

STUDIES ON THE TRANSMISSION OF MALIGNANT CATARRHAL FEVER IN EXPERIMENTAL ANIMALS: BALI CATTLE IN CLOSE CONTACT WITH SHEEP

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(Received by the editor 5 January 1999)

ABSTRAK

WIYONO, A. dan R. DAMAYANTI. 1999. Studi penularan buatan *malignant catarrhal fever* pada hewan percobaan: sapi Bali kontak langsung dengan domba. *Jurnal Ilmu Ternak dan Veteriner* 4(2): 128-135.

Sebuah studi tentang penularan buatan *malignant catarrhal fever* (MCF) secara kontak langsung antara sapi Bali yang dikandangkan berdekatan dengan kandang domba bunting dan domba baru melahirkan dilaksanakan pada dua flock sapi dan domba pada kurun waktu berbeda. Berdasarkan kelainan klinik dan pemeriksaan histopatologi serta uji *polymerase chain reaction* (PCR), penularan buatan MCF berhasil diperoleh pada empat ekor sapi Bali yang berdekatan dengan domba, dan pada satu ekor sapi Bali yang berjarak 100 meter dari kandang domba. Hasil uji PCR menunjukkan bahwa agen penyebab MCF dapat dideteksi pada *peripheral blood leucocytes* (PBL) dan sekresi mata, hidung dan vagina domba-domba tersebut. Virus pada sekresi domba tersebut diduga dapat menyebar dan mengakibatkan kelima sapi Bali tersebut tertulari MCF. Sungguhpun demikian cara penularan yang pasti masih memerlukan penelitian lebih lanjut terutama tentang waktu dan cara penularan dari hewan reservoir ke hewan peka.

Kata kunci : *Malignant catarrhal fever*, penularan buatan, kontak langsung , sapi Bali, domba

ABSTRACT

WIYONO, A. and R. DAMAYANTI. 1999. Studies on the transmission of malignant catarrhal fever in experimental animals: Bali cattle in close contact with sheep. *Jurnal Ilmu Ternak dan Veteriner* 4(2): 128-135.

A study on experimental transmission of malignant catarrhal fever (MCF) in Bali cattle that were closely penned to a flock of pregnant sheep and lambing sheep were conducted in two different periods and flocks of Bali cattle and sheep. On the basis of clinico-pathological findings and polymerase chain reaction (PCR) tests, the study revealed that MCF transmission has been successfully achieved in four Bali cattle that were in contact with lambing sheep, and in one Bali cow that was penned 100 metres away from lambing sheep pen. The polymerase chain reaction (PCR) detected the causal agent of MCF in peripheral blood leucocytes (PBL) and nasal, ocular and vaginal secretions of sheep. It is assumed that viruses in the secretion were spread by sheep causing five Bali cattle suffered with MCF. However, further studies on exact mode of transmission needs to be clarified particularly on the time and mode of viral transmission from reservoir to susceptible animals.

Key words : Malignant catarrhal fever, experimental transmission, in-contact, Bali cattle, sheep

INTRODUCTION

Malignant catarrhal fever (MCF) is a fatal disease of domesticated large ruminants as well as many other wild Bovidae (PLOWRIGHT, 1981) and pigs (LØKEN *et al.*, 1998). There are two types of MCF i.e. wildebeest-associated MCF (WA-MCF) and sheep-associated MCF (SA-MCF). The WA-MCF occurred in association with wildebeest (*Connochaetes* sp) from which the causal agent was Alcelaphine Herpesvirus-1 (AHV-1) (PLOWRIGHT *et al.*, 1960; ROIZMAN *et al.*, 1996). The SA-MCF occurred in the absence of wildebeest (*Connochaetes* sp), and sheep has been epidemiologically suggested as an important role in

causing of SA-MCF disease (PLOWRIGHT, 1981; DANIELS *et al.*, 1988) this was designated as sheep-associated MCF (SA-MCF). Clinically and pathologically, SA-MCF and WA-MCF are indistinguishable (PLOWRIGHT, 1981). Although AHV-1 is easily isolated from wildebeest, the exact mode of natural transmission from reservoir animals to susceptible animals is not yet clearly known (RWEYEMAMU *et al.*, 1974.). Similar pathogenesis is suggested to occur in SA-MCF (PLOWRIGHT, 1981). However, the causal agent of SA-MCF has yet to be isolated.

