HAYATI Journal of Biosciences 23 (2016) 160–167

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Contents lists available at ScienceDirect

HAYATI Journal of Biosciences

journal homepage: http://www.journals.elsevier.com/hayati-journal-of-biosciences

Original research article

# The *Meq* Gene Molecular Profile of Marek's Disease Virus Serotype 1 From Kampung and Arabic Chicken Farms in Sukabumi, West Java, Indonesia

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ARTICLE INFO

*Article history:*
Received 8 March 2016
Received in revised form
16 November 2016
Accepted 8 December 2016
Available online 3 January 2017

*KEYWORDS:*
Arabic chicken,
Kampung chicken,
MDV-1,
*meq* gene,
Sukabumi

ABSTRACT

There is an increasing trend to use molecular approaches to the study of Marek's disease virus serotype 1 (MDV-1), the causative agent for the neoplastic syndrome of chickens known as Marek's disease. The *meq* gene is of particular interest as it is the principal oncogene of the virus. This study aimed to characterize the *meq* gene of field strains of MDV-1 circulating in Kampung and Arabic chicken farms in Sukabumi, West Java, Indonesia during 2014. This study detected circulating MDV-1 strains in Kampung chickens in extracts from feather, blood and dust samples. In comparison only the MDV-1 vaccine strain CVI988/Rispens was identified in Arabic chickens in feather samples. Although the MDV-1 field strain, SMI14-KampungCk was detected in healthy Kampung chicken. Its *meq* gene is identical with Marek's disease virus causing outbreak in layer farms in North Sumatera in 2013. Our result shows that the *meq* gene of Marek's virus field strain from Sukabumi has the closest proximity with G2, a very virulent Marek's disease virus from China. The molecular analyses of the *meq* gene indicated that SMI14-KampungCk has pathotype trait of virulent or very virulent.



## 1. Introduction

Marek's disease is a neoplastic disease in poultry (*Gallus* sp.) caused by Marek's disease virus serotype 1 (MDV-1), which is also called as Gallid herpesvirus 2 (GaHV-2). This double-stranded linear DNA virus belongs to *Mardivirus* genus, Alphaherpesvirinae subfamily (Davison 2010). The clinical signs of Marek's disease are related to T cell tumorigenesis in several tissues that can be manifested into several appearances, including neurologic, visceral, ocular and cutaneous forms (Schat and Nair 2008). In the past this disease caused massive economic loss; however, vaccination programs have reduced the outbreaks significantly (Biggs and Nair 2012).

Despite that the disease has been already well controlled, Marek's disease still poses a serious threat to the poultry industry. The evolution of MDV-1 into more pathogenic has been acknowledged (Witter 1997). Moreover, Marek's disease outbreaks have been reported even in the vaccinated flocks from different regions all over the world (Gong *et al.* 2013; Hassanin *et al.* 2013; Wozniakowski *et al.* 2011). Thus, surveillance in the farm environment is important to monitor this disease.

The molecular approach using gene sequencing seems to be a useful tool to investigate disease circumstance in the field. Several genes have been targeted in numerous studies, including *meq*, *vIL-8*, *pp38*, etc. (Cui *et al.* 2005; Parcells *et al.* 2001; Shamblin *et al.* 2004). The Marek's EcoRI-Q (*meq*) gene is generally 1020 bp long encoding 339 amino acid (aa) bZIP transactivator (Jones *et al.* 1992). This gene is associated with oncogenicity and is one of the preferred targets of analysis (Hassanin *et al.* 2013; Tian *et al.* 2011; Wajid *et al.* 2013). Unfortunately, the data about Marek's disease in Indonesia are limited. Actually, the cases of Marek's disease occurred in the past (De Boer and Djaenoedin 1951). Moreover, several recent cases in layer flocks were unofficially reported. Therefore, the main objective of this study was to characterize *meq* gene of MDV-1 circulated in Kampung and Arabic chicken in Sukabumi district, West Java Province, Indonesia during 2014. Kampung chicken is backyard chicken raised for meat production. Commercial Kampung farms generally do not conduct vaccination for Marek's disease. On other hand, the Arabic chicken is specific

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Peer review under responsibility of Institut Pertanian Bogor.

http://dx.doi.org/10.1016/j.hjb.2016.12.004

breed of native chicken for layer purpose. Marek's disease vaccination has been routinely applied in this type of farm, especially with strain CVI988/Rispens. Subsequently, we included spleen samples from Marek's disease outbreak at Medan, North Sumatera in 2013 for comparison analysis.

