Genetic Variation of the Causal Agent of Sheep-Associated Malignant Catarrhal Fever in Buffalo in Indonesia: Using a Repeat-Sequence Polymerase Chain Reaction

Agus Wiyono[1(✉)], Muharam Saepulloh[1], and Rini Damayanti[2]

[1] Research Center for Veterinary Science, Research Organization for Health, National Research and Innovation Agency, Bogor, Indonesia
aguswrinid@gmail.com

[2] Indonesian Research Center for Veterinary Science, Ministry of Agriculture, Bogor, Indonesia

Abstract. A study on the genetic variation of Ovine Herpesvirus-2 (OVH-2), the causal agent of sheep-associated malignant catarrhal fever (SA-MCF), in buffalo in Indonesia using a repeat-sequence polymerase chain reaction (RS-PCR) has been conducted. Samples of heparinized blood and/or pre-scapular or pre-femoral lymph nodes were received from buffaloes that were occasionally slaughtered in private slaughterhouses in the District of Bogor. Butchers were requested to collect samples from buffaloes showing at least one of the clinical signs of an MCF such as febrile, diarrhoea, conjunctivitis, serous to mucopurulent ocular and nasal discharges and enlargement of superficial lymph nodes. A total of 21 specimens of white blood cells (WBC) were extracted and tested by a conventional SA-MCF polymerase chain reaction (PCR) to diagnose SA-MCF and subsequently tested by an RS-PCR. Based on the PCR and RS-PCR results, genetic variation of the causal agent of SA-MCF in buffalo was visually observed. The RS-PCR results were then confirmed using Southern blot hybridization analysis and also using restriction enzyme profiling. The results showed that genetic variation of the causal agent of SA-MCF in buffalo was found in 21 buffalo that were tested. The variables were grouped into two main groups A and B consisting of 12 and 9 buffalo respectively. Group A was further divided into two sub-groups A1 and A2 consisting of 9 and 3 buffalo respectively. Southern blot hybridization analysis and restriction enzyme profiling confirmed the RS-PCR results. The study concludes that genetic variation of the causal agent of SA-MCF was observed in 21 buffaloes that were tested. However, the significance of the genetic variation of the causal agent of SA-MCF in the pathogenesis of the disease in buffaloes has yet to be studied.

Keywords: SA-MCF · genetic variation · OHV-2 · buffalo · Indonesia

1 Introduction

Malignant Catarrhal Fever (MCF) based on its reservoir host consisted of two major groups, namely sheep-associated MCF SA-MCF with sheep and goats as reservoir hosts;

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and Wildebeest-Associated MCF (WA-MCF) with wildebeest as reservoir host [1]. The clinical sign of MCF is divided into four forms, namely per-acute, intestinal, the head and eye and mild forms. It is however considered that there are no clear-cut divisions since the clinical manifestations of MCF are more often a combination of the forms [2]. In terms of the occurrence of each form, head and eye forms are reported to occur more frequently than the other three. It is characterised by sudden high fever up to 42 °C and profuse oculo-nasal discharges from serous at first becoming sero-mucoid to mucopurulent with time. Eye lesions include conjunctivitis and corneal opacity. Corneal opacity extends centripetally and sometimes results in blindness in chronic cases. Superficial lymph nodes such as pre-femoral and pre-scapular lymph nodes are often enlarged. There may be bloody diarrhoea, dry and cracked muzzle and other skin lesions. Central nervous system disorders such as tremors, incoordination, twitching of the ears even torticollis may be found [3]. However, there is considerable variation in clinico-pathological findings reported from the field [4], and severe clinico-pathological findings such as diarrhoea, mucopurulent ocular-nasal discharges, corneal opacity and fibrinoid vasculitis are not always observed. Despite the clinico-pathological features of MCF which are well characterised, the causative agent is still not established for SA-MCF.

