STUDIES ON THE TRANSMISSION OF MALIGNANT CATARRHAL FEVER IN EXPERIMENTAL ANIMALS: A SERIAL INFECTION OF CATTLE AND BUFFALO BY MEANS OF WHOLE BLOOD INOCULATION

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ABSTRAK

WIYONO, A. dan R. DAMAYANTI. 1999. Studi penularan buatan *malignant catarrhal fever* pada hewan percobaan: Penularan berseri pada sapi dan kerbau dengan menggunakan inokulum darah. *Jurnal Ilmu Ternak dan Veteriner* 4 (4): 264-272.

Malignant catarrhal fever (MCF) adalah penyakit yang hampir selalu bersifat fatal, terutama menyerang sapi dan kerbau. Suatu studi penularan buatan secara berseri dilaksanakan dengan menyuntikkan darah hewan yang terserang MCF kepada 9 ekor hewan percobaan berupa sapi dan kerbau. Diagnosis penyakit ditegakkan berdasarkan gejala klinis, kelainan patologis dan uji *polymerase chain reaction* (PCR). Penyakit dapat ditularkan kepada 6 ekor hewan percobaan yang dipakai, yaitu masing-masing 3 ekor sapi Bali dan kerbau, tetapi penyakit tersebut tidak berhasil ditularkan kepada seekor sapi Bali-cross dan dua ekor sapi Peranakan Ongole (PO). Gejala klinis dan kelainan patologis anatomi hewan yang diinfeksi terlihat sangat beragam. Berdasarkan hasil penelitian ini terlihat bahwa urutan kepekaan adalah sebagai sapi Bali dan kerbau sangat peka (masing-masing 3 dari 3 tertular MCF), sedangkan sapi Bali-cross (0 dari I) dan sapi PO (0 dari 2) tidak peka, terhadap penularan buatan MCF dengan menggunakan darah. Di samping itu, terlihat kecenderungan bahwa bila sapi Bali dipakai sebagai donor inokulum, maka kerbau lebih parah terserang MCF dibandingkan dengan sapi Bali. Sebaliknya, bila kerbau bertindak sebagai donor inokulum, maka sapi Bali lebih parah dibandingkan dengan kerbau. Diagnosis MCF dengan pemeriksaan mikroskopis dan uji PCR dalam penelitian ini menunjukkan kesesuaian hingga 100% pada percobaan pertama, sedangkan pada percobaan kedua PCR terlihat lebih peka. Berdasarkan uji *restriction endonuclease* (RE) terlihat bahwa secara genetik tidak ada perbedaan agen penyebab MCF pada penularan buatan ini. Kesimpulan dari penelitian adalah bahwa penularan buatan MCF secara berseri dengan menggunakan .inokulum darah telah berhasil dilakukan pada sapi Bali dan kerbau, tetapi tidak berhasil pada sapi PO dan Bali-cross, dan bahwa hasil uji PCR menunjukkan kesesuaian dengan hasil pemeriksaan mikroskopis dengan uji PCR cenderung lebih peka.

Kata kunci: MCF, penularan berseri, darah, sapi, kerbau, Indonesia

ABSTRACT

WIYONO, A. dan R. DAMAYANTI. 1999. Studies on the transmission of malignant catarrhal fever in experimental animals: A serial infection of cattle and buffalo by means of whole blood inoculation. *Jurnal IImu Temak dan Veteriner* 4 (4): 264-272.

Malignant catarrhal fever (MCF) is a fatal disease especially affecting cattle and buffaloes. A study on the serial blood transmission of MCF was conducted by injecting whole blood of MCF animals into 9 experimental animals. Diagnosis of MCF was based on the clinico-pathological fmdings and polymerase chain reaction (PCR) test. The disease has successfully, been achieved in six animals of three Bali cattle and three buffaloes but not in a Bali-cross breed and two *Bos indicus* (Ongole) cattle. Wide range of clinical signs and gross-pathological features were observed. The study showed the degree of susceptibility of experimental animals: Bali cattle and buffalo were highly susceptible (3 out of 3 affected with MCF), Bali-cross breed and *Bos indicus* (Ongole) cattle seemed not susceptible to whole blood experimental transmission. It shows that when Bali cattle acted as inoculum donor, buffalo tended to be clinically more severe than Bali cattle. On the other hand, when buffalo acted as inoculum donor, Bali cattle suffered from MCF more severe than buffalo. The diagnosis of MCF by histopathological examination and the PCR test bad positive correlation (100%) in the first experiment, while in the second experiment the PCR test tends to be more sensitive. Based on the restriction endonuclease (RE) test, the MCF causal agent in this study appeared to be genetically similar in each case. It is concluded that the serial experimental transmission of MCF by means of whole blood inoculation has been successfully achieved in Bali cattle and buffalo but not in Bali-cross breed and Ongole cattle, and there is a positive correlation between the PCR test and histopathological examination with the PCR test tends to be more sensitive.