## 2. Materials and Methods

### 2.1. Field sampling

Field sampling was conducted in three Kampung and two Arabic chicken farms in Sukabumi District, West Java Province in July 2014. In each farms, bird feathers, blood and dust of farm cages were collected. The feather tip pulps were taken from the axillaries, spinal and cervical region and cut about 5 cm from the proximal region, then collected into a sterile 1.5 mL microcentrifuge tubes. The blood samples were taken from axillaries vein using 3 mL syringe with anticoagulant and later were transferred into a sterile 2 mL microcentrifuge tubes. The dust were sampled from the birdcage and the barn wall and collected in plastic bags. In total of 140 feather pool samples, 140 blood samples and 33 dust samples were collected. All samples were preserved by refrigeration at 4°C until further analyses.

### 2.2. *Mardivirus* serotypes screening

The screening for three *Mardivirus* serotypes; (1) MDV-1, (2) GaHV-3, (3) HVT; was done using polymerase chain reaction (PCR) test with multiplex platform (mPCR) as described by Hartawan and Dharmayanti (2013). The DNA extraction was done using phenol chloroform isoamyl alcohol (25:24:1). Meanwhile, the extraction from the blood was done using DNeasy minikit (Qiagen). Subsequently, the mPCR assay was carried out using HotStarTaq Plus (Qiagen).

### 2.3. Amplification of the *meq* gene

The *meq* gene amplification was only applied for the field samples that are positive for the MDV-1 in the previous screening test. The *meq* gene PCR products were amplified at 972 bp with set of primers of meqF26 (5′-CTATGCCCTACAGTCCCGCT-3′) and meqR998 (5′-GGAAACCACCAGACCGTAGA-3′) designed by primer-BLAST software (National Center for Biotechnology Information [NCBI] GenBank). The PCR assay was carried out using HotStarTaq Plus (Qiagen) in the thermal cycler machine Applied Biosystem 9700. Briefly, the 50 μL of mixture contain 1x HotStarTaq Plus master mix, 0.5 mg/mL of bovine serum albumin, 0.8 μM of primer meqF26, 0.8 μM of primer meqR998 and 18.5 μL of DNA template.

Table 1. Screening test of Marek's disease serotypes using mPCR of the field samples from Kampung and Arabic chicken farms in Sukabumi District in 2014