Transmission experiments of SA-MCF has long been reported by DAUBNEY and HUDSON (1936).

However, due to lack of viral isolation or detection, studies on SA-MCF has been relied on epidemiological data and similar studies of the WA-MCF including studies on its transmission. SA-MCF has a similar pattern, where epidemiological evidence suggests that domestic sheep play an important role in disease transmission.

Recently, polymerase chain reaction (PCR) techniques have been developed for diagnosis of WA-MCF (HSU *et al.*, 1990) and of SA-MCF (BAXTER *et al.*, 1993). Before the PCR was developed the role of sheep in the transmission of SA-MCF cases was based on epidemiological data (HOFFMANN *et al.*, 1984; DANIELS *et al.*, 1988), and extrapolation of similar studies of the WA-MCF virus isolated from wildebeest.

Malignant catarrhal fever is considered to be an important disease in Indonesia. Domestic animals that are susceptible to MCF infection include Bali cattle (*Bos javanicus*) and the swamp buffalo (*Bubalis bubalis*) (HOFFMANN *et al.*, 1984; DANIELS *et al.*, 1988). These animals are both important as draught power and as a source of wealth for many Indonesian farmers in rural areas. Bali cattle show excellent productivity under dry conditions. However, because of its susceptibility to MCF, this species can not be kept in areas where sheep are commonly present such as in West Java. On the other hand, in areas where Bali cattle are presence in large numbers such as in West Timor of East Nusa Tenggara Province, keeping of sheep is prohibited. For the same reason farmers who receiving Bali cattle under government developing programs must be forbidden to keep sheep. This means, that MCF also restricts the development strategies for livestock distribution, and limits choices of farming systems at small holder farmer level.

The aim of this study is to investigate the transmission of MCF by means of experimentally close contact between Bali cattle and pregnant sheep and lambing sheep.

MATERIALS AND METHODS

Association between Bali cattle and lambing sheep were conducted in two experiments involving different flocks. In the first experiment, Bali cattle were penned underneath lambing sheep and another Bali cow 005 was kept approximately 100 metres away from the sheep pen. In the second experiment, domesticated large ruminants including Bali cattle were kept 3 to 10 metres distance away from lambing sheep and this was conducted at different time.

Experiment 1. Three Bali cattle (*Bos javanicus*) were used in this study. Two Bali cattle (designated as Bali cattle HM and MM) aged approximately three years which had been at the Research Institute for

Veterinary Science (RIVS) for more than six months were penned underneath three lambing sheep (Nos. 56, 58 and 59) with three lambs (Nos. 56A, 58A and 59A). Another Bali cow No.005 was kept approximately 100 metres from the sheep pen.

Experiment 2. Five domesticated large ruminants were used in this experiment consisting of three Bali cattle, a swamp buffalo (*Bubalus bubalis*) and a Friesian cow (*Bos taurus*). These animals were kept approximately 3 - 10 metres away from three lambing sheep (Nos. 34, 35, 45) and four lambs (34A-1, 34A-2, 35A and 45A).

The detection of SA-MCF agent in experimental animals

Peripheral blood leucocytes (PBL) of all domesticated large ruminants except Bali cow 005 were tested by the PCR (BAXTER *et al.*, 1993) prior to use in the experiment. Samples of PBL were subsequently collected from Bali cattle MM and HM on the weekly basis.

Nasal secretion and PBL samples of lambs from experiment 1 were collected weekly. DNA were extracted from the samples and tested to the PCR (SAMBROOK *et al.*, 1989).

Ocular, nasal and vaginal secretions and PBL samples of ewes and lambs from experiment 2 were taken and tested to the PCR (SAMBROOK *et al.*, 1989).