The disease has been recognised sporadically throughout most of the Indonesian archipelago [5] and is considered to be an economically important disease of Bali cattle and swamp buffalo since these animals are the main source of draught animals and wealth for most of the Indonesian farmers [6]. Bali cattle (*Bos javanicus* / *sondaicus*) have been reported to be very susceptible to MCF in Indonesia [5] and in the USA [4]. Buffalo was reported to be susceptible to MCF in Indonesia [1], Thailand [7], Switzerland [8] and Brazil [9]. In Indonesia, a variation in clinical signs of SA-MCF cases was observed in several breeds of large ruminants with different degrees of sensitivity and clinico-pathological severity [1].

The aim of this study was to identify the potential genetic variation of clinical cases of OHV-2 infection by RS-PCR among buffaloes slaughtered in private slaughterhouses in the district of Bogor.

2 Materials and Methods

2.1 Sample

Samples of heparinized blood and/or pre-scapular or pre-femoral lymph nodes were received from buffaloes that were occasionally slaughtered in private slaughter houses in the District of Bogor. Butchers were requested to collect samples from buffaloes which were shown one of the following clinical signs of a malignant catarrhal fever (MCF) such as febrile, diarrhoea, conjunctivitis, serous to mucopurulent ocular and nasal discharges and enlargement of superficial lymph nodes [10]. These samples were accompanied by information on buffaloes' health status. A total of 21 samples were analysed for this study.

Table 1. Primer pairs of RS-PCR

Primers	Sequence
3762	5'-GACAATTCAAATGCAGGGCATACGTGGCCT-3'
6142	5'ACTAATACTGACGGCGACGACGATGGCAAG-3'

2.2 Conventional MCF PCR

DNA was extracted from samples according to [11] followed by amplified by conventional polymerase chain reaction as previously described by [12].

2.3 Repeat Sequence PCR (RS-PCR)

Repeat sequence PCR was performed for all positive results using conventional PCR using primers developed by HW Reid (pers. Com), ie. 3762 and 6142 (Table 1). The repeat PCR contained 0.5 mg template DNA target, 2 units of Taq Polymerase, 200μM of dATP, dCTP, dGTP and dTTP, and also 1.0μM of each primer.

2.4 Southern Blot Hybridisation as Confirmatory Test

The agarose gel was incubated in 0.2 N HCl for 15 min before being incubated in a denaturing buffer solution containing 0.5 M NaOH and 1.5 M NaCl for one hour with three changes of solution. The gel was then put into a neutralization buffer solution of pH 7.5 containing 1 M Tris 7–9 and 1.5 M NaCl for one hour with three changes of solution. DNA on agarose gel was transferred to nylon paper (Amersham Hybond N) capillary [11] using a buffer solution of 10x Standard saline citrate (SSC) pH 7.0 containing 1.5 M NaCl and 0.15 M Tri sodium citrate (Na3H2O7). Furthermore, the nylon paper was washed with 6 × SSC buffer pH 7.0 containing 1 M NaCl and 0.1 M Tri sodium citrate (Na3H2O7) before being fixed using ultraviolet radiation at 254 nm for 10 min. The paper was stored in a plastic bag tightly at 4 °C until it was time for hybridization.

2.5 Labelling of DNA Fragments with Digoxigenin and Hybridization

DNA fragments on nylon paper were detected by Southern blot hybridization analysis using an 1170 oligonucleotide probe labelled with digoxygenin (DIG). This marker is specific for detecting the OHV 2 major internal repeat sequence. After the DNA fragment was labelled with DIG (Boehringer Mannheim Germany), then the fragment was further hybridized [11].

2.6 Immunological Detection of DIG Markers

DIG markers were detected immunologically by the manufacturer's procedure (Boehringer Mannheim Germany).

2.7 Extraction and Purification of DNA from Agarose Gel

DNA extraction and purification from agarose gel containing ethidium bromide were carried out using The Geneclean II kit (Bio 101 Inc, La Jolla, CA, USA) and carried out according to the manufacturer's procedure. The presence of DNA that has been successfully obtained and purified was checked by inserting it into 1.8% agarose gel and electrophoresed at 60 V for 1 h and viewed on a UV transilluminator.

