THE DETECTION OF OVINE HERPESVIRUS-2 IN RESERVOIR HOST OF MALIGNANT CATARRHAL FEVER IN INDONESIA BY MEANS OF POLYMERASE CHAIN REACTION

AGUS WIYONO

Balai Penelitian Veteriner
Jalan R.E. Martadinata 30, P.O. Box 151, Bogor 16114, Indonesia

(Received by the editor 5 January 1999)

ABSTRAK

WIYONO, A. 1999. Deteksi ovine herpesvirus-2 pada hewan reservoir *malignant catarrhal fever* di Indonesia menggunakan *polymerase chain reaction*. *Jurnal Ilmu Ternak dan Veteriner* 4(2): 121-127.

Malignant catarrhal fever (MCF) merupakan penyakit fatal pada ruminansia besar, beberapa hewan liar dan babi. Ovine herpesvirus-2 (OHV-2) adalah agen penyebab *sheep-associated MCF* (SA-MCF). Di Indonesia, secara epidemiologik domba dikaitkan dengan kejadian MCF, kemudian setelah dikembangkan uji biologi molekuler yang sensitif dan spesifik, yaitu *polymerase chain reaction* (PCR), maka aspek biologi molekuler MCF banyak terungkap. Uji PCR tersebut telah berhasil digunakan untuk mendeteksi fragmen OHV-2 pada sampel domba seperti *peripheral blood leucocytes* (PBL) domba, sekresi hidung anak domba dan organ anak domba. Fragmen OHV-2 tersebut juga dapat dideteksi pada PBL kambing yang berasal dari beberapa daerah di Indonesia, tetapi tidak ditemukan pada PBL babi yang berasal dari Bali. Hasil penelitian ini dapat diduga bahwa domba dan anak domba merupakan hewan reservoir yang potensial bagi MCF, sedangkan keterkaitan kambing sebagai hewan reservoir MCF belum dapat ditetapkan.

Kata kunci : Ovine herpesvirus-2, *polymerase chain reaction*, hewan reservoir, *malignant catarrhal fever*, Indonesia

ABSTRACT

WIYONO, A. 1999. The detection of ovine herpesvirus-2 in reservoir host of malignant catarrhal fever in Indonesia by means of polymerase chain reaction. *Jurnal Ilmu Ternak dan Veteriner* 4(2): 121-127.

Malignant catarrhal fever (MCF) is a fatal disease affecting large ruminants, some wild animals and pigs. Ovine herpesvirus-2 (OHV-2) is the causal agent of sheep-associated MCF (SA-MCF). In Indonesia, sheep is epidemiologically implicated in the occurrence of MCF. Providing a sensitive and specific molecular biological technique, i.e. polymerase chain reaction (PCR), a wide range of MCF aspects on molecular level have been developed. The PCR has been successfully used to detect OHV-2 fragment in sheep samples such as peripheral blood leucocytes (PBL) of sheep, nasal secretion of a sheep lamb, and organs of some sheep lambs. Fragment of OHV-2 was also detected in PBL of goats from some areas in Indonesia but not in PBL of pigs from Bali. These results suggest that sheep and sheep lambs are the potential reservoir hosts of MCF in Indonesia, while the role of goats as reservoir host of MCF has yet to be determined.

Key words : Ovine herpesvirus-2, polymerase chain reaction, reservoir host, malignant catarrhal fever, Indonesia

INTRODUCTION

Malignant catarrhal fever is a lymphoproliferative sporadic fatal disease of domestic cattle and buffalo as well as many other wild Bovidae (PLOWRIGHT, 1981) and pigs (LØKEN *et al*., 1998). Ovine herpesvirus-2 (OHV-2) is the causal agent of sheep-associated malignant catarrhal fever (SA-MCF) (BRIDGEN and REID, 1991; ROIZMAN *et al*., 1996). SA-MCF is one of MCF types that occurs in the absence of wildebeest (*Connochaetes* sp), and epidemiological evidence suggest that sheep are the reservoir animals and it is reported world wide including in Indonesia. The other type of MCF is wildebeest-associated MCF (WA-MCF) and its causal agent is alcelaphine herpesvirus-1 (AHV-1) (PLOWRIGHT *et al*., 1960; ROIZMAN *et al*., 1996). Wildebeest-associated MCF occurs when susceptible animals have close contact with wildebeest (*Connochaetes* sp) which carry the causal virus without any clinical signs. Both types of MCF are indistinguishable clinically and pathologically (PLOWRIGHT, 1981).