Key words: MCF, serial transmission, blood, cattle, buffalo, Indonesia

INTRODUCTION

Malignant catarrhal fever (MCF) is a fatal lymphoproliferative and degenerative disease of cattle, buffaloes, deer and some Wild ruminants (PWWRIGHT, . 1968). Recently, MCF was reported to occur in pigs (L0KEN *et al.,* 1998). The disease epidemiologically exists in at least two forms namely sheep-associated MCF (SA-MCF) with the causal agent has not yet been isolated and wildebeest-associated MCF (WA-MCF) with the agent is alcelaphinae herpesvirus-l (AHV-l) (PLOWRIGHT, 1968). Both types of MCF are clinically and pathologically indistinguishable (PLOWRIGHT, 1968). Epidemiological evidences showed that MCF cases in Indonesia is SA-MCF form (DANIELS *et al.,* 1988), and it is recently confirmed by molecular biological tests (WIYONO *et al.,* 1994).

The disease has been recognised sporadically throughout most of the Indonesian archipelago (PARTADIREDJA *et al.,* 1988), and is considered to be an economically important disease of Bali cattle and swamp buffalo since these animals are the main source of draught animal and wealth for most of Indonesian farmers (DANIELS *et aI.,* 1988). Bali cattle *(Bos javanicus/ sondaicus)* has been reported to be very susceptible to MCF in Indonesia (RAMACHANDRAN *et al.,* 1982) and in the USA (HATKIN, 1980). Buffalo were reported to be susceptible to MCF in Indonesia (HOFFMANN *et al.,* 1984), India (SINGH *et al.,* 1979) and New Zealand (HILL *et al.,* 1993).

The transmission experiments of SA-MCF were first reported by DAUBNEY and HUDSON (1936), and later transmission experiment was suggested as the diagnosis to confirm the disease (JUBB *et al.,* 1985). In addition, experimental transmissions of MCF by inoculation of whole blood were conducted in a number of experimental animals such as buffalo (SINGH *et al.,* 1979), bison (LIGGITT *et al.* 1980), rabbits (BUXTON and REID, 1980), cattle (RAMACHANDRAN *et al.,* 1982), deer (OLIVER *et al.,* 1983), hamsters, rats and guinea pigs (JACOBY *et al.,* 1988), and even to sheep (BUXTON *et al.,* 1985).

It has been known that the causal agent of MCF is highly cell-associated (PLOWRIGHT, 1968), particularly T lymphocytes (MUSHI *et al.,* 1984). It is likely that tile source of cell-associated materials to be experimentally transmitted to susceptible animals will be whole blood.

The aim of this study is to investigate tile serial transmission of MCF in experimentally inoculated Bali, Bali-cross and Ongole cattle and buffalo using whole blood from animals which were confirmed as having MCF and to study the diagnostic aspects of MCF.

MATERIALS AND METHODS

Source of infective agents

Whole blood of two MCF animals were used namely buffalo No.505 and Bali cow No.05. Buffalo No.505 was an MCF suspected case from Research Institute for Animal Production (RIAP), while Bali cow No.05 was an MCF suspected case from Research Institute for Veterinary Science (RIVS).

Experimental animals

In the first experiment, the animals were consisted of three Bali cattle *(Bos javanicus/ sondaicus)* (Nos. 63,66 and 55), two Indonesian swamp buffalo *(Bubalus bubalis)* (Nos. 36 and 38), one Bali-cross cow (No.27) and two Ongole cattle (Nos.29 and 23).

In the second experiment, another Indonesian swamp buffalo (No.15) was used as an experimental animal .

Peripheral blood leucocytes (PBL) of all experimental animals were collected and tested by the PCR (BAXTER *et al.,* 1993) to prove that the animals were MCF-free prior to inoculation.

