Identification of Mardivirus Serotypes Circulating in Poultry Farms in Sukabumi and Cianjur District, West Java, 2011 using Multiplex Polymerase Chain Reaction (mPCR) Approach

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ABSTRAK

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Tiga serotipe kelompok Mardivirus yang beredar pada peternakan ayam komersial adalah Marek's disease virus serotipe 1 (MDV-1), Gallid herpesvirus 3 (GaHV3) dan herpesvirus of turkey (HVT). Tetapi hanya MDV yang paling sering mendapatkan perhatian karena sifatnya yang patogenik, menyebar luas secara cepat dan sangat persisten di lingkungan. Virus Marek's menyebabkan penyakit yang ditandai dengan pembentukan tumor pada berbagai organ disertai dengan kelumpuhan yang dapat mengakibatkan kegagalan siklus produksi. Meskipun program vaksinasi telah sukses menekan kejadian penyakit, tetapi beberapa strain telah mengalami mutasi menjadi lebih ganas yang dapat menyebabkan vaksinasi menjadi tidak efektif. Tujuan penelitian ini adalah mengidentifikasi ketiga serotipe Mardivirus yang beredar pada peternakan ayam di Kabupaten Sukabumi dan Cianjur. Pendekatan yang dilakukan dengan multipleks PCR pada sampel bulu ayam yang dikoleksi pada bulan April sampai Juni 2011. Keberadaan ketiga serotipe Mardivirus terdeteksi pada sampel yang dianalisa, tetapi penerapan vaksin hidup MDV yang dilemahkan menyebabkan kesulitan diagnosa penyakit untuk membedakan antara *strain* vaksin dan *strain* lapangan. Uji coba marker attenuasi dengan identifikasi motif pengulangan 132 bp pada bagian *terminal* dan *inverted repeats* dari *long region* (TRL & IRL) memberikan hasil yang kurang memuaskan. Oleh karena itu, masih diperlukan penelitian dengan pendekatan lain untuk menyelesaikan permasalahan yang ada di lapangan seperti seleksi marker yang lainnya, restriction fragment length polymorphism (RFLP), high-resolution melt curve analysis (HRM) maupun sekuensing gen.

Kata Kunci: Serotipe Mardivirus, MDV, GaHV3, HVT, multipleks PCR

ABSTRACT

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Three serotypes of Mardivirus had been circulating in the farm environments, these being Marek's disease virus serotype 1 (MDV-1), Gallid hepesvirus 3 (GaHV3) and herpesvirus of turkey (HVT). However, only MDV-1 poses a significant hazard to the poultry farm. The virus causes a neoplastic syndrome that inflicting severe economic loss to the affected farms. Although vaccination has successfully reduced the frequency and severity of outbreaks, the threat does not disappear since several more pathogenic strains have evolved, and these can overcome protection by vaccination. The aim of this study was to investigate the circulation of three Mardivirus serotypes in commercial poultry farms in Sukabumi and Cianjur district using mPCR approach for the feather samples. A low prevalence of these three serotypes was detected. However, the practice of vaccinating using live attenuated MDV-1 caused difficulty in the investigation. Differentation between virulent field strains and CVI988 vaccine strain using the 132 bp repeat motif attenuation marker within the terminal and inverted repeats flanking the unique long region generated an ambiguous result. Thus, other approaches are required to address this issue, such as selection of other markers, restriction fragment length polymorphism (RFLP), high-resolution melt curve analysis (HRM) and gene sequencing.

Key Words: Mardivirus serotype, MDV-1, GaHV3, HVT, multiplex PCR

INTRODUCTION

Mardiviruses are double-stranded linear DNA viruses that belong to Alphaherpesvirinae subfamily (Davison 2010). Three members of the group have circulated in poultry farms, including gallid herpesvirus 2 (GaHV2), gallid herpesvirus 3 (GaHV3) and herpesvirus of turkey (HVT). While the GaHV3 (also known as Marek's disease virus serotype 2 (MDV-2)) and HVT (also known as Melleagrid herpesvirus 1

(MeHV1)) are naturally non-pathogenic, the GaHV2 (also known as Marek's disease virus serotype 1 (MDV-1)) causes significant economic losses in poultry farms, especially layers and breeders (Bublot & Sharma 2004). The virus causes neoplastic disease with clinical signs related to lympho-proliferative disorders of T cells such as immuno-suppression, polyneuritis and tumor development in lymphoid tissues, internal organs and ectoderm-derived tissues (Baigent & Davidson 2004; Calnek 2001). Paralysis may occur because of progressive tumor formation in the brachial and sciatic nerves (Calnek 2001). The clinical signs often occur during the rearing period at 12-30 weeks but can also be seen at 3-4 weeks of age (Nair et al. 2008).