Polymerase chain reaction techniques

Sample collections

Heparinised blood and nasal, ocular and vaginal secretion samples were collected from sheep. Similarly, samples of heparinised blood or lymph node were taken from Bali cattle. PBL samples were obtained by lysing heparinised blood with ammonium chloride (0.85% NH₄Cl) solution. The PBL fraction was collected by pelleting at 500 g and washed twice with PBS and then lysed with DNA extraction buffer. Nasal, ocular and vaginal secretion samples were collected using sterile cotton swabs in 1 mL of PBS. The PBS suspension was pelleted at 12,000 rpm for 10 minutes. The pellet was dissolved in DNA extraction buffer.

DNA extraction

DNA fragment were extracted from PBL and secretion as described elsewhere (SAMBROOK *et al.*, 1989; BAXTER *et al.*, 1993; WIYONO *et al.*, 1994).

Briefly, DNA fragment were extracted from PBL by phenol-chloroform extraction and were ethanol precipitated. Samples of secretions were added with 100 µg of Proteinase K and incubated at 50°C overnight to extract the DNA. The DNA concentrations were then quantified by ultra-violet spectrophotometry.

The PCR techniques were performed as described by BAXTER *et al.* (1993). Briefly, two amplification of reaction were performed. The first amplification step used the primer pairs 556/755. It was pre-cycled at 99°C for three minutes, and set up for 25 cycle amplification reaction as follows: 94°C for 20 seconds, 60°C for 30 seconds, 72°C for 30 seconds and was followed by a final extension of 72°C for five minutes. The second amplification employed the primer pairs 556/555. The reaction programme was set up for 30 cycles as above without pre-cycling. The amplified DNA fragment were visualized by 1.8% agarose gel in Tris Borat EDTA (TBE) or Loening E buffer (SAMBROOK *et al.*, 1989).

Histopathological examination

At necropsy, samples of Bali cattle organs were collected and stored in 10% Neutral-Buffered Formalin. Organs such as *rete mirabile*, brain, trachea, lung, heart, liver, spleen, kidney, abomasum, small intestine, urinary ladder and superficial lymph nodes were taken. The tissues were processed and stained by Hematoxylin and Eosin (H&E) for histopathological examination as described elsewhere (PLOWRIGHT, 1981).

RESULTS

PCR based detection of SA-MCF agent in experimental cattle

Before being used in the experiments, all samples from domesticated large ruminant were tested by the PCR except Bali cow 005. Based on the PCR test, the causal agent of SA-MCF was not detected from PBL of the cattle and buffaloes. These animals were then used in the experiments. PCR based longitudinal study on the detection of the casual agent of SA-MCF in PBL of domesticated large ruminant showed that OHV-2 were found from Bali cattle HM and MM (Table 1). The table showed that fragment of Ovine Herpesvirus-2 were not detected a month prior to the death of both cattle.

Table 1. Longitudinal PCR analysis of PBL of Bali cattle HM and MM during a contact transmission experiment

Table 2. PCR based detection of the causal agent of SA-MCF in nasal swab and PBL of sheep lambs

Duration of contact with sheep	PCR results	
	Bali cow HM	Bali cow MM
1 month	-ve	-ve
2 months	-ve	-ve
10 weeks	-ve	-ve
11 weeks	-ve	-ve
3 months	-ve	-ve
5 months	-ve	-ve
6 months	+ve*	+ve**

Note :

* Bali cow HM died

** Bali cow MM died

PCR based detection of SA-MCF agent in nasal secretions and PBL of lambs in experiment 1

OHV-2 fragment could be detected by the PCR as early as one day old in samples from one of the lambs (No. 59A) (Table 2). Samples from the other two lambs started to react to the PCR at the 9th days of age (lamb No. 56A) and at the 8th day of age (lamb No. 58A).

In addition to the detection of OHV-2 in nasal secretion of lambs, PBL samples that were collected at the same time as the nasal secretions appeared that to have a higher prevalence of OHV-2 in PBL than that of nasal secretion in all lambs except lambs No. 56A.

