumbawa	27 252	86 892	26 626	4 559
Dompu	6 189	20 955	14 287	
Bima	26 578	55 491	54 124	5 303
Totals	291 708	208 590	258 100	43 556

Source: Development Program, Livestock Services, NTB.

Table 3. Clinical diagnoses of MCF in NTB (1981–85).

Kabupaten	Number of cases					
	1981	1982	1983	1984	1985	Total
West Lombok						
cattle	33	18	67	30	47	195
buffalo	–	3	11	8	–	22
Central Lombok						
cattle	14	25	66	62	45	212
buffalo	–	2	1	14	13	30
East Lombok						
cattle	49	23	34	75	78	25
buffalo	4	5	–	11	5	25
Sumbawa						
cattle	9	–	–	–	–	–
buffalo	–	–	–	–	–	–
Dompu						
cattle	127	113	128	97	90	555
buffalo	43	44	19	41	31	178
Bima						
cattle	165	146	107	82	77	577
buffalo	4	11	12	10	1	38
Annual Totals						
cattle	397	325	402	346	337	1807
buffalo	51	65	43	84	50	293

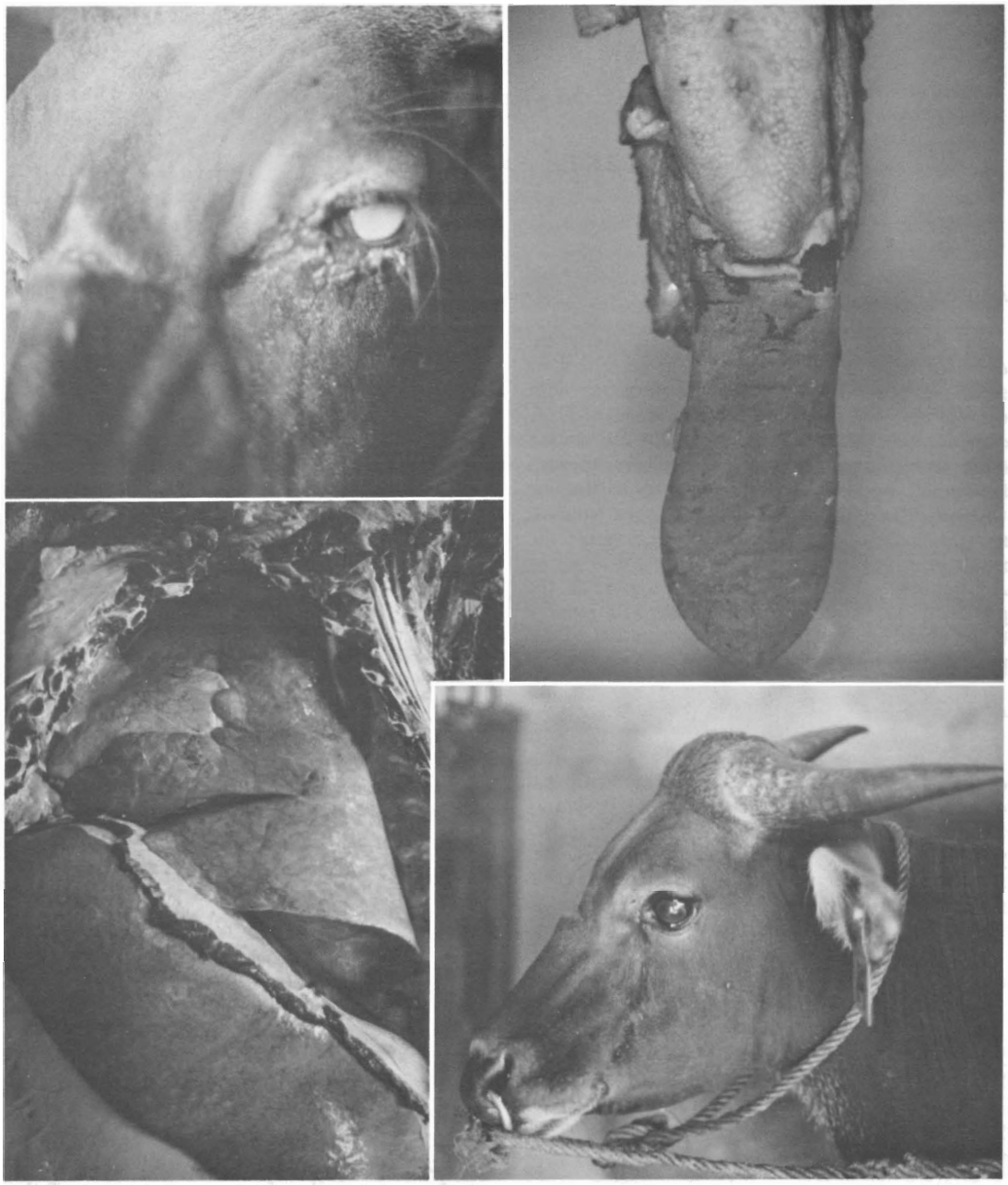
Source: Animal Health Section, Livestock Services, NTB

Discussion

Based on field observations, the incidence of MCF in NTB is relatively high, with an average 420 cases/year. In the last 5 years, 86% of the affected animals have been cattle. This high proportion of cattle is attributed to the fact that cattle are reared intensively and are kept with sheep, while the rearing of buffalo is more extensive with many being kept in forest areas. During the observation period, only 10% of suspected cases were submitted for histological confirmation of diagnosis. Logistical problems and lack of budget were the reasons.

MCF in NTB occurs both sporadically and enzootically, and is closely associated with sheep. The low level of reports from Kabupaten Sumbawa may be attributable to a lower level of surveillance in this district. An interesting exception to the general rule is seen in Kabupaten Dompu, where official figures indicate there are no sheep present, but the prevalence of MCF is still quite high. There should be a specific investigation of this apparent anomaly.

As noted, the planning of the future livestock development of NTB is based on the principle of keeping cattle and sheep separate. This approach has the support of government and farmers. Control of outbreaks is supervised by field officers of the Livestock Services Section, who oversee the compulsory slaughter of affected animals. Such animals lose 75% of their cash value, and so the disease represents a serious financial loss to the farmer.



Pathology and Differential Diagnosis

The Pathology of Malignant Catarrhal Fever

R.S.F. Campbell*

Abstract

As the agent for sheep-associated malignant catarrhal fever is not yet isolated or identified by serological or other means, many problems with respect to the disease remain unsolved. A consideration of the pathology helps to define these problems and indicates areas where research is required. In particular, it is necessary to discover whether differences in pathology between animals, species, breeds and geographical regions are the result of host factors or of the aetiological agent. Application of modern biotechnologies to the problems of identifying the agent will resolve these questions and allow an appreciation of the pathogenesis of the disease.

Abstrak

Agen penyebab penyakit MCF yang berkaitan dengan domba sebagai sumber infeksi belum dapat diisolasi atau diidentifikasi secara serologis atau uji lainnya, karena masih banyak masalah dari penyakit ini yang belum dapat dipecahkan. Perubahan patologi yano ada guna membantu menerangkan masalah diatas dan membantu mengarahkan penelitian sangat dibutuhkan. Terutama dalam mempelajari perbedaan patologi diantara ternak, species, bangsa dan letak geografisnya sehingga lebih menjelaskan adanya faktor induk semang atau agen penyebab penyakitnya. Penggunaan biotehnologi canggih pada penanggulangan masalah diatas dalam menyidik agen penyebab penyakit akan menjawab pertanyaan diatas dan menjadi jelas patogenesis dari penyakit MCF ini.

Introduction

Sheep-associated malignant catarrhal fever (SA-MCF) remains one of the major mysteries of livestock disease in the world today. While the African wildebeest-associated syndrome (WA-MCF) is linked conclusively with a bovine herpesvirus (BHV-3 or AHV-1), research workers in all other parts of the world have so far failed to unlock the secret of the non-African forms.

Current evidence and opinion leans to the view that the disease is not a simple host-response to infection but a complex infectious agent–host cell–immune system equation that will need the weight of contemporary technology to solve.

General Considerations

Although a superficial reading of the literature might suggest that the pathology of MCF was generally of the same character and pattern, a closer analysis shows that significant differences occur in published accounts. These do not depend on an association with either WA-MCF or SA-MCF. Within the so-called SA-MCF group, some differences are indicated that could be related to different geographic areas, different ruminant species or breeds and, even more fundamentally, to different pathogens.

It should be noted that SA-MCF in Asia has been observed in *Bos taurus, Bos indicus, Bos javanicus,* and *Bubalus bubalis*. The pathology in buffalo has shown some striking differences from that in cattle (Hoffmann et al. 1984). We may therefore ask if genetic factors and, in particular, the genetic characteristics of the immune system may be responsible for some of the variations. There is little basic information on the comparative immune responses of the ruminant tribe.

General Pathology of MCF

Common factors in the syndrome are, in probable order of pathogenetic significance, as follows:

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(i) lymphoreticular changes;
(ii) vascular lesions which are clearly dependent to some extent on (i); and
(iii) epithelial lesions.

Descriptions from all parts of the world contain elements of this triad, although the degree of involvement of these tissues and the precise nature of the changes may vary from outbreak to outbreak. Good general accounts are given by Berkman et al. (1960), Selman et al. (1974), and in buffalo by Hoffmann et al. (1984).

Macroscopic Pathology

MCF can often be diagnosed clinically on the basis of the following criteria:

(i) contact with small ruminants;
(ii) usually a low morbidity rate;
(iii) a very high case mortality rate;
(iv) respiratory catarrh, conjunctivitis and corneal opacity;
(v) enlarged lymph nodes; and
(vi) erosions which may be present in the respiratory and alimentary mucosae.

Of course, in some areas it may be necessary to differentiate foot-and-mouth disease, rinderpest, mucosal disease, infectious rhinotracheitis, Jembrana disease, and lantana toxicity among others, but the case mortality rate, usually 100%, is particularly striking.

Most writers comment on a complex of postmortem changes that includes the lesions already listed plus swelling of internal organs, foci of interstitial 'inflammation' in solid organs such as kidney and liver, and haemorrhages in the intestine and bladder. Arthritis may be present.

To emphasise that sharp differences can occur it should be noted that an Indonesian study (Hoffmann et al. 1984) found a high (100%) prevalence of epicardial haemorrhages with myocarditis. A comparative study of MCF in the United States, Europe and Australia of material from *Bos taurus, Bos javanicus,* bison and deer did not show similar severe lesions (Campbell, unpublished data).

Histopathology

It is in the area of microscopic pathology that the similarities and differences between outbreaks are most apparent. Only the principal changes will be described here:

a. Lymphorecticular tissues: Several groups of workers (Selman et al. 1974; Hoffmann et al. 1984) have commented on the lack of follicular hyperplasia and germinal centre formation in lymph nodes, while at the same time finding marked activity in the paracortical T-lymphocyte areas and intense macrophage activity in the sinusoids. An occasional finding in Indonesian material was focal necrosis and fibrinoid deposition in the deeper cortical areas.

b. Cardiovascular system: In the Indonesian study of buffalo by Hoffmann et al. (1984), emphasis was given to cardiac haemorrhage, pericarditis, myocarditis and calcification, features that were usually not marked in material examined by most authors. The extent of myocardial degeneration and lymphoreticular infiltration was sometimes very severe in that species.

All reports of WA-MCF and SA-MCF, however, gave emphasis to changes in the peripheral circulation. This generalised vasculitis of arterioles and venules usually involved lymphocytic infiltration, although one account of the disease in *Bos taurus* in Britain (Selman et al. 1974) emphasised polymorphonuclears. Some descriptions (Liggitt and de Martini 1980a; Hoffmann et al. 1984) indicated that the adventitial layer was most severely affected, although medial and intimal lesions may occur and endothelial cells can hypertrophy or even proliferate. Some differences emerge from descriptions of fibrinoid necrosis which was most marked in the material of Selman et al. (1974). Such lesions are widespread throughout the body, though greatest attention has been given to those in the brain, kidney, liver, heart, serosae and carotid rete. In the parenchymatous organs they may be associated with degenerative and infiltrative changes, hence the focal lesions that may be visible in the kidneys at autopsy as pale areas a few millimetres in diameter.

c. Epithelial membranes: The upper and lower alimentary tract, biliary system, urinary bladder and choroid plexus are the preferred sites of change. Skin lesions may be present in natural

but not experimental cases (Liggitt and de Martini 1980b).

This is another aspect of pathology that may vary widely in different outbreaks. Erosive lesions were common in Holstein cattle in the United States (Liggitt and de Martini 1980b) but relatively uncommon, only in about 8–14%, in affected Indonesian buffalo. The basic epithelial lesion is a multifocal intraepithelial necrosis that may take the form of a microvesicle and lead to frank superficial erosions. The predominant reactive cell is the lymphocyte.

Like all other MCF lesions the erosions intensify as the disease progresses.

Pathogenesis

In the absence of a known pathogen it is difficult to understand the pathogenesis of SA-MCF. A knowledge of general pathology may offer some clues, but even these may be confused by differences in the immunological responses of different species and breeds of ruminant. This possibility has received little attention.

Common to all descriptions of MCF are certain anatomical distributions and cellular responses. A number of questions are easily raised.

(i) Does SA-MCF result from direct viral cytolysis?

Despite intensive cultural, electron-microscopic and serological investigations, no consistent evidence of viral activity has been obtained from European, American, Asian or Australian studies.

(ii) Does SA-MCF contain an immunopathological component?

The majority of researchers emphasise a lymphocytic response in the perivasculitis, and possibly stimulation of T-lymphocyte sets judging by the distribution of activity in the paracortical areas of lymph nodes. The role of T-lymphocytes has been further supported by Reid et al. (1983), who isolated a cytotoxic T-lymphocyte line from a rabbit infected with SA-MCF material. The possible role of the abundant macrophages in lymph nodes and other lesions is still unknown.

A variation to this pattern was found by Selman et al. (1974) whose MCF material contained vascular lesions dominated by polymorphonuclear leucocytes. Careful consideration of the cytology of MCF was given by Liggitt and de Martini (1980a), who compared the lesions with Arthus reactions, host–graft rejection phenomena and lymphocyte-associated vasculopathies. On a cytological basis alone, they concluded that SA-MCF most closely resembled host–graft rejection lesions and some viral infections with marked lymphoid responses rather than other reactions which are dominated by acute neutrophil and plasma cell infiltrations.

Such an interpretation takes no account of possible species or breed differences in immunological phenomena and it may be premature to assume the nature of the basic immunopathology of SA-MCF.

(iii) Do ephithelial lesions result from direct viral action?

There is no evidence to support this view and the predominance of lymphoid cells indicates the possibility of some immunological process (Liggitt and de Martini 1980b). Alternatively, epithelial degeneration could relate to post-vasculitis infarction (Rweyemamu et al. 1976).

(iv) Is SA-MCF an expression of cell-associated virus?

Some of Koch's postulates can be confirmed with MCF. The disease can be transmitted and even passaged not only to large ruminants but also to rabbits. Only isolation and appropriate serological identification remain elusive.

Current knowledge suggests that we must dig more deeply at the molecular level to solve the mystery of SA-MCF pathogenesis. The virologist must team up with the biochemist and explore the genome of lymphocyte populations and epithelia to determine if viral sequences can be found in cellular DNA. Experience suggests that herpesviruses are prime candidates but it should be noted that retroviruses (the notorious AIDS agents) have occasionally been isolated from MCF cases.

The technology now available will probably ensure that we will understand the pathogenesis of SA-MCF within 5 years. It should not be forgotten, however, that sheep and possibly other small ruminants are an essential link in the epidemiological chain of MCF, acting as subclinical carriers of the pathogenic agent.

The key to the control of MCF may therefore be not in the use of vaccines, but in the simple management of large and small ruminants as separate production systems.

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Malignant Catarrhal Fever in Bali Cattle

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Abstract

Sheep-associated malignant catarrhal fever (SA-MCF) in Bali cattle *(Bos javanicus)* follows the broad clinicopathological course described for SA-MCF in other breeds, being characterised by fever, depression, lymph node enlargement and lymphoproliferative changes, variably severe involvement of the respiratory, gastrointestinal and urinary tracts, consistently severe conjunctivitis, and 100% mortality. In a study in which SA-MCF was induced by placing cattle in contact with lambing small ruminants, and then by blood transfusions from affected cattle to other cattle, somewhat different syndromes developed in the two groups of animals. In the spontaneous cases as compared with the inoculated group, the diagnostic features of corneal opacity, profuse mucopurulent nasal discharge and necrotising vasculitis were not prominent, while the gastrointestinal changes, particularly of the abomasum and intestines, were more severe. SA-MCF occurs with a high prevalence in Bali cattle in Indonesia, and familiarity with the full range of possible syndromes is necessary to allow accurate diagnoses to be reached.

Abstrak

Gambaran kliniko-patologi SA-MCF pada sapi Bali *(Bos javanicus)* mirip dengan apa yang ditulis untuk hewan lain seperti demam, depresi, pembesaran kelenjar pertahanan dan perubahan proliferasif, perubahan yang bervariasi pada alat pernafasan, gastro intestinal dan saluran urinaria, konjungtivitis hebat secara konsisten, dan diahiri dengan kematian. Dalam studi SA-MCF dengan cara menempatkan sapi agar kontak dengan domba beranak, kemudian dengan transfusi darah hewan penderita ke sapi lain, timbul sindroma yang berlainan pada kedua kelompok hewan percobaan tersebut. Kalau dibandingkan antara hewan yang sakit spontan dengan hewan percobaan gejala opasitas kornea, leleran hidung profus mukopurulenta dan vaskulitis nekrotikan tidak begitu jelas; sementara itu perubahan gastro intestinal, terutama pada abomasum dan usus halus terjadi sangat parah pada yang sakit spontan. Mengingat kejadian SA-MCF pada sapi Bali prevalensinya tinggi, karenanya mengenal sindrom penyakit secara baik sangat diperlukan agar diagnosa tepat dapat diperoleh.

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Introduction

A clinicopathological syndrome of Bali cattle *(Bos javanicus), Bos indicus* cattle and swamp buffalo *(Bubalus bubalis)* consistent with sheep-associated malignant catarrhal fever (SA-MCF) has been recognised in Indonesia for many years (Mansjoer 1954; Ginting 1979; Anon. 1982; Ramachandran et al. 1982; Hoffmann et al. 1984). The disease is usually of low population morbidity and a reported 100% case mortality. In most cases, an association with sheep has been demonstrated. Features of the disease in Bali cattle have been described by Ginting (1979), and a brief description given by Ramachandran et al. (1982). SA-MCF may be readily transmitted to susceptible Bali cattle by blood transfusion (Ramachandran et al. 1982; Sudarisman et al. 1985).

Sudarisman et al. (1985) described MCF in Bali cattle placed in contact with lambing small ruminants, and the successful passage of the disease to other Bali cattle by intravenous inoculations of large volumes of blood collected in the early stages of the febrile response. It was believed that the development of MCF after

exposure to the sheep represented natural disease. The clinical and postmortem findings are summarised in this paper as an aid in the differential diagnosis of MCF and similar diseases in Bali cattle.

Clinical Signs

In the four spontaneous cases of SA-MCF, the incubation period ranged from 9 to 15 weeks following exposure to the lambing small ruminants. Fever, inappetence and depression were the initial and most commonly observed clinical signs. Superficial lymph nodes were enlarged and readily palpable throughout the disease course. In a few cases, lymph nodes were visible as protuberances beneath the skin.

Ocular lesions were among the most prominent features of the clinical disease. There was reddening and oedema of the conjunctiva, congestion of scleral blood vessels, photophobia and a mild serous ocular discharge. Corneal opacity developed later in the course of the disease in one animal, beginning as a narrow white line at the limbus. It did not progress to involve the entire cornea. Profuse yellow mucopurulent nasal discharges were not seen in these animals.

Congestion of the nasal and oral mucosae and mild erosive lesions of the tips of buccal papillae were also observed, becoming more severe in some cases to involve large areas of the oral mucosa. Saliva accumulated at the commissures of the lips. These Bali cattle died or became moribund between 4 and 11 days after the onset of clinical signs.

The animals in the whole blood transmission experiments had a shorter incubation period of 13–29 days, but a more variable clinical course of disease ranging from 5–21 days. Corneal opacity was observed in four of five cases resulting from first blood passages, progressing to staphyloma in one case. Mucopurulent nasal discharges were common and were profuse and yellow in two cases. Sloughing of the skin of the muzzle occurred in one animal.

Gross Pathology

There was marked variation in both distribution and severity of gross lesions among the animals, and for most lesion categories the severity was not related to the source of infection, either spontaneous or blood inoculation. Lesions were most consistently observed in the lymphoreticular, urinary, respiratory and alimentary systems, in that order. All lymph nodes were enlarged, oedomatous, congested and locally haemorrhagic. Peyer's patches and the palatine tonsils were prominent. The spleen was slightly enlarged and the lymphoid follicles were prominent in all cases.

The mucosa of the urinary bladder and ureters was reddened, thickened and oedomatous with petechial and ecchymotic haemorrhages. Renal lesions were not always present. Infarcts or discrete, raised, pale foci up to 2 mm in diameter were observed in some cases.