The causal agent of WA-MCF could easily be isolated from wildebeest's organs (PLOWRIGHT *et al*., 1960) as well as from nasal and ocular secretions (RWEYEMAMU *et al*., 1974; MUSHI *et al*., 1980; MUSHI and WAFULA, 1983). Based on epidemiological evidences, sheep have long been considered to be the

reservoir animals for SA-MCF (GOTZE and LIESS, 1929). Using indirect immunofluorescence and viral neutralisation test, ROSSITER (1981b) reported that sheep sera were collected from Kenya, Austria, United Kingdom and Australia had antibody to AHV-1. In addition to this, HERRING *et al*. (1989) tested sheep sera using western blotting concluded that a virus antigenically similar to AHV-1 was prevalent in sheep. These results showed that sheep carry a related but not identical virus to AHV-1 (ROSSITER, 1981a; HERRING *et al*., 1989).

Sheep-associated MCF occurs in Indonesia, and sheep has long been implicated in the occurrence of MCF cases (DANIELS *et al*., 1988; PARTADIREDJA *et al*., 1988), but very little knowledge about the reservoir hosts is known. In some areas of Indonesia region, MCF has occurred in large ruminants on many occasions without direct or indirect association with sheep such as in West Nusa Tenggara (MUTHALIB, 1988) and Bali (DHARMA *et al*., 1985). In such cases, it has been postulated that goats are the reservoir hosts of MCF (DHARMA *et al*., 1985; MUTHALIB, 1988). Apart from this, epidemiological findings suggested that pigs may be involved in MCF cases in large ruminants in Bali (DHARMA *et al*., 1985).

Recent studies on MCF has reached a molecular level in which the construction of oligonucleotide primers for OHV-2, and the polymerase chain reaction (PCR) specific and sensitive for SA-MCF was successfully developed (BAXTER *et al*., 1993). The PCR method has been adapted in Indonesia (WIYONO *et al*., 1994a and b) and used in United Stated of America (LI *et al*., 1994) in Kenya (MIRANGI and KANG'EE, 1997), Australia (ELLIS and MASTERS, 1997) and in Europe (MULLER *et al*., 1998).

This paper describes the application of the PCR to detect OHV-2 fragment in peripheral blood leucocytes (PBL) of sheep, nasal secretions of a sheep lamb, organs of some sheep lambs, PBL of goats from some areas in Indonesia and PBL of pigs from Bali.

MATERIALS AND METHODS

Samples

Peripheral blood leucocytes (PBL) of sheep, goats and pigs

Peripheral blood leucocytes samples of sheep were collected from various areas of Indonesia (West, Central and East Java, Lampung, Bali, West Nusa Tenggara and South-East Sulawesi). Peripheral blood leucocytes of goats were collected from West Java, Lampung and Bali Provinces. PBL of pigs were also taken from Bali.

Tissues of sheep

Three thin-tailed breed of sheep (sheep Nos. 34, 35, 45, and four lambs i.e. lambs Nos. 34A-1, 34A-2, 35A and 45A) that were penned at Research Institute for Veterinary Science (RIVS) were used. At necropsy, a range of tissues were collected in a sterile manner and stored at -20°C before tested in the PCR. The tissues collected were turbinate, cornea, nasal epithelium and lachrymal gland.

Nasal secretion of a sheep lamb

In a longitudinal PCR based detection of OHV-2 in nasal secretion and PBL of a sheep lamb (lamb No.132A) that was penned at Research Institute for Animal Production (RIAP), Ciawi, Bogor, Indonesia, was used. Samples were collected on a weekly basis, processed (SAMBROOK *et al*., 1989) and tested in the PCR (BAXTER *et al*., 1993).