Clinical cases of MCF in experiment 1

At the first experiment, two Bali cattle HM and MM were suffered by MCF after being penned underneath a flock of lambing sheep (ewes No.56, 58 and 59, and lambs No.56A, 58A and 59A). The other Bali cow No. 005 which was kept approximately 100 metres from the lambing sheep pen died first and it was followed by Bali cattle HM and MM 43 and 53 days later respectively. The cow 005 was the first to be affected, and showing clinical signs such as sudden high fever (40.2°C at day 1 of the onset of the clinical signs), anorexia, serous to mucopurulent nasal discharges, conjunctivitis, oral lesions, corneal opacity, and was killed at the third day. At necropsy, it showed haemorrhagic inflammation of liver, kidney, urinary bladder, abomasum and large intestine. Histopathological examination concluded that the cow was caused from MCF (Table 4). This infection was proofed by OHV-2 agent confirmed by the PCR.

No	Lamb No.56A		Lamb No.58A		Lamb No.59A	
	Age-days	PCR	Age-days	PCR	Age-days	PCR

		PBL	swab			PBL	swab			PBL	swab
1	1	ND	-ve	0	-ve	-ve	1	ND	+ve		
2	9	+ve	-ve	8	+ve	-ve	10	ND	+ve		
3	16	+ve	-ve	17	+ve	-ve	17	+ve	-ve		
4	23	+ve	+ve	23	-ve	-ve	24	ND	+ve		
5	31	+ve	+ve	30	-ve	+ve	32	+ve	-ve		
6	40	+ve	+ve	37	ND	+ve	41	+ve	ND		
7	46	-ve	-ve	42	+ve	-ve	47	+ve	-ve		
8	53	-ve	-ve	49	+ve	+ve	54	+ve	+ve		
9	60	-ve	ND	56	+ve	+ve	59	+ve	+ve		
10	65	-ve	+ve	65	+ve	-ve	66	-ve	-ve		
11	72	-ve	+ve	76	-ve	-ve	73	-ve	-ve		
12	79	+ve	+ve	79	-ve	ND	80	+ve	+ve		
13	88	-ve	-ve	88	-ve	-ve	89	ND	+ve		
14	99	+ve	+ve	93	-ve	ND	93	-ve	ND		
15	102	-ve	-ve	146	+ve	+ve	102	-ve	-ve		
16	111	-ve	-ve				105	-ve	ND		
17	170	+ve	+ve				114	+ve	ND		
18							119	-ve	ND		
19							171	+ve	-ve		
		8/16	8/16			7/14	5/13			9/15	7/14
		50%	50%			50%	38%			60%	50%

Bali cow HM died suddenly without showing any clinical signs. The gross lesions were found to be widespread of inflammation in abomasum, small and large intestine, gall bladder and urinary bladder. Histopathological examinations showed an un-usual distribution of MCF lesion, but it was diagnosed as MCF (Table 4). The PCR demonstrated that the cow was infected by the causal agent of SA-MCF.

Clinical signs of Bali cow MM showed high fever (maximum 40.9°C day 1 of the disease onset), very depressed, loss of appetite, serous ocular discharge, and very weak and finally died at day 2. Gross-pathological examinations showed moderately hemorrhages of trachea, small and large intestine and urinary bladder, and severe haemorrhagic inflammation of abomasum. Microscopic lesion of MCF were found to be unusually distributed, but it was diagnosed as MCF (Table 4). OHV-2 DNA fragment was detected from this cow by the PCR which confirmed that the cow was infected by MCF virus.

PCR based detection of SA-MCF agent in nasal, ocular and vaginal secretion and PBL of sheep and lambs in experiment 2

This experiment was conducted in a group of sheep consisting three ewes (Nos.45, 35 and 34), and

their four male lambs (Nos.45A, 35A, 34A-1 and 34A-2). Three of the lambs were observed until they were six months of age (lambs Nos. 35A, 34A-1 and 34A-2), and the other one (lamb No.45A) was observed until it was eight months of age.