This worldwide disease has been distributed in many regions and caused significant economic loss. The infection spreads horizontally, via the respiratory route, through either direct or indirect pathways, including infected chickens, premises, litter, dust and broken feathers (Baigent & Davidson 2004). The virus within the feather follicle epithelium (FFE) debris is extremely stable so the infection is persistent in the contaminated environment. However, outbreaks have been controlled by vaccination in the hatchery using several types of vaccines including attenuated MDV-1, GaHV3 and HVT (Biggs & Nair 2012). Moreover, the evolution of viruses having more pathogenic character can cause vaccination breakdown, so the outbreaks may still occur even in vaccinated flocks (Arulmozhi et al. 2011; Gimeno 2008). Therefore, a reliable detection technique is indispensible for monitoring circulation of virus in the farm environment.

The objective of this study was to investigate the circulation of three serotypes of Mardivirus in poultry farms in Sukabumi and Cianjur districts of Java, using multiplex polymerase chain reaction (mPCR) approach. PCR offers a fast and reliable test for detection of Mardivirus serotypes (Baigent et al. 2005; Handberg et al. 2001; Islam et al. 2006; Islam et al. 2004; Renz et al. 2006). The multiplex approach is expected to be more economical to process a large quantity of samples. Moreover, the investigation to distinguish between field and vaccine strain was trialed using recognition of the 132 bp repeat motif attenuation marker in *Bam*HI region within the terminal and inverted repeats flanking the unique long region (Becker et al. 1992).

MATERIALS AND METHODS

Standard DNA

Standard DNA for PCR positive controls are vaccine strains that are obtained from PT. ROMINDO, which are live vaccine Marek's serotype 1 MDV CVI988 Rispens, Merial JA199 (Rispens et al. 1972) and live vaccine Marek's serotype 3 HVT strain FC 126, Merial A9333 (Okazaki et al., 1970). Since the vaccine of GaHV3 is no longer used in Indonesia, the PCR assay for this serotype was performed directly with the field samples.

Field sampling

The sample collection was conducted in several commercial chicken farms in Sukabumi and Cianjur Districts, West Java Province from April to June 2011. The samples collected were feathers, since these are easy to collect and proven as suitable sample for detection of high levels of MDV. The feathers were taken from spinal and/or cervical region. Although the preferred feather sample is from the axillary region, the wing feathers are difficult to collect without stress to the bird. Moreover, there is no statistical difference in MDV detection between feather tracts (Baigent et al. 2005).

The field investigations were undertaken in accordance with the local agricultural services. Briefly, at least 5-10 feathers were collected from each bird. The feathers were collected in clean plastic bags and transferred to the Virology Laboratory, Indonesian Research Center for Veterinary Science (IRCVS), Bogor. Subsequently, the feather tip pulps were chopped about 5 cm from the proximal region, collected into a sterile 1,5 ml microcentrifuge tubes and preserved at 4°C until further analysis.

DNA isolation for standard virus samples and field samples

The extraction of genetic material (DNA) of the standard virus samples was performed using a QIAamp DNA mini kit (Qiagen) according to the manufacturer's instructions. However, the DNA of field samples (FFE tissue) was extracted using phenol chloroform extraction approach (Baigent et al. 2005; Handberg et al. 2001). The extracted DNA was preserved at -20°C until further analysis.

Primer sets for Mardivirus detection

The three serotypes of Mardivirus share extensive homology in the genomic content and organization. However, each serotype still retains distinctive genes that can be utilized for identification. The meq gene encodes a 339-amino acid bZIP transactivator associated with oncogenicity. This gene is an excellent marker for detection of MDV-1 since this gene is absent in the other serotypes (Kung et al. 2001). Meanwhile, part of the DNA pol gene (UL30) is specific for identification for GaHV3 because with the sequence is dissimilar in other serotypes (Islam et al. 2004). The sorf1 gene is a unique putative gene within HVT

genome that suitable for HVT detection (Kingham et al. 2001). Therefore, sets of primers for MDV, GaHV3 and HVT detection were selected from previous studies (Islam et al. 2006; Renz et al. 2006). These set of primers are suitable for multiplexing format in term of gene target and size of amplification (Table 1). Moreover, identification for the 132 bp repeats motif was performed as previously described methodology (Becker et al. 1992; Davidson et al. 1995).