Congestion and oedema, and serous exudation from the nasal mucosa, septum and turbinates were observed in most animals. In more severely affected animals, there was a more copious mucopurulent nasal exudate and the pharyngeal, laryngeal and tracheal mucosae were congested and oedomatous. Erosive and ulcerative lesions were observed. Pulmonary changes were variable and non-specific, including slight focal consolidation, emphysema and oedema.

The conical papillae of the cheek were congested and the tips eroded, ulcerated or necrotic. Similar changes were observed on the inside of the lips and on the gingiva and palates. Ulceration and congestion of the mucosae of the lower alimentary tract, particularly of the abomasum, were important features of the disease. In this aspect the spontaneous disease tended to be more severe than the experimental. A higher proportion showed oedema, ulceration and severe inflammation of the abomasum. In the experimental cases petechial haemorrhages were seen rather than inflammation. Inflammation and ulceration were more severe in the small intestine of the spontaneous cases, and inflammation of Peyer's patches more severe.

Small reddened fibrous tags were present on the superficial part of the greater omentum. Similar tags were present on the epicardium along the longitudinal and coronary grooves. Livers were congested and petechia were commonly found on the gall bladder mucosae. Focal petechial haemorrhages were observed at the corticomedullary junction of the adrenal gland in some cases. Slight cerebral and

cerebellar oedema and congestion of meningeal vessels was common.

Histopathology

The characteristic lesions of SA-MCF in Bali cattle were found in arteries and arterioles of most organs, lymphoid tissues and epithelial surfaces throughout the body.

Blood vessels. Small and medium-sized arteries, arterioles, capillaries and companion veins in most tissues and organs were affected. Changes were similar in all organs, but were particularly severe in the rete mirabile epidurale, brain, pampiniform plexus of the testes, kidney, adrenal and urinary bladder. Mild perivascular cuffing, oedema, haemorrhage and the accumulation of mononuclear cells in the adventitia were the most commonly observed changes. Vasculitis with mononuclear cell infiltration of the media, haemorrhage and medial fibrinoid necrosis was rarely observed in the spontaneous cases of SA-MCF, but was frequent in experimental transmissions. Intimal lesions including subintimal oedema, subendothelial accumulation of mononuclear cells, fibrinoid material and inflammatory debris, and endothelial cell hypertrophy were generally observed in association with either adventitial or medial involvement in experimental transmissions.

Lymph nodes. Oedema, congestion and moderate to marked vasculitis of the hilar and capsular vessels were observed in lymph nodes. Degenerative and necrotic changes of the capsule and trabeculae were present in a few cases. Cortical and paracortical zones of lymph nodes were obscured by an active proliferation of small lymphocytes, lymphoblasts and reticulum cells. In the medullary and subcapsular sinuses there were well-differentiated macrophages, and lymphoblasts.

Epithelial lesions. These were present in the oral and nasal mucosae, gastrointestinal tract, gall bladder, urinary bladder and ureters, skin, joints, ducts of salivary glands and pancreas, and lung. Perivascular and focal interstitial mononuclear cell accumulations, interstitial oedema and vasculitis were present in the lamina propria. Focal accumulations of mononuclear cells were present in the basal layers of the epithelium. Epithelial necrosis and sloughing, hydropic degeneration, focal acantholysis and ballooning degeneration of epithelial cells accompanied the mononuclear infiltrate in the squamous epithelia in more severe cases.

Central nervous system. Nearly all cases were affected, but with varying degrees of severity unrelated to mode of infection. In less severely affected cases, lesions were confined mainly to the white matter, but were present throughout the brain. Perivascular cuffing with mononuclear cells of mixed type consisting of small lymphocytes, lymphoblasts and large pleomorphic mononuclear cells was the most evident lesion. Oedema, focal gliosis and perivascular haemorrhage were also present. Involvement of larger vessels was seen only in the meninges and supporting tissues, and there necrosis of the tunica media sometimes occurred. Non-suppurative meningitis and focal mononuclear infiltration of the choroid plexus were observed in many cases.

Heart. Vasculitis, serofibrinous epicarditis and myocardial degeneration were found to a variable degree in most cases.

Other organs. Lesions in the joints were characterised by mild perivascular accumulations of mononuclear cells together with mild hyperplasia of the synovial epithelium. Mononuclear cell infiltration was also observed in the adrenals, hypothesis, kidney and liver.

Discussion

Animals in the series showed clinical signs and pathological changes as reported in classical descriptions of MCF (Barker and Van Dreumel 1985). The case fatality rate was 100%, and fever, lymph node enlargement, ocular changes and, to some extent, upper respiratory tract involvement, were consistently observed. The abomasum and the urinary bladder showed gross pathological changes in nearly every case. Lymphoproliferative changes were a histological feature, and necrotising vasculitis was present in some tissues in some cases. Hence, the diagnosis of MCF can be substantiated.

However, there was a tendency for the two modes of exposure to be associated with variations in the clinicopathological syndrome. In particular, differences in the severity of the ocular and nasal lesions and the lesions in the abomasum and intestines were observed between

the two groups. The importance of the observation lies in the fact that corneal opacity and inflammation of the gastrointestinal tract are readily visible changes clinically and at necropsy, and so considerable reliance may be placed upon these features when reaching a diagnosis. A statistically significant difference between groups is not implied, but the results do illustrate clearly that MCF need not be accompanied by corneal opacity, and that it may show a severely haemorrhagic gastrointestinal tract.

The usefulness of studying the vessels of the rete mirabile as recommended by Liggitt and de Martini (1980) has been confirmed. It is recommended that this tissue be examined in all cases where MCF is suspected or where it could be considered in the differential diagnosis. It was noted that the histological changes in the blood vessels were less severe in the spontaneous cases. Fibrinoid lesions were seen only in cases induced by blood transfusion, as was occlusion of vessel lumens. Necrosis and mononuclear cell infiltration of vessel walls were not features of several cases. Hence, reliance on necrosis and cellular infiltration of the tunica media in the vasculature of parenchymal organs as a pathognomonic lesion in the histological diagnosis of MCF may lead to confusion. Not only may this lesion be difficult to demonstrate in some cases of SA-MCF, but it also occurs in some cases of bovine virus diarrhoea (BVD) viral infections in other bovine species (Barker and Van Dreumel 1985).

The animals described in this report were only a small sample of the hundreds that die each year in Indonesia of SA-MCF. The syndromes reported were derived from the one source of infection under two different regimes of exposure. The range of syndromes that may be observed in association with other sources of infection under varying conditions of exposure cannot be predicted. The factors that lead to variation among cases are not known, but may include strain differences in the infectious agent, effects of dose, intercurrent infections, genetic susceptibility, and immunological or stress factors. Spontaneous disease may not always show less obvious clinical signs than textbook descriptions, but it should be appreciated that this may happen. A register of cases would be a useful step in defining the spectrum of manifestations of this disease throughout the country.

Variations in syndromes among series of SA-MCF cases have occurred elsewhere. For example, in the United States one series of cases was characterised by severe upper respiratory tract involvement, lesions of the abomasum and corneal opacity (Berkman and Barner 1958; Berkman et al. 1960), while another series from New Zealand showed marked diarrhoea, and corneal opacity in only a very few cases (James et al. 1975). Both series of cases had a fibrinoid necrotising vasculitis. In the buffalo described by Hoffmann et al. (1984), the vasculitis had no fibrinoid component and, as in the present spontaneous cases from the same location, was not usually marked by necrosis and infiltration of the vessel walls. The differences may be breed or species effects, but a wide range of cases from various locations is needed for study. Identification of the causes of variation between cases may give information on the factors contributing to the disease itself.

Acknowledgments

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Malignant Catarrhal Fever as Compared with Other Diseases of Bali Cattle, with Special Reference to Jembrana Disease

Soeharsono*

Abstract

Bali cattle have a reputation in Indonesia for superior productivity under many of the environmental conditions found in that country. For this reason, they are distributed widely in agricultural development projects. However, Bali cattle are susceptible to certain diseases, particularly Jembrana disease, malignant catarrhal fever (MCF) and haemorrhagic septicaemia. Problems of differential diagnosis can arise, but if attention is paid to the epidemiology, the clinical signs and gross pathology, and to laboratory findings, these diseases can be distinguished.

Abstrak

Sapi Bali di Indonesia terkenal unggul dalam hal produktifitas dibawah kondisi-kondisi lingkungan setempat. Karena itu sapi-sapi tersebut disebar secara meluas pada proyek-proyek pengembangan pertanian. Tetapi sapi Bali peka terhadap penyakit tertentu, khususnya penyakit Jembrana, malignant catarrhal fever (MCF) dan haemorrhagic septicaemia. Permasalahan dalam diagnosa banding yang muncul meminta kita untuk mencurahkan perhatian pada epidemiologi, gejala klinik dan gambaran patologi, sehingga penemuan laboratorium terhadap penyakit-penyakit tersebut dapat dibedakan.

Introduction

Bali cattle, which are believed to be directly descended from the wild banteng *(Bos sondaicus)*, are regarded as the best of the indigenous cattle in Indonesia in terms of reproductive performance and ability to thrive in poor conditions. The cattle are said to possess 'pioneering vigour', and survive in the feral condition. The presence of wild Bali cattle elsewhere in Indonesia and in the Northern Territory of Australia is an indication of their adaptability. The rapid increase in numbers of Bali cattle distributed by the Small Holder Cattle Development Project (an IFAD/World Bank Project) in transmigration areas in Sumatra revealed that this breed is the most highly prized of Indonesian cattle.

The superior productive capacity of Bali cattle is balanced a little by a susceptiblity to several diseases. In the order of perceived economic importance the diseases are Jembrana disease (JD), malignant catarrhal fever (MCF) and haemorrhagic septicaemia (HS). MCF should be carefully differentiated from JD, bovine ephemeral fever (BEF) and HS. This paper describes some differences between MCF and other diseases of Bali cattle on the basis of epidemiology, pathology and aetiology.

Jembrana Disease Compared with MCF

Epidemiology

(i) Distribution

The first outbreak of JD was reported in the district of Jembrana in Bali in December 1964 (Pranoto and Pudjiastono 1967). Within a few months, the disease spread to neighbouring districts and, by August 1965, all eight districts of the island were affected. In 1976, a disease affecting Bali cattle that could not be differentiated from JD appeared in Lampung, Sumatra. The disease was first called Seputih Raman disease (Soeharsono and Darmadi 1976), but subsequently became known as Rama Dewa disease. In 1978, a similar disease was reported

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among Bali cattle and Rambon cattle (Bali–*Bos indicus* crossbred cattle) in Banyuwangi, East Java. Further severe outbreaks of JD in Bali were reported in 1974 and 1981. JD is at present confined to the island of Bali.

Nowadays JD is endemic in Bali. In Lampung, sporadic cases of Rama Dewa disease are still reported, but no more information is available concerning the disease resembling JD in Banyuwangi. The distribution of MCF among Bali cattle is wider than that of JD. It has been recorded in Sumatra (including Lampung), West Jawa, Kalimantan, Sulawesi, West Nusa Tenggara and East Nusa Tenggara.

(ii) Susceptible Animals

Only Bali cattle are susceptible to JD. During the first outbreak in 1964, buffalo were reported to have died, but no postmortem or laboratory examination was carried out (Pranoto and Pudjiastono 1967). To date, no laboratory animal has been found to be susceptible to JD.

Bali cattle are perhaps the animals most susceptible to MCF. Their inability to survive in West Jawa (where sheep are abundant) suggested that Bali cattle are more susceptible to MCF than ongoles and buffalo. The outbreak of MCF among wild Banteng in San Diego Wild Animal Park (Hatkin 1980) revealed that these animals are also very susceptible to wildebeest-associated MCF. However, all breeds of cattle are apparently susceptible to MCF to some degree. MCF has also been diagnosed in deer in Indonesia by the DIC Region VI, Denpasar, in cases from Surabaya zoo and from Kupang.

(iii) Mode of Transmission

Observation of field cases of JD suggests that the disease is most probably transmitted by vectors. The role of a vector is still under investigation by the BCDIU, Denpasar, with the help of an entomology team from the Bogor Veterinary School (IPB). Artificial transmission of JD can be carried out easily by inoculation with heparinised blood, or suspensions of spleen or lymph nodes from diseased animals, by either the subcutaneous, intramuscular, intraperitoneal or intravenous routes. Macrophage cultures from Jembrana-diseased animals can also transmit JD parentally.

In contrast, MCF is very difficult to transmit artificially. Artificial transmission from a sick animal to a healthy one is possible by inoculation with large amounts of blood through the intravenous route. The evidence suggests that MCF is usually associated with the presence or close association with either sheep or wildebeest. In JD those animals are not necessarily present.

(iv) Morbidity and Case Fatality Rate

In the early outbreak of JD in the 1960s, the morbidity rate as well as the case fatality rate was very high. However, JD is no longer a mass-killing disease, but it still occurs enzootically with a morbidity rate of 10–40% and a case fatality rate of 10–20%. Prolonged observation of JD experimental animals in the 1970s disclosed that relapses can occur 2–5 times. In the field, the second relapse often caused sudden death.

In Bali, MCF is sporadic. In the rural areas, the morbidity rate is perhaps less than 1%. Recovery of animals with clinical MCF has never been reported, and the case fatality rate is therefore nearly 100%.

Pathology

The gross pathology of JD as seen in the 1960s and 1970s was characterised by extensive haemorrhages in various organs, leading to early descriptions of the disease as 'rinderpest-like disease'. At present, the pathological changes are much milder. The differences between JD and MCF are summarised in Table 1.

Aetiology

The causal agent of JD is currently thought to be an *Ehrlichia* sp. The agent can be isolated in macrophage cultures of diseased cattle. Attempts to isolate the agent through laboratory animals and various other cell cultures have been unsuccessful. Serum samples from an *Ehrlichia bovis* endemic area in Zimbabwe, when tested against JD antigen from macrophage culture, showed positive complement-fixing antibody. Attempted isolation of the agent through murine macrophage cultures is still under study. The aetiology of MCF has been clearly defined as a bovine herpesvirus.

Bovine Ephemeral Fever Compared with MCF

BEF occurs sporadically among Bali cattle in Bali. During the field investigations of JD by the BCDIU team, some cases which were reported as JD by animal health assistants were revealed to be BEF by close clinical observations. The rise of temperature and anorexia of BEF could not be differentiated from early signs of JD and MCF. The stiffness or lameness seen in BEF was not found in MCF. Mortality due to BEF in Bali cattle has never been reported. The affected animals recover spontaneously within 3–4 days.

Haemorrhagic Septicaemia Compared with MCF

In Bali, HS is endemic, affecting buffalo, pigs and Bali cattle. An acute case of HS followed by sudden death in Bali cattle in Bali could be confused with JD or MCF. In HS, the postmortem changes in the lungs are prominent (bronchopneumonia, hepatisation), whereas in JD the changes in the lungs are minimal. A definitive diagnosis of HS can be made by isolation of Pasteurella multocida from blood, oedema fluid and organs of affected animals.

Conclusions

Bali cattle, which are very productive for farmers, are very susceptible to certain diseases such as JD, MCF, and HS. Knowledge about disease management for JD and MCF is still minimal. As a result those two diseases are difficult to control.

Lack of knowledge of epidemiology, clinical signs and pathology of Bali cattle diseases by animal health assistants in the field could lead to misdiagnosis. Careful examination of clinical signs is useful for separating BEF from JD and MCF. Correct specimens for histopathological examination are necessary to differentiate MCF from JD or HS. For bacterial isolation, especially in HS-suspected cases, immediate submission of specimens should be attempted by the investigator. The laboratory at the DIC, Denpasar, is able to diagnose MCF, HS and JD. As Bali cattle have been chosen by the government as 'pioneering cattle' for distribution to new areas, they should receive more attention, especially with respect to disease prevention.

Table 1. The comparative pathology of Jembrana disease and malignant catarrhal fever.

Clinical signs and postmortem observations	JD	MCF
Corneal opacity	No	Yes
Haemorrhage, anterior chamber of the eye	Occasionally	No
Oral ulceration	Yes	Yes
Nasal discharge	Sero-mucous No smell	Muco-purulent Bad smell
Blood sweating	Yes	No
Enlargement of spleen and lymph nodes	Yes	Yes
Endocardial and sub-endocardial haemorrhage	Yes	No
Haemorrhage of serosa of abomasum, rumen and intestine	Yes	No
Linear haemorrhages in rectal mucosa	Yes	Yes
Encephalitis	No	Yes
Vascular wall changes	No	Yes
Endotheliosis	Yes	No

Source: BCDIU progress report January–April 1985 (Ressang et al. 1985).

Recommendations

To improve the health care of Bali cattle the following recommendations are made.

1. Research on Bali cattle diseases should be a continuing program carried out by a permanent institute.

2. The priorities for Bali cattle disease research are JD, MCF and HS, in that order.

3. The place for the Bali cattle Research Unit should be close to the diseases. Denpasar would be the correct place.

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The Differential Diagnosis of Malignant Catarrhal Fever in Indonesia

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Abstract

The differential diagnosis of diseases with similarities to malignant catarrhal fever (MCF) in Indonesia is presented. The subject is approached on the basis of clinical, macropathological and histopathological features. Some of the current problems associated with histological diagnosis are discussed, with special reference to MCF and trypanosomiasis. Studies on field material are presented.

Abstrak

Diferensial diagnosa dari penyakit-penyakit yang mempunyai persamaan dengan MCF di Indonesia didiskusikan. Pendekatannya melalui dasar-dasar gambaran klinis, gambaran neuropatologi dan gambaran histopatologi. Beberapa risalah baru sehubungan dengan diagnosa secara histologis didiskusikan, dengan tekanan pada MCF dan trypanosomiasis. Pengamatan material lapangan juga disajikan.

Introduction

The feature that binds veterinarians involved in research and veterinarians involved in diagnosis is an interest in the scientific process of establishing cause-and-effect relationships. The difference between these two groups is in the approach to establishing cause-and-effect relationships. Veterinary researchers usually work first from hypothesised causes which they experimentally try to control and then study the effects. Veterinary diagnosticians, on the other hand, working either in the field or in the laboratory, usually move in the reverse direction. They usually study effects first and then try to establish cause. The effects may be any of clinicopathological, population, or production effects. Based on the most prominent of these effects, several potential causes are hypothesised (differential diagnoses). These potential causes are differentiated and eliminated on the basis of evidence at hand, to come to a specific cause for the effects observed. Confirmation for cause may be sought by seeking further evidence or carrying out specific laboratory tests.

Both groups, those involved in research and those involved in diagnosis, are interdependent. The researcher is dependent on the diagnostician for information on effects that are being observed in the field and the laboratory, particularly for those effects for which the cause is not clearly established, in order to establish the direction of research. The diagnostician on the other hand is dependent on the researcher for information on new causes and their specific effects in order to improve his ability to differentiate diseases in the field. This paper, besides presenting some differential features of MCF, will attempt to illustrate the need for interaction between the two groups.

MCF: the Clinicopathological Entity on Java

MCF is a familiar disease to veterinary laboratory staff and prominent among field submissions to the Disease Investigation Centre (DIC) at Jogjakarta, as indicated in the proportional histopathological diagnoses of large ruminant submissions (Tables 1 and 2).

MCF in Indonesia can be described in relation to its various effects. These are usually as classically described.

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Population Effects

MCF as described in European and North American literature is said to occur sporadically with a very high case fatality rate. The latter appears to be true on Java as well. Our experience indicates that the occurrence of the disease in Indonesia is more than sporadic. We have at different times seen several cases of MCF occurring in a small radius of a few kilometres, at the same point in time. In one instance, all buffalo (2) on one farm were clinically affected. Tables 1 and 2 indicate that the proportional mortality rate due to MCF is around 8%, which also suggests that the disease is more than sporadic on Java. A possible better description of the population effect on Java is low to moderate incidence rather than sporadic.

From our data from Java (Table 2), we can say little about differences in breed susceptibilities, as the quantity of our diagnostic records is still too limited. Table 2 may indicate that there are differences in susceptibilities between local buffalo and buffalo imported from Australia. However, in the imported group there are disproportionately high numbers of stress-related conditions, and local diseases to which the imported animals had not previously been exposed. This in all likelihood accounts for the relatively low proportional mortality rate in the imported group.

Table 1. Distribution of cattle and buffalo MCF cases by province on Java Island.

Province	Cattle			Buffalo		
	Total Sub-missions	MCF	%	Total Sub-missions	MCF	%
E. Java	131	15	12	143	11	8
C. Java	13	0	0	14	0	0
W. Java	14	1	7	2	0	0
Jogjakarta	4	0	0	6	0	0
Jakarta	6	0	0	0	0	0

(Based on Pathological Submissions to DIC Jogjakarta, July 1985 to July 1986).

Table 2. Distribution of MCF cases by species and breed.

Species/ breed	Total submissions	MCF cases
Local *Bos indicus*	93	8
Bali and Madura Cattle	12	1
Friesian–Holsteins	26	0
Brahman	9	0
Local Buffalo	66	8
Buffalo ex Australia	97	

(Based on Pathological Submissions to DIC Jogjakarta November 1985 to July 1986).