2.8 DNA Cutting with a Restriction Endonuclease Enzyme

The purified DNA fragments were cut with the restriction endonuclease AvaII enzyme according to the procedure issued by the manufacturer. The AvaII cuts OHA-2 DNA at major repeat element resulting super molar repeat fragments.

2.9 Polyacrylamide Gel Electrophoresis

DNA fragments that had been cut with the restriction endonuclease AvaII enzyme were then analysed directly by non-denaturing continuous polyacrylamide gel electrophoresis (PAGE) [11].

2.10 PAGE Staining with Silver Staining

This staining was carried out according to the method presented by [13].

3 Results

A total of 21 samples of heparin blood and/or buffalo lymph nodes were obtained from an emergency slaughterhouse in Bogor Regency. The causative agent of SA-MCF could be detected from all the buffaloes by conventional PCR MCF assay. Furthermore, all of the buffalo reacted in the RS-PCR test by producing a minimum of two fragments. Based on the size and number of fragments observed visually on agarose gel, the genetic diversity of MCF-causing agents found in the 21 buffaloes can be grouped into two main groups A and B, which consist of 12 and 9 buffaloes, respectively. Furthermore, into group A by visual observation, it can be divided into two sub-groups A1 and A2 consisting of 9 and 3 buffalo, respectively (Table 2).

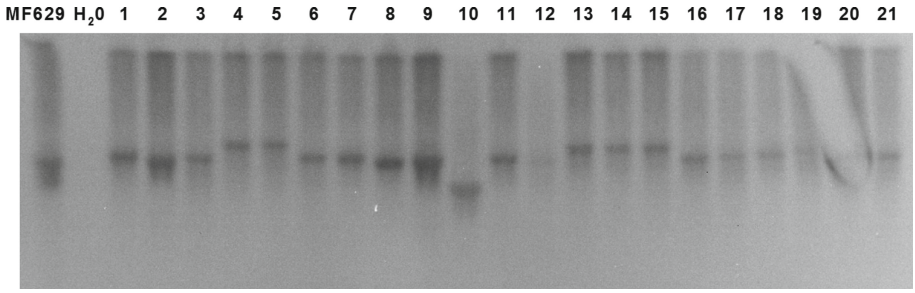

Fig. 1. Southern blot hybridization analysis results using 1170 labelled with DIG confirming RS-PCR test. 1.PAS 44; 2.PAS 83; 3.PAS 93; 4.PAS 95; 5.PAS 108; 6.PAS107; 7.PAS 124; 8.PAS 125; 9.PAS 149; 10. PAS 148; 11.PAS 5; 12.PAS 160; 13.PAS 163; 14.PAS 166; 15.PAS 4; 16.PAS 6; 17.PAS 29; 18.PAS 31; 19.PAS 32; 20.PAS 36 and 21.PAS 37

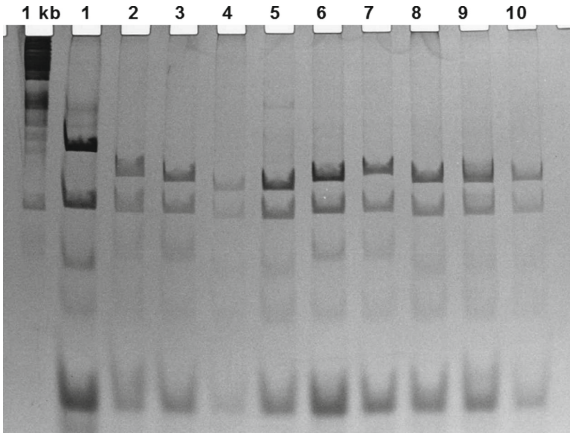

Fig. 2. Results of restriction endonuclease AvaII enzyme which cuts OHV 2 DNA on major repeat elements at 12% PAGE stained with Silver staining: 1.PAS 6; 2.PAS 93; 3.PAS 32; 4.PAS 31; 5.PAS 29; 6.PAS 149; 7.PAS 5; 8.PAS 124; 9.PAS 160; 10.PAS 148