Multiplex PCR protocol for Mardivirus serotype identification

The mPCR assay for three Mardivirus serotypes was performed using HotStarTaq® Plus (Qiagen) as per manufacturer's instruction. Briefly, the mPCR was carried out in a 20 µl mixture containing 10 µl of 2x HotStarTaq® Plus Master mix, 2 µl of 10x CoralLoad concentrate, 1 µl of bovine serum albumin BSA (10 mg/ml), 0.5 µl of each forward and reverse primer (20 µM) for meq, DNApol and sorf1 gene and 4 µl of DNA template. The amplification was performed in thermal cycler machine either AB 9700 or AB 9800. Temperature profile was designed in several steps, including initial denaturation at 95°C for 5 min, 30 cycles of amplification (denaturation at 94°C for 90 s, annealing at 60°C for 60 s & extension at 72°C for 60 s), and final extension at 72°C for 3 min. The products were visualized by electrophoresis (100 Volts, 30 min) in 2% agarose gel stained with ethidium bromide in 1xTBE buffer. The molecular weight marker for fragment analysis was a 100 bp DNA ladder (Qiagen).

Differentiation between MDV natural infection and CVI988 vaccine strain

The PCR assay for amplification of 132 bp repeats in the BamH1-H region was performed only for samples that were positive for MDV-1 in the multiplex PCR. Amplification was carried out using HotStarTaq® Plus (Qiagen) as per manufacturer's instruction. Briefly, the PCR was carried out in a 20 µl mixture containing 10 µl of 2x HotStarTaq® Plus Master mix, 2 µl of 10x CoralLoad concentrate, 1 µl of BSA (10 mg/ml), 0.5 µl of each forward mdvF and reverse mdvR primer (20 µM) and 6 µl of DNA template. The thermal cycling profile was designed at 95°C for 5 min (initial denaturation), 31 cycles of amplification (denaturation at 94°C for 45 s, annealing at 55°C for 45 s & extension at 72°C for 45 s), and final extension at 72°C for 3 min. The products were visualized by electrophoresis (100 Volts, 30 min) in 2% agarose gel stained with ethidium bromide in 1xTBE buffer using 100 bp DNA ladder (Invitrogen™) as marker.

RESULTS

Field sampling

The sample collection was conducted from several commercial poultry farms in Sukabumi and Cianjur. In Sukabumi, the samples were collected from a total of 100 birds from 3 layer and 2 kampung chicken farms. Meanwhile the sampling in Cianjur District was carried out for a total of 90 birds from 4 layer and 2 broiler farms.

Isolation of genetic material for Mardivirus

The DNA from MDV vaccines was successfully extracted using a QIAamp DNA mini kit (Qiagen). As the viruses are highly cell associated, the outcome of extraction is a total DNA of both host cell and virus (Calnek 2001). Therefore, phenol chloroform extraction approach was successfully applied to extract the DNA from feather samples.

Multiplex PCR for Mardivirus for standard virus samples

The mPCR protocol for three serotypes of Mardivirus was only optimized using standard antigens for MDV-1 and HVT. The mPCR assay successfully amplified target genes with expected size of products that are 196 bp and 350 bp for meq and sorf1 gene, respectively. Since there was no standard control available for GaHV3, product in between 196 and 350 bp was considered as GaHV3, since the expected PCR product size was 283 bp. The successful use of mPCR assay with standard viruses for MDV-1 and HVT is illustrated in Figure 1. Moreover, identification of the 132 bp repeats motif of MDV-1 strain CVI988 resulted in amplification of many copies (Figure 2). While the PCR product with length of 2 copies was observed as a thick and sharp band, PCR products equivalent to multiple copies of this marker were observed as more diffuse bands.