The detection of OHV-2 in nasal, ocular and vaginal swabs, and PBL of sheep were summarised in Table 3. OHV-2 agent was detected in the nasal, ocular and vaginal swabs of ewes and lambs. A total of 367 samples of nasal, ocular and vaginal swabs which were tested during the study resulted 38 (10.4%) reacted in the OHV-2 PCR. It is likely that the viral genome could be detected more frequently in samples collected from the ewes than that from the lambs with the prevalence of 16.4% and 2.5% respectively. In terms of animals tested samples from ewe No.45 reacted most frequently in the PCR (24.6%). In addition lambs No.45A was one of two lambs from which did not have nasal or ocular secretions reacting in the PCR. The vaginal and nasal swabs collected from the ewes had frequency in the PCR of 23.3% and 11.4% respectively. Viral genome was less frequently detected in the ocular swabs (3.4%).

Clinical cases of MCF in experiment 2

The contact transmission experiments was conducted in a group of animals which consisted of three Bali cattle, a buffalo (*Bubalus bubalis*) and a Friesian cow (*Bos taurus*), and penned approximately 3 10 metres away from three lambing sheep (Nos. 34, 35, 45) and four lambs (Nos. 34A-1, 34A-2, 35A and 45A).

Six and ten days after ewe No.45 was lambing, two Bali cattle (Nos.NN1 and 006) were seen to be

affected and died due to MCF infection. Their clinical signs, gross and histopathological findings were shown at Table 4, and the PCR test results confirmed MCF. The other large ruminants penned in the vicinity all remained un-infected. One week after the onset of MCF cases were observed PBL samples from these animals were none reacted in the PCR.

Table 3. Summary of detection of OHV-2 in ocular, nasal and vaginal swabs of sheep and lambs by the PCR at the RIVS

Sheep	Ocular swab		Nasal swab		Vaginal swab		Total
	No. Tested	No. PCR +ve	No. Tested	No. PCR +ve	No. Tested	No. PCR +ve	
Sheep No.45	23	2	23	8	23	7	17/69 (24.6%)
Sheep No.35	23	2	23	4	23	3	9/69 (13.0%)
Sheep No.34	23	0	23	2	23	6	8/69 (11.6%)
Sub-total	69	4 (5.8%)	69	14 (20.3%)	69	16 (23.3%)	34/207 (16.4%)
Lamb No.45A	23	0	23	0	ND	ND	0/46
Lamb No.35A	19	0	19	1	ND	ND	1/38 (2.6%)
Lamb No.34A-1	19	0	19	0	ND	ND	0/38
Lamb No.34A-2	19	1	19	2	ND	ND	3/38 (7.9%)
Sub-total	80	1 (1.3%)	80	3 (3.8%)	ND	ND	4/160 (2.5%)
Total	149	5 (3.4%)	149	17 (11.4%)	69	16 (23.3%)	38/367 (10.4%)

Note :

ND = samples were not available because the lambs were male

Table 4. Histopathological examination on in-contact experimental transmission of MCF in Bali cattle

No.	Bali cattle	Histopathological lesions											
		Rete mirabile	Brain	Trachea	Lung	Heart	Liver	Spleen	Lymph node	Abomasum	Intestine	Kidney	Urinary bladder
1	005	+++	+	-	-	-	-	-	+++	+	-	-	+
2	HM	+	-	-	-	+	+++	+++	+	+	+++	+++	+
3	MM	-	-	-	++	+	+++	++	+++	+++	+++	++	-
4	NN1	+	+	+++	-	-	++	-	-	+	-	++	++
5	006	++	++	++	-	-	++	-	-	+	+	+++	++

Notes:

- : no lesion

+: mild lesion

++ : moderate lesion

+++ : severe lesion

Type of lesion were mainly vasculitis and non-suppurative inflammation of affected organs

Bali cow NN1 had clinical signs of high fever (41.0°C), corneal opacity, depression and anorexia. Gross pathological lesions showed severe haemorrhagic of trachea, abomasum and urinary bladder. While in epicard and intestine has less haemorrhagic

areas. Histopathological observations presented at Table 4 and PCR test confirmed that it had MCF.

Bali cow 006 clinically showed high fever (40.9°C), depression, anorexia, conjunctivitis, serous ocular and nasal discharges, and partial corneal opacity. At necropsy observations, it had mild haemorrhagic

areas in the trachea, abomasum, small intestine and urinary bladder. MCF was diagnosed based on the histopathological lesions (Table 4) and PCR test.