Experimental inoculation

Experiment 1

Buffalo No.505 was tile source of infective agents in this experiment. It was naturally suffered from MCF. At the second day of the clinical onset of the buffalo, approximately I litre of its whole blood was collected from the jugular vein and was kept at 37EC for approximately 30 minutes before intra-venously inoculated into Bali cow No.63.

Bali-cross breed cow No.27, Bali cow No.66, buffalo No.36, and Ongole cow No.29 each of which were inoculated intra-venously with approximately 1 litre of whole blood of the Bali cow No.63 when the cow showed MCF clinical signs at 21 days post infection (dpi).

Subsequently, whole blood of buffalo No.36 was experimentally inoculated intra-venously into buffalo No.38 and Bali cow No.55, each of which received approximately I litre of whole blood. The whole blood were taken from the buffalo No.36 when it was affected by MCF at 32 dpi.

Finally, Ongole cow No.23 was experimentally inoculated intra-venously with approximately 1 litre of whole blood of Bali cow No.55 when it showed clinical signs ofMCF after 37 dpi.

Experiment 2

Bali cow No.05 was a spontaneous MCF case at the RIVS and it was acting as the source of infective agents. Approximately 1 litre of its whole blood was collected from the jugular vein when it was suffered from MCF. The whole blood was stored at 37EC before transmitted to buffalo No.15.

Diagnosis

Clinico-pathology

Clinical signs of the experimentally infected animals were observed and recorded on the daily basis. The animals were sacrificed at extremis and necropsy were performed whenever the animals died and/or killed. Gross-pathological findings were recorded at necropsy.

Samples of *rete mirabile,* brain. trachea, lung, heart, liver, spleen. kidney, abomasum, small intestine, urinary bladder and superficial lymph nodes were taken from clinically affected animals at necropsy and stored in 10% neutral-buffered formalin. The samples were processed and stained by hematoxylin and eosin (H&E) for histopathological examination (PLOWRIGHT, 1968). They were diagnosed as MCF by the presence of patognomonic lesions consisting of non-suppurative inflammation and vasculitis in the affected organs as described by LIGGITT and DEMARTINI (1980). There were four classification of lesions in organs and they were coded as follows: - (no lesion), + (mild lesion), ++ (moderate lesion), and +++ (severe lesion).

Polymerase chain reaction

Samples of heparinised blood or superficial lymph node (pre-scapular or pre-femoral) were collected from clinically affected animals for PCR test (BAXTER *et al.,* 1993). Samples of PBL were obtained by lysing red blood cells of heparinised blood with ammonium chloride (0.85% NH_4Cl) solution (SAMBROOK *et al.,* 1989).

Extraction of deoxy-ribonucleic acid (DNA) from PBL and lymph nodes were carried out by phenol-chloroform extraction and were ethanol precipitated as described elsewhere (SAMBROOK *et al.,* 1989; BAXTER *et al.,* 1993; WIYONO *et al.,* 1994). The DNA were quantified by ultra-violet spectrophotometry.

The PCR was performed using two amplifications, namely primer pairs 556/755 and 556/555 for the first and second reaction respectively. It was conducted as described by BAXTER *et al.* (1993). The final product of the reaction were visualized by 1.8% agarose gel in tris borate EDTA (TBE) buffer (SAMBROOK *et al.,* 1989).

Restriction endonuclease profiling of PCR amplified DNA fragments

The amplified DNA fragments in ethidium bromide-agarose gels were excised and purified using the Geneclean II Kit based on the methods as suggested by the manufacturer (Bio 101 Inc, La Jolla, CA, USA). The DNA was then digested with RsaI (Boehringer Mannheim, Germany). The cut DNA fragments were separated using continuous polyacrylamide gel electrophoresis (PAGE) and visualized by staining with a silver stain (SAMBROOK *et al.,* 1989).

RESULTS

Clinico-pathological findings and the PCR test

Clinical signs and gross-pathological findings of the experimental animals were presented in Table 1, while histopathological examination results were shown in Table 2 and were presented in Figures 1, 2 and 3. Details of each case were given as follows.

Buffalo No.505 that was used as a source of infected blood in the study had been placed for several years in close contact with sheep. It showed clinical signs of sudden high fever (maximum 41.2°C), very mild serous oculo-nasal discharges, and loss of appetite. It was killed at the 5th days of sickness. Gross pathological features showed a very mild petechial haemorrhages of the abomasums, trachea and urinary bladder (Table 1). Severe lesion in the lung and moderate lesions in the *rete mirabile* were found in microscopic examinations of this buffalo, suggesting that it was affected by MCF. This was confirmed by the PCR test (Table 2).