Preparation of DNA template

The DNA were extracted from sheep PBL and tissues samples with phenol-chloroform solution and then followed by chloroform:isoamyl alcohol as described by SAMBROOK *et al*. (1989). They were extracted from nasal secretion by using 100µg proteinase K at 50^{o}C over night (SAMBROOK *et al*., 1989), and were quantified (SAMBROOK *et al*., 1989) prior to testing in the PCR (BAXTER *et al*., 1993).

The polymerase chain reaction

The PCR techniques that was described by BAXTER *et al*. (1993) was used in this experiment. Two phases of amplification reactions were prepared using positive displacement pipettes which were performed in a 50 µL of amplification reaction, and were added with 50 µL of mineral. The first amplification employing 556/755 primer pairs. The reaction contains 1 mM of each primer, PCR buffer (50 mM of KCl, 10 mM of Tris pH 8.3, 0.01% of gelatin, 2 mM of $MgCl_2$), 10% of DMSO, 0.2 mM of dNTPs, 2 µg of DNA sample and 1.25 units of Taq DNA polymerase. The following cycling programme was used : Pre-cycled at 99^{o}C for three minutes after which a mixture of dNTPs and Taq polymerase was added to the reaction. A 25 cycle of amplification reaction was set up as follows 94^{o}C for 20 second, 60^{o}C for 30 second, 72^{o}C for 30 second. At the last cycle, it was followed by a final extension of 72^{o}C for five minutes and at 25^{o}C until the second set of PCR amplification started. The second set of reaction used primer pair 556/555. The 50 µL reaction was consisted of 44 µL of master mix, 1 µL of amplified DNA from

first amplification and 5 μL of mixture of dNTPs and Taq polymerase as above. The mixture was overlayed with 50 μL of mineral oil before put into the thermal machine. The reaction programme was set up for 30 cycles as above but without pre-cycling.

Visualisation of PCR amplified DNA by agarose gel

Amplified DNA was extracted with equal volume of chloroform-isoamyl alcohol (24:1) and a minimum DNA loading dye (SAMBROOK *et al*., 1989). Five percent of them were direct analysed by 1.8% agarose gel electrophoresis.

RESULTS

PBL of sheep and goats were collected from 13 areas in six provinces in Indonesia which were tested by the PCR demonstrated that 68 of 115 (59%) samples reacted in the PCR (Table 1). The highest prevalence of PCR reactive sheep (86%) was originally from the Sindangbarang village, Bogor district, West Java. Samples that were collected from the RIAP and the RIVS, and Banjarsari village in the Bogor district from which the prevalence of PCR reactive animals were 79%, 68%, and 60% respectively. Samples of PBL which were collected from villages of Sindangbarang and Banjarsari were taken from sheep which were penned close to buffaloes. MCF cases were frequently occurred in buffalo in these areas (data not shown). While PBL samples that were taken from sheep from the RIAP and the RIVS from which epidemiologically implicated in the occurrence of MCF cases (HOFFMANN *et al*., 1984).

High prevalence of PCR reactive PBL of sheep samples were also found in districts of Lombok, West Nusa Tenggara (83%), Central Lampung, Lampung (81%), and Kendari, South-East Sulawesi (56%). PBL samples from Central Lampung were taken from sheep that were kept in close contact with Bali cattle which were affected by Rama Dewa disease. These samples were collected during the outbreak of the disease. PBL samples from Lombok and Kendari were taken from sheep in contact with cattle and buffalo which had previously suffered from MCF. But, there were no active cases at the time of sample collection.

Table 1. PCR studies of the SA-MCF agent in PBL of sheep and goats from various areas of Indonesia