DISCUSSION

During this experiment five Bali cattle were believed to have died due to MCF infection (Bali cattle 005, HM, MM, NN-1, and 006). These cases are considered to be the first spontaneous MCF cases at the Research Institute for Veterinary Science (RIVS), since all of the MCF cases before were from blood transmission experiments (WIYONO *et al.*, 1994; DAMAYANTI, 1995a; DAMAYANTI, 1996a). In this study all of the Bali cattle showed typical MCF clinical signs as well as gross pathology except Bali cow HM which died suddenly. All of the Bali cattle had histological lesions consistent with characteristic MCF. However, Bali cattle HM and MM both had an un-usual distribution of lesions.

The severity of the lesions that were observed in this study showed a wide range of variation. This findings were in accordance with previous reports (DAMAYANTI, 1995a; DAMAYANTI, 1995b; DAMAYANTI, 1996a). The gross and histopathological lesions usually manifest the severity of the disease (DAMAYANTI, 1996b). In addition, it was also observed that there were variation in the incubation period and the course of the disease (SELMAN *et al.*, 1978)

Regarding the detection of OHV-2 DNA fragments in sheep PBL and secretion, the related virus of WA-MCF the AHV-1 can be isolated from nasal secretion of four day old wildebeest calves (MUSHI *et al.*, 1980), and blood of seven day old wildebeest (PLOWRIGHT, 1965). The virus was also isolated from wildebeest fetuses (PLOWRIGHT, 1965) suggesting that there is a trans-placental transmission. In this study, OHV-2 virus was detected by the PCR more frequently in samples collected from ewes rather than lambs and the highest percentage of samples reacting in the PCR were vaginal swabs followed by nasal and ocular swabs. This study showed that the shedding of OHV-2 can occur from a variety of secretion from ewes. This support the previous reports that most wildebeest calves became infected during the 2nd, 3rd or 4th month of life (PLOWRIGHT, 1965; MUSHI and RURANGIRWA, 1981). BARNARD *et al.* (1989) reported that 61% of wildebeest calves 1-2 months had mean titre virus of 9.8×10^4 cytopathic forming foci per mL in their ocular fluid, and less than 2% in wildebeest aged more than six months. The virus is still shed by wildebeest calves from their nasal and ocular secretions until three months old (MUSHI and RURANGIRWA, 1981).

From the experiment 1 seems likely that the three Bali cattle designated as 005, HM and MM received the

infectious agent from sheep and lambs and possibly from ewe No.45 which gave birth six days before the first cow showing clinical signs of MCF. Samples of PBL and secretions collected from the ewe consistently reacted in the PCR. However, the exact mechanism of transmission is not known. In WA-MCF, RWEYEMAMU *et al.* (1974) reported that adult wildebeest which were treated with corticosteroids or under the stress of confinement and change of nutrition shed AHV-1 in their nasal secretions. This was later confirmed by MUSHI *et al.* (1980) that the virus can be demonstrated in both secretion as cell-free virus. KALUNDA *et al.* (1981) have shown that aerosol transmission to cattle was possible using cell-free virus. In the present study it is very likely that the virus shed by the sheep flock was cell-free OHV-2 virus. However, until virus can be isolated this hypothesis may remain unproven.

Interestingly, Bali cow No. 005 which was penned approximately 100 metres from the sheep suffered from MCF earlier than did the other two (Bali cattle MM and HM) which were penned close to the sheep. This cow was not tested by the PCR prior to experimental transmission. Due to this reason, there was no information whether this cow was actually infected by MCF virus originally from the sheep. There are several possible modes of transmission. The first possibility is that the cow may have been infected in utero (PLOWRIGHT *et al.*, 1972). Secondly, the transmission can also be due to the movement of sheep from infected areas to other areas (SINGH *et al.*, 1979). This is unlikely to be the case in this experiment since there were no sheep moved during the experiment. Thirdly, there is the possibility of contamination of cattle feed or pastures by lambs and lambing ewes (SELMAN *et al.*, 1974). In this experiment, the sheep and cattle were fed separately, and the experimental cattle were fed first and then the sheep. The fourth possibility is that there are alternative hosts or intermediate hosts capable of biological transfer of the virus (PIERCY, 1954; BARNARD *et al.*, 1989). The fifth possibility is that transmission is by aerosols (KALUNDA *et al.*, 1981), but there are no published reports yet to confirm this hypothesis. If this was the case, this study is the first report of airborne transmission of MCF. It has to be noted that PLOWRIGHT (1965) found that the virus in cattle or wildebeest blood is entirely cell-associated MCF and extremely fragile. Finally, this cow may have been sub-clinically infected by MCF virus before being used in this experiment. Sub-clinical infection has been reported to occur among Bali cattle in Indonesia (DAMAYANTI, 1995a). However, sub-clinical infection is unlikely to occur in this cow, because this cow was born at RIVS. The most likely infection of MCF virus to this cow was from the closest sheep to the cow.