Bali cow No.63 was experimentally inoculated by whole blood taken from Buffalo No.505 above. The cow showed severe clinical MCF at 21 days post infection (dpi). The clinical signs were sudden high fever (maximum 40.5°C at day 3 of the disease onset), loss of appetite, very depressed, diarrhoea, serous to mucopurulent ocular discharge, and mucopurulent nasal discharge. Gross pathological findings of the cow included mild haemorrhages of the epicardium. gall bladder, abomasum, mesenteric lymph nodes and small and large intestine, and severe haemorrhagic inflammation of oesophagus, trachea and urinary bladder (Table 1). Histopathological examination revealed that the buffalo had severe lesions in the *rete mirabile,* lung, liver and lymph nodes, suggesting that it was infected by MCF. The PCR detected MCF causal agent from this cow (Table 2). Figure 1 showed severe non-suppurative vasculitis in the *rete mirabile.*

Table 1. Summary of clinical signs and gross-pathological findings of experimental animals infected with MCF

Clinical signs/ Gross-pathological findings	Experimental animals										
	B 505	BC 63	BC66	B36	BX27	PO 29	B38	BC55	PO23	BC05	B 15
lncub. Period (days)		21	14	32			31	7			58
Clinical signs											
Fever (^{0}C}	41.2	*4O.S*	41	39.7			41	40,5		40.2	
Ocular discharges	SM	SM	SM	SM				S			
Nasal discharges	SM	M	SM	SM				M		SM	
Anorexia	X	X	X	X			X	X		X	
Depressed		X	X								
Diarrhoea		X									
Conjunctivitis	.			X						X	
Corneal opacity				X						X	
Oral lesion				X						X	
Killed (months post infection)					11	11			7		
Sudden death											X
Gross lesions: Haemorrhage of:											
Abomasum	v mild	mild	mild	severe			mild			Mild	
Trachea	v mild	severe	mild	severe			mild	severe		Mild	
Urinary bladder	v mild	severe	mild	severe			mild			Mild	
Epicardium		mild								Mild	
Gall bladder		mild		severe							
Lymph node		mild									
Small intestine		mild	mild					severe		Mild	
Large intestine		mild	mild					severe			

Remark:

SM : serous to mucopurulent: mild haemorrhages
M : mucopurulent
X : clinical signs that were observed
v mild: very mild haemolThages
mild : mild haemorrhages
severe:severe haemorrhages

B : buffalo
DC : Balicow
BX : Bali-cross breed cow
PO : *Bos indicus* (Ongole) cow
blank. : no obvious changes were observed

Table 2. The severity degree of histopathologica1 lesions and the PCR results in ruminants experimentally infected with whole blood of MCF affected animals

	B505	BC63	BC66	B36	PO29	BX27	B38	BC55	PO23	BCO5	Bl5
Rete mirabile	++	+++	-	+	-	-	-	++	-	+++	-
Brain	+	-	++	++	-	-	++	+	-	+	-
Lung	+++	+++	+++	-	-	-	-	-	-	-	-
Heart	-	-	-	-	-	-	-	-	-	-	-
Liver	++	+++	+++	+	-	-	-	+++	-	-	-
Spleen	-	-	-	-	-	-	-	++	-	-	-
Abomasum	-	-	-	-	-	-	-	-	-	-	-
Intestine	-	-	-	-	-	-	-	-	-	-	-
Kidney	-	-	-	+++	-	-	-	-	-	-	-
Urinary bladder	-	-	-	-	-	-	-	-	-	-	-
Lymph node	-	+++	-	-	-	-	-	-	-	+++	-
HP Diagnosis	MCF	MCF	MCF	MCF	I	I	MCF	MCF	I	MCF	I
PCR Diagnosis	MCF	MCF	MCF	MCF	N	N	MCF	MCF	N	MCF	MCF

Remark:

B	: buffalo	++: moderate lesion
Be	: Bali cow	+++: severe lesion
BX	: Bali-cross breed cow	HP: histopathology
PO	: *Bos indicus* (Ongole) cow	PCR: polymerase chain reaction
-	: no lesion	I: inconclusive
+	: mild lesion	N: MCF causal agent was not detected by the PCR