Mardivirus detection in field samples

Mardivirus identification in field samples in several commercial poultry farms in Sukabumi and Cianjur is illustrated in Tables 2 and 3 and Figure 3. In general, the study revealed low detection of all three serotypes in both Sukabumi and Canjur. The MDV-1 identification in layer farms showed low incidence in about 11,7% (Sukabumi) and 16,7% (Cianjur) even though this kind of farm usually practices vaccination. Interestingly, breeding of kampung chicken with no history of vaccination showed quite high occurrence of MDV in about 32,5% of birds. Despite the fact that GaHV3 is no longer used as vaccine in Indonesia, this

Table 1. The oligo-primers used within the study.

Serotype	Target gene	Primer name	Sequence (5'-3')	Primer location	Genbank Accession	Size of amplicon
MDV-1	meq	meqF	GAATCTTCCCTGCATTGTGTC	135624 – 135664	AF147806.2	196 bp
	(Islam et al. 2006)	meqR	ATCTGGCCCGAATACAAGGAA	135799 – 135819		
	132 bp repeat motif	mdvF	TACTTCCTATATAGATTGAGACGT (24	129378 – 129401	DQ530348.1	copies of 132 bp
	(Becker et al. 1992)		mer)	129008 – 129031	NC00229.3	(434 bp, 566 bp,
		mdvR	GAGATCCTCGTAAGGTGTAATATA	130578 – 130601	DQ530348.1	Et seq)
			(24 mer)	129418 – 129441	NC00229.3	
GaHV3	DNApol	dnapolF	GTCTGCCCTCGTCTTAGC	84550 – 84567	AB024414.1	283 bp
	(Renz et al. 2006)	dnapolR	ACTCGCTTCCTCCAATTCG	84814 – 84832		
HVT	sorf1	sorf1F	AAGCGCTTGTATGTGTAGG	130888 – 130906	AF282130.1	350 bp
	(Islam et al. 2006)	sorf1R	TATGGACGTCATGCAGTTGG	131218 – 131237		

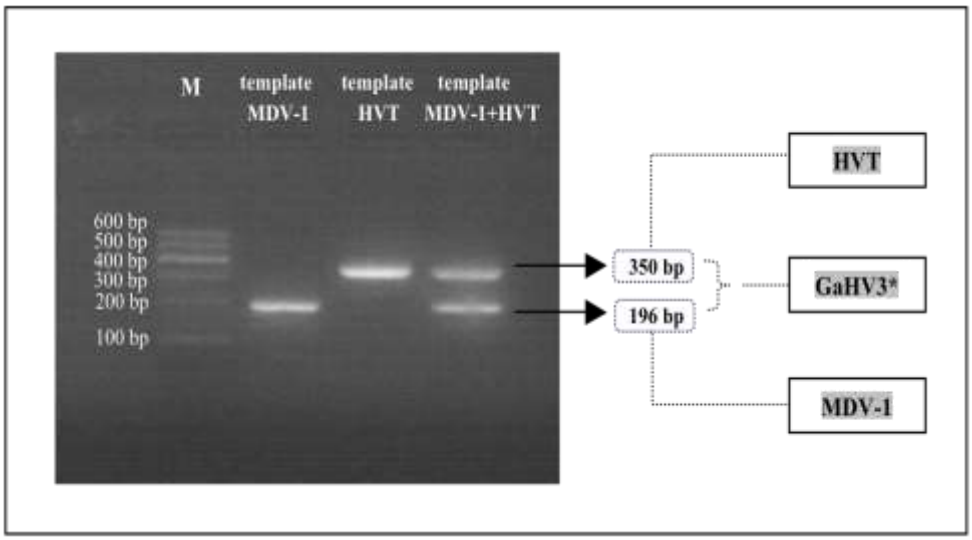

Figure 1. The multiplex PCR assay for identification of Mardivirus serotypes, including MDV-1, GaHV3 and HVT. *The GaHV3 is identified if there is product between 196-350 bp. Molecular weight (M) is 100 bp DNA ladder.