No	Area	Sheep		Goats	
		No. tested	No. reacting in PCR	No. tested	No. reacting in PCR
1	Lampung, Southern Sumatra	11	9 (81%)	16	12 (75%)
2	RIVS-Bogor-West Java	19	13 (68%)	5	1 (20%)
3	Sindangbarang-Bogor, West Java	7	6 (86%)	ND	ND
4	Banjarsari-Bogor, West Java	5	3 (60%)	ND	ND
5	RIAP-Bogor-West Java	19	15 (79%)	20	2 (10%)
6	Tegal-Central Java	10	1 (10%)	ND	ND
7	Brebes-Central Java	5	1 (20%)	ND	ND
8	Pemalang-Central Java	6	0	ND	ND
9	Batang-Central Java	6	0	ND	ND
10	Karangasem-Bali	ND	ND	15	0
11	Denpasar-Bali	ND	ND	30	0
12	Lombok-West Nusa Tenggara	18	15 (83%)	ND	ND
13	Kendari-South-East Sulawesi	9	5 (56%)	ND	ND
	Total	115	68 (59%)	86	15 (17%)

ND = not detected
No. = numbers

Relatively low prevalence of OHV-2 was detected in PBL of sheep from districts of Tegal (10%), Brebes (20%), Pemalang (0%) and Batang (0%) of Central Java. These PBL samples were collected from sheep which were penned close to or were grazing together with buffalo from villages where some buffalo died of MCF.

PBL of goats were only available from the district of Central Lampung (Lampung), the RIVS, the RIAP, the districts of Karangasem and Denpasar of Bali Province each of which had a prevalence of 75%, 20%, 10%, 0% and 0% respectively (Table 1). These samples were collected whenever goats were available except in Bali where sheep were difficult to be found.

PCR studies of the SA-MCF agent in PBL of pigs from Bali island demonstrated that out of forty samples that were collected from a slaughter house in Denpasar, none of them were reacted to the PCR.

The longitudinal study of OHV-2 in nasal swabs and PBL collected from lambs at the the RIAP using the PCR showed that an OHV-2 fragment could be detected by the PCR as early as two days old in samples from the lamb (Table 2). In addition to the detection of OHV-2 in nasal secretion of lambs by the PCR, samples of PBL were also tested in the PCR, these samples were collected at the same time as the nasal secretions. It appeared that there was a higher prevalence of OHV-2 in PBL than in nasal secretion. Whenever OHV-2 was detected in the PBL samples it was frequently found in the corresponding nasal secretions.

PCR studies of the SA-MCF agent in four tissues of lambs i.e. turbinate, cornea, nasal epithelium and lachrymal gland showed that at least one organ from each animal reacted in the PCR (Table 3). Three out of the four tissues of the ewes reacted. Viral genome was detected in three organ samples from one lamb and in one sample from each of the other two lambs. Six samples of each organ were tested. Five turbinate samples reacted, and viral genome was detected in four samples of nasal epithelium, three gland samples and two cornea lachrymal samples.

Table 2. Results of a longitudinal study of OHV-2 in nasal swabs and PBL of lamb No.132 at the the RIAP using PCR

No	Age- days	PCR	
		PBL	swab
1	2	ND	+ve
2	9	ND	+ve
3	17	-ve	+ve
4	24	+ve	-ve
5	31	+ve	-ve
6	38	ND	-ve
7	46	+ve	ND
8	53	+ve	-ve
9	59	+ve	-ve
10	67	-ve	+ve
11	74	+ve	+ve
12	81	+ve	+ve
13	89	+ve	+ve
14	96	+ve	+ve
15	110	+ve	ND
16	117	+ve	-ve
17	124	+ve	ND
18	128	+ve	+ve
19	139	+ve	+ve
20	144	+ve	+ve
21	152	ND	-ve
22	158	+ve	ND
23	162	+ve	ND
		17/19 (89%)	11/18 (61%)

-ve = negative
+ve = positive

Table 3. PCR based detection of the SA-MCF agent in variety of tissues from sheep at the RIVS

Tissues	Ewes			Lambs			PCR reactions per tissue
	45	34	35	35A	45A	34A-1	
Turbinate	+ve	+ve	+ve	+ve	+ve	-ve	5/6
Nasal epithelium	+ve	-ve	+ve	+ve	-ve	+ve	4/6
Lachrymal gland	+ve	+ve	+ve	-ve	-ve	-ve	3/6
Cornea	-ve	+ve	-ve	+ve	-ve	-ve	2/6
Animals reacting in the PCR	3/4	3/4	3/4	3/4	1/4	1/4	