Clinicopathological Effects

Based on our field observations of MCF cases in Indonesia, we would divide the clinicopathological effects into two groups: those that are more prominent and those that are less.

(a) The most prominent clinicopathological changes (those most frequently present in field cases or most easily observed by field personnel on either clinical or postmortem examination)

(i) Ocular:

- photophobia
- lachrymation leading to mucopurulent discharge
- severe conjunctival hyperaemia
- keratitis (corneal opacity)

(ii) Upper Respiratory:

- mucoid to mucopurulent discharge
- erosions of the muzzle
- stertor (ngorok is the Indonesian term often used)
- rhinitis and laryngitis which is often necrotising

(iii) Upper Gastrointestinal:

- mucoid hypersalivation
- erosive stomatitis
- glossitis
- pharyngitis
- oesophagitis

(iv) Lymphoid:

- prominent lymph node enlargement with evidence of hyperplasia (in our experience this is less prominent in buffalo)

(v) General:

- high fever
- severe depression

(b) The less prominent clinicopathological changes (those not as frequently present, or more difficult to observe in the field)

(i) Nervous:

- hypermetria, terminal convulsions

(ii) Cutaneous:

- exanthema
- cornetitis of the hoof

(iii) Reproductive:

- vaginal erosions (usually superficial)

(iv) Urinary:

- haemorrhagic cystitis
- focal infiltration in the kidneys

(v) Gastrointestinal:

- erosive gastroenteritis

Histological Effects

In our laboratory, in the absence of other diagnostic aids for MCF (which we assume to be sheep-associated), confirmation is still based on histopathology. The diagnostic feature used is vasculitis which can be described as necrotising with fibrinoid or hyaline degeneration of vessel walls. In addition, there is infiltration of mononuclear cells around vessels. These changes can be seen in many organs. We see it most readily in the brain, but also encounter it in the lymph node medullas, spleen and kidney. Additionally, there is noted lymphoreticular hyperplasia in lymph nodes as well as nonsuppurative infiltration in liver and kidney. Necrotising vasculitis remains, however, the most characteritic change that we use to confirm MCF in our laboratory. We are relatively conservative in this regard.

Problems of Differential Diagnosis: the Clinicopathological Approach

In Indonesia, we must realise that a majority of diagnoses are still done at field level without laboratory backup, and are based on field observations of population and clinicopathological effects. Moreover, because of shortages of veterinarians and for other reasons, diagnostic observations are often made by veterinary assistants, whose abilities to observe and interpret changes are not as good as those of veterinarians. Hence, there is often a problem of differential diagnosis and mistaken diagnosis. In the writers' experience, a variety of diseases have caused problems of differentiation with regard to MCF for field personnel. Many of these problems can be related to incomplete clinical observations. Table 3 is a list of diseases that occur or potentially occur in Indonesia that have caused confusion with MCF at field level. This list is based on the most prominent clinical and pathological changes of MCF. Included are the differential features of these diseases which, it is hoped, might be of use to field veterinarians in the future.

Problems of Differential Diagnosis Based on Histopathology

In our laboratory, we find that the changes of MCF are most consistently and clearly seen in the brain, although changes may be seen also in other organs. As mentioned previously, these can best be described as a nonsuppurative encephalitis with vasculitis.

In the past 2 years, we have encountered three diseases that cause nonsuppurative encephalitis in cattle and buffalo, which could cause confusion with MCF. Two have been toxoplasmosis and rabies. Although encephalitis is present in both diseases, the distinguishing features of toxoplasma pseudocysts and tachyzooites in the former and Negri bodies in the latter are present and can be used for differentiation.

A third, relatively large group of 29 cases has presented some difficulty in terms of diagnosis. This group has the following characteristics. It has been confined mostly to imported buffalo that have been resident in the country for several months. There is mononuclear cell infiltration in meninges and Virchow Robins space. The cell types consist of lymphocytes and macrophages with plasma cells and Russel bodies (cells of Mott) often being present. This meningoencepahalitis ranges from mild to

Table 3. Differential diagnosis of MCF: a clinicopathological approach.

	MCF	Disease with oral and upper gastrointestinal changes						Disease with eye lesions		Disease with upper respiratory changes		
		Jembrana	BVD	Rinderpest	Ibaraki	FM	Ulcerative stomatitis	IBK	Tryps	IBR	HS	Dipth
Population Effects												
1. Morbidity rate	Lo	Lo–Hi	Lo	Hi	Lo–Hi	Med–Hi	Lo	Med–Hi	Lo–Hi	Lo–Hi	Lo–Hi	Lo
2. Case fatality rate	Hi	Med–Hi	Hi	Hi	Lo	Lo	Lo	0	Lo–Hi	Lo	Hi	Lo
Clinicopathology												
1. Clinical course	Ac–Chr	Var	Ac–Chr	Ac–Chr	Ac	Ac–Chr	Chr	Ac–Chr	Chr	Ac	Ac	Chr
2. General												
(a) Temperature	Inc	Inc	Inc	Inc	Inc	Inc	Nor	Nor	Var	Inc	Inc	Var
(b) Depression	++	++	++	++	+	+	–	–	+	+	++	–
3. Oral												
(a) Hyper salivation	++	++	++	++	++	++	+	–	–	–	++	–
(b) Erosive stomatitis	++	++	++	++	++	–	–	–	–	–	–	–
(c) Vesicular stomatitis	–	–	–	–	–	+	–	–	–	–	–	–
(d) Ulceration	–	–	–	–	–	–	++	–	–	–	–	+
4. Ocular												
(a) Photophobia	++	–	–	–	–	–	–	++	+	–	–	–
(b) Mucopurulent discharge	++	–	–	–	–	–	–	++	–	–	–	–
(c) Conjunctivitis	+++	+	++	++	+/–	–	–	++	+	+	–	–
(d) Keratitis	+++	–	–	–	–	–	–	++	–	–	–	–
5. Respiratory												
(a) Stertor (Ngorok)	++	–	–	–	–	–	–	–	–	++	++	+
(b) Mucopurulent discharge	++	–	–	–	–	–	–	–	–	++	–	–
(c) Pulmonary congestion/oedema	+/–	+/–	+/–	+/–	+/–	–	–	–	+/–	+/–	+++	–
(d) Rhinitis	+++	–	–	–	–	–	–	–	–	++	+/–	–
(e) Tracheitis	++	–	–	–	–	–	–	–	–	++	–	+
6. Cutaneous												
(a) Blood sweating	-	+	–	–	–	–	–	–	–	–	–	–
7. Foot lesions												
(a) Digital vesicles	–	–	–	–	–	+	–	–	–	–	–	–
(b) Coronitis	+/–	–	–	–	–	–	–	–	–	–	–	–
8. Enteric												
(a) Necrotic peyerpatches	–	–	+++	+++ –	–	–	–	–	–	–	–	–
(b) Haemorrhagic mucosae	+/–	–	+	+	–	–	–	–	–	–	+	–
9. Renal												
(a) Focal white spots	+	–	–	–	–	–	–	–	–	–	–	–
(b) Haemorrhagic cystitis	+	–	–	–	–	–	–	–	–	–	–	–
10. Generalised												
(a) Petechiation and haemorrhage		++	+/–	+/–	+/–	–	–	–	–	–	++	–

Ac = acute; Chr = chronic; Inc = increased; Var = variable; – = usually not present; +/– = sometimes present; + = usually present, mild; +++ = usually present, severe.

severe. Moderate lymphoid infiltration is often present in other organs, such as kidney, spleen and heart. Necrotising vasculitis, however, is absent. On the other hand, characteristics of anaemia are almost always present. This includes centrilobular degenerations and necrosis in the liver, accumulation of bile pigment in the liver and haemosiderosis in the spleen.

When the first cases were received in the laboratory they were classified as suspect MCF cases on the basis of a nonsuppurative encephalitis. Subsequent visits to the field indicated that the clinical picture was not consistent with MCF. Field veterinarians felt the major problem was trypanosomiasis, based on blood examination and clinical signs of circling, incoordination and the eating of soil. Further submissions were of brain tissue along with other organs from dead animals. The observed changes were as described in the previous paragraph and in Table 4.

At present we are tentatively regarding these cases as *Trypanosoma evansi* infections rather than MCF. Our reasons for this tentative classification are as follows:

1. The *Trypanosoma brucei* group of trypanosomes which includes *T. brucei, T. rhodensii* and *T. gambiense* has received extensive pathological study (Losos and Ikede 1972). Encephalitis is commonly found in experimental cases as well as in natural cases. This includes human trypanosomiasis (African sleeping sickness) where cells of Mott (Russel bodies) along with encephalitis are considered relatively pathognomonic. Studies of the pathological changes due to *T. evansi* appear sparse. We have found one paper by Ikede et al. (1983) which describes analogous encephalitic changes in three horses infected with *T. evansi.*

2. The clinical histories from the field lend some support to this diagnosis. Unfortunately, we have been unable to get specific information on each pathological submission regarding the presence of trypanosome infection prior to death. This would potentially lend strength to the diagnosis.

3. We have been able to reproduce a nonsuppurative encephalitis in three guinea pigs which we inoculated with a trypanosome strain from the area where the buffalo cases occurred. Further experimental work is needed.

It is of interest that almost all such cases observed have been in imported buffalo, although we get a similar number of routine local buffalo submissions. A number of possible explanations could be considered. The buffalo imports come from a trypanosome-free country and have therefore not been exposed to natural selective pressure of trypanosome exposure as has the local population, and are therefore genetically more susceptible. Additional transportation and adaptational stress from a wild to a domesticated environment may also be predisposing factors.

An alternative explanation for the nonsuppurative encephalitis is that it is virus-induced. An unusual type of MCF reaction cannot be entirely ruled out (Hoffmann et al. 1984).

Discussion

The above problems of differential diagnosis raise some questions that can really be answered only by research.

1. Does the MCF agent, whatever it is, cause

Table 4. Histopathology in imported buffalo (38 of 97 cases in which brain was received).

Encephalitic changes	Group I (n=9) No enceph-alitis	Group IIA (n=15) mild enceph-alitis	Group IIB (n=14) moderate to severe enceph-alitis
Mononuclear infiltration	0	15	14
Plasma cells	0	7	11
Russel bodies	0	0	10
Oedema	0	8	12
Gliosis and satellitosis	0	12	13
Suppurative changes	0	1	0
Meningitis only	0	3	0
Encephalitis only	0	2	0
Meningitis and encephalitis	0	10	14

clinical and pathological changes that have a wider spectrum than has originally been described for this disease?

2. The clinical and pathological changes associated with *T. evansi* infection appear to be incompletely studied, and poorly defined. What are these changes? Are these changes different in imported, previously unexposed, buffalo?

3. Agent identification for trypanosomiasis is usually done on material collected while animals are alive. Are there better methods for identifying trypanosomes or trypanosome antigens in either semi-fresh or formalised tissue to confirm the presence of antigens?

4. The identification of the agent of sheep-associated MCF is a goal we all seek. We would hope that diagnostic tests would follow, so we would not have to rely on only histopathology.

These are questions that the diagnostic laboratory veterinarians cannot answer unless they step into the role of research. As stated in the introduction, there is a need for the veterinary diagnostician and the veterinary researcher to interact, and this is an area where such interaction could be usefully applied here in Indonesia.

At the same time, diagnosticians at field and laboratory level cannot expect the researchers to find all the answers. The adage by Virchow still holds true: 'The study of things caused (effects) must precede the study of the cause of things.' The people in the best position to study the clinicopathological effects of disease are veterinarians in the field. This is the reason for the constant request for better clinical histories and records of clinical and pathological changes by laboratory diagnosticians. There is still a definite need for training of field veterinary personnel in order to improve their skills in differentiating disease at field level. Laboratory diagnosticians on the other hand too frequently become agent-oriented with an emphasis on collecting many samples, but without relation to a clinicopathological effect. This gap needs to be bridged in order for us to sort out some of the diagnostic problems that relate to MCF, trypanosomiasis and other diseases.

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The Differential Diagnosis of Malignant Catarrhal Fever: Unusual and Difficult Cases

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Abstract

Malignant catarrhal fever (MCF) can occur with any of a range of clinical signs, and so mild, peracute, gastrointestinal, 'head and eye' and chronic forms have been described. Mortality is nearly 100% in the last four forms. The disease processes are based on, or accompanied by lymphoid proliferation, with invasion of many tissues including blood vessels by lymphoid cells. It is usually accepted that the damage to blood vessels causes the severe clinical manifestations. Because of the variability in clinical signs and variability in the severity of lymphoid and blood vessel changes among affected animals, similarities with other diseases can occur and problems in differential diagnosis arise. This paper reviews briefly some of the variation in naturally occurring sheep-associated MCF and also the pathology of several diseases that may complicate the diagnosis of some MCF syndromes in Indonesia. Disease associated with *Trypanosoma evansi,* bovine virus diarrhoea virus, several arboviruses, rickettsia and large ruminant retroviruses are discussed in relation to case material. Some of these agents can establish chronic or persistent infections, and so the possibility of dual infections may have to be considered on occasions. Recommendations are made on some aspects of submitting specimens to a diagnostic laboratory.

Abstrak

Malignant catarrhal fever (MCF) dapat terjadi dalam berbagai bentuk. Bentuk seperti ringan, perakut, gastro intestinal, kepala dan mata, serta knonik, adalah bentuk yang telah lama dikenal. Angka kematian dari ke empat bentuk terahir itu sampai mencapai 100%. Perjalanan penyakit bergantung pada atau diikuti oleh proliferasi limfoid, dengan invasi sel limfoid ke jaringan-jaringan tubuh termasuk juga pembuluh darah. Karenanya, kerusakan-kerusakan yang terjadi pada pembuluh darah akan menentukan juga kehebatan gejala klinik yang timbul. Karena pada hewan terserang, sering ditemukan perbedaan-perbedaan dalam gejala klinik, kehebatan perubahan yang ditemukan pada limfoid dan pembuluh darah; dan juga terdapat persamaan dengan penyakit lain, maka masalah diferensial diagnosis pun muncul. Makalah ini mencoba mengadakan telaahan beberapa variasi dalam kejadian SA-MCF alami dan tentang gambaran patologi beberapa penyakit yang dapat mempersulit dalam diagnosis sindroma MCF di Indonesia. Penyakit yang terkait dengan *T. evansi,* bovine virus diarrhoea, beberapa arbovirus, rickettsia, dan retrovirus hewan besar, dibahas sehubungan dengan kasus. Beberapa dari agen penyakit tersebut dapat menimbulkan infeksi kronis atau menahun, sehingga kemungkinan infeksi ganda adakalanya harus dipertimbangkan. Beberapa rekomendasi tentang aspek tertentu dalam pengiriman spesimen untuk laboratorium diagnostik juga diulas.

Introduction

The clinicopathological syndrome of bovine malignant catarrhal fever (MCF) has been well described, both in original reports (Plowright 1953; Berkman et al. 1960; Selman et al. 1974; Liggitt et al. 1978; Liggitt and de Martini 1980a, b), and also in pathology texts (Barker and Van Dreumel 1985), where the microscopic changes are said to be highly characteristic. A similar syndrome has been described in the swamp buffalo *(Bubalus bubalis)* by Hoffmann et al. (1984).

Unfortunately for the diagnostic pathologist, the manifestations cover a wide spectrum of possibilities including peracute, gastrointestinal, head and eye, and mild clinical forms. A fifth form—chronic MCF—has also been described (Snowdon 1985). Consistently, there is nearly 100% mortality except, of course, in the mild

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form. The unifying feature of the disease as so far described is the histopathology.

The effect of the disease is pantrophic. Although there are lesions of the mucosal and epithelial surfaces, the features that are consistently present and on which diagnosis is usually based are a characteristic lymphoid proliferation and a vasculitis. Most descriptions of the lymphoid element emphasise the presence of large, immature lymphoblasts frequently with a high mitotic index. The vasculitis is described as necrotising. The essential feature is an invasion by mononuclear cells that usually contain some lymphoblasts. The infiltrate is nearly always perivascular in the adventitia, and in every MCF case it should be possible to find infiltration of the vessel wall and necrosis of the smooth muscle in a variable number of vessels. Mononuclear cells can also accumulate under the endothelium causing some constriction of the lumen. Severely affected animals may have fibrin deposited in the vessel wall, but this is not a consistent finding.

Although these changes together are highly characteristic of MCF, none is unique to the disease. Given the highly variable clinical syndrome and the variable expression of the characteristic histology in some animals, problems of differential diagnosis in diseases with similar features arise that are particularly important to those attempting to evaluate the result of experimental infections for research purposes or to reach a diagnosis on field material.

This paper will examine and discuss several cases that have posed problems or that illustrate certain features of the diagnostic dilemma. In most of them, the investigation from which the case was derived is not complete and will be described in full elsewhere. Firstly, the disease agents known to be present in Indonesia which could produce clinical signs or pathology similar to those described in the cases presented are briefly reviewed so a clear appreciation of the range of probabilities can be developed.

Naturally Occurring Sheep-associated MCF

Descriptions of naturally occurring SA-MCF in cattle *(Bos taurus)* are available from the USA (Berkman and Barner 1958; Berkman et al. 1960), Canada (Murray and Blood 1961), the United Kingdom (Selman et al. 1974) and New Zealand (James et al. 1975). Although all authors emphasised variability in the syndrome among the cases in their series, it can also be noted that there is variation between reports in the typical syndrome described. Hence, respiratory distress and severe pathology in the upper respiratory tract were emphasised in all except the report from New Zealand. The abomasum showed oedema or inflammation in all cases except the New Zealand series. Corneal opacity was a prominent feature in only the cases from the USA (Berkman and Barner 1958) and the UK (Selman et al. 1974). In the series of Murray and Blood (1960) and James et al.(1975), without significant corneal opacity, diarrhoea was a prominent feature of the latter but not the former. Whether such differences reflect different susceptibilities in the affected populations or different strains of infectious agent or intercurrent infection is not known. In all cases a fibrinoid necrotising vasculitis was reported as the diagnostic feature.

In buffalo with MCF, conjunctivitis with hyperaemia and serous discharge has been reported as a unifying clinical feature in one series of cases in Indonesia, with corneal opacity developing to some degree only late in the course of the disease in 30 of 50 animals. Diarrhoea was seen in half the cases. Erosions of the mouth and rhinitis were rarely encountered, and inflammation of the upper respiratory tract at postmortem was not a feature. Hence, the syndrome in these buffalo differed from some of the bovine series. Vasculitis was present in all cases but was never fibrinoid, and marked necrosis and infiltration of the vessel wall was not usually encountered. Hence, even the essential diagnostic lesion differed in degree in these buffalo (Hoffmann et al. 1984). Myocarditis was, however, severe.

In the neighbouring country of Malaysia, MCF has been reported in buffalo and cattle (Omar 1971) and in cattle *(Bos indicus)* (Vaneslow 1980). Twenty-one of 35 cases reported by Omar (1971) were in buffalo. Of these, details of clinical signs were given for 14. Respiratory distress was observed in most, as was conjunctivitis with serous discharge that progressed to mucopurulent in about half the cases. Corneal opacity was observed in three

animals which tended to have had a longer illness than others in the series. Diarrhoea was reported in 50% of cases.

Gross pathological changes included erosions and ulceration in the oral cavity, inflammation of the mucosa of the upper respiratory tract, and catarrhal inflammation of the abomasum and intestines in those showing diarrhoea. A large range of changes affected various animals within the series, emphasising the variability of the syndrome.

Histologically, no distinction was made between buffalo and cattle cases. There was vasculitis in the CNS which was fibrinoid in a few cases. Similar lesions were present in many organs, including the kidney. Changes in the intima were prominent in some cases. Mononuclear cells with prominent lymphoblast and primitive reticular cell types infiltrated parenchymal organs as well as comprising the vascular infiltrates.

From the above description it seems that the buffalo in Malaysia showed more severe respiratory involvement and pathology in the upper respiratory tract than in Indonesian cases, but that the ocular pathology and the incidence of diarrhoea were similar. In the bovine cases of Vaneslow (1980), breed differences were noted in the speed with which the disease progressed, but not in the range of clinical signs. Early disease was characterised by diarrhoea, and corneal opacity developed later. Respiratory distress was again not a feature, in contrast to the reports from North America (Berkman and Barner 1958; Murray and Blood 1961) and the UK (Selman et al. 1974). Prominent vasculitis with marked mononuclear cell involvement of the media was reported, but it was not described as fibrinoid.

Deer are very susceptible to MCF, but variability in syndromes has again been noted in reports from the Australasian region. In Indonesia, MCF in *Cervus timorensis* has presented differently from Australian cases. In Indonesia, diarrhoea and rapid death were prominent manifestations (Sudarisman et al. unpublished), while in Australia oculonasal discharge and corneal opacity were noted more frequently (Denholm and Westbury 1982). The cases of MCF in *C. timorensis* in Indonesia more closely resembled MCF of *C. elaphus* in New Zealand (McAllum et al. 1982; Oliver et al. 1983). Most reports emphasise a fibrinoid vasculitis, but this change was not noted in the two Indonesian cases available for study.