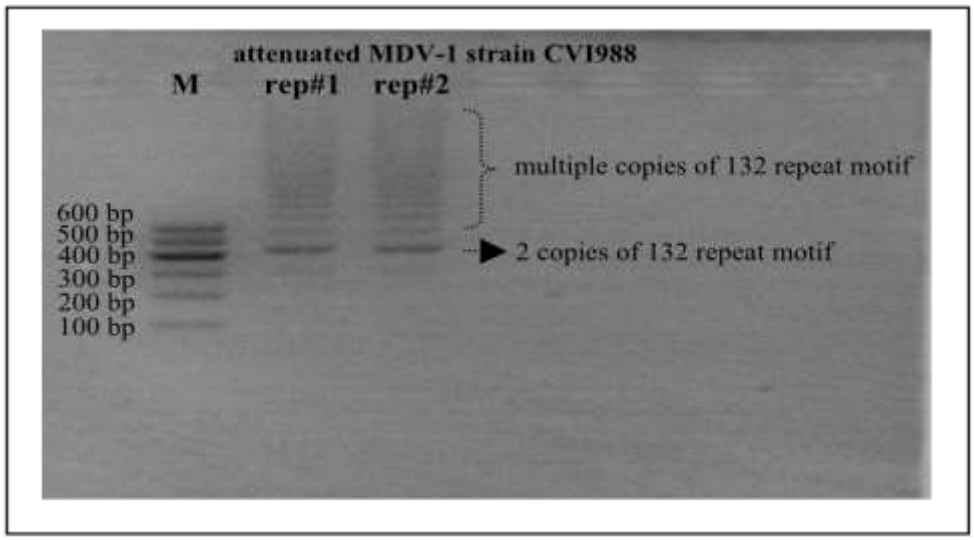

Figure 2. Amplification of 132 repeats motif of BamH1-H region of the live vaccine attenuated MDV-1 strain CVI988 Rispens. Molecular weight (M) using 100 bp DNA ladder.

study revealed occurrence of this serotype in both layer and kampung chicken farms. The GaHV3 detection in layers revealed 16,7% and 19,4% for Sukabumi and Cianjur, respectively. Meanwhile, detection in kampung chicken in Sukabumi showed only 2,5% of incidence. Moreover, HVT was only detected in layers, in only 5% birds and 2,7% birds for Sukabumi and Cianjur, respectively. No Mardivirus of any serotype was detected in broiler farms.

The identification of 132 bp repeat motif from MDV-1 positive samples showed different motifs compared with attenuated MDV CVI988 (Table 4 & Figure 4). Unfortunately, the PCR to amplify this marker seemed less sensitive than meq gene amplification. As a consequence, only 15 from 33 samples showed amplification of this marker. Most samples from the Sukabumi region showed only two copies, whereas samples from the Cianjur region showed more than two copies.

Table 2. Detection Mardivirus serotypes in the commercial farms in Sukabumi district, 2011

No	Flock code	Breed of bird	Age	Number of sample	Identification of Mardivirus serotype using mPCR							Recapitulation of Mardivirus serotype		
					MDV-1	GaHV3	HVT	MDV-1 + GaHV3	MDV-1 + HVT	GaHV3 + HVT	MDV-1 + GaHV3 + HVT	MDV-1	GaHV3	HVT
1	A.1.1	layer	32 weeks	10	-	2	-	3	-	1	-	4 of 10	6 of 10	1 of 10
2	A.1.2	layer	32 weeks	10	-	-	-	-	-	-	-	0 of 10	0 of 10	0 of 10
3	A.3.1	layer	30 weeks	10	-	-	-	-	-	-	-	0 of 10	0 of 10	0 of 10
4	A.3.2	layer	40 weeks	10	-	1	-	-	-	-	1	1 of 10	2 of 10	1 of 10
5	A.4.1	layer	14 weeks	10	-	1	-	-	-	-	-	0 of 10	1 of 10	0 of 10
6	A.4.2	layer	unknown	10	1	1	-	1	-	-	-	2 of 10	1 of 10	1 of 10
Total percentage for layer farm												11,7%	16,7%	5%
7	A.2.1	kampung	4 weeks	10	-	-	-	-	-	-	-	0 of 10	0 of 10	0 of 10
8	A.2.2	kampung	8 weeks	10	-	-	-	-	-	-	-	0 of 10	0 of 10	0 of 10
9	A.5.1	kampung	4 weeks	10	3	-	-	-	-	-	-	3 of 10	0 of 10	0 of 10
10	A.5.2	kampung	8 weeks	10	9	-	-	1	-	-	-	10 of 10	1 of 10	0 of 10
Total percentage for kampung chicken farm												32,5%	2.5%	0%

Table 3. Detection Mardivirus serotypes in the commercial farms in Cianjur district, 2011