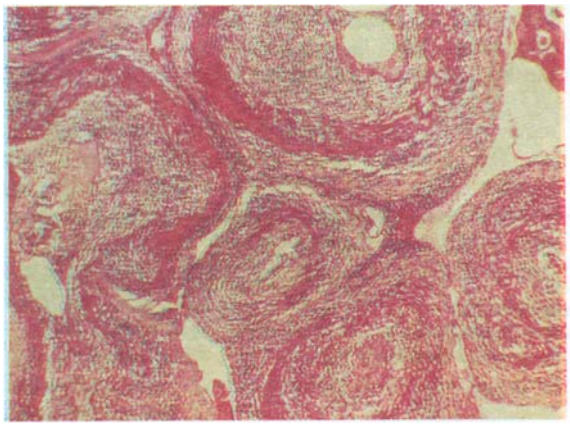

Figure 1. *Rete mirabile* with severe non suppurative vasculitis associated with hyperplastic blood vessels and lumen obstruction. (H&E Staining, X 85)

Blood taken from diseased cow No.63 was inoculated into Bali cow No.66, buffalo No.36 and Bali-cross cow No.27 and Ongole cow No.29. Bali cow No.66 showed clinical MCF at 14 dpi with sudden high fever (maximum 41°C at day 2 of the clinical onset), loss of appetite, very depressed, serous to sero-mucous oculo-nasal discharges. Mild haemorrhages of the trachea, abomasum, urinary bladder, small and large intestine were found at necropsy (Table 1). The cow showed microscopic changes of severe lesions in the lung and liver and moderate lesion in the brain indicating an infection of MCF. This was confirmed by the PCR test (Table 2). Figure 2 indicated severe non-suppurative hepatitis including vasculitis. Buffalo No.36 was found to be affected by MCF at 32 dpi. The buffalo showed fever (maximwn 39.7°C at day 5 of the onset of the clinical signs), loss of appetite, conjunctivitis leading to corneal opacity, serous oculo

nasal discharges to mucopurulent ocu1o-nasal discharges. The temperature was shown to be sub-normal and it was killed at the 18th days of the clinical signs onset Severe haemorrhages of the trachea. abomasum, gall bladder and urinary bladder were recognised at *post-mortem* examination (Table 1). Severe lesions in the kidney, moderate lesion in the brain and mild lesions in the *rete mirabile* and liver were examined under microscope showing MCF infection. The PCR confirmed the MCF infection (fable 2). Severe, non-suppurative interstitial nephritis with vasculitis was presented in Figure 3.

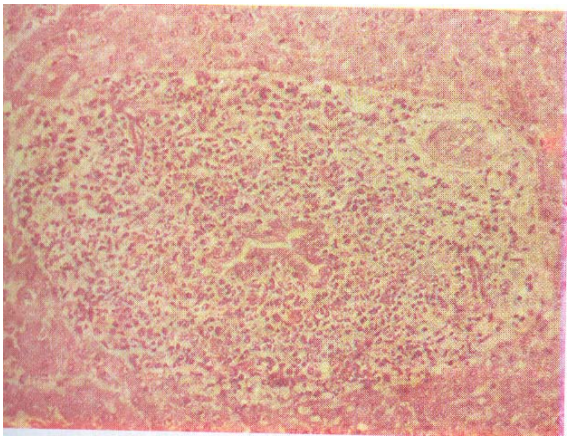

Figure 2. Non-supurative hepatitis associated with diffuse vasculitis in the portal area of the liver. (H&E Staining, X115)

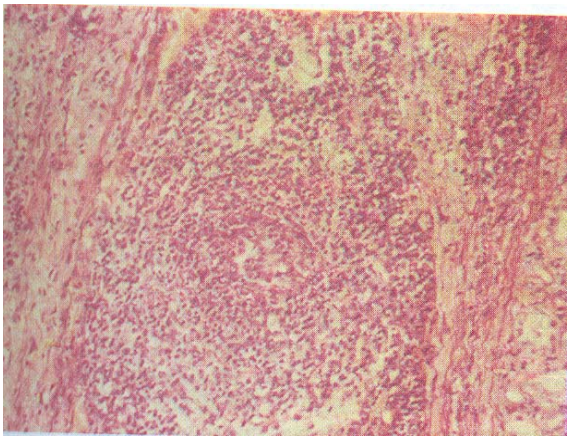

Figure 3. Non-suppurative intestinal nephritis with severe vasculitis of the kidney. (H&E staining, X115)

Bali-cross cow No.27 and Ongole cow No.29 remained healthy for 11 months post infection. They were slaughtered at 11 months post infection without showing any clinical signs. The gross and histopathological examinations showed that there were no specific lesion found, and the PCR test failed to detect any MCF causal agent from these cattle (Table 2).