Only reports of naturally occurring SA-MCF have been considered. A previous report in this volume (Young et al. 1988) has shown how experimental MCF can differ from naturally occurring MCF. In the Bali cattle *(Bos javanicus)* under study, the pathology was less severe in the naturally induced cases than in those resulting from blood transmissions. The latter more closely resembled the description of cases from North America (Berkman et al. 1960; Murray and Blood 1961) and the UK (Selman et al. 1974), but the natural cases resembled a little the reports of MCF in buffalo (Hoffmann et al. 1984) and in cattle in New Zealand (James et al. 1975). It is clear that the severe clinicopathological picture of 'classical' MCF is not observed in all episodes of MCF, particularly the diarrhoea, mucopurulent oculonasal discharge, corneal opacity and the fibrinoid component of the vasculitis. The constant features have been the massive lymphoid proliferation in lymphoid tissue and infiltration of other organs, and the associated vasculitis.

Trypanosomiasis

Reports of the pathology of *Trypanosoma evansi* infections in cattle and buffalo are limited. Mohan (1968) in reviewing the diseases of buffalo stated that *T. evansi* infections are mostly latent and subclinical, but that pathogenic epizootics can occur for undefined reasons that may include strain differences in parasites or stress or other concurrent infections in affected animals. Naive animals may be more susceptible. *Trypanosoma evansi* spreading to new areas has been reported to cause severe disease and death in wild animals (Losos 1986). Verma and Gautam (1978) infected buffalo and cow calves by inoculation. Eight of 10 buffalo calves died 30–96 days post infection, while two recovered and remained carriers. Other experiments in which a high proportion of experimentally infected buffalo died were reviewed. It seems that under certain circumstances *T. evansi* can kill buffalo.

However, comprehensive descriptions of histopathology are lacking. Verma and Gautam (1979) reported on the histopathology of their experimentally infected animals, but did not

examine the central nervous system, the tissue that seems to be part of the current problem of differential diagnosis. In lymph nodes, the corticomedullary junction was obscured and germinal centres were absent, changes that are also seen in MCF. Sinuses of the spleen were dilated and contained reticular cells, and there was variably 'hyalinisation' and haemosiderosis. The changes reported in other organs were non-specific. In studies at RIVS, weight loss and anaemia have been the main clinical signs noted, and myocarditis and haemosiderosis were prominent histopathologically (Wilson et al. 1985).

Early Dutch literature from Indonesia, reviewed by Dieleman (1983) and Partoutomo (pers. comm.), indicated that both acute and chronic infections were observed in buffalo, and that affected buffalo showed signs of central nervous system involvement. Emaciation occurred in spite of a good appetite.

Other clinical signs of surra in buffalo recorded in early literature included fever, rapid respiration, ocular discharge, mucopurulent nasal discharge, scale formation on the nose, salivation, swollen lymph nodes and crusty excema, all signs that have been reported in MCF. In addition, there were the more commonly cited signs of trypanosomiasis, including anaemia, cachexia, icteric mucous membranes and oedema in the laryngeal region and the distal parts of the legs (Dieleman 1983). The suspicion arises that the problem of differential diagnosis is not entirely recent. Indeed, some investigators are reported to have said that surra was usually similar to MCF, and that they both could resemble haemorrhagic septicaemia and anthrax. No histological observations were reported, the diagnosis of trypanosomiasis being based on the demonstration of parasites in the blood, body fluids or tissue smears or by laboratory animal inoculation. It must be remembered that the presence of a ubiquitous parasite such as *T. evansi* in a sick animal is not proof of the cause of its disease.

As a guide to changes that might be expected, the pathology of reported natural trypanosomiasis in more susceptible species can be considered. In horses, there may be petechial or ecchymotic haemorrhages on the epicardium and endocardium, in the intestines, the capsule of the spleen, the kidneys and the bladder. The stomach may show erosions and ulcerations (Stephen 1986). Histopathology observed in horses in Malaysia included a variably severe nonsuppurative meningoencephalitis with perivascular cuffs of large lymphocytes, histiocytes and plasma cells and some foci of gliosis. Large morular cells with eccentric nuclei and large eosinophilic globules in the cytoplasm were present in inflammatory infiltrates. Additionally, there were lymphoid hyperplasia in nodes and spleen, mononuclear periportal infiltrates in most cases and mild nonsuppurative myocarditis in a few cases (Seiler et al. 1981). Essentially similar findings were reported by Ikede et al. (1983) in other Malaysian ponies.

MCF experiments should ideally be conducted in animals either uninfected by *T. evansi* or treated with Naganol. Although parasitaemias can be monitored, it is noted that *T. evansi* is regarded as a tissue parasite (Stephen 1986) which may leave the circulation and which also may be difficult to locate in histological sections. Hence, doubts could remain about the possible involvement of this parasite under some circumstances. There is a need for experimental work to clarify the pathology of *T. evansi* infection in buffalo.

Arboviruses

The tropical climate of Indonesia could be expected to support substantial populations of arthropod vectors of arboviruses and other infectious agents. The possibility of arbovirus infection causing transient fevers and subclinical encephalitis must be considered. Little work has been done on the effects in livestock of viruses of low pathogenicity and their specific effects in buffalo and Bali cattle are unknown. There is already evidence for some arbovirus infections of cattle and buffalo in Indonesia.

Of the flaviviruses, serological evidence has been reported for infections with Japanese encephalitis B (JBE) in cattle and buffalo (Van Peenen et al. 1974a; Olson et al. 1983), and Sepik virus (Olson et al. 1983). Different flaviviruses cause encephalitis among a range of species. JBE was the first arbovirus to be isolated in Indonesia (Van Peenen et al. 1974b). Intranasal inoculation in calves has produced a fatal encephalitis (Sullivan 1985), and the virus

has been isolated from cattle in Indonesia (Bartz, pers. comm.).

The alphavirus group also contains viruses that cause fever and neurological disease. The group is represented in Indonesia by Chikungunya virus, confirmed by viral isolation. Infections have been identified serologically in cattle (Bartz, pers. comm.). Its effect in cattle is not known. Other members of the group identified on serological grounds in Indonesia are Ross River virus and Geta.

Of the bunyaviruses, there is serological evidence that Batai virus and bunyavirus or an antigenically similar virus infect cattle and buffalo (Olson et al. 1983). Infections by Akabane (AKA), a Simbu group virus, have been identified by serum neutralisation tests in Bali and East Java (Sudana and Miura 1982), and in southern Sumatra and Lampung by HI tests (Marfiatiningsih 1983). AKA is noted for teratogenic effects, but can cause a nonsuppurative encephalitis in calves (Beveridge 1986). Its effect in adult buffalo and Bali cattle is not known. Another group member, Ingwavuma (ING), has been isolated in Indonesia and antibodies found in 35 of 79 (44%) buffalo sera sampled (Converse et al. 1985). Its clinical significance is unknown.

Of the orbiviruses, there is serological evidence for the presence of several serotypes of bluetongue (BLU) and epizootic haemorrhagic disease virus (EHD) (Sendow et al. 1986). BLU may cause inapparent infections in cattle and also mild fever with salivation, nasal discharge and anorexia.

Ephemeral fever, well known as a transient fever in cattle, has been suspected clinically by the authors on Java and serological evidence for its presence has been presented (Soeharsono et al. 1982).

Hence, among the arboviruses it is possible to list several known pathogens of cattle, as well as some of unknown clinical significance that are present in Indonesia and that have infected cattle and buffalo, as demonstrated either serologically or by viral isolation. The capacity to diagnose infections with these viruses should be established by laboratories conducting investigations of febrile diseases of unknown aetiology. It cannot be assumed without reservation that an animal with a fever in an experiment is showing a mild form of the disease under study.

Other Bovine Viral Diseases

Trypanosomiasis and arboviral infections are considered of special interest in Indonesia, and possibly constitute the main potential problems in differential diagnosis. However, other bovine diseases usually considered in the differential diagnosis of MCF should not be ignored, especially rinderpest, bovine virus diarrhoea-mucosal disease (BVD-MD) and infectious bovine rhinotracheitis (Barker and Van Dreumel 1985). Rinderpest is not present in Indonesia and from a general description (Barker and Van Dreumel 1985) it seems that rinderpest should be easily diagnosed. It would differ epidemiologically from MCF by its high morbidity and mortality rates. Syncytial giant cells with characteristic cytoplasmic inclusions are visible in histological sections and the virus can be isolated readily.

BVD infections have been identified serologically in East Java (Putra 1985), and may be widespread. These present a more difficult problem, for not only can the clinical syndrome and gross pathology be similar to that of MCF, but a fibrinoid necrotising vasculitis may be present in blood vessels of the gastrointestinal tract and the heart, brain and other organs. Massive lymphoid proliferation is not a feature, however. Animals with inapparent persistent infections may have mild perivascular cuffing and hypertrophic endothelial cells in the vessels of the brain, and show satellitosis. Although it should be possible to histologically differentiate BVD infection from MCF when associated with acute disease, it could possibly be associated with chronic infections or inapparent infections.

Other Agents

Protozoa such as *Theilaria* spp. and bacteria such as the rickettsias can cause diseases similar in some respects to MCF. As well as careful comparison of the histopathology described in association with such agents with that of MCF, careful examination of blood smears and histological sections should be conducted to ensure that the presence of agents with visible tissue forms is not overlooked. The presence in Indonesia of disease agents from these groups has not been confirmed, although an *Erlichia* sp. is suspected of being involved in Jembrana

disease (Hardjosworo and Budiarso 1973). This syndrome is not associated with clinical respiratory signs but may show fever, lymphadenopathy due to lymphoproliferative changes, and diarrhoea. Histologically, widespread lymphoreticular infiltration occurs in the liver, kidneys and other organs. Apparently, however, the central nervous system is spared.

Case Reports

Case 1. Spontaneous MCF in a *Bos indicus* (Ongole) from Banyuwangi

The animal was an adult male showing clinical signs of the head and eye form of MCF. It was sent for emergency slaughter, photographs taken of gross pathology by field staff, and tissues submitted in formalin to RIVS. The case showed typical clinical signs of moderate severity and is of local interest in Indonesia where MCF is rare in *Bos indicus* breeds.

There was profuse ocular discharge staining the cheeks, a mucoid nasal discharge and salivation. The corneas showed a peripheral rim of opacity. Prescapular and prefemoral lymph nodes were visible under the skin. At postmortem examination the lymph nodes were enlarged, there were epicardial haemorrhages along the coronary groove, but no other gross changes were recorded.

In the CNS were multiple lesions of vasculitis, perivasculitis and gliosis in proximity to vessels, progressing in places to lesions of granulomatous encephalitis.

In the lymph nodes, the cortex was prominent and there were numerous germinal centres showing depletion of cells and protein deposition. Paracortical areas were moderately expanded, and medullary cords were prominent and contained lymphoblasts. Sinuses contained prominent lymphoblasts, macrophages with haemosiderin, and histiocytes with prominent eosinophilic cytoplasm. There was marked mononuclear cell invasion of the capsule and trabeculae, and perivasculitis of medium-sized arteries. There was marked vasculitis of the vessels of the hilus. The splenic capsule was invaded by mononuclear cells and the red pulp was highly cellular.

Vasculitis and perivasculitis affected the vessels of the corticomedullary junction in the kidney, which also had an interstitial nephritis and a mononuclear cell infiltrate with plasma cells adjacent to the pelvis. The lung showed obvious vasculitis and perivasculitis, interstitial thickening and a subpleural mononuclear cell infiltrate. There was a periportal mononuclear cell infiltrate in the liver. The heart had an interstitial mononuclear cell infiltrate with blast cells. There was perivasculitis and mononuclear cell infiltration at the angle of the cornea.

The skin of the muzzle showed perivascular and papillary mononuclear cell infiltrates, and focal necrosis of the epithelium. Lesions of perivasculitis were present in the submucosa of the abomasum, and vasculitis and perivasculitis in the serosa of the intestine. Mononuclear cell infiltration of the mucosal epithelium of the bladder had occurred, and also of the smooth muscle. In the submucosa vessels showed perivasculitis, and vasculitis as evidenced by mononuclear cell infiltration of the vessel walls.

Case 2. Buffalo from an MCF transmission experiment

This buffalo was a young male inoculated with more than 1 litre of blood from a buffalo with spontaneous MCF. The animal developed a fever several weeks postinoculation, suffered loss of appetite and became progressively emaciated over a 12-month period. It became weak, was reluctant to stand, and died. There were no other marked clinical signs.

The case is presented because of the unusual clinical course. The diagnosis is important, for the case may be an indication of a cause of a wasting disease in buffalo in the field, and hence have epidemiological and disease control implications.

The brain showed perivascular cuffing and vasculitis with mixed mononuclear cells, and foci of gliosis. The vessels of the rete mirabile were mostly unaffected, some showed perivascular foci of mononuclear cells and a few a segmental vasculitis with mononuclear cell infiltration of the vessel wall.

Lymphoid tissues were reduced in size but contained active follicles. The paracortical area was usually not prominent. Subcapsular, trabecular and medullary sinuses were

comparatively empty, containing a few histiocytes and a small number of lymphocytes. Lymphoblastic cells were infrequent.

The heart showed an interstitial mononuclear cell infiltrate and periarteritis and arteritis, particularly of larger vessels. The liver had periportal infiltrates of mononuclear cells with blast cells showing occasional mitoses. The kidney showed a mixed mononuclear cell infiltrate with vasculitis and perivasculitis, particularly of vessels at the corticomedullary junction. The lung had an interstitial infiltrate of mononuclear cells including blast cells that was more marked perivascularly.

There was vesicle formation in the epithelium of the rumen. Necrotic papillae showed vascular congestion and hyaline necrosis while vessels of the submucosa showed a mild vasculitis and perivasculitis. In the serosa was a mild segmental vasculitis. Giant cells were present in the muscle. In the abomasum there was a mixed mononuclear infiltrate of the mucosa and submucosa, perivasculitis with lymphocytes, macrophages and blast cells, and marked lesions of vasculitis. One affected vessel showed a segmental necrotising vasculitis, while another was completely infiltrated by mononuclear cells with partial occlusion of the lumen.

Case 3. Bali breed male, experimental contact with sheep

The calf was 6–12 months old when placed in contact with sheep associated previously with MCF in deer. After a contact period of 100 days, the calf developed nasal discharge and diarrhoea. After 11 months of contact it was again ill for 4 days with fever of 41.2°C. Twenty-one days after the onset of this incident it was killed for postmortem examination. Its temperature was then 39.1°C.

Lymph nodes were slightly enlarged, there was ulceration of the abomasal mucosa on the folds, and there were foci of mild haemorrhage in the small and large intestines.

Histological changes were mild but present in several organs. In the CNS there were mononuclear cells adjacent to blood vessels without definite cuff formation, and distinct foci of gliosis.

Lymph nodes showed prominent germinal centres. Paracortical areas showed tingible body macrophages, but were not greatly expanded. Medullary cords showed proliferation of lymphoid cells with lymphoblasts in some foci. Subcapsular sinuses were filled with a mixed population of mononuclear cells, but the medullary sinuses were not highly cellular although containing significant numbers of lymphoblasts in some areas.

The heart had a mixed mononuclear cell infiltrate, and the kidney a mild interstitial nephritis with infiltrates of mixed mononuclear cells adjacent to blood vessels and glomeruli. In the lung there was a marked mononuclear cell infiltrate particularly around blood vessels. In the eye, a focus of perivascular infiltration at the angle of the cornea contained mitotic figures.

Sections of intestine showed lymphoid infiltrates in the serosa and submucosa, with some lymphoblastic cells. Foci with erosions or ulceration contained polymorphs also. The abomasum contained mucosal and submucosal infiltrates of mononuclear cells.

Cases 4 and 5. Clinically normal buffalo

These animals were involved in a trypanosomiasis experiment in which the effects of high and low planes of nutrition on immunological responses during *T. evansi* infections were compared (Partoutomo, unpublished data). At necropsy, both animals, which had been kept on a high plane of nutrition, were clinically normal and showed no gross pathological changes.

Case 4 had been experimentally infected with *T. evansi*.

In the brain, mild vasculitis and perivasculitis were evident, with mononuclear cells in the lumen of small vessels and also foci of mild nonsuppurative meningitis. In other tissues there were also mild to moderate changes based on infiltration of mononuclear cells. Interstitial nephritis, periportal involvement in the liver, and an intertitial pneumonia were evident. Germinal centres were active in the lymph nodes which also showed mononuclear infiltration of the capsule and trabeculae and extension of lymphoid cells beyond the capsule. Subcapsular and trabecular sinuses were highly cellular, and the medullary sinuses were becoming progressively affected. Cells in sinuses were

predominantly histiocytic, giving an appearance of early sclerotic change. Small lymphocytes were plentiful, and plasma cells and some lymphoblasts were present. The red pulp of the spleen was highly cellular, showing histiocytosis.

Case 5 was a *T. evansi*-free control

In the brain were vasculitis, perivasculitis and foci of gliosis with granuloma formation with syncytial giant cells. One such cell contained basophilic-staining rod-shaped material. There were severe foci of interstitial nephritis, a mild periportal mononuclear cell infiltrate in the liver, perivascular mononuclear cell infiltrates in the lung and focal lymphoid accumulations in the mucosa, submucosa and serosa of the abomasum. Lymph node germinal centres were active. The trabeculae were infiltrated with mononuclear cells, and the sinuses showed a marked histiocytosis progressing to sclerosis, as described in the previous case.

Discussion

This series of cases included an animal that died of spontaneous MCF, one that showed fever and recovery, another that died in an MCF transmission experiment, and others that were clinically normal during other experiments. Of the cattle and buffalo that died, the first mentioned showed a normal clinicopathological course for MCF and the other a chronic course without the characteristic clinical signs. Hence, the cases cover a wide spectrum of possibilities.

All the animals except one showed variably severe vasculitis in the CNS and infiltration of several tissues by lymphoid cells. On the basis of these changes, a diagnosis of MCF could initially have been considered. However, the full clinicopathological spectrum must be taken into account in each case, and consideration given in the differential diagnosis to other diseases as reviewed.

Case 1 was characterised by clear lesions of vasculitis involving mononuclear cell infiltration of vessel walls, and by lymphoproliferative changes, and so was diagnosed as MCF. However, there was no fibrinising vasculitis, and haemorrhagic and necrotic changes at postmortem were minimal. Corneal opacity was restricted to the periphery, and nasal discharge was mucoid rather than mucopurulent. Hence, the case more closely resembled the syndrome described by Hoffmann et al. (1984) in buffalo. Lymph node changes were not in exact conformity with the picture usually seen in MCF.

In the authors' experience, the capsule and trabeculae are frequently infiltrated by mixed lymphoid cells during MCF. Germinal centres are few or absent, and the cortex is expanded with pronounced paracortical areas due to lymphoid proliferation that histologically extends into the medullary cords. The sinuses are filled with a mixed population of mononuclear cells in which pleomorphic lymphoblasts with a high mitotic index are prominent, and also macrophages with a clearly delineated cytoplasm that may contain phagocytosed material.

The sections of node examined in the current case differed from this description in having active germinal centres and a greater proportion of histiocytic cells in the sinuses. The possibility that the lymphoproliferative changes were superimposed on another disease process may be considered, or note taken of the variability.

Case 2 also showed lesions of vasculitis and lymphoid infiltration of organs, but contraction of the lymphoid organs and an unusual clinical course involving a progressive wasting disease over a 12-month period. MCF could be suspected on the basis of the history of infection by blood transfusion followed by lesions of severe, necrotising vasculitis in several organs, but Barker and Van Dreumel (1985) have warned that such vascular lesions with an organ distribution as in the present case and unaccompanied by lymphoproliferative changes may occur in mucosal disease.

Laboratory examination of such cases should check their virological and serological status for evidence of superinfection with cytopathic BVD virus in an immunotolerant animal, as described by Brownlie et al. (1984). BVD antigen should be detectable in tissue sections by immunohistochemistry.

CNS changes and progressive emaciation have also been reported associated with a retrovirus infection of cattle, now designated bovine immunodeficiency virus (BIV) (Gonda et al. 1987). The full range of pathological changes associated with BIV infections has not been described. Suspect retroviruses have been

isolated from buffalo in Indonesia (Sudarisman et al. 1986), and serological results indicate infections may be reasonably common. Wasting disease is seen frequently in buffalo in that country (Unruh, pers. comm.). Firm identification of the agent and a study of its effects in buffalo are urgently needed. Investigations of wasting diseases in buffalo should consider not only this virus but also BVD, MCF and trypanosomiasis in the differential diagnosis.

Case 3, on the basis of its exposure to sheep implicated in a previous outbreak and clinical signs at various times of fever, nasal discharge and diarrheoa, invites a diagnosis of mild MCF. Gross pathological changes of some lymph node enlargement, abomasal lesions and mild haemorrhagic lesions in the intestines could be consistent with this hypothesis, but the histological changes were not sufficient to support the diagnosis. CNS lesions were of focal gliosis rather than primary vascular changes. There were interstitial and perivascular infiltrates in the lung, heart, kidney and eye, but these were mild. Lymph node histology, although not conforming to that expected in fulminating MCF, did show proliferative changes in the medullary cords and an increased number of lymphoblasts in the sinuses. These early lymphoproliferative changes were not accompanied by evidence of vasculitis.