No	Flock code	Breed of bird	Age	Number of sample	Identification of Mardivirus serotype using mPCR							Recapitulation of Mardivirus serotype		
					MDV-1	GaHV3	HVT	MDV-1 + GaHV3	MDV-1 + HVT	GaHV3 + HVT	MDV-1 + GaHV3 + HVT	MDV-1	GaHV3	HVT
1	B.1.1	layer	23 weeks	10	-	-	-	-	-	-	-	0 of 10	0 of 10	0 of 10
2	B.1.2	layer	60 weeks	10	5	-	-	1	-	-	-	6 of 10	1 of 10	0 of 10
3	B.1.3	layer	40 weeks	10	1	-	-	-	-	-	-	1 of 10	0 of 10	0 of 10
4	B.2.1	layer	pullet	10	1	2	-	-	-	-	-	1 of 10	2 of 10	0 of 10
5	B.2.2	layer	unknown	2	1	-	-	1	-	-	-	2 of 2	1 of 2	0 of 2
6	B.3	layer	unknown	10	-	8	-	1	-	-	1	2 of 10	10 of 10	1 of 10
7	B.4.1+2	layer	20 weeks	10	1	-	-	-	-	-	-	1 of 10	0 of 10	0 of 10
8	B.4.3	layer	90 weeks	10	-	-	1	-	-	-	-	0 of 10	0 of 10	1 of 10
	Total percentage for layer farm											16,7%	19,4%	2,7%
9	B.5	broiler	21 days	10	-	-	-	-	-	-	-	0 of 10	0 of 10	0 of 10
10	B.6	broiler	17 days	8	-	-	-	-	-	-	-	0 of 8	0 of 8	0 of 8
	Total percentage for broiler farm											0%	0%	0%

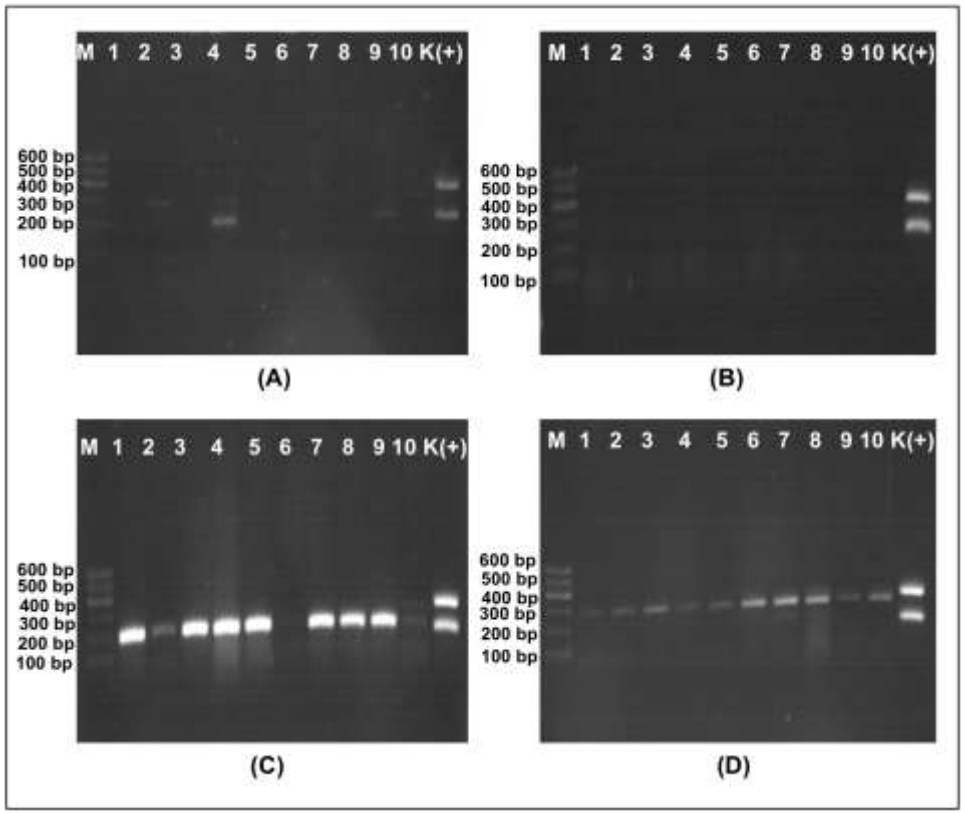

Figure 3. Identification of Mardivirus serotypes from field samples. (A) Flock A.1.1. (B) Flock A.2.1. (C) Flock A.5.2. (D) Flock B.3. Lanes 1-10 represent samples from individual chickens in the flock. Positive control (K+) is MDV-1 Rispens CVI988 and HVT FC126. Molecular weight (M) is 100 bp DNA ladder.