Whole blood taken from buffalo No 36 was inoculated into Bali-cross cow No.27, buffalo No 38 and Bali cow No 55. Buffalo No.38 showed clinical sign of sudden high fever (maximum 41°C at the first day of clinical onset) at 31 dpi, Other clinical signs were loss of appetite and severely depressed. It died at the 2nd - day of sickness, and it showed gross pathological features of mild petechial haemorrhages in the trachea, gall bladder and urinary bladder (Table 1). Moderate lesion in the brain was demonstrated by microscopic examination of the buffalo confirming MCF infection, and it was supported by the PCR test (Table 2),

Bali cow No.55 suffered from the disease after 37 dpi with clinical signs of fever (maximum 40.5°C at the third day of the clinical signs onset), loss of appetite, severe depressed, bloody diarrhoea, serous ocular discharge, and mucopurulent nasal discharge. At the 5th day of sickness, it was unable to stand, very weak, and killed. Severe haemorrhages of the trachea and small and large intestine were found at necropsy (Table 1). The cow microscopically showed severe lesion in the liver, moderate lesion in the *rete mirabile* and spleen and mild lesion in the brain demonstrating MCF infection. The PCR test confirmed the MCF infection (Table 2).

Ongole cow No.23 which was inoculated with blood from Bali cow No.55 remained healthy when slaughtered 5 months after infection without showing any clinical signs and gross-pathological changes. No specific lesion were found under histopathological examination of the cow, and the PCR test failed to detect any MCF causal agent (Table 2).

The second set of blood transmission experiment was conducted using blood from Bali cow No 05. This cow was a spontaneous MCF case at the RIVS. Briefly, Bali cow No.05 was penned approximately 100 m from lambing sheep pen for 6 months before it showed clinical signs of fever (maximum 40.2°C at day the first day of clinical onset), anorexia, serous to mucopurulent nasal discharges, conjunctivitis, oral lesions, and corneal opacity. Gross-pathological examination showed haemorrhagic liver, kidney, urinary bladder, abomasum and large intestine (Table 1). The cow showed severe lesion in the *rete mirabile* and lymph node and mild lesion in the brain suggesting that it was suffered from MCF, and it was confirmed by the PCR (Table 2).

Whole blood taken from Bali cow No.05 was experimentally inoculated into buffalo No 15. This buffalo died suddenly after 58 dpi. without showing any clinical sign. Mild haemorrhages of the epicard, trachea, abomasum, small intestine and urinary bladder were observed at necropsy of this cow (Table 1). No visible lesion were found histologically. However, the PCR detected MCF causal agent from this cow (Table 2).

The *RsaI* digestion profiles of PCR amplified DNA from clinical MCF.

The RsaI digestion fragments of amplified DNA from the animals (buffalo Nos.505, 36 and 38 and Bali cattle Nos.63, 66 and 55) demonstrated the same pattern of DNA fragments that were characterised by the full length PCR products refractory to *RsaI* restriction (data not presented).

DISCUSSION

A serial transmission infection of whole blood from two MCF donor animals to normal animals has been successfully conducted under laboratory condition and produced clinico-pathological changes similar to those of the natural disease Prior to use, the animals were tested by the PCR and revealed that they did not have MCF causal agent. It means the diseases were resulted from tile whole blood experimental infection. Similar results have been reported by SELMAN *et al.* (1978), SINGH *et al.* (1979) and HOFFMANN *et al.* (1984).

Wide range of clinical signs, gross and histological findings were achieved in the study. These features were similar to field cases reported by DANIELS *et al.* (1988) in which there were considerable variation in clinico-pathological findings of diarrhoea, mucopurulent oculo-nasal discharges, corneal opacity and fibrinoid vasculitis. The incubation period in experimental animals ranged between 14 to 58 dpi, except two animals that survived until 7 and 11 months post infection. Similar results have been previously reported by SELMAN *et al.* (1978) and HOFFMANN *et al.* (1984).