In the first instance, alternative diagnoses should be sought. A range of infectious agents known to be present in Indonesia has been reviewed in the introduction. Pre-exposure and convalescent sera are available from this animal, and the immediate task is to develop the serological capacity to investigate infections with the agents listed. It is probable that episodes of fever and diarrhoea in Bali cattle have varied aetiologies.

Cases 4 and 5 involved clinically normal buffalo that showed essentially similar histopathological changes, although infected and uninfected with *T. evansi*, respectively. Although the cerebral granuloma associated with rod-shaped material may indicate a specific cause of the CNS changes in the control animal, the changes in other organs, particularly the lung, kidney, spleen, and lymph nodes were similar in each animal. Similar changes were present in other buffalo in this experiment, although the CNS changes were the least pronounced (Damayanti, unpuplished data).

The changes were not sufficiently severe to indicate MCF, but it should be remembered that the animals were clinically healthy. The question must be raised whether subclinical infections with SA-MCF agent occur, with clinical disease being precipitated in only a few instances by presently undefined factors. This would be consistent with epidemiological observations that, although many animals of susceptible species are exposed to the reservoir host, only sporadic cases of MCF are seen. However, in the case of WA-MCF, serological surveys have shown that infections by AHV-1 are not widespread in normal cattle populations exposed to wildebeest (Rossiter et al. 1980). It has not yet been shown whether it is appropriate to extrapolate from such observations of WA-MCF and AHV-1 infections to SA-MCF.

Lymph node changes showing germinal centre activity and sinus histiocytosis leading to sclerosis were not consistent with those normally seen in MCF, a lymphoproliferative disease. Rather, the lymph node changes were as described in trypanosomiasis (Ladds 1986). Changes in other organs were also consistent with *T. evansi* infections as reviewed above. Trypanosoma-free animals for the experiment were obtained by chemotherapy of animals with Naganol (Suramin, Bayer), and so there is the possibility that the lymph nodes continued to receive antigenic stimulation from phagocytosed material, and that the cerebral granuloma was in response to dead parasites in the tissue rather than to an undiagnosed bacterial infection.

Other possible viral aetiologies can also be considered. The changes resemble a little those reported in persistent BVD infections (Barker and Van Dreumel 1985). In that case, future immunohistochemical studies should identify the presence of the virus. As also discussed in the previous case, possible effects of retrovirus infection must also be investigated.

The discussion of the pathological changes recorded in this paper has not yet been supported by broadly based serological studies or attempted identification of a range of viruses to clarify the status of the animals under review, and so is somewhat speculative. However, such a discussion constitutes a necessary step in the

process of differential diagnosis. A number of avenues for future investigations have been identified.

Laboratory Submissions in Suspected Cases of MCF

A prerequisite for the diagnosis of any disease is a thorough investigation and analysis of all the circumstances surrounding its occurrence, the history, including observations of epidemiological importance, the clinical signs and the findings at necropsy, the histopathological changes and the results of other appropriate laboratory investigations. These notes are not intended as a substitute for normal procedures, but are intended to highlight some aspects to which particular attention should be paid.

Epidemiological Factors

In all suspected cases of MCF, note should be made of whether contact with sheep or wildebeest was possible in the year preceding the onset of disease. It is of value to note movements of groups of livestock at the site, either suspect reservoir species or the affected species, and any births. Notes may be made of the seasonal conditions, and whether the animals involved were under any stress, such as nutritional, climatic, or associated with work or production.

Clinical Signs

Full descriptions of clinical signs may be of help not only in reaching a provisional diagnosis, but also in helping others to subsequently analyse the significance of the disease event in relation to other similar events. The progression of signs during the course of the disease should be noted. Where MCF is common or where reliance must be placed on paraveterinary field staff, it may be helpful to have a checklist of necessary observations.

The Postmortem Examination

Again a detailed and systematic protocol of observing and recording is necessary. Different diagnostic laboratories may vary in their requirements for specimens from necropsies conducted in the field, but the following recommendations can be offered, bearing in mind that a diagnosis can be made on consideration of lymphoproliferative changes and vasculitis.

A consideration of bovine lymph node drainage patterns (Ladds 1986) shows that the prescapular (superficial cervical) node and the prefemoral (subiliac) node do not drain visceral organs either directly or indirectly, and may therefore contain primary changes of the lymphoid tissue rather than changes secondary to inflammation elsewhere. An added advantage of these nodes is their superficial location making them easy to collect early in the course of the postmortem examination. For routine examination, a transverse section 1 cm wide through the whole node at its mid-point has been found a useful specimen for formalin fixation.

The brain must always be sampled, for the vascular lesions in this organ can be of diagnostic importance. If the whole brain cannot be fixed in formalin for submission to the diagnostic laboratory, then sections through the whole organ at various levels should be made. Sections through the anterior cord, the cerebellum and brain stem, the posterior calliculis (the swelling anterior to the cerebellum lying beneath the posterior aspects of the cerebral hemispheres), and the cerebrum give a representative sample.

Experience has shown that the vascular plexus on either side of the pituitary gland—the rete mirabile—is an extremely useful tissue in which to examine vascular lesions (Liggitt and De Martini 1980a). The tissue is very easy to collect, requiring only a pair of forceps and a scalpel, and is easy to locate, lying on the floor of the cranial cavity immediately beneath and attached to the fascia that contains the pituitary foramen, immediately posterior to the optic chiasma. It can be dissected free from the floor of the cranial cavity by making incisions 1 cm laterally to the foramen, and anteriorly and posteriorly to it, followed by separation of the vascular plexus from the underlying bone (Fig. 1). The plexus is removed attached to the pituitary gland, with both supported by the fascia, and placed in fixative in this fashion.

Fixed tissue is also needed from the parenchymal organs including lung, heart, liver,

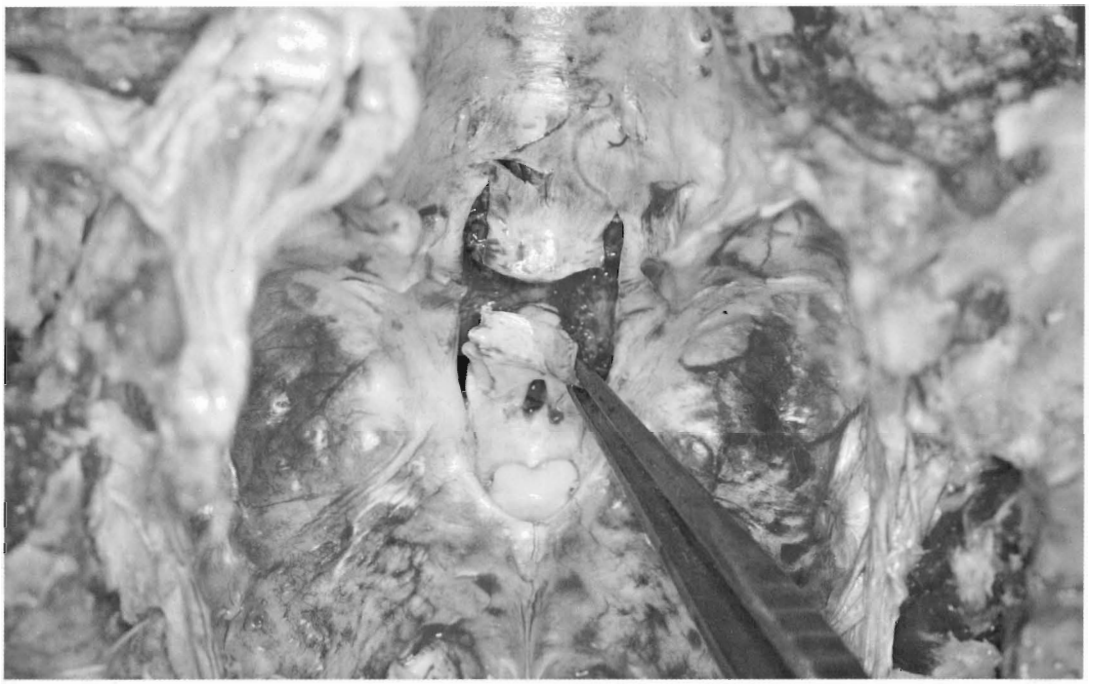

Fig. 1. Removal of the pituitary gland and associated vascular plexus from beneath the fascia of the floor of the cranial cavity.

adrenal and kidney, even if gross changes are not evident. The section of kidney should include a wedge of tissue from the capsule through to the pelvis. This is considered important as in some species the vascular changes are not distributed evenly throughout the organ. Following examination of abomasum, intestines and urinary bladder, specimens should be collected where appropriate, and from other tissues showing gross lesions. Within the constraints as discussed for certain organs, small pieces of tissue that the fixative will penetrate quickly are best, and make for the most economical submission.

Histological Examination

For diagnostic purposes tissues will be examined, noting particularly evidence of vasculitis. The vascular plexus removed with the pituitary gland is very useful, for here the vessels can be observed supported only by a fine connective tissue stroma, facilitating the examination of primary vessel changes free of the influence of other tissue changes (Fig. 2).

Note must also be made of lymphoproliferative changes. The patterns of infiltration in various organs can be examined, and of the cellular composition of the infiltrates which in MCF are normally described as containing lymphoblastic cells.

The range of pathological changes that can occur in bovine lymph nodes under various influences has been extensively described by Ladds (1986). In examining nodes histologically, the following routine has been found helpful. Using alternatively low and high power magnification, the features of the node are examined in turn, beginning with the supporting structures, the capsule, trabeculae and major blood vessels. The features of the lymphoid component are noted, including germinal centre activity and features of the paracortex. Finally, the subcapsular, trabecular and medullary sinuses are examined for cellular content, and the cell types described.

Serology

The agent of SA-MCF has not yet been identified or isolated, and so there is not yet a serological test that can aid in diagnosis. However, whenever possible it is recommended

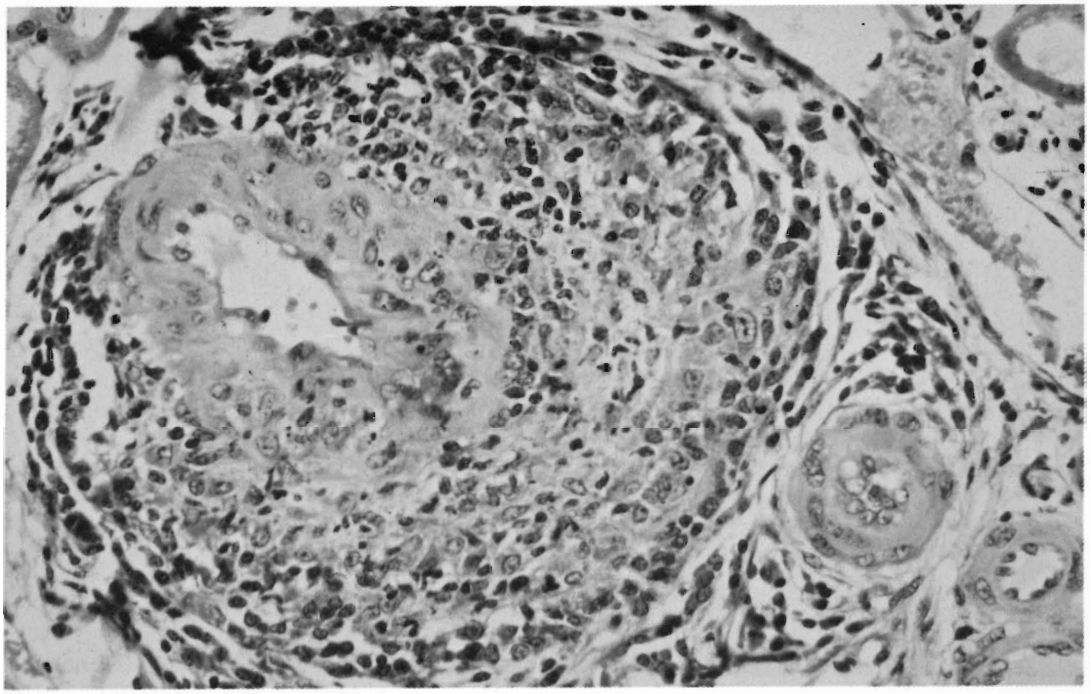

Fig. 2. Histological section through the vascular plexus removed with the pituitary gland: this tissue is useful for determining the presence of MCF-associated vasculitis.

that in areas where MCF is recognised frequently sera from clinically affected animals be submitted to the regional laboratory. Depending on laboratory policy such sera can be stored or fowarded to a reference laboratory for research purposes involving the seeking of serological evidence for the involvement of suspected infectious agents. Field staff should not feel that the collection of such sera is a waste of time, for when collected in some numbers over a period of time they constitute a valuable resource in the task of unravelling the mystery of MCF.

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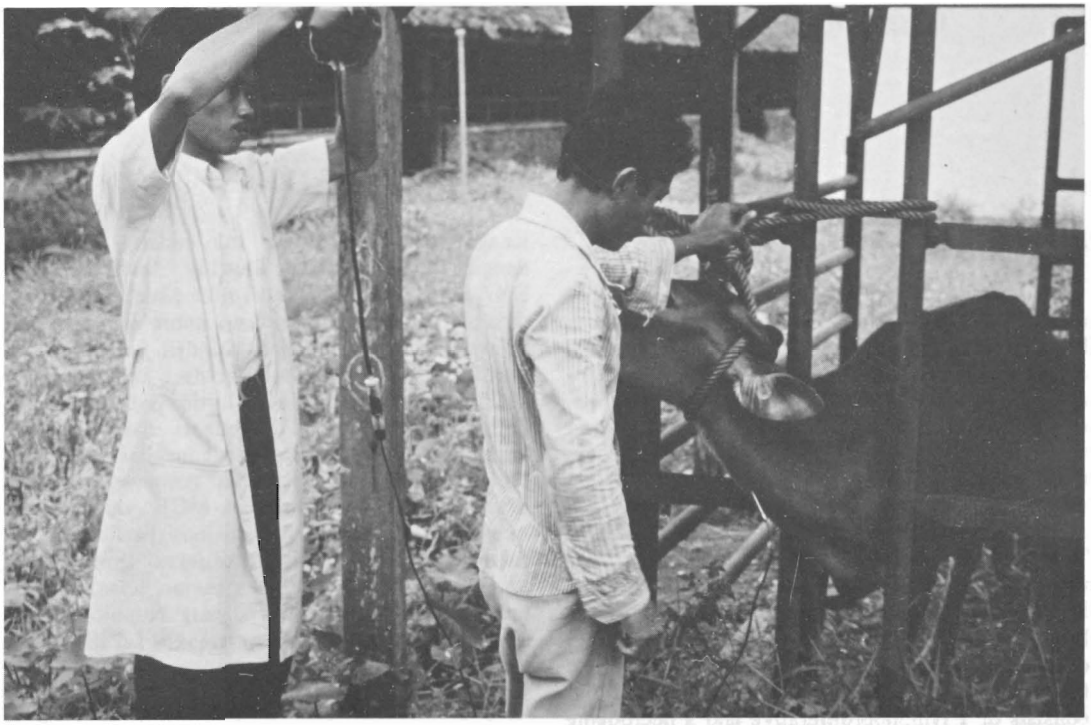

Aetiology

Current Malignant Catarrhal Fever Research in the United Kingdom

H.W. Reid*

Abstract

Malignant catarrhal fever (MCF) has a worldwide distribution. Research in the United Kingdom has been directed towards defining the similarities and differences of the wildebeest associated (WA-MCF) and sheep-associated (SA-MCF) forms. Transmission of both forms to laboratory animals has been achieved. Certain aspects of WA-MCF infections in these animals resemble SA-MCF in cattle. Sheep and goats have some antibodies to the WA-MCF agent, as do some cattle infected with SA-MCF. These serological reactions are being analysed using the Western Blotting technique. The virus DNA of the WA-MCF agent has been cloned, and is being used in hybridisation studies to demonstrate cross-reacting DNA in SA-MCF infected cells and tissues. The pathology of both forms of MCF consists of a lymphoproliferative and a necrotising component. The pathogenesis of these effects may be explained by the properties of large granular lymphocytes isolated from cases of SA-MCF, which can transmit the disease. These cells produce a factor that stimulates lymphoid proliferation, and also show marked cytotoxic activity. These cells appear to contain DNA homologous to the WA-MCF agent, and are probably the key to understanding this difficult disease.

Abstrak

Malignant catarrhal fever (MCF) tersebar luas di dunia. Penelitian penyakit ini di Inggris yang ditujukan untuk penentuan persamaan dan perbedaan antara wildebeest associated (WA-MCF) dengan sheep associated (SA-MCF) telah dilaksanakan. Penularan kedua bentuk penyakit tadi ke hewan percobaan telah berhasil. Beberapa aspek dari infeksi WA-MCF pada hewan-hewan tadi mirip dengan SA-MCF pada sapi. Domba dan kambing mempunyai zat kebal terhadap WA-MCF, demikian juga beberapa sapi terhadap SA-MCF. Reaksi-reaksi serologis ini sedang dianalisa dengan tehnik 'Western Blotting'. Demikian juga DNA virus WA-MCF telah diklonkan dan sedang dipakai dalam studi hibridisasi untuk menunjukkan infeksi silang DNA dalam sel dan jaringan yang terinfeksi SA-MCF. Patologi dari kedua bentuk MCF tersebut terdiri atas limfo proliferasi dan komponen nekrosi. Patogenesis dari efek ini mungkin dapat dijelaskan dengan sifat-sifat yang terdapat pada 'large granular lymphocites' yang diasingkan dari kasus SA-MCF yang dapat menularkan penyakit. Sel ini menghasilkan suatu faktor yang merangsang proliferasi limfoid dan juga aktivitas sitotoksisitas secara jelas. Sel-sel tersebut mengandung DNA yang homoloh untuk agen WA-MCF, karenanya mungkin sekali hal ini merupakan kunci penjelasan penyakit yang sulit ini.

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Introduction

This contribution concentrates on laboratory aspects, for until the aetiology is identified diagnosis cannot be precise and optimal control measures cannot be found. After an overview of the malignant catarrhal fever (MCF) situation worldwide, serological data will be examined for information they may yield on the possible aetiology. Viruses isolated from cases of MCF will be considered and an attempt made to construct a unifying hypothesis that could account for some of the the enigmas of this disease.

The World Situation

MCF has a worldwide distribution, and is encountered wherever cattle husbandry is practiced. In some countries, logistical problems in the veterinary diagnostic services may have led to the disease not being widely reported. Through most of its distribution it is a sporadic disease of cattle affecting only a few animals,

but in most countries so affected, such as Australia, the United Kingdom (UK) and the United States (USA), outbreaks involving many animals have been reported. Up to 200 animals have been involved on a single farm in Australia, and there have been several outbreaks in the UK involving over 10 animals.

MCF has a peculiar importance in East Africa, in the great grasslands where wildebeest share grazing with cattle. In this situation, large numbers of cattle die. The wildebeest have been shown to harbour a herpesvirus which can be transmitted to cattle and cause MCF. Throughout most of the world, however, wildebeest are an uncommon species, and epidemiological data indicate that sheep is the host.

This sheep-associated form of the disease is a particular problem in New Zealand where deer farming is important. Deer have an extreme susceptibility and MCF has been recognised as the most serious infectious disease of these animals. From the information presented in this volume, it seems that the Bali cattle of Indonesia have a similar susceptibility.

Aspects of the Natural Disease

The wildebeest is the natural host of one form of the disease caused by Alcelaphine herpesvirus 1 (AHV-1). The sheep-associated agent has not yet been identified. AHV-1 may cause disease naturally in a wide range of ruminants, as can the sheep-associated agent. The wildebeest-associated form (WA-MCF) occurs not only in Africa but also in zoological gardens keeping wildebeest, and the sheep-associated form (SA-MCF) occurs throughout the world, including areas of Africa where the former occurs.

Transmission

Experimentally, WA-MCF can be transmitted from affected cattle and from clinically normal wildebeest to cattle, deer, rabbits, hamsters, guinea pigs and rats. Virus may be recovered from diseased animals other than hamsters or rats.

Transmission of SA-MCF is difficult. Cattle to cattle transmission is usually not successful, although work in Indonesia shows Bali cattle are more readily infected, as are deer. Using blood inoculation, SA-MCF can be transmitted from deer to rabbits, and from rabbit to rabbit. Only rarely can the disease be transmitted from cattle to rabbits although, once achieved, rabbit to rabbit passage is readily maintained.

Once WA-MCF experimental infections in guinea pigs, hamsters and rats, established from rabbits, are adapted to these rodent species, disease can be passaged from animal to animal, although it is not possible to passage back to the rabbit or to recover virus. Thus, in these cases the virus has adapted itself to the new host species, possibly in an incomplete form. The presence of the virus can be demonstrated only by passage of disease from animal to animal, with histopathological confirmation.

This may be considered analogous to the situation seen with SA-MCF. From cattle, disease can be passaged only irregularly, but from deer it can be passaged readily to other deer and to rabbits, and from rabbits to hamsters, but not to other rodent species. SA-MCF is not readily transmitted from rabbits back to deer. Transmission from cattle to rabbits is relatively unusual, presumably because the virus in cattle is incomplete.