The results of the RS-PCR test when confirmed by Southern blot hybridization analysis showed that the DNA fragments of the MCF-causing agent in buffalo on nylon paper successfully hybridized with marker 1170 labelled with DIG (Fig. 1). The genetic diversity of the MCF causative agent found in the 21 buffaloes were seen in the size (location) and the number of lines that were successfully hybridized by the 1170 marker.

All of 21 buffaloes that were sampled and tested by RS-PCR and tested for confirmation by analysis using Southern blot hybridisation, all of them were taken from RS-PCR DNA samples to be cut with the restriction endonuclease AvaII enzyme which cuts OHV 2 DNA on major repeat elements to produce a pattern of fragments containing super molar repeat fragments. The diversity of fragments in Figs. 2 and Fig. 3 showed the differences between the viruses that cause MCF from one pattern to another.

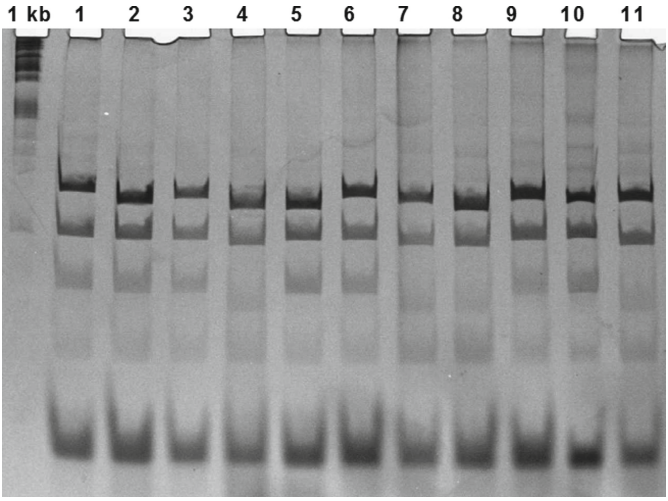

Fig. 3. Results of restriction endonuclease AvaII enzyme which cuts OHV 2 DNA on major repeat elements at 12% PAGE stained with Silver staining: 1.PAS 166; 2.PAS 83; 3.PAS 107; 4.PAS 44; 5.PAS 95; 6.PAS 125; 7.PAS 37; 8.PAS 4; 9.PAS 163; 10.PAS 108; and 11.PAS 36

Table 2. Genetic variation pattern of SA-MCF virus by RS-PCR detected from 21 buffaloes samples received from Private Slaughter House in District of Bogor

Group of buffaloes		
A1	**A2**	**B**
PAS 032	PAS 005	PAS 004
PAS 093	PAS 083	PAS 006
PAS 107	PAS 095	PAS 029
PAS 108		PAS 031
PAS 124		PAS 036
PAS 125		PAS 037
PAS 149		PAS 044
PAS 163		PAS 148
PAS 166		PAS 160

[1] PAS = Private Abattoir Survey

4 Discussion

In discussing the results, it has to be noted that there were some limitations to the study to be considered. In these abattoir studies, some bias is faced due to the rules applying to the abattoirs such that most animals should be male or very old females, and abattoirs should only slaughter healthy animals. However, the abattoirs in this study also received unhealthy buffalo that were used for the study. The second limitation is that it was agreed

from the beginning that certain data and samples were requested but all were managed by the owner of the private abattoirs.