-ve = negative; +ve = positive; ND = not detected

DISCUSSION

Study on the PCR based on the detection of OHV-2 in PBL of sheep and goats that were collected from 13 areas in seven provinces of Indonesia revealed that OHV-2 fragment was detected in both sheep and goats in some areas where MCF cases were frequently reported to occur (PARTADIREDJA *et al*., 1988). Cases of MCF were reported to be endemic in West Java (GINTING, 1979; HOFFMANN *et al*., 1984; SUHARYA, 1988), Lampung (PRABOWO and ISHITANI, 1984), Banyuwangi (TRANGGONO, 1988), Lombok (MUTHALIB, 1988), Kendari (MARDIJONO, 1988) and Central Java. In the Province of Bali, MCF was reported occur in Bali cattle without the existence of sheep (DHARMA *et al.*, 1985).

The PCR results revealed that the highest prevalence of PCR reactive sheep were in West Java in particular in the Sindangbarang village followed by the RIAP, the RIVS, and the Banjarsari village. In these areas, cases of MCF frequently occur in buffalo (GINTING, 1979; HOFFMANN *et al*., 1984; SUHARYA, 1988). It means PCR confirms epidemiological data that sheep in these areas have been implicated in the epidemiology of MCF. These have long been suggested by GOTZE and LIESS (1929).

In terms of molecular epidemiological aspect, these results demonstrated that high percentage of sheep which involved in MCF cases were infected by OHV-2. The causal agent of SA-MCF was also detected by the PCR in PBL samples collected from goats, suggesting that some cases of MCF in the Province of Lampung were introduced not only by sheep but also goats. However, the infected goats are not clear yet whether they are acting as carriers or as susceptible species in a similar manner to the large ruminants. Goats with clinical signs of MCF were not observed in this study. There are some reports of WA-MCF, (AHV-1) or a similar virus being isolated from hartebeest (*Alcelaphus buselaphus cokei*, Gunther) (REID and ROWE, 1973) and topi (*Damaliscus korrigum*, Ogilby) (MUSHI *et al*., 1981), but there was no epidemiologic evidence that these viruses were naturally transmitted to cattle (MUSHI *et al*., 1981; PLOWRIGHT, 1981). The goat OHV-2 virus may play a similar role to these viruses. However, this has yet to be determined.

With regard to the absence of sheep during MCF cases in Bali, DHARMA *et al*. (1985) suggested that in such cases other reservoir animals such as pigs may be involved. Involvement of pigs as a susceptible animals to MCF has recently been described by LØKEN *et al*. (1998) in Norway. However, OHV-2 fragment was unable to be detected by the PCR from PBL of pigs that were collected from a slaughter house in Denpasar, suggesting that pigs in this study were not infected by SA-MCF agent, but this data has to be considered as a preliminary results.

The detection of OHV-2 in sheep lamb's nasal secretion showed that OHV-2 fragment was detected as early as two days old of the lambs. The PCR results revealed that OHV-2 is prevalent in nasal secretion of the lamb suggesting that sheep is a potential reservoir of MCF. Similar results were reported in WA-MCF. The aetiological agent of WA-MCF can be isolated from nasal secretion of four day old wildebeest calves (MUSHI *et al*., 1980), and from blood of seven day old wildebeest (PLOWRIGHT, 1965). The virus was also isolated from wildebeest foetuses (PLOWRIGHT, 1965) suggesting that there is a trans-placental transmission. In relation to this, it was suggested that the virus was excreted by wildebeest in nasal secretions (RWEYEMAMU *et al*., 1974).