The diagnosis of the MCF cases were based on histopatological examinations and they were confirmed by the PCR test. Both methods detected MCF in the experimental transmission animals. These mean that there were a very good correlation between histopatological examination and the PCR. The PCR tends to be more specific and sensitive. Moreover, the PCR reduces the confusion over a wide range of clinical, gross-pathlogical and histopatological findings. The PCR is relatively a new technique for detection of SA-MCF causal agent (BAXTER *et al.,* 1993). This technique has been used to confirm the diagnosis of MCF in spontaneous MCF cases (WIYONO *et al.,* 1994).

The clinical, gross and histopatological findings and PCR test of MCF was demonstrated in 5 out of 8 animals in the first experiment of tile present studies While in the second experiment, the infected buffalo No.15 was suddenly died and was diagnosed as MCF by PCR. However, it had insufficient patognomonic lesions to be diagnosed as MCF microscopically, suggesting that the PCR was more sensitive than the histopathological examination. BAXTER *et al.* (1993) reported that the PCR was a very sensitive test to detect as little as 6.4 pg OHV-2 DNA which was estimated to correspond to one diploid bovine cell.

Vasculitis is a patognomonic lesion of MCF histopathologically (LIGGITT and DEMARTINI, 1980). However, the present study revealed that vasculitis was not always found in all organs of MCF affected animals and the degree of the severity was varied in every organ in each case. In other word, vasculitis is not always found in every organs in MCF experimentally infected animals. For this reason, in the point of view of MCF diagnosis, it is recommended that as many as possible related organs to be submitted for microscopic examination (DAMAYANTI, 1996).

Three out of 9 aninmls of the serial blood transmission study failed to show any clinical signs of MCF. It was suggested that SA-MCF was relatively difficult to be experimentally transmitted. Similar results was reported previously by PIERSON *et al.* (1979). DAMAYANTI (1995) reported the occurrence of sub-clinical infection of MCF in Bali cattle that were slaughtered in abattoirs by means of histopathological examination. It is unlikely that these three animals were sub-clinically infected by MCF since the PCR test failed to detect MCF causal agent.

The difficulties to produce SA-MCF by blood inoculation were observed in two Ongole cattle and one Bali-cross breed cow. These findings were very likely due to the susceptibility of each breed to the infectious agent. This supports tile previous report (DANIELS *etal.;* 1988) that Bali cattle are the most susceptible animals to MCF, followed by buffalo and Ongole cattle, but not Bali-cross cattle. However, due to the limited number of animal used in the present study these findings may be considered as a preliminary result.

The results in the first experiment showed that there were differences in the severity of the disease. The *RsaI* profiles of buffalo Nos.505, 36 and 38 and Bali cattle Nos.63, 66 and 55 demonstrated the same pattern of DNA fragments. This confirmed that these animals were infected with the same aetiological agent. It is very likely that variation of the degree of the disease severity of each animals was due to an individual factor. This result supported the previous studies reported by SHIH *et al.* (1989) that on the basis of restriction endonuclease DNA cleavage pattern *(Hind* III and *Eco* RI) of eight isolates of MCF were assigned to two

distinct groups. Unfortunately, restriction endonuclease profiling of the present study was not conducted in the second experiment.

The infective inoculums were used in relatively large volume (I litre) of whole blood of MCF infected animals. This was similar to those reported by PLOWRIGHT (1968) in which transmission of the SA-MCF form might have been attained by intravenous transmission of large volumes (500 to 1,500 ml) of whole blood. He suggested that the causal agent of MCF in susceptible animals was highly cell-associated, and the present results demonstrated that the transmission of SA-MCF might be achieved by large inoculation of whole blood.

The clinical signs associated with the serial transmission in the present study were said to be an interesting finding because if the blood inoculum donor was a Bali cow and the normal recipient were a buffalo and a Bali cow, the buffalo was more severely affected than the Bali cow. But if the inoculum donor was a buffalo and the normal recipient were a buffalo and Bali cow, the more severely affected would be the Bali cow. Similar observation has never been reported elsewhere. However, the exact explanation of this mechanism needs to be further studied.

CONCLUSION

A serial transmission infection of MCF using whole blood as inoculums and inoculated intra-venously has been successfully achieved in six out of nine normal large ruminants. Wide range of clinical signs, gross and microscopic findings were observed. The incubation period ranged between 14 and 58 days. The study proved that Bali cattle and buffalo are highly susceptible to MCF infection but not for Bali-cross breed and Ongole cattle. There is a good correlation between histopathological examination and the PCR test. The PCR test tends to be more sensitive and reduces the confusion over a wide variety of clinical, gross and histopathological findings.

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