These sheep was the ones that were used in experiment 1.

In experiment 2, viral genome in nasal, ocular and vaginal secretions from one lamb (No.45A) was not observed up to eight months. However, samples of PBL from this animal reacted in the PCR. In studies on WA-MCF, it was concluded that wildebeest calves received the virus either *in utero* from the dam or horizontally from other calves (PLOWRIGHT, 1981). All wildebeest become infected by AHV-1 by the age of nine months (REID and BUXTON, 1985). A similar situation appears to occur in the case of reservoir animals for SA-MCF as shown in this experiment. In fact, the lamb No.45A was the only animal from which nasal, ocular and vaginal secretion did not react in the PCR, but the secretions collected from the dam of this lamb frequently reacted in the PCR. This result suggests that individual animal at this flock could not be ascertained secreting the virus. Studies on WA-MCF demonstrated large differences in the prevalence of MCF in susceptible species, and DE KOCK and NEITZ (1950) considered that the infective agent was not necessarily present in every herd of wildebeest. Interestingly epidemiological evidence showed that two cases of MCF in Bali cattle (Nos. NN-1 and 006) occurred six days after the ewe No.45 lambd while the other sheep at this stage had not given birth. It is very likely that both Bali cattle received the virus from this ewe, while viral genome was not detected in secretions collected from her lamb. Viral genome was only detected in 10.4% (38/367) of the secretion samples. This may indicate that it does not require a heavily infected area to produce a case of SA-MCF.

From the experiment 2, apart from two Bali cattle Nos. NN-1 and 006, there were another Bali cow, one buffalo, and one Frisian cow. These animals remained healthy and their PBL samples failed to react in the PCR suggesting that they were remained uninfected until the end of the observation period. Based on the studies of contact transmission between lambing sheep and large ruminants concluded that Bali cattle was the most susceptible SA-MCF species followed by Bali-cross cattle, buffalo and *Bos indicus* cattle (DANIELS *et al.*, 1988). However, in some cases of MCF in mixed herds including Bali cattle, some Bali cattle survived while other animals became affected.

With regard to incubation period of affected Bali cattle, based on the PCR data of Bali cattle HM and MM, these Bali cattle probably had an incubation period of one month or less, since OHV-2 fragments were not detected by the PCR from PBL from these Bali cattle one month before they died. PLOWRIGHT (1981) reported that the incubation period of MCF varied from nine to 77 days, in experimental transmission of WA-MCF.

CONCLUSION

On the basis of clinico-pathological findings and PCR tests, the studies of in-contact transmission of MCF to five Bali cattle has successfully been achieved. The virological agent was assumed to be spread by sheep from which OHV-2 fragment was detected by the PCR from their PBL and secretion. It is assumed that OHV-2 in PBL and secretion was spread by sheep causing five Bali cattle suffered by MCF. This study is a preliminary study on understanding MCF transmission. It confirmed that Bali cattle is not recommended to be penned in close contact with sheep particularly lambing sheep. Further studies on exact mode of viral transmission needs to be clarified particularly on the time and mode of viral transmission from reservoir to susceptible animals. It is hoped that the exact control measure may be considered.

ACKNOWLEDGMENT

The authors wish to thank the technicians of Virology and Pathology Department for their excellent technical assistance during the study. Part of this study was sponsored by the British Overseas Development Administration (ODA) for which gratefully acknowledged.

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