This research is the result of a preliminary study on the genetic diversity of MCF-causing agents in buffaloes in Indonesia using RS-PCR. The PCR amplified fragment was confirmed by restriction enzyme profiling using AvaII. This enzyme cuts within the major repeat element of OHV 2 to yield a fragment pattern containing super molar repeat fragments. This result was the first data recording the prevalence of histological changes consistent with MCF in samples collected from sick buffaloes that were slaughtered in the private abattoirs. However, this is a preliminary study with some limitations. The observations were based on the size and number of fragments in agarose gel and not the actual sequence, and also a limited number of samples was involved in the study. Even so, this finding may well be a genetic expression of severity and duration in clinical signs as well as gross lesions and histopathology of MCF in buffaloes and probably in other large ruminants as reported by [4]. However, they are not related to whether the source of infection is natural or experimental [4].

Concerning the genetic variation of SA-MCF causal agent, the RS-PCR proved that the causal agent of SA-MCF in buffalo that were slaughtered at the private abattoirs produced at least two or possibly three different patterns. In addition, the difference did not vary with the origin of the buffalo. In WA-MCF, [14] reported that there was some strain diversity within the herpesviruses based on DNA restriction endonuclease analysis. Further studies on Indonesian isolates of OHV-2 using sequencing may provide additional information. The results of the PCR have been confirmed by the hybridisation of PCR amplified DNA with a SA-MCF probe which showed the same size between PCR product and hybridized one. In WA-MCF different strains of AHV-1 were reported [15]. Unfortunately, there is no data on the strain variation in latently infected MCF cases. Genetic variation of OvHV-2 has been reported by [16] in Tukey and Iraq [17]. Similarly, genetic variation of AHV-1 of wildebeest placenta samples was reported by [18].

In this study, there were two sub-districts of Cisarua and Ciawi, Bogor, West Java in which a reasonably high prevalence of OHV-2 infected buffalo was recorded. It was not surprising therefore that these sub-districts contributed to most of the unhealthy buffalo. Interestingly, the other sub-districts that contributed only a small number of unhealthy buffalo to the abattoirs, showed a high percentage of OHV-2 infected buffalo. It means buffalo that were sold from sub-districts other than Cisarua and Ciawi tended to be unhealthy and carried OHV-2 DNA in their lymph nodes. It was also obvious that the private abattoirs were one of the solutions used to avoid having buffalo die in the field. Unfortunately, there was no data on the exact population of sheep and buffalo in each sub-district. However, West Java was well known as the main source of sheep in Indonesia [19]. Therefore, Bali cattle were not allowed to be kept in this province due to the high probability of death from MCF infection [1]. The existence of the private abattoirs in Bogor that slaughter healthy as well as unhealthy animals without being reported to the Livestock Services proved that the private abattoirs contribute to the phenomena of under-reported and/ or under-diagnosed disease in Indonesia, especially MCF. The occurrence of MCF among unhealthy slaughtered buffalo in the private abattoirs was reported [20].

In the future, some aspects will need to be clarified such as the real geographical and seasonal prevalence of MCF and genetic variation of SA-MCF based on DNA sequencing.