In experiments in the UK, transmission of SA-MCF was achieved three times from 16 bovine field cases. In the first case, transmission was to a bovine but not to rabbits. Attempted second passages to cattle, rabbits and deer were not successful. In the second case, transmission to cattle and deer was unsuccessful, but successful to rabbits. Further passages to rabbits and hamsters were achieved and once the disease was established it could be readily passaged through rabbits but not back from the rabbit to cattle. On the third occasion, passage was to a second and then a third bovine, from which it was passaged to rabbits but not to deer.

These experiences are interpreted as indicating that the virus may be in a form that does not represent the whole virus; perhaps the portion of the virus retained determines whether transmission can be achieved or not. No infectious virus can be identified in these situations.

Serology

All wildebeest have neutralising antibodies to

AHV-1. From this information, certain deductions can be made. The virus must be readily transmitted in this species. Wildebeest never show disease associated with this infection, either in captivity or in open grazing, so there is a very efficient and stable host–parasite relationship.

Other species of large antelope also have neutralising antibodies to AHV-1, indicating that they also harbour this or a closely related agent. In confirmation, herpesviruses similar to the WA-MCF virus have been isolated from some of these animals, and these isolates also cause MCF on inoculation into cattle. However, there is no evidence that transmission of these viruses from other antelope species to cattle occurs naturally. MCF has never been observed to occur naturally where cattle are grazed with these other antelope species.

There is a large body of other African species of ruminants that have no neutralising antibody to AHV-1 but are positive using the indirect immunofluorescent (IIF) test, a test known to be highly cross-reactive. In cattle, a certain amount of nonspecific cross-reactivity has been found, but most sheep appear to have antibody (Rossiter 1981). The sheep that were negative in Rossiter's test were from specific pathogen-free sheep produced at the Moredun Institute by caesarian delivery and reared in isolation.

Subsequent work at Moredun has confirmed Rossiter's observation. Sera from many countries, including Iceland, Peru, USA, Greece, Africa and the UK, have shown low levels of antibody to the WA-MCF virus. This has been interpreted as evidence that there may be an antigen infecting sheep which is antigenically related to AHV-1. Not only sheep have this antibody, but also goats, oryx and various other species related to sheep, indicating that these other species may also be infected with related herpesviruses.

The observations have been further augmented using the Western Blotting technique. Wildebeest sera clearly detect a number of AHV-1 antigens, while sheep sera detect a proportion of antigens detected by wildebeest sera. This is powerful evidence that sheep are infected with a herpesvirus related to AHV-1. If serum from a bovine vaccinated with an inactivated form of AHV-1 and then experimentally infected with AHV-1 is similarly examined, after having suffered clinical MCF, only a few of the antigens detected by the wildebeest sera react with this bovine serum.

A large number of bovine sera from clinical cases of SA-MCF have been tested in the Western Blotting Test. Most give no reaction, but a few detect one of the AHV-1 antigens. Deer with SA-MCF in the UK have not shown any such antibody. One deer in New Zealand that apparently recovered from clinical MCF and suffered a fulminating relapse 3 months later did develop some antibody against the same antigen as detected by the bovine with SA-MCF.

These observations demonstrate an important point in analysing the antibody response of animals with SA-MCF, particularly those that are extremely susceptible. They may not be reacting with an antibody response to any of the antigens present on the AHV-1 virus. They may, however, be reacting with antibody to other antigens on the sheep-associated agent not shared by AHV-1.

More evidence pointing to the nature of the agent has been found in serological examinations of experimentally infected rodents. All rabbits reacting to AHV-1 infection produce antibody to the virus, but no antibody to AHV-1 has been found in rabbits successfully infected with SA-MCF.

However, hamsters reacting with SA-MCF develop IIF antibody to AHV-1. Confidence can be placed in this observation because the fluorescence appears quite specific, with antibody attaching to the nucleus of infected cells. So there is evidence that a proportion of rodents infected with SA-MCF react to an antigen common to the WA-MCF agent. This further confirms that, in the UK at least, the SA-MCF agent is antigenically related to the wildebeest virus.

Possible Nature of the Sheep-associated Agent

Based on the observations as outlined it can be hypothesised that the SA-MCF agent belongs to the gamma group of herpesviruses. These include Epstein-Barr virus, the cause of infectious mononucleosis in humans as well as Burkitt's lymphoma in Africa and

nasopharyngeal carcinoma in Asia. Other viruses in this group are Mareks disease virus, a virus of rabbits, simian herpesvirus and AHV-1. A consideration of the histopathology suggests that SA-MCF is lymphotrophic, which supports inclusion of the agent in this group.

Work is progressing on proving this association by studies of the viral DNA. Analysis of AHV-1 DNA shows it to be very similar in nucleotide patterns to other gamma herpesviruses. Most of the AHV-1 viral DNA has been cloned. When some of these clones were used in hybridisation studies with tissue of animals dying of MCF, preliminary results indicated the presence of DNA homologous to that of AHV-1. Development of these studies is necessary to prove that a similar herpesvirus is present in sheep. In the process, a very potent diagnostic tool, able to detect the presence of virus in field samples, will be developed.

Pathogenesis and Pathology

When animals are infected with AHV-1 there is lymphoproliferation from the time of inoculation. There is no evidence of the necrotising lesions until the animal reacts clinically, with a febrile response. However, if animals are treated with Cyclosporin A, a potent T-cell suppressor, from the time of inoculation the lymphoproliferative response is prevented, but the animal still develops MCF with the necrotising lesions after the same incubation period.

Hence, it seems that the lymphoproliferative component of WA-MCF is separate from the necrotising component, and is essentially benign, while the necrotising lesions are the important part of the disease.

Virus Isolations

A number of viruses have been isolated around the world from cases of SA-MCF but it must be stressed that none of these has been able to reproduce disease. It will probably turn out that these agents have been isolated gratuitously, and that they are not aetiologically associated with the disease. At Moredun, no viruses considered to be important in the study of the disease have been isolated.

Cell Cultures

There has been considerable success in the study of SA-MCF in the culturing of cells from infected laboratory animals, deer and cattle. The cells, which cannot be isolated from control animals, have the morphology of large granular lymphocytes, and have been recovered from a large range of tissues including cerebrospinal fluid. In the UK, this has emerged as a consistent phenomenon of SA-MCF, that large granular lymphocytes can be isolated in culture from the tissues of affected animals.

In many cases, these cells can transmit the disease, and there is evidence of DNA homologous with AHV-1 in these cells. It is considered that granular lymphocytes may play an important role in the development of MCF.

Large granular lymphocytes are also known as natural killer (NK) cells, and are now thought by immunologists to be a primitive type of T-cell active in the early stages of immune responses, being cytotoxic to virus-infected cells and transformed cells. The activity of NK cells is regulated by interleukin 2 (IL-2), one of the substances recognised as having a vital role in the control of the immune system. Activated T-cells and NK cells generate IL-2, which then acts on T-cells to give T-cell proliferation. It also acts back on the NK cell further activating it to become cytotoxic for transformed cells. The supernatant from cultures of cells isolated from animals with MCF causes a blastogenic response in normal bovine lymphocytes, indicating that the isolated cells are secreting a substance with activity similar to IL-2. Also, the cultures have been demonstrated to have natural killing activity against cultures of red deer testes cells and lamb kidney cells. Chromium release assays show effective killing in a short time at relatively low effector to target cell ratios. So these presumed virus-infected NK cells are killing normal cells, which is an unusual situation.

NK activity in the lymphoid tissues of normal and SA-MCF infected rabbits has been examined. Cells from a normal adult show no such activity, but cytotoxic cells can be found in animals starting to react clinically. If it is remembered that the onset of clinical disease coincides with the development of necrotising lesions, there is therefore an association with the

development of such lesions and the onset of NK activity.

A Hypothesis

In developing a hypothesis for the pathogenesis of MCF, the wildebeest- and sheep-associated forms have been considered as being essentially the same. It seems that the virus preferentially infects NK cells, which then secrete a lymphoproliferative factor, presumably IL-2. This drives the proliferative component of the disease from the start of the incubation period. As stated, this is an essentially benign response that can be controlled with cyclosporin A. However, the infection goes on to affect the NK cells in such a way that they become deregulated and kill normal cells. Experimentally, high levels of IL-2 have induced this effect in NK cells, and it may be that this also occurs in the diseased animal.

Discussion

Does such a hypothesis answer the enigmas found in MCF? Although virus replication can be found in WA-MCF, it cannot be detected in SA-MCF. In terms of the above hypothesis, this may be acceptable, if the situation is comparable to that seen with other herpesviruses. *Herpesvirus saimiri* also affects large granular lymphocytes, and as little as 30% of the virus genome has been shown to be retained in *H. saimiri* transformed cells.

There is no lateral transmission from affected cattle to other cattle and no infective virus can be demonstrated.

Only with lymphoid cells of affected animals can the disease be transmitted, and here only irregularly, suggesting that only a portion of the virus is present in infected cattle. In spite of the lack of virus, there is widespread cell proliferation which is similar in some respects to an oncogenic response, but can be seen to be a hyperplastic normal immune reaction probably driven by IL-2. We should therefore be seeking to identify a small piece of viral genome within only a proportion of cells if we are to resolve MCF.

This summarises the direction MCF research is taking at the Moredun Institute, and the direction that is most likely to be profitable in defining the SA-MCF agent. It is hoped that these thoughts will be of benefit to the research currently being conducted in Indonesia.

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Virological Investigations of Malignant Catarrhal Fever in Indonesia

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Abstract

As malignant catarrhal fever (MCF) in Indonesia is a disease with serious economic consequences, it is important to isolate and identify the causative agent to allow improved diagnosis, control and prevention. Transmission experiments have proven the disease to be infectious. Viral isolation has been attempted from clinically affected animals and in-contact sheep. Syncytia-forming viruses have been isolated from buffalo with MCF, and subsequently also from healthy buffalo. Their significance is not yet determined. Many buffalo have antibody to this virus, but usually not Bali cattle, including those with MCF. Antibody to the agent of wildebeest-associated MCF has not been detected in animals with MCF in Indonesia.

Abstrak

Malignant catarrhal fever (MCF) di Indonesia mempunyai arti ekonomi yang sangat besar. Karenanya usaha isolasi dan identifikasi agen penyakitnya untuk pengembangan diagnosis, pengendalian dan pencegahan perlu diusahakan. Dari hasil percobaan penularan telah terbukti bahwa penyakit ini infeksius. Isolasi virus penyebab penyakit telah dicoba dari hewan yang secara klinis sakit MCF dan hewan yang kontak dengan domba. Percobaan isolasi, baik dari kerbau sakit MCF atau kerbau sehat, telah berhasil menemukan virus sinsisial. Namun sampai saat ini pentingnya virus tadi dalam MCF belum ditentukan. Banyak kerbau yang dalam tubuhnya mengandung zat kebal terhadap virus tersebut, tetapi sebegitu jauh tidak dalam tubuh sapi Bali, walaupun sapi itu terserang MCF. Sampai saat ini, hewan-hewan MCF di Indonesia, sebegitu jauh belum ada yang mengandung zat kebal terhadap virus WA-MCF.

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Introduction

Malignant catarrhal fever (MCF) is an economically important disease in Indonesia, and in epidemiological terms is the sheep- associated form (SA-MCF) (Plowright 1984). The agent of SA-MCF has not yet been isolated and identified anywhere in the world, although Plowright (1984) has discussed the serological evidence that an agent similar to alcelaphine herpesvirus 1 (AHV-1) that causes wildebeest-associated MCF (WA-MCF) in Africa may be involved.

Various viruses have been isolated from cases of SA-MCF, including bovine syncytial virus (BSV) (Clarke et al. 1973; Storz et al. 1976), enteroviruses and a parvovirus (Storz et al. 1976), morbilliviruses (Coulter and Storz 1979), bovine herpesviruses different from bovine rhinotracheitis virus (BHV-1) (Liebermann et al. 1967; Storz et al. 1976; Storz 1978), and a herpesvirus believed to be closely related to AHV-1 (Hamdy et al. 1978). Another herpesvirus was isolated from a bison with MCF, and this isolate as well as others reported previously (Liebermann et al. 1967; Storz et al. 1976) was considered to have the properties of cytomegaloviruses (Todd and Storz 1983). No isolated virus from cases of SA-MCF has produced the disease on inoculation into susceptible animals. Togavirus-like particles have been seen by electron microscopy in MCF-affected deer tissues (Clark and Adams 1976), and reoviruses, adenoviruses and syncytiogenic viruses have been isolated from sheep associated with cases of MCF in cattle (Snowdon and French 1975; Snowdon 1985).

Adequate control measures for infectious diseases are usually based on a thorough knowledge of the aetiological agent, its

epidemiology and pathogenesis. In some cases, isolation of the agent opens the possibility of developing a vaccine. Hence, isolation of the agent of SA-MCF in Indonesia has been attempted from both natural and experimental cases of MCF, and associated serological studies conducted. The intention in this paper is to give an overview of the techniques used, and a preview of results, some of which are being used by the senior author for a PhD program at Institut Pertanian Bogor.

Transmission Experiments

The transmission experiments have been outlined in a earlier paper in this volume. MCF occurred spontaneously in Bali cattle *(Bos javanicus)* penned in close proximity with lambing small ruminants. The disease was passaged by blood transfusion to other Bali cattle, and to a smaller number of swamp buffalo *(Bubalus bubalis)* and ongole cattle *(Bos indicus)*. In each case, sera were collected prior to infection, weekly postinfection and at regular intervals during the course of the disease, and stored at −22°C. Buffy coat cells were also collected and stored in a viable state in a freezing medium containing 20% foetal calf serum and 10% DMSO.

The experiments have yielded valuable information regarding the aetiology by confirming the involvement of an infectious agent, confirming the disease under examination to be MCF, and providing material for subsequent laboratory studies.

Viral Isolation

Cell Culture

A prerequisite for viral isolation is a cell culture system in which the agent under study will grow. The agent of WA-MCF can be isolated in primary bovine thyroid cultures, but isolation in conventional cell culture of the agent of SA-MCF has not yet been reported. Hence, the first phase of the investigation was not only to produce primary bovine cells as used successfully for the isolation of other viruses, but also to attempt the development of other culture systems that might be useful.

In cattle, the agent is present in the blood. This is known from the results of the transmission experiments. The pathology in cattle is associated with proliferative changes in mononuclear cells, which are represented in the blood by lymphocytes and monocytes. Hence, it was hypothesised that the agent may be present, and may even replicate in such cells in the affected animal, and may be adaptable to such cells in culture. Monocytes growing as monolayers is a system that is easy to propagate once established (Asagba et al. 1981) and so was considered for isolation attempts.

In sheep, the presumed reservoir host, excretion of the agent is not accompanied by the development of clinical signs. Hence, there is at present no suggestion of a susceptible ovine tissue. Presumed periods of alternating latency and excretion suggest that it is in equilibrium with the immune system of the sheep. A virus may achieve this biological state by infecting cells of the immune system, and so appropriate cell cultures were again sought by developing cultures from lymphoid tissues, in particular foetal sheep thymus cells, sheep macrophages and foetal sheep macrophages.

The slaughter of female cattle is prohibited in Indonesia, and so bovine foetuses are not readily available. This largely precluded the establishment of primary bovine cells, and so most of the viral isolation work was attempted in ovine cells, although blood macrophage cultures from a Bali cross bull were propagated and used. One buffalo foetus became available, and primary cultures including thyroid cultures were established.

Collection and Storage of Samples

WBCs and sera were collected before, during and at the termination of transmission experiments, and from spontaneous cases of MCF. Samples for sera were allowed to clot at room temperature (approximately 27°C) overnight, separated, clarified by centrifugation, and stored in the RIVS serum bank. Blood for WBCs was collected in heparin, and the cells separated by ammonium chloride lysis of erythrocytes, followed by washing in PBS or physiological saline. Cells were suspended in medium (MEM with 5% FCS) for immediate use, or stored in ampoules in MEM containing

10% FCS and 20% DMSO in liquid nitrogen or at −70°C.

Cocultivation and Inoculation

Virus isolations were attempted by cocultivation of white blood cells from MCF cases with monolayers and cell suspensions as described above.

WBCs were those taken from MCF cases during the febrile period when the disease was transmissible. Cell suspensions from lymph node, thyroid and spleen were prepared postmortem and cocultivated with monolayers as above.

On two occasions, from sheep in Kupang, West Timor, and from the sheep involved in the natural transmission experiment, nasal swabs were used in viral isolation attempts.

From five field cases of MCF in buffalo and from the buffalo in the natural contact transmission experiment during its febrile period (Daniels et al. 1988), syncytia-forming agents were isolated from WBCs. The cytopathic effect in each case was of multinucleate syncytia formation with little or no vacuolation of cytoplasm. A range of cell culture types was subsequently infected (Table 1).

No similar isolates have been obtained to date from similar samples taken from Bali cattle with MCF. One strain of syncytial virus has apparently become cell-free after the 13th passage, an observation which has yet to be confirmed. No viruses other than the syncytial viruses were isolated. Viable WBCs from cases are still being stored for subsequent attempts.

Autocultures

Cell culture techniques were also used in attempts to grow the agent directly, by establishing cultures of cells from MCF cases. No success has been achieved in attempts to grow monocytes from clinical cases in monolayer cultures. Primary cultures of testes, spleen and lymph node cells were established from some Bali cattle, but did not survive.

Electron Microscopy (EM)

EM has been investigated as a means of locating the agent. Suspensions of white blood cells from animals with clinical disease were examined for the presence of viral particles. In one Bali bull, particles resembling retroviruses were seen.

Serology

Cattle with WA-MCF develop IIF antibodies to AHV-1 and antibodies have been detected in nearly all wildebeest examined (Plowright 1984). Hence, there is interest to know whether animals with SA-MCF have serological evidence of infection with this or a related virus, for there would then be a means of confirming diagnoses. Detection of similar antibodies in the suspect reservoir host may give a means of identifying potential disseminators of the disease.

Indirect Immunofluorescence (IIF)

An adaptation of the IIF test for antibody to the WC-11 strain of AHV-1 as described by Rossiter et al. (1977) was used. WC-11 virus (a gift from Dr Neil Edington) was grown in MDBK cells on 12-well teflon-coated spot slides until CPE was observed. Slides were fixed in acetone and stored at −20°C until use.

Table 1. Isolations of syncytiogenic viruses.

Animal	Clinical status	Primary cell inoculated	CPE at passage
Buffalo (Ciawi)	MCF	Sheep foetal thymus	3
Buffalo (Boyolali)	MCF	Sheep foetal thymus	2 (in sheep macrophages)
Buffalo (Ciawi)	MCF	Sheep foetal thymus	2
Buffalo (Ciawi)	MCF	Buffalo foetal thyroid	1
Buffalo (Balitvet)	Conjunctivitis Nasal discharge	Sheep thymus	2

Sera were screened at a dilution of 1:20. After an hour of incubation, slides were washed and spots incubated with a commercial fluorescein-conjugated rabbit anti-IgG (Miles) for 30 min. After washing and mounting in glycerol saline the slides were viewed by fluorescence microscopy. No reactive sera from cases of MCF were found (Sudarisman et al. 1986). Occasional sheep sera and some goat sera showed variably repeatable nonspecific or weak reactions which may indicate a cross-reaction with a similar herpesvirus, or which may be of no significance.

Animals with WA-MCF show seroconversion to AHV-1 detectable by IIF before the onset of clinical signs in most cases (Rossiter et al. 1977). The lack of any reactions in cases of SA-MCF in Indonesia shows AHV-1 to be an unlikely cause of MCF in that country and, in turn, the IIF test based on the WC-11 strain of AHV-1 cannot be considered useful as a diagnostic aid or seroepidemiological tool.

It remains a valid research objective to investigate nonspecific reactions, in order to determine whether they are associated with a viral infection or whether they are artifacts of the test system. Although this may be achieved by absorptions of test sera another serological test such as the Western Blotting technique may offer more specificity. The latter approach is currently being employed.

Western Blotting

This test allows a more detailed analysis of serological reactions. Antigens were prepared by the method of Sculley et al. (1984) by growing viruses in cell cultures and lysing the cells with an SDS buffer when CPE was at a suitable stage of development. Uninfected cell cultures were similarly prepared to serve as controls. Proteins in the preparations were separated on the basis of their molecular weight by SDS polyacrylamide gel electrophoresis (SDS-PAGE) and electro-blotted onto nitrocellulose paper. Papers were incubated with test antibody and control antisera, and antibody–antigen reactions detected by demonstrating the presence of bound antibody using a commercial biotin-streptavidin horseradish peroxidase detection system (Amersham®). The methodology for the test was developed using bovine herpesvirus 1 (BHV-1) antigen and antiserum, and the technique applied in three different experiments.