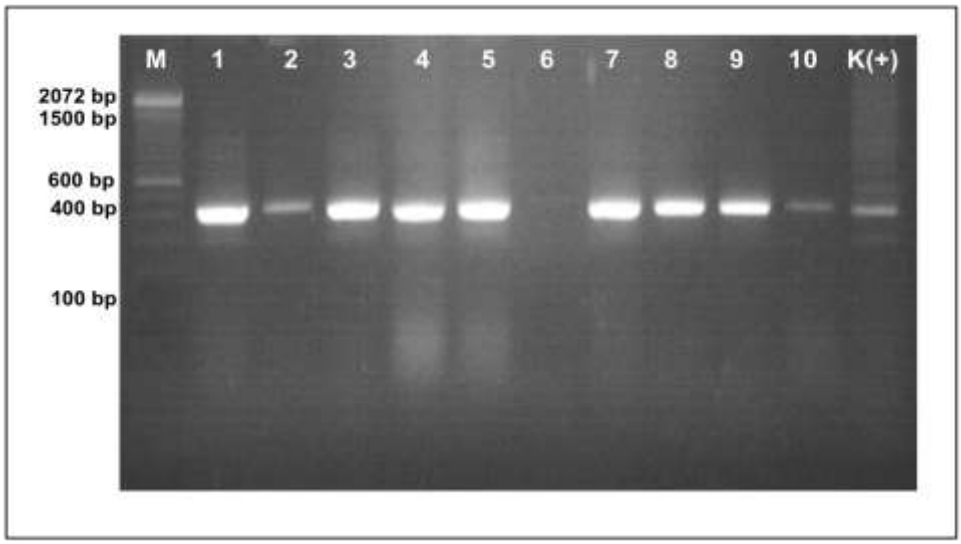

Figure 4. Identification of 132 bp repeat motif of MDV positive samples in flock A.5.2. Lanes 1-10 represent samples from individual chickens in the flock. Positive control (K+) is attenuated MDV Rispens CVI988. Molecular weight (M) is 100 bp DNA ladder.

Table 4. PCR analysis of 132 bp repeats motif BamH1-H region for positive MDV-PCR samples from Sukabumi and Cianjur

No.	Sample	Motif of 132 bp repeat
1	A.1.1.2	undetected
2	A.1.1.4	2 copies
3	A.1.1.8	undetected
4	A.1.1.9	undetected
5	A.3.2.8	undetected
6	A.4.2.2	undetected
7	A.4.2.9	undetected
8	A.5.1.2	undetected
9	A.5.1.8	undetected
10	A.5.1.10	2 copies
11	A.5.2.1	2 copies
12	A.5.2.2	2 copies
13	A.5.2.3	2 copies
14	A.5.2.4	2 copies
15	A.5.2.5	2 copies
16	A.5.2.6	2 copies
17	A.5.2.7	2 copies
18	A.5.2.8	2 copies
19	A.5.2.9	2 copies
20	A.5.2.10	2 copies
21	B.1.2.1	undetected
22	B.1.2.2	more than 2 copies
23	B.1.2.4	undetected
24	B.1.2.6	undetected
25	B.1.2.7	undetected
26	B.1.2.8	undetected
27	B.1.3.9	more than 2 copies
28	B.2.1.3	more than 2 copies
29	B.2.2.2	undetected
30	B.2.2.7	undetected
31	B.3.1.7	undetected
32	B.3.1.8	undetected
33	B.4.1&2.9	undetected

DISCUSSION

The monitoring of dynamics of virus in the field is one of the key elements in a disease management program. As for Marek's disease, identification of circulating viruses in the environment will significantly influence decisions on disease control. The diagnostic tool for monitoring should not only be reliable (high sensitivity and specificity), but it should also straightforward and time-cost effective. The multiplex approach will facilitate a large-scale investigation with a high number of samples (Huang & Wang 2008). For MDV-1 detection, feather tip pulp is proven as a suitable sample for MDV detection (Baigent et al. 2005; Handberg et al. 2001). However, the FFE tissue is unique. The DNA isolation may require strongly extraction approach such as phenol chloroform extraction method (Baigent et al. 2005; Handberg et al. 2001). Moreover, the PCR assay also requires supplementation with BSA to enhance its sensitivity, especially in the presence of the PCR inhibitor melanin, which is found in the feathers of brown strains of layer (Baigent et al. 2005).