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References

1. DamayantiR. Penyakit Malignant Catarrhal Fever di Indonesia dan Upaya Pengendaliannya (Malignant Catarrhal Fever in Indonesia and Its Control Strategy). Wartazoa. 2016;26(3):103–14.
2. RussellGC, StewartJP, HaigDM. Malignant catarrhal fever: A review. Vol. 179, Veterinary Journal. 2009. p. 324–35.
3. PatelJR, DidlickS. Epidemiology, disease and control of infections in ruminants by herpesviruses - An overview. J S Afr Vet Assoc. 2008;79(1):8–14.
4. O'TooleD, LiH. The pathology of malignant catarrhal fever, with an emphasis on ovine herpesvirus 2. Vet Pathol. 2014;51(2):437–52.
5. WiyonoA, DamayantiR. Wabah Penyakit Ingusan (Malignant Catarrhal Fever) Pada Sapi Bali Di Pulau Lombok, Nusa Tenggara Barat, Indonesia (Outbreak of Malignant Catarrhal Fever in Bali Cattle in Lombok Island, West Nusa Tenggara, Indonesia). J Vet. 2018;19(3):419–29.
6. WiyonoA, NuradjiH, SanamMU, SimarmataYT, DamayantiR. Detection of ovine herpesvirus-2 in clinical cases of sheep-associated malignant catarrhal fever in balinese cattle and apparently healthy sheep in East Nusa Tenggara. In: BIO Web of Conferences. 2021. p. 06006.
7. TeankamK, TantilertcharoenR, BoonsermT. MALIGNANT CATARRHAL FEVER IN SWAMP BUFFALOES (BUBALUS BUBALIS) : A RETROSPECTIVE. Thai J Vet Med. 2006;36(1):19–30.
8. DettwilerM, StahelA, KrugerS, GerspachC, BraunU, EngelsM, et al. A possible case of caprine-associated malignant catarrhal fever in a domestic water buffalo (Bubalus bubalis) in Switzerland. Bmc Vet Res. 2011;7:1–6.
9. CostaÉ a., BastianettoE, VasconcelosAC, BomfimMRQ, FonsecaFGDa, GomesAD, et al. An outbreak of malignant catarrhal fever in Murrah buffaloes in Minas Gerais, Brazil. Pesqui Veterinária Bras. 2009;29(5):395–400.
10. OIE World Organization for Animal Health. Malignant Catarrhal Fever. 2013. p. 1–12.
11. CostaEA. Ovine Herpes Virus 2. Emerg Infect Dis. 2009;15(5):844–5.
12. WiyonoA, BaxterS, DharsanaR, DamayantiR, SaepullohM, PearceM, et al. SHEEP ASSOCIATED MALIGNANT CATARRHAL FEVER IN BUFFALO : AN ABATTOIR SURVEY IN WEST JAVA , INDONESIA . Kenyan Vet. 1994;18(2):464–6.
13. ChevalletM, LucheS, RabilloudT. Silver staining of proteins in polyacrylamide gels. Nat Protoc. 2006;1(4):1852–1858.
14. WambuaL, WambuaPN, RamogoAM, MijeleD, OtiendeMY. Wildebeest-associated malignant catarrhal fever: perspectives for integrated control of a lymphoproliferative disease of cattle in sub-Saharan Africa. Arch Virol. 2016;161(1):1–10.
15. LiH, DyerN, KellerJ, CrawfordTB. Newly recognized herpesvirus causing malignant catarrhal fever in white-tailed deer (Odocoileus virginianus). J Clin Microbiol. 2003;38(4):1313–8.

16. TuranT, IsidanH, AtasoyMO, Sozdutmazİ, BulutH. GENETIC DIVERSITY of OVINE HERPESVIRUS 2 STRAINS OBTAINED FROM MALIGNANT CATARRHAL FEVER CASES in EASTERN TURKEY. Virus Res. 2020;276(October 2019).
17. KhudairY, AyyezH, HussainM. Phylogenetic analysis of ovine herpes virus-2 (OHV-2) in malignant catarrhal fever infected cattle in Al-Qadisiyah governorate of Iraq. Iraqi J Vet Sci. 2019;33(1):51–8.
18. LankesterF, LugeloA, MnyambwaN, NdabigayeA, KeyyuJ, KazwalaR, et al. Alcelaphine herpesvirus-1 (malignant catarrhal fever virus) in wildebeest placenta: Genetic variation of ORF50 and A9.5 alleles. Vol. 10, PLoS ONE. 2015.
19. BPS Jawa Barat. Hasil Survey Pertanian Antar Sensus (SUTAS) 2018 Provinsi Jawa Barat. AliF, editor. BPS Provinsi Jawa Barat; 2018. 1–81 p.
20. DamayantiR. Prevalensi Histopatologik Malignant Catarrhal Fever pada Kerbau yang Dipotong di tempat Pemotongan Hewan Perseorangan di Kabupaten Bogor. J Ilmu Ternak dan Vet. 1995;I(3):206–11.