The detection of OHV-2 in lambs's organs showed that OHV-2 fragment were detected in turbinate, cornea, nasal epithelium and lachrymal gland. This confirms that in reservoir animals these organs were identified as a probable site of replications of OHV-2. Similar results had been reported by BAXTER *et al*. (1997) that OHV-2 fragment were detected in sheep lamb samples of turbinate, cornea, lachrymal gland, nasal epithelium, tonsil, soft palate, larynx, buccal epithelium, tongue, urinary bladder and lymph nodes, but not salivary gland and retropharyngeal lymph node. In WA-MCF, MUSHI *et al.* (1981) concluded that the turbinate and cornea were sites of replication of AHV-1 since they were able to isolate the virus from explant cultures of cornea and turbinate of wildebeest calves less than four months of age. The study reported by MUSHI *et al.* (1981) indicated that there was similarity between SA-MCF and WA-MCF in terms of site of replication of the causal agent suggesting a similar pathogenesis between both type of MCF in the reservoir. In addition to this, close relationship between SA-MCF and WA-MCF has been suggested on the basis of serological studies in WA-MCF. The serological studies in WA-MCF revealed that antibody to AHV-1 were prevalent among sheep, goats and other wild ruminant using indirect immunofluorescence (ROSSITER, 1981b), viral neutralisation (PLOWRIGHT, 1967; SCHULLER *et al*., 1990), and Western blotting (HERRING *et al*., 1989).

Prior to the development of the PCR for SA-MCF the evidence for the existence of virus in infected tissues was very limited (ROSSITER, 1980). Evidence of viral DNA was demonstrated by BRIDGEN *et al*. (1992) using *in situ* hybridisation in infected rabbits, where a small number of virus-infected cells were consistently detected in samples of sub-mandibular lymph nodes and no other tissues.

CONCLUSION

This study confirmed that sheep and goats in Indonesia carry OHV-2 fragment in their PBL. Sheep and probably goats are the reservoir animals of MCF in various areas of Indonesia. The significant achievement of molecular study of OHV-2 in reservoir animals indicated that turbinate, cornea, nasal epithelium and lachrymal gland of lambs were identified as a probable site of replications of OHV-2.

ACKNOWLEDGEMENT

The author wishes to thank to Muharam Saepulloh and Mansyur for their excellent technical assistance during the study. Thank you is also extended to Directors and staffs of Livestock Services of West, Central and East Java, Lampung, Bali, West Nusa Tenggara and South-East Sulawesi for the access of field sample collections. Part of this study was sponsored by Oversease Development Administration of United Kingdom.

REFERENCES

BAXTER, S.I.F., I. POW, A. BRIDGEN, and H.W. REID. 1993. Polymerase chain reaction detection of the sheep-associated agent of malignant catarrhal fever. *Arch. Virol.* 132:145-159.

BAXTER, S.I.F., A. WIYONO, I. POW, and H.W. REID. 1997. Identification of ovine herpesvirus-2 infection in sheep. *Arch. Virol.* 142: 823-831.

BRIDGEN, A. and H.W. REID. 1991. Derivation of a DNA clone corresponding to the viral agent of sheep-associated malignant catarrhal fever. *Res. Vet. Sci.* 50:38-44.

BRIDGEN, A., R. MUNRO, and H.W. REID. 1992. The detection of alcelaphine herpesvirus-1 DNA by in situ hybridization of tissues from rabbits affected with malignant catarrhal fever. *J. Comp. Path.* 106:351-359.

DANIELS, P.W., SUDARISMAN, A. WIYONO, and P. RONOHARDJO. 1988. Epidemiological aspects of malignant catarrhal fever in Indonesia. *In : Malignant Catarrhal Fever in Asian Livestock*. P. W. Daniels, Sudarisman and P. Ronohardjo (Eds.). Australian Centre for International Agricultural Research. Canberra. p.20-31.

DHARMA, D.N., D. DARMADI, I.G. SUDANZA, and K. SANTHYA. 1985. Short communication on comparative study of Jembrana disease and malignant catarrhal fever in Bali cattle. *Annual Report on Animal Disease Investigation in Indonesia During Period of 1983-1984.* Directorate General of Livestock Services, Jakarta, Indonesia.

ELLIS, T.M. and A.M. MASTERS. 1997. Use of magnetic particles to improve the diagnosis of sheep-associated malignant catarrhal fever by polymerase chain reaction. *Aust. Vet. J.* 75:520-521.

GINTING, N.G. 1979. Kasus penyakit ingusan (bovine malignant catarrh) pada sapi Bali di Jawa Barat. *Bul. LPPH* 11:7-22 (Indonesian, English Abstract).