In the first study, soluble antigens were prepared from lysates of cells infected with various isolates of the syncytial viruses, and from uninfected control cells. After SDS-PAGE and electroblotting onto nitrocellulose paper, detection of antigens was attempted with antisera to known ruminant viruses, and case sera. Antisera to AHV-1, BHV-1, bovine virus diarrhoea (BVD) and bovine spumavirus (BSV) did not react with antigens in the lysates. Case sera from buffalo detected two antigens in infected cells. Sera from other species of bovine with MCF were unreactive. The molecular weight of one antigen was approximately 45 000 and that of the other 26 000. Both buffalo with MCF and buffalo presumed unexposed to MCF were subsequently found to have reactive antibody. One buffalo with experimental MCF had no detectable antibody (Table 2). Hence, these studies failed to show a clear relationship between the isolates and MCF in Indonesia, and failed to identify the isolates as any of the four viruses for which type antisera were available.

In the second study, lysates of cells infected with the WC-11 strain of AHV-1 were tested. In the standardisation of the test, 5 of 6 sera—a gift from Dr Rossiter, from cases of WA-MCF in Kenya—reacted with virus-associated antigens. Indonesian case sera from Bali cattle in the blood transmission experiments were unreactive. The work will be extended to examine all sera giving low grade or nonspecific reactions in the IIF test.

Table 2. Antibody to buffalo syncytial virus detected by immunoblotting.

Source of Test Sera	No. tested	No. reactive
Uninfected Bali cattle	5	0
Bali cattle dying of MCF	4	0
Buffalo unexposed to MCF	16	10
Buffalo surviving exposure to MCF	3	2
Buffalo dying of MCF	6	5
AHV1 reactive serum	1	0
BHV1 reactive serum	1	0
BVD reactive serum	1	0
BSV reactive serum	1	0

Techniques are also being developed to detect novel antigen and antibody reactions in Indonesian MCF case material. Lysates were made of stored buffy coat cells from before and after infection of the animal with MCF, and antigens sought using type antisera to known viruses and case sera before and after infection. In two experiments to date, antigens in presumed infected cells have not been detected by antisera to AHV-1. No consistent patterns were observed in other test reactions. Strategies to increase antigen presentation in WBCs are to be investigated. Given the valuable stores of WBCs from MCF cases, and sera from before and after infection, there is scope for the development of this approach using various strategies to enhance antigen presentation in infected material.

Agar Gel Precipitation Test (AGPT)

A commercial test (Leukassay B, Pitman–Moore®) has also been used to test for antibody to bovine leucosis virus (BLV) in buffalo with antibody to syncytial virus, in all animals in transmission experiments in Bogor, and in sheep from the natural transmission experiments. Reactive sera were obtained from two animals in the contact transmission experiment, a buffalo and an ongole from South Sulawesi. There was no evidence that BLV is associated with MCF, or that the isolates are BLV.

Partial Characterisation of Isolates

AHV-1 antigens were not detected in acetone-fixed cell cultures of the isolates by antisera to WC-11. Antigenic preparations from isolates did not react with antisera to AHV-1, BHV-1, BVD or BSV in western blot tests.

Discussion

The information presented confirms that the aetiological agent of SA-MCF in Indonesia, as in other countries, will not be isolated and identified easily.

A syncytial virus was isolated from buffalo with MCF, which was the first isolation of this virus in Indonesia (Sudarisman et al. 1985). However, it is noted that Adiwinata (1967) reported that material from Bali cattle with Jembrana disease induced multinucleate giant cell formation in calf kidney cell cultures 9 days after inoculation. Rinderpest was suspected, but the diagnosis was not confirmed and the isolates were lost. Peranginangin et al. (1986) reported the isolation of an apparently similar virus from buffalo with MCF in North Sumatra.

The isolates have not been fully identified. Retroviruses can produce a cytopathic effect similar to that observed, one of simple syncytia production without intracytoplasmic or intranuclear inclusions. Spumaviruses (BSV) of the retrovirus group, as isolated in northern Australia (Johnson et al. 1983), give syncytia with vacuolated cytoplasm, a feature not seen with the present isolates. A reference serum against BSV from northern Australia did not react with the isolate antigens in the Western Blotting test. Neither do sera with antibody reactive to the isolates react with bovine leucosis antigen in the Leukassay B test (Pitman–Moore®). If the isolates are confirmed as retroviruses, their relationship to other bovine retroviruses remains to be established.

The significance of the isolates is also not resolved. The original isolates were from buffaloes with MCF, but subsequently buffaloes with no history of clinical MCF have also yielded virus. Some have been exposed to MCF either by contact with sheep or by blood inoculation, while others have had no experimental contact with MCF. To date, the virus has been isolated only from buffalo, and is consequently referred to at the present time as buffalo syncytial virus. It is noted that some ovine fibroblast cell cultures may be infected with retroviruses in a latent form (Barban et al. 1984). However, it is considered unlikely that the cytopathic effect was derived from an endogenous virus in the sheep cells, because not all cocultivations produced CPE, induction of CPE could be reproduced by stored original WBCs, and primary isolations were achieved in buffalo foetal thyroid cells as well as sheep foetal thymus cells (Table 1).

Serological testing using the syncytial virus as antigen has shown that none of four Bali cattle with classical MCF developed antibody, but that 10 of 16 buffalo with no history of MCF did have antibody. Therefore, there is no simple serological association of the isolates with clinical MCF. SA-MCF is obviously a complex disease because its aetiology remains a

mystery, and further serological studies involving more cases must be completed before any of a range possible hypotheses is developed.

As none of the MCF cases tested to date developed antibody to AHV-1, as detected in the IIF test to WC-11, the disease appears to have a different aetiology, for cases of WA-MCF routinely develop antibody prior to the onset of clinical signs (Rossiter et al. 1977). There was no evidence of cross-reactivity in the case sera tested to suggest infection with a related virus. The possibility of weak cross-reactions in sera from some sheep and goats must be considered separately until such time as natural infection of these animals with a herpesvirus is proved more conclusively and its ability to infect cattle and buffalo demonstrated.

Work has started on development of a test system in which a presumed infectious tissue—white blood cells—is being used as the antigen in the Western Blotting test. The first experiment did not yield an identifiable pattern of reaction. Strategies to increase the concentration of antigen in such cells must be attempted by culturing cells with various cell and virus growth promoters.

If such investigations fail to identify the antigen of the agent, consideration must be given to the hypotheses that in bovine tissue it does not express itself in antigenic form, or that large ruminants do not produce antibody to the SA-MCF agent. Another avenue of study may be to use sheep tissues and sera in a semi-blind manner, from sheep known to have been associated with cases of MCF in cattle, and this approach is in progress.

Another approach is to use the techniques of biotechnology to identify the nucleic acid of the agent in tissue or WBC samples. Opportunities to develop such an approach are being investigated.

Acknowledgments

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Isolation of a Virus from Buffalo Infected with Suspect Malignant Catarrhal Fever

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Abstract

A disease with clinical signs resembling those of MCF occurred in buffalo in Deli Serdang District, North Sumatra, in 1978. The annual incidence was 6.26 per 1000 during the period 1978–84. Isolation of the disease agent was attempted by inoculating white blood cells, lymph node cells and spleen cells onto primary cell cultures of bovine foetal thyroid and bovine foetal kidney. Syncytia were formed 3–4 days after inoculation. The inoculations were carried out by either the feeder layer method or the cocultivation method, and both methods produced the same results. Six isolates grew in cell culture.

Abstrak

Kejadian penyakit yang menunjukkan gejala klinik malignant catarrhal fever (MCF) pada kerbau, telah terjadi di Kabupaten Deli Serdang Sumatra Utara. Dari hidung dan mata keluar cairan mucopurulen, konjungtivi, opasitas kornea, erosi mukosa mulut, suhu badan lebih 41 C dengan angka kematian sangat tinggi; spesimen diambil dari 3 ekor kerbau di lapangan; buffy coat dari hewan hidup lymphoglandula dan limpa dari hewan mati. Virus sinsitiogenis tumbuh pada biakan sel Bovine Embryo Kidney (BEK) pada hari ke 3-9.

Introduction

Malignant catarrhal fever (MCF) is a disease that has frightened the farmers in Indonesia for a long time. Paszotta in 1894 found cases of infected buffaloes in Kediri district, and since then the disease has been found to occur in many places in Indonesia. Sensitive animals have been Bali cattle, Madura cattle and buffalo, while sheep have been suspected as the carrier (Mansjoer 1954). The diagnosis of the disease has been based on the clinical signs and pathological changes (Mansjoer 1954; Ginting 1979; Hoffmann et al. 1984), and attempts at isolation of the disease agent have not yet succeeded.

The cases of suspected MCF in the Deli Serdang District, North Sumatra, were observed for the first time in February 1978 in the Lubukpakae subdistrict. The clinical signs were mucopurulent discharge from nostrils and eyes without crust formation as well as production of a particularly bad smell, conjunctivitis, corneal opacity, mucosal erosion of nostrils and the oral cavity, high body temperature (above 40°C), tiredness and, in the end, death. Total cases in that year numbered eight: seven buffaloes and one ongole *(Bos indicus)* cross.

The annual incidence increased each year from 1978 to 1984, except in 1980, when only six cases occurred. The incidence in 1984 was 62 cases. In that year, the disease spread to neighbouring subdistricts, to Tanjungmurawa subdistrict and Perbaungan subdistrict, with one case each (Table 1). The most susceptible animals in the infected areas were buffalo, with a 6.26 in 1000 average annual incidence during the observation period. Ongole crosses showed relative resistance with a 0.03 incidence in 1000 during the same period (Table 1).

There were large populations of sheep and goats within the affected areas (Table 2), allowing a close relationship with the buffalo. This report is part of a thesis concerning the isolation and identification of a virus from buffalo infected with suspect malignant catarrhal fever.

Materials and Methods

Cell Cultures

Primary bovine foetal cell cultures of thyroid (BFT) and kidney (BFK) were prepared by trypsinisation of bovine foetal organs obtained

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Table 1. Incidence of MCF within the infected area in Deli Serdang District, North Sumatra Province (1978–84).

	Buffalo			*Bos indicus* cattle		
	Population	MCF cases	MCF cases/ 1000	Population	MCF cases	MCF cases/ 1000
1978	4 501	7	1.56	11859	1	0.08
1979	326	17	5.25	116	-	
1980	4 286	6	1.40	11519	-	
1981	4 581	24	5.24	4977	-	
1982	4 531	22	4.85	7654	-	
1983	4 521	43	9.29	4521	-	
1984	3 308	61	20.08	8224	-	2.12

from a slaughterhouse. The cells were cultivated in 11 oz. (*c.* 310 mL) flasks containing Eagle's minimum essential medium supplemented with 10% bovine or sheep serum, and incubated at 37°C. The cell concentration was 300 000/mL of medium. In addition, a continuous cell line, Martin-Derby bovine kidney (MDBK) obtained from the National Institute of Animal Health (NIAH), Japan, was used.

Specimens for Viral Isolation

White blood cells (WBC)

Peripheral whole blood (1–2 mL) was collected from clinical cases into sterilised test tubes containing 0.01–0.02 mL of anticoagulant (EDTA, 1%). The WBC were prepared by centrifugation at 400 *g* for 15 min. in a refrigerated centrifuge after being mixed with 1–2 mL of Ficoll-Paque (Pharmacia Fine Chemicals®). The separated WBC were suspended in medium supplemented with 10% bovine or sheep serum and stored at 4°C.

Table 2. Population of sheep and goats in Deli Serdang District, North Sumatra Province (1978–84).

	Sheep	Goats
1978	1 358	12219
1979	1 394	12545
1980	1 533	13799
1981	766	6889
1982	1 538	13839
1983	1 560	14038
1984	1 190	10714

Lymph node and spleen cells

The cells were prepared as for primary cell culture, and stored at –40°C.

Viral Isolation

Feeder layer method

Samples (0.5 mL) were inoculated onto preformed primary monolayer cultures of BFT and BFK cells and also MDBK cells in 11 oz. flasks containing cover slips, then incubated at 37°C. Three days after inoculation, cover slips were taken, one a day, and stained with Giemsa.

Cocultivation method

Samples (0.5 mL) were cultivated together with suspended cells of BFT, BFK and MDBK in growth medium (4.5 mL) in 11 oz. flasks that were equipped with cover slips.

Results

Source of Specimens

Samples were taken from seven buffalo: one from Tanjungmurawa subdistrict and six from Lubukpakam subdistricts, at different times (Table 3).

Cell Cultures

Six days after seeding the tissue culture flasks of BFT and BFK were ready to be used for virus

isolation by either the feeder layer or cocultivation methods. MDBK cells were used 2 days after being subcultured. The BFT and BFK were used 2–3 days after being subcultured and were used at up to seven subcultures. The MDBK cells in this study were used from the 90th subculture.

Viral Isolations

Six of seven samples studied produced a cytopathic effect characterised by syncytia (Table 4). Most of the syncytia were formed on the third day after inoculation, except for the cells inoculated with K2/AP and K6/AP (fourth day) and K1/AP (ninth day).

Discussion

In this study, seven viruses were isolated from cases of MCF. Syncytia are formed not only by herpesviruses but also by para-influenza (PI-3) infection. Other ruminant herpesviruses besides MCF, such as infectious bovine rhinotracheitis (IBR) virus, could also be involved. For identification and differentiation, it is proposed to employ antiserum to IBR and to the WC-11 strain of alcelaphine herpesvirus 1 that causes wildebeest-associated MCF, in immunofluorescence tests, and also to do haemadsorption tests that will indicate the presence or otherwise of a PI-3-like agent. The isolated viruses were stored and cultivated for further studies of biologic characteristics, and for making diagnostic reagents and vaccine should they be confirmed as the agent of MCF in Indonesia.

Table 3. Derivation of specimens for viral isolation.

Location	No. of buffalo	Code no. of isolation materials	Nature of isolation materials
Tanjungmurawa	1	K1/AP	WBC,Ln,S
Lubukpakam I	1	K2/AP	Ln,S
Lubukpakam I	1	K3/AP	WBC
Lubukpakam III	1	K4/AP	Ln,S
Lubukpakam IV	1	K5/AP	Ln,S
Lubukpakam V	1	K5.1/AP	WBC
Lubukpakam VI	1	K6/AP	Ln,S

WBC = white blood cells, Ln = lymph node, S = spleen.

Table 4. Results of viral isolation attempts in various cell cultures.

Code of isolation materials	FT cells		BFK cells		mDBK cells	
	FL SC	CC SC	FL SC	CC SC	FL SC	CC SC
KI/AP	+	+	+	+	-	-
K2/AP	+	+	+	+	-	-
K3/AP	+	+	+	+	-	-
K4/AP	+	+	+	+	-	-
K5/AP	0	0	+	+	-	-
K5.1/AP	-	-	-	-	-	-
K6/AP	0	0	+	+	-	-

FL = feeder layer method, CC = cocultivation method, SC = syncytia, 0 = not applied

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Problems in Developing a Rabbit Model of Malignant Catarrhal Fever

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Abstract

Experiments to transmit malignant catarrhal fever (MCF) to rabbits are summarised, in which pathological changes induced included vasculitis, perivasculitis and granulomas in the brain, interstitial infiltration of parenchymal organs with lymphoid cells and an accompanying perivasculitis and vasculitis, and lymphoblastic proliferation in lymphoid organs and lymphoid infiltrates. A review of experimental MCF in rabbits revealed that others had reported similar findings. However, the changes observed were also similar to those associated with *Encephalitozoon cuniculi* infections in rabbits and other species. It is suggested that histological criteria may be an unreliable index of successful passage of MCF to rabbits, and that *Encephalitozoon* sp.-infected rabbits should not be used in pathogenesis studies. *Encephalitozoon* sp. is a ubiquitous parasite, and so the status of experimental animals should be confirmed using serological tests, the most sensitive indicator of the parasite infection.

Abstrak

Pada makalah ini, percobaan penularan MCF pada kelinci diulas. Gambaran patologis akibat hasil penularan tadi antara lain ialah vasculitis, perivasculitis dan granuloma dalam otak, infiltrasi intertistialis sel limfoid pada jaringan parenkhim alat tubuh yang diikuti oleh vasculitis dan perivasculitis serta proliferasi lymphoblastik dalam kelenjar limfa dan infiltrasi limfoid. Hasil tersebut tadi sesuai dengan laporan peneliti lain. Namun demikian perubahan-perubahan ini mirip dengan gambaran yang didapat pada kelinci dan hewan lain akibat infeksi *Encephalitozoon cuniculi*. Karena itu untuk studi patogenesis, kriteria histologi pada kelinci MCF dan *Encephalitozoon* sp. sebaiknya jangan dipakai. Sedang untuk *Encephalitozoon* sp. pada hewan percobaan sebaiknya diperkuat dengan uji serologi sebagai indikator yang sangat sensitif untuk infeksi parasit tersebut.

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Introduction

From early in the study of malignant catarrhal fever (MCF) until the present time, transmission experiments have played an important role in defining the disease, including its pathogenesis and, to some extent, its aetiology. As well as transmission to naturally susceptible species, passage to other animals has been attempted, including rabbits. Daubney and Hudson (1936) reviewed the early work in this field and reported successful transmission to rabbits of wildebeest-associated MCF (WA-MCF) and passage of disease from rabbits back to cattle.

Plowright (1968) emphasised the relative difficulty of transmitting sheep-associated MCF (SA-MCF). Transmissions of SA-MCF from deer to rabbits have since been reported (Buxton and Reid 1980), and from such rabbits cell lines were established capable of transmitting disease to rabbits, but not back to deer (Reid et al. 1983). The ability to routinely establish SA-MCF infections in rabbits would offer a significant advance towards isolation of the SA-MCF agent and demonstrate the value of rabbit models.

WA-MCF in Rabbits

The histopathology associated with experimental WA-MCF and AHV-1 infections in rabbits has been well described (Plowright 1953; Piercy 1955; Edington et al. 1979). The three reports cited are consistent in their descriptions. There was lymphoid necrosis, lymphoblastic proliferation with excess mitoses in all

lymphoid organs, perivascular infiltration by lymphoid cells and accompanying vasculitis with lymphoid infiltration and destruction of the smooth muscle. Similar necrosis was seen in the capsules and trabeculae of the spleen and lymph nodes. There were also interstitial lymphoid cell accumulations in liver, kidney, adrenal, myocardium and lung, and meningoencephalitis with vasculitis, perivascular cuffing and mononuclear cell infiltration of the choroid plexus. The lymphoid infiltrate in the lung was perivascular and peribronchiolar. Ocular structures and bone marrow were also invaded by lymphoblasts. No syncytial giant cells were mentioned in these reports.

SA-MCF in Rabbits

The first comprehensive report of passage of SA-MCF to rabbits was that of Buxton and Reid (1980). Clinical signs included fever, dullness, anorexia and frequently serous or catarrhal ocular and nasal discharges. Lymph nodes showed haemorrhage and necrosis with proliferation of lymphoblasts. Such cells were present in lymphoid infiltrates in the periportal spaces, perivascular and peribronchiolar spaces in the lung, ocular structures, as well as the lamina propria of stomach and intestines and in the kidney and heart to a lesser degree. Focal necrosis of hepatocytes occurred. Brains showed perivascular cuffing, and one had a focus of necrosis in the cerebellar folia.

Similar changes were reported in subsequent pathogenesis experiments (Buxton et al. 1984). Proliferation of lymphoblasts was prominent. There was interstitial thickening of alveolar walls and mild accumulations of cells in the kidneys. Of particular note was focal necrosis in the liver characterised by necrotic cells surrounded by increased numbers of Kupffer cells. Large multinucleate giant cells were noted in some animals. Hepatic granulomas with necrotic centres and accompanied by multinucleated giant cells had been reported previously in attempted transmission of SA-MCF to rabbits (Pattison 1946).

Well-documented responses following inoculation of SA-MCF infected blood and tissues to rabbits have also been reported by another group, in which rabbits developed pyrexia, depression, anorexia, mucopurulent ocular and nasal discharges and diarrhoea. Microscopic changes were essentially as described above. There was necrosis of lymphoid tissue and lymphoblast proliferation. Necrosis of vessel walls was seen in many tissues as well as perivascular cuffing and vasculitis in the brain. There were lymphoid infiltrations in the liver, kidney and alveolar septae. Multinucleated giant cells were present in the follicles of some intestinal lymphoid tissue. Control rabbits had perivascular cuffing in the brain and a lymphoid infiltrate in the kidney attributed to *Encephalitozoon* sp. (Westbury and Denholm 1982).

Encephalitozoonosis

Encephalitozoonosis is a protozoal infection of rabbits and other laboratory animals. One species is recognised, *Encephalitozoon cuniculi*. Infection is common and is usually chronic or latent, but disease and deaths have been recorded.

Microscopically, it is characterised by granulomas and nonsuppurative inflammation. The typical granuloma consists of an area of central necrosis surrounded by epithelioid and lymphoid cells. There is perivascular cuffing and gliosis in the brain, an interstitial mononuclear cell infiltrate in the kidney, a mild focal nonsuppurative hepatitis, epicarditis and lymphoid hyperplasia especially of the mesenteric node. There may also be lymphoid infiltration of the myocardium, adrenal glands and retina. Interstitial pneumonia and perivascular lymphoid infiltrates have been reported in the lung of affected mice. Organisms can frequently be seen in tissue sections, but not always (Shadduck and Pakes 1971). Multinucleated giant cells have been reported in brain lesions (Jortner and Percy 1978; Koller 1969).