Field investigation revealed a relatively low occurrence of MDV-1 in the FFE tissue in both Sukabumi and Cianjur. Detection of MDV-1 was expected at high frequency in layers since all birds were vaccinated with CVI988 in hatcheries (Bublot & Sharma 2004) but the study showed a different outcome. As the DNA extraction and PCR assay were considered successful (Baigent et al. 2005; Islam et al. 2006; Renz et al. 2006), several explanations maybe attributed for this phenomenon. The FFE tissue taken within the study may contain a low titer of virus. Thus, feathers from the spinal area need to be compared with feathers from the axillary region (Baigent et al. 2005). Comparison with other types of sample such as spleen, peripheral blood lymphocytes (PBL) and dust should also be analyzed (Islam et al. 2006). Another possible explanation is the failure of MDV vaccination in the hatchery so no virus can be detected.

Interestingly, MDV-1 was highly detected in kampung chicken farms in Sukabumi despite the fact that these flocks have no history of vaccination. This MDV-1 could originally be derived from natural infection and/or vaccine carry-over. Moreover, all three serotypes of Mardivirus were unable to be detected in broilers. MDV vaccination is rarely practiced for broilers because of their short lifespan. However, studies by Islam et al. (2001, 2002) indicated immunosuppressive effects of MDV-1 infection in broilers, which may reduce immunity to other diseases. Thus, vaccination should be considered in broilers since MDV-1 infection already was found in many regions around the world.

Another interesting finding is the presence of GaHV3 in several farms in both Sukabumi and Cianjur; in spite of the fact that this serotype is no longer used as a vaccine in Indonesia. It is possible that the circulation of this strain in the farm environment is due to either natural infection or carry-over from the time when GaHV3 was last used for vaccination. Therefore, further study is needed to identify the significance of

this serotype in the environment. Meanwhile, the level of HVT was very low compared with other serotypes. This could be due to low virus titer in the samples. The horizontal transmission of HVT via FFE tissue is naturally very limited (Baigent & Davidson 2004; Calnek et al. 1970; Witter et al. 1976). As a consequence, it was difficult to detect HVT in the samples by the PCR assay. Moreover, vaccination using HVT is being replaced gradually by vaccination using attenuated MDV-1 CVI988, because of the emergence of novel more pathogenic strains (Gimeno 2008).

The differentiation between field pathogenic strain of MDV and vaccine carry-over was difficult to accomplish based on 132 bp repeat motif. Despite high numbers of serial passage in cell culture causing expansion from two copies to many copies (Silva et al. 2004), the PCR assay for this marker resulted in an ambiguous result. Indeed, another study acknowledged that the extensive copies would revert to original two copies after *in vivo* passage (Young and Gravel 1996). By excluding the prior thesis, all positive samples from the Sukabumi had 2 copies of the repeat that may possibly indication of field strain virus, especially in kampung chicken with no history of MDV-1 vaccination. In the other hand, positive samples from the Cianjur all had multiple copies that suggested as manifestation of vaccine carry-over of MDV-1 CVI988. However, the confirmatory tests, such as gene sequencing, are required to verify this premise. Therefore, more studies are required on the selection of suitable markers for either attenuation or pathogenic characteristics. Moreover, other approaches such as restriction fragment length polymorphism (RFLP), high resolution melt curve analysis (HRM) and gene sequencing may become appropriate alternatives to develop investigative tests for MDV infection.

CONCLUSION

Proper diagnostic methods are essential for monitoring Marek's disease situation in farm environment in the field. The test should be accurate, straightforward and time-cost effective. The multiplex PCR method developed within this study is effective and efficient to differentiate three Mardivirus serotypes (MDV-1, GaHV3 & HVT) of chicken feather sample in a single test. Subsequently, all three Mardivirus serotypes were detected in the commercial chicken farm in Sukabumi and Cianjur districts, West Java Province, in year 2011. The trial of the 132 bp repeat motif approach in the field samples demonstrated imprecise result in differentiation between field strain of MDV-1 and attenuated vaccine MDV-1 strain CVI988. Therefore, further studies are urgently required to develop better diagnostic approach for distinguish between the field and vaccine strain of MDV-1.

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