GOTZE, R. and LIESS. 1929. Erlogreiche Ubertragungsver-suche des bosart. Katarrhalfiebers von Rind zu Rind. Identitat mit der sud-afrikanischen Snotsiekte. *D.T.W.* 37:433.

HERRING, A.J., H.W. REID, N. INGLIS, and I. POW. 1989. Immunoblotting analysis of the reaction of wildebeest sheep and cattle sera with structural antigens of alcelaphine herpesvirus-1 malignant catarrhal fever virus. *Vet. Microbiol.* 19:205-215.

HOFFMANN, D., SOERIPTO, S. SOBIRONINGSIH, R.S.F. CAMPBELL, and B.C. CLARKE. 1984. The clinicopathology of a malignant catarrhal fever syndome in the Indonesian swamp buffalo (*Bubalus bubalis*). *Aust. Vet. J.* 61:102-112.

LI, H., D.T. SHEN, D.P. KNOWLES, J.R. GORHAM, and T.B. CRAWFORD. 1994. Competitive inhibition enzyme-linked immunosorbent assay for antibody in sheep and other ruminants to a conserved epitope of malignant catarrhal fever virus. *J. Clin. Microbiol.* 32:1674-1679.

LØKEN, T., M. ALEKSANDERSEN, H.W. REID, and I. POW. 1998. Malignant catarrhal fever caused by ovine herpesvirus-2 in pigs in Norway. *Vet. Rec*. 143:464-467.

MARDIJONO, H. 1988. Malignant catarrhal fever in South-East Sulawesi. *In : Malignant Catarrhal Fever in Asian Livestock*. P. W. Daniels, Sudarisman and P. Ronohardjo (Eds.). Australian Centre for International Agricultural Research. Canberra. p.49-50.

MIRANGI, P.K. and F.M. KANG'EE. 1997. Detection of ovine herpesvirus 2 in Kenyan sheep by polymerase chain reaction. *Vet. Rec*. 141:176-177.

MULLER, U.U., H. LI, B. HAUSER, H. ADLER, and M. ACKERMANN. 1998. Field validation of laboratory tests for clinical diagnosis of sheep-associated malignant catarrhal fever. *J. Clin. Microbiol.* 36:2970-2979.

MUSHI, E.Z., L. KARSTAD, and D.M. JESSETT. 1980. Isolation of bovine malignant catarrhal fever virus from ocular and nasal secretions of wildebeest calves. *Res. Vet. Sci.* 29:168-171.

MUSHI, E.Z., P.B. ROSSITER, D.M. JESSETT, and L. KARSTAD. 1981. Isolation and characterization of a herpesvirus of topi (*Damaliscus korrigum*). *J. Comp. Path.* 91:63-68.

MUSHI, E.Z. and J.S. WAFULA. 1983. Infectivity of cell-free malignant catarrhal fever virus in rabbits and cattle. *Vet. Res. Comm.* 6:153-155.

MUTHALIB, A. 1988. A report on the malignant catarrhal fever disease situation in West Nusa Tenggara. *In : Malignant Catarrhal Fever in Asian Livestock*. P. W. Daniels,

Sudarisman and P. Ronohardjo (Eds.). Australian Centre for International Agricultural Research. Canberra. p.59-63.

PARTADIREDJA, M., I.G. SUDANA, and SUSILO. 1988. Malignant catarrhal fever in Indonesia. *In : Malignant Catarrhal Fever in Asian Livestock*. P. W. Daniels, Sudarisman and P. Ronohardjo (Eds.). Australian Centre for International Agricultural Research. Canberra. p.14-18.

PLOWRIGHT, W. 1965. Malignant catarrhal fever in East Africa. I. Behaviour of the virus in free-living populations of blue wildebeest (*Gorgon taurinus taurinus*, Burchell). *Res. Vet. Sci.* 6:56-67.

PLOWRIGHT, W. 1967. Malignant catarrhal fever in East Africa. III. Neutralising antibody in free living wildebeest. *Res. Vet. Sci.* 8:129-136.

PLOWRIGHT, W. 1981. Herpesvirus of wild ungulates, including malignant catarrhal fever virus. *In : Infectious Diseases of Wild Mammals,* 2nd Edition. (Eds. Davis JW, Karstad LH and Trainer DO), Iowa State University Press, Ames, Iowa, USA. pp.126-146.