Another susceptible species is the blue fox *(Alopex lagopus)* (Bjerkas and Nesland 1987). In this species, the vessels of the brain and kidney were the main tissues affected, although foci of granulomatous inflammation in the parenchyma were seen. Parasites were observed in endothelial and smooth muscle cells, macrophages and neurones among other cell types. Vascular lesions resembled polyarteritis nodosa in humans, and although variable in severity, comprised lesions including subendothelial

mononuclear cell infiltration with partial occlusion of lumens, necrosis and mononuclear cell infiltration of smooth muscle walls, and infiltration of the adventitia by mononuclear cells. These changes were said to be pathognomonic of *Encephalitozoon cuniculi* infection in blue foxes.

Experimental Studies

From naturally occurring and experimental cases of SA-MCF in Indonesia, transmission of MCF to pairs of rabbits was attempted by intraperitoneal inoculations of blood from Bali cattle and buffalo. Initially, 20 mL volumes were used, but subsequently this was increased to 50 mL. Rabbits were either transported to the case and inoculated immediately after the collection of the blood in heparin, or the blood was held at 37°C for transportation to the animal house and until subsequent use, normally within 1 hour of collection. On one occasion, rabbits were taken by air and road transport to a field case in a distant province, and on another the veterinary field services purchased rabbits locally, inoculated them and forwarded them by air freight to the research laboratory.

The clinical responses, gross pathology and histopathology were highly variable. Microscopically, vasculitis, perivasculitis and focal granulomas were seen frequently in the CNS. Some CNS granulomas had foci of central necrosis which occasionally contained rod-shaped parasitic forms and which were surrounded by epithelioid cells with a large open cytoplasm with other mononuclear cell types peripherally. Interstitial lymphoid infiltrates or fibrous scarring occurred in the kidneys. Livers frequently showed periportal mononuclear infiltrates and occasional multinucleated cells. Lungs showed vasculitis, perivasculitis, subendothelial infiltrates in vessels, and interstitial pneumonia. In some cases, lymphoid tissue showed blast cell proliferation with pleomorphic forms and excess mitotic figures, and in a few instances suspected syncytia formation. There was sometimes interstitial myocarditis with infiltrates of mononuclear cells including lymphoblasts.

Subsequent work was directed at defining the response to blood inoculation more accurately by using groups of 10 rabbits on each occasion and performing a necropsy examination on pairs at 1, 2, 3 and 12 weeks post inoculation (PI), or at the time of death. One rabbit was necropsied at the beginning of the observation period and one at the end.

This approach in groups treated with blood from two natural cases of SA-MCF or with normal buffalo blood has also shown variable responses. In one series, the central nervous system (CNS) was progressively affected over the period, showing vasculitis and perivasculitis in inoculated animals but not in controls, while both controls and inoculated rabbits showed interstitial nephritis. Only inoculated rabbits showed periportal mononuclear cell infiltrates in the liver and foci of myocarditis.

In the series inoculated with blood from the second case, changes were milder. Control rabbits showed periportal mononuclear cell infiltration of the liver and one died showing pneumonia at 4 weeks. One inoculated rabbit showed interstitial nephritis, two had periportal infiltrates, another two interstitial pneumonia, and a total of three showed some myocarditis. However, similar changes were seen in the series inoculated with uninfected blood.

Discussion

The descriptions of encephalitozoonosis and MCF show considerable similarity. Many of the lymphoid infiltrates and granulomas described in MCF experiments could be attributable to *Encephalitozoon* sp. infections. Westbury and Denholm (1982) were the only authors to report on the *Encephalitozoon* status of their rabbits. The clinical signs associated with a CNS disturbance as described by Pattison (1946) are consistent with those reported for rabbits with severe encephalitozoonosis (Jortner and Percy 1978). Concurrent infections such as 'snuffles' are said to exacerbate the severity of the *Encephalitozoon* lesions (McCartney 1923), as does immunosuppression with corticosteroids or cyclophosphamide (Shadduck and Pakes 1971).

It is therefore conceivable that the stress of inoculations could exacerbate the subclinical encephalitozoonosis in rabbits. It is further possible that a disease such as MCF could be functionally immunosuppressive, either through lymphoid necrosis or some other mechanism. It thus cannot be assumed that mild lesions in

control animals are attributable to the parasite and florid lesions in treated animals are attributable solely to the treatment. Westbury and Denholm (1982) have identified an important aspect of MCF research in laboratory animals.

The interference the infection can cause to experimental work has been described (McCartney 1923; Shadduck and Pakes 1971; Jortner and Percy 1978), but the similarity in the pathology compared with that expected in MCF makes the infection of even greater significance in MCF investigations. However, the strategies available for eliminating the parasite from rabbit colonies in the past were limited. Howell and Edington (1968) described a technique based on the selection of progeny of dams diagnosed as being free of the parasite retrospectively on the basis of histological examination. As late as 1972, there was no reliable method for detecting infection in living rabbits (Pakes et al. 1972). These authors developed a delayed-type hypersensitivity skin test which detected infected animals, while Cox et al. (1972) developed immunofluorescence tests including a serological test. Serological examination of several rabbit colonies and rabbit sera from around the world frequently showed the presence of infected animals, often at high prevalence (Cox and Pye 1975). Serology is a sensitive measure of infection, antibodies being detectable 2 weeks before organisms appear in urine and 5 weeks before histological lesions appear in the kidney (Cox and Gallichio 1978). In that study, CNS lesions were rarely seen, and then only 8 weeks after seroconversion.

Although the pathological changes observed in the current series are similar to those described by others (Buxton and Reid 1980; Westbury and Denholm 1982), it cannot yet be said with certainty whether any of the lesions are attributable to MCF. The syncytia in liver and lymphoid tissue are particularly interesting and have been reported previously in SA-MCF transmission experiments, in the liver (Pattison 1946; Buxton et al. 1984) and in an intestinal lymphoid follicle (Westbury and Denholm 1982). In rabbits with encephalitozoonosis, such cells have been reported in the brain, but it cannot be assumed that in exacerbated encephalitozoonosis they would be restricted to that tissue. It is clear that definitive studies are needed in rabbits specifically free of *Encephalitozoon* sp., in order to accurately describe the range of pathology that can be expected in transmitted MCF. Only then will it be possible to attempt interpretation of the results obtained to date.

Clearly, any pathogenesis experiments should be conducted in parasite-free animals. Until the situation is clarified the status of rabbits used in any MCF work should be recorded, based on serology which is the most sensitive measure of the parasite infection (Cox and Gallicho 1978). There is even one report (Hunt et al. 1972) of gnotobiotically derived rabbits harbouring the parasite.

The presence of parasites should not preclude successful infection of a rabbit by the SA-MCF agent. Indeed, some of the pathology reported in the present series and by other authors may be the result of dual infections. Further research may also reveal that some of the pathology associated with SA-MCF and similar diseases in Indonesian ruminants is the result of dual infections involving other agents. A potential problem with *Trypanosoma evansi* and other undiagnosed infections in buffalo has already been noted (Daniels et al. 1988, Unruh et al. 1988).

It is in attempting to use histological criteria for the diagnosis of successful passage of SA-MCF to rabbits, and in attempting to use *Encephalitozoon* sp.-infected rabbit models for MCF pathogenesis studies, that problems arise. Serial transmission studies in which the disease is successfully passaged back to susceptible ruminants obviously remain valid as demonstrations of the partial isolation of the agent, the identification of which is the primary goal of current research.

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Malignant Catarrhal Fever Research in Queensland

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Abstract

A small population of sheep in Queensland was identified as a potential carrier of the sheep-associated malignant catarrhal fever (MCF) agent by its association with buffalo dying of MCF. Extensive protocols attempting virus isolation in cell culture and in laboratory animals failed to isolate a disease agent. A Bali cow penned with these sheep and their progeny developed clinical MCF, but attempted blood transmissions and virus isolations were not successful. It was concluded that the techniques of conventional virology may not be adequate to identify the MCF agent.

Abstrak

Sekelompok kecil dari domba di Queensland diduga keras mempunyai peranan penting sebagai pembawa (carrier) agen penyebab penyakit MCF, sehubungan dengan matinya kerbau dengan gejala MCF. Dengan cara kerja yang terus menerus dalam isolasi agen penyebab penyakit pada biakan sel dan pada hewan percobaan ternyata gagal mengisolasi agen penyebabnya. Seekor sapi Bali yang dikandangkan bersama domba diatas dan progeninya menunjukkan gejala klinis MCF tetapi dengan transmisi lewat darah dan isolasi virusnya belum berhasil. Disimpulkan bahwa tehnik virologi konvensional (lama) mungkin tidak cukup untuk menyidik agen MCF.

Introduction

Two distinct forms of malignant catarrhal fever (MCF) have been proposed: the wildebeest-associated MCF (WA-MCF) and the sheep-associated MCF (SA-MCF). Alcelaphine herpesvirus 1 (AHV-1) causes WA-MCF while the cause of SA-MCF has not been identified. Both agents produce no disease in their respective natural hosts, wildebeest and sheep, but cause a fatal lymphoproliferative disease following transmission to certain other species of ruminants. Only with AHV-1 is there any information on the immune response of the natural host to infection. Epidemiological studies support the view that all wildebeest become infected between 3 and 6 months of age and virus can be isolated periodically from some animals, particularly pregnant females and young calves. The presumptive agent of sheep probably follows a similar epidemiological pattern.

The proposition that the sheep-associated agent behaves in sheep similarly to AHV-1 in wildebeest is supported both by circumstantial evidence and by data suggesting that a virus sharing antigenic determinants with AHV-1 is prevalent in sheep. This proposition has been the main basis for the direction of research carried out at the Oonoonba Veterinary Laboratory (OVL). As numerous unsuccessful attempts to isolate the agent from the end host have been reported, it was felt that a concerted effort directed towards the natural host might be more rewarding. Nine sheep known to be associated with SA-MCF transmission to buffalo in southeast Queensland were purchased in 1983 and transported to OVL for intensive studies including a transmission experiment. Australia's quarantine regulations and the lack of wildebeest in this country necessitated overseas study to compare the serology of the two diseases. The opportunity to do this was provided in both Indonesia and the United Kingdom.

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Materials and Methods

Animals

(a) Sheep

One ram and eight ewes associated with SA-MCF in buffalo were purchased for the studies. They arrived at OVL from southeast Queensland in December 1983.

(b) Rabbits

Animals from OVL's colony of laboratory rabbits were used.

(c) Animals for natural transmission experiments

(i) Cattle *(Bos taurus)*

Two 6-month-old castrated males from the OVL herd were used.

(ii) Buffalo *(Bubalus bubalis)*

Two males, 6 months old, were obtained from the Northern Territory Department of Primary Production. They arrived at OVL in April 1985.

(iii) Bali cattle *(Bos javanicus)*

A male and a female, 6 months old, were obtained from the Northern Territory Department of Primary Production. They arrived at OVL in April 1985.

(iv) Rusa deer *(Cervus timorensis)*

A male and a female were obtained as young adults from Hamilton Island, Queensland, in March 1985.

(v) Chital deer *(Axis axis)*

A male and a female were obtained as young adults from a property 120 km northwest of Charters Towers in Queensland in March 1985.

Cell Cultures

(a) Cell dispersal

To establish cultures from organs and tissues, suspensions of single cells were prepared by treating finely cut material with trypsin at 37°C. To passage established cell cultures, an activated trypsin-versene solution (ATV) warmed to 37°C was used to remove cell monolayers from culture vessel surfaces as suspensions of single cells.

(b) Media

Minimum essential medium (Eagle) with Earl's salts and nonessential amino acids was used for all cell cultures except the attempts to grow lymphocytes. For the lymphocyte culture attempts RPMI 1640 was used.

Foetal calf serum, which was not heat-inactivated, was used as a supplement in all media. For the lymphocyte culture attempts and for macrophage cultures the supplementation rate was 20%. For all other cultures it was 10%.

(c) Incubation

Cultures were incubated at 37°C, except in the bromodeoxyuridine experiment where each culture was duplicated so that two incubation temperatures—37°C and 33°C—could be used.

Cell Fusion

Cell fusions were accomplished by polyethylene glycol as described by Castro et al. (1983).

Leucocyte Separation

A wide range of centrifugation conditions was investigated to develop the following procedure for extracting leucocytes from sheep blood suitable for culturing. The technique effectively removed erythrocytes, and the percentage viability of recovered leucocytes was high. An important feature of this procedure was the washing of the buffy coat three times with large volumes of Dulbecco's phosphate buffered saline solution A (PBSA) before layering it over Ficoll-Paque (Pharmacia). This washing removed most thrombocytes and thus prevented clotting when the leucocytes were suspended in culture medium supplemented with foetal calf serum.

Twenty millilitres of blood were collected by jugular venipuncture into a 1-oz. (*c.* 30-mL) bottle containing anticoagulant. It was centrifuged at 1000 *g* for 9 min. The buffy coat was collected and made up to 20 mL with PBSA. Cells were washed three times in PBSA, being pelleted by centrifugation at 250 *g* for 10 min and being resuspended in 20 mL in PBSA each time. After the last wash, cells were

resuspended in PBSA to 2 mL and layered over 3 mL of Ficoll-Paque in 13 mm diameter centrifuge tubes, and centrifuged at 1000 *g* for 20 min. The band of cells at the interface was collected, resuspended to 20 mL in 1 oz. universal bottles and washed once in PBSA as had been done prior to use. All centrifugations were performed at 4°C using a swing-out rotor. The *g* forces quoted are *g* maximum, as measured at the bottom of the tube.

Serology

An agar gel diffusion precipitin test (AGDPT) was standardised for the spumavirus isolated from a sheep in a cortisone stressing experiment. The antigen was made by disrupting infected cell cultures either by ultrasonication or repeated freezing and thawing, and the agar used was a high salt agar (10N NaCl). Plates were incubated in a humid atmosphere at room temperature for 3 days before reading.

Summary of Investigations and Results

History of Imported Sheep

Two buffalo were introduced to a property in southeastern Queensland in late 1979 and were pastured with sheep. Early in 1981, a bull calf born on the property died without postmortem examination. In February 1983, the original cow died suddenly, again with no necropsy. In June 1983, the original bull and a heifer born on the property died suddenly, and necropsies were performed. The clinical history, gross pathology and histopathology confirmed a diagnosis of SA-MCF. One ram and eight ewes, the total flock, were purchased and transported to OVL in December 1983.

In vitro Methods Used to Investigate Sheep

The following investigations were performed:

a. Weekly cultivation of macrophages from the sheep.

b. Polyethylene glycol fusion of leucocytes with a range of cell cultures, namely:

calf testis	lamb testis
rabbit testis	foetal lamb thyroid
rabbit kidney	foetal lamb adrenal
rabbit macrophage	sheep macrophage

c. Cocultivation of leucocytes with a range of cell cultures, namely:

calf testis	rabbit testis	foetal lamb lung
lamb testis	rabbit kidney	

d. Cultivation of lymphocytes.

e. Inoculation of swab material into calf testis cell cultures.

f. Cocultivation of a range of organs and tissues with calf testis cell cultures.

g. Immunoperoxidase staining of lymphocytes using terminal serum from the Bali cow as a possible source of specific antibody.

Results: no virus was isolated or detected.

Cortisone Stressing Experiment

Seven lambs, 6 months old, the progeny of the imported sheep, were intramuscularly inoculated with 2 mg/kg of a corticosteroid (Opticortenol) daily for 5 days, and were slaughtered 2 days later. Eye, nose and throat swabs and blood were collected for virus isolation before, during and after the cortisone treatment. A range of organs and tissues was collected for virus isolation at slaughter.

Results: virus was isolated from only one lamb. It caused multinucleated syncytia, a CPE identical to that of bovine spumavirus.

Spumavirus Investigations

An AGDPT for serological studies was standardised. This AGDPT could not distinguish between the sheep virus and bovine spumavirus. The effect of the isolated spumavirus on seronegative lambs less than 1 week old was studied.

Results:

a. 3/3 lambs inoculated intravenously seroconverted.

b. 1/3 lambs inoculated intranasally seroconverted.

c. Virus was recovered 40 and 74 days after inoculation.

5-Bromodeoxyuridine (5-BDU) Experiment

The leucocyte fraction was extracted from blood collected from each of five sheep and divided into three aliquots, one for each of the following treatments:

5 μg/ml 5-BDU for 3 days

25 μg/ml 5-BDU for 3 days

100 μg/ml 5-BDU for 1 day

Results:

a. The higher the 5-BDU concentration, the greater was the damage done to the leucocytes.

b. After 5-BDU treatment, the cells and culture fluids were tested for virus by growing rabbit testis cells with them.

c. No CPE was seen.

In-Contact Transmission Experiment

The cattle, buffalo and deer listed previously were penned with the sheep implicated in carrying the disease.

Result: only the Bali cow died. MCF was diagnosed on the basis of clinical signs, gross pathology and histopathology.

Experimental Transmission from the Bali Cow

The following inoculations were made to cattle and rabbits:

a. 10% spleen suspension into two *Bos taurus*

b. 10% spleen suspension into two rabbits

c. citrated blood into two rabbits

d. 5% thymus suspension into two rabbits.

Result: no transmission was achieved.

Virus Isolation Attempts from the Bali Cow

1. Autocultures of thymus, lymph nodes, spleen and kidney were prepared.

2. Cocultivation of thymus, lymph nodes, spleen and kidney was performed with rabbit testis cells and sheep macrophages.

3. Polyethylene glycol fusion of thymus and lymph node cells with sheep macrophages was attempted.

Result: no virus was isolated.

Attempts to Transmit SA-MCF to Rabbits from Sheep

Rabbits were inoculated with:

a. blood buffy coats

b. leucocytes separated by Ficoll-Paque

c. eye, nose and vaginal swab material

d. lymph node material

e. 3rd passage of cell cultures inoculated with swab material

f. 5th passage of polyethylene glycol fusions of leucocytes with cell cultures.

Result: no transmission was achieved.

Serology—WC-11

Sera from the nine sheep purchased and 49 sera from sheep originating in western Queensland were tested for antibodies to WC-11 in the United Kingdom. The test was conducted blind.

Result: only one serum reacted in the test. It was from a sheep purchased as a suspected carrier of MCF.

The above work was repeated in Indonesia using similar sera with negative results.

Serology—Spumavirus

a. In Indonesia

Selected sera from a serum bank were tested against spumavirus using the AGDPT. The test was done blind.

Result: 2/11 goats, 0/10 buffalo, 0/28 Bali cattle, 0/17 deer, and 2/117 sheep were found positive. One of two *Bos indicus* cattle had a faint reaction. It is interesting that the four positives and one weak positive were from animals at Ciawi, a known focus of SA-MCF infections (Hoffmann et al. 1984). The significance of the finding is unknown.

b. In Australia

Sheep were tested for antibodies to spumavirus using AGDPT.

Result: antibody prevalence of 25% was found in sera of sheep from western Queensland. No positive sera were detected in purchased sheep or their lambs.

Conclusions

The inability to isolate the putative causative agent from either carrier sheep or sick cattle was most frustrating. The reasons could be many.

Sheep may not be carriers of the agent. However, in view of the overwhelming evidence, albeit circumstantial, it is difficult to believe that they do not play a major role in the disease. If sheep are carriers of the agent, failure could be due to the agent not being present in the tissues or excretions examined, or being present only in a form that precluded isolation. The use of many techniques would suggest that the methods used may not be suitable for isolation of this virus. Isolation of MCF virus of wildebeest origin has been possible if only from a limited range of bovine cell cultures. It equally cannot be ruled out that the agent is in a form which defies standard methods of isolation. There are many examples in virology where a successful cell culture system has not been found for agents that can be visualised under the electron microscope. The situation may be even more difficult with the sheep-associated agent since the agent has not yet been so visualised.

Workers at Moredun Institute in Scotland have characterised a lymphocyte cell line from rabbits infected with the tissues of a deer with SA-MCF and found that inoculation of as few as 100 of these cells induced typical MCF (Reid et al. 1983). This work has led to a hypothesis that the lesions of MCF may arise through parasitism by the causal virus of a subpopulation of T-lymphocytes known as large granular lymphocytes, and that virus may persist in these cells in an incomplete form, possibly as episomal DNA, and cause a profound dysfunction of this cell type. This could result in both benign and polyclonal T-lymphocyte hyperplasia arising through the absence of suppressor cell activity. Subsequent terminal tissue destruction could be caused by indiscriminate natural killer activity.

If the agent in sheep exists only as DNA, it is difficult to see how it could be transferred from sheep to sheep and from sheep to other ruminants. Methods are now available utilising DNA probes to attempt to detect incorporated DNA in specimens. There is, however, the need to have a probe that might show some homology with the unisolated sheep-associated agent. The obvious probe to try would be one prepared from the wildebeest-associated agent. Until the Australian Animal Health Laboratory in Geelong obtains permission to import the wildebeest-associated agent, this work must be conducted outside Australia.

Such a probe would also allow a detailed examination of sheep to determine where the agent resides, how it is excreted and also the proportion of sheep that carry the agent.

These are very exciting times for an increased understanding of MCF.

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