PLOWRIGHT, W., R.D. FERRIS, and G.R. SCOTT. 1960. Blue wildebeest and the aetiological agent of bovine malignant catarrhal fever. *Nature* 188:1167-1169.

PRABOWO, H. and R. ISHITANI. 1984. Studies on Rama Dewa, the enzootic disease of cattle occuring in Lampung province of Sumatra, Indonesia-its histopathology and critical views on name of the disease. Japan International Cooperation Agency. Jakarta.

REID, H.W. and L.W. ROWE. 1973. The attenuation of a herpesvirus (malignant catarrhal fever virus) isolated from hartebeest (*Alcelaphus buselaphus cokei*, Gunther). *Res. Vet. Sci.* 15:144-146.

ROIZMAN, B., R.C. DESROSIERS, B. FLECKENSTEIN, C. LOPEZ, A.C. MINSON, and M.J. STUDDERT. 1996. Family Herpeviridae. *In : International Committee on Taxonomy of Viruses (ICTV),* Classification and Nomenclature of Viruses. Sixth Report of the ICTV.

ROSSITER, P.B. 1980. A lack of readily demonstrable virus antigens in the tissues of rabbits and cattle infected with malignant catarrhal fever virus. *Brit. Vet. J.* 136:478-483.

ROSSITER, P.B. 1981a. Antibodies to malignant catarrhal fever virus in sheep sera. *J. Comp. Path.* 91:303-311.

ROSSITER, P.B. 1981b. Immunoperoxidase and immuno-fluorescence techniques for detecting antibodies to malignant catarrhal fever virus in infected cattle. *Trop. Anim. Health Prod.* 13:189-192

RWEYEMAMU, M.M., L. KARSTAD, E.Z. MUSHI, J.C. OTEMA, D.M. JESSETT, L.W. ROWE, S. DREVEMO, and J.G. GROOTENHUIS. 1974. Malignant catarrhal fever virus in nasal secretions of wildebeest: a probable mechanism for virus transmission. *J. Wildlife Dis.* 10:478-487.

SAMBROOK, J., E.F. FRITSCH, and T. MANIATIS. 1989. *Molecular Cloning-A Laboaratory Manual.* 2nd Ed. Cold Spring Harbor Laboratory Press. New York, USA.

SCHULLER, W., S. CERNY-REITERER, and R. SILBER. 1990. Evidence that the sheep associated form of malignant catarrhal fever is caused by a herpes virus. *J. Vet. Med. Series B*. 37:442-447.

SUHARYA, E. 1988. Malignant catarrhal fever in West Java. *In : Malignant Catarrhal Fever in Asian Livestock*. P. W. Daniels, Sudarisman and P. Ronohardjo (Eds.). Australian Centre for International Agricultural Research. Canberra. p.37-38.

TRANGGONO, M. 1988. A high prevalence of malignant catarrhal fever in Banyuwangi. *In : Malignant Catarrhal Fever in Asian Livestock*. P. W. Daniels, Sudarisman and P. Ronohardjo (Eds.). Australian Centre for International Agricultural Research. Canberra. p.52-54.

WIYONO, A., S.I.F. BAXTER, M. SAEPULLOH, R. DAMAYANTI, P. DANIELS, and H.W. REID. 1994a. PCR detection of ovine herpesvirus-2 DNA in Indonesian ruminants - normal sheep and clinical cases of malignant catarrhal fever. *Vet. Microbiol.* 42: 45-52.

WIYONO, A., S.I.F. BAXTER, M. SAEPULLOH, SUDARISMAN, R. DAMAYANTI, P.W. DANIELS, and H.W. REID. 1994b. Diagnosis malignant catarrhal fever di Indonesia dengan menggunakan teknik reaksi berantai polimerase (PCR). Prosiding Seminar Nasional Teknologi Veteriner untuk Meningkatkan Kesehatan Hewan dan Pengamanan Bahan Pangan Asal Ternak. Bogor, 22-24 Maret 1994. Balai Penelitian Veteriner, Bogor. p.112-120.