| No. | Sample code | Type of sample | Number of sample | Number of positive sample in mPCR test | | |
|---|---|---|---|---|---|---|
| | | | | MDV-1 | GaHV-3 | HVT |
| Kampung chicken farm | | | | | | |
| 1. | A.A1_(PBL1-16) | Blood | 16 | 6 | 0 | 0 |
| 2. | A.A1_(FFE1-16) | Feather | 16 | 4 | 0 | 0 |
| 3. | A.A1_(DFE1-3) | Dust | 3 | 1 | 0 | 0 |
| 4. | A.A2_(PBL17-31) | Blood | 15 | 10 | 0 | 0 |
| 5. | A.A2_(FFE17-31) | Feather | 15 | 11 | 0 | 0 |
| 6. | A.A2_(DFE4-6) | Dust | 3 | 1 | 0 | 0 |
| 7. | B.B1_(PBL32-46) | Blood | 15 | 5 | 0 | 0 |
| 8. | B.B1_(FFE32-46) | Feather | 15 | 8 | 0 | 0 |
| 9. | B.B1_(DFE7-9) | Dust | 3 | 1 | 0 | 0 |
| 10. | B.B2_(PBL47-61) | Blood | 15 | 8 | 0 | 0 |
| 11. | B.B2_(FFE47-61) | Feather | 15 | 3 | 0 | 0 |
| 12. | B.B2_(DFE10-12) | Dust | 3 | 2 | 0 | 0 |
| 13. | C.C1_(PBL62-71) | Blood | 10 | 2 | 0 | 0 |
| 14. | C.C1_(FFE62-71) | Feather | 10 | 0 | 0 | 0 |
| 15. | C.C1_(DFE13-15) | Dust | 3 | 0 | 0 | 0 |
| 16. | C.C2_(PBL72-80) | Blood | 9 | 0 | 0 | 0 |
| 17. | C.C2_(FFE72-80) | Feather | 9 | 2 | 0 | 0 |
| 18. | C.C2_(DFE16-18) | Dust | 3 | 0 | 0 | 0 |
| Percentage for PBL sample | | | | 31 of 80 (38.8%) | 0 of 80 (0%) | 0 of 80 (0%) |
| Percentage for FFE sample | | | | 28 of 80 (35%) | 0 of 80 (0%) | 0 of 80 (0%) |
| Percentage for DFE sample | | | | 5 of 24 (20.8%) | 0 of 24 (0%) | 0 of 24 (0%) |
| Arabic chicken farm | | | | | | |
| 19. | D.D1_(PBL81-90) | Blood | 10 | 0 | 0 | 0 |
| 20. | D.D1_(FFE81-90) | Feather | 10 | 2 | 1 | 0 |
| 21. | D.D1_(DFE19-21) | Dust | 3 | 0 | 0 | 0 |
| 22. | D.D2_(PBL91-100) | Blood | 10 | 0 | 0 | 0 |
| 23. | D.D2_(FFE91-100) | Feather | 10 | 2 | 1 | 0 |
| 24. | D.D2_(DFE22-24) | Dust | 3 | 0 | 0 | 0 |
| 25. | D.D3_(PBL101-100) | Blood | 10 | 0 | 0 | 0 |
| 26. | D.D3_(FFE101-100) | Feather | 10 | 3 | 0 | 0 |
| 27. | D.D3_(DFE25-27) | Dust | 3 | 0 | 0 | 0 |
| 28 | E.E1_(PBL111-126) | Blood | 16 | 0 | 0 | 0 |
| 29. | E.E1_(FFE111-126) | Feather | 16 | 2 | 1 | 0 |
| 30. | E.E1_(DFE28-30) | Dust | 3 | 0 | 0 | 0 |
| 31. | E.E2_(PBL127-140) | Blood | 14 | 0 | 0 | 0 |
| 32. | E.E2_(FFE127-140) | Feather | 14 | 0 | 0 | 0 |
| 33. | E.E2_(DFE31-33) | Dust | 3 | 0 | 0 | 0 |
| Percentage for PBL sample | | | | 0 of 60 (0%) | 0 of 60 (0%) | 0 of 60 (0%) |
| Percentage for FFE sample | | | | 9 of 60 (15%) | 3 of 60 (5%) | 0 of 60 (0%) |
| Percentage for DFE sample | | | | 0 of 15 (0%) | 0 of 15 (0%) | 0 of 15 (0%) |

PBL = peripheral blood lymphocyte; FFE = feather follicle epithelium; DFE = dust of farm environment; MDV-1 = Marek's disease virus-1 as serotype 1; GaHV-3 = Gallid herpesvirus-3 as serotype 2; HVT = Herpesvirus of turkey as serotype 3.

The PCR temperature profile was designed in the several steps, including initial denaturation at 95°C for 5 minutes, 40 cycles of gene amplification (denaturation at 94°C for 30 seconds, annealing at 55°C for 90 seconds and extension at 72°C for 120 seconds) and final extension at 72°C for 10 minutes.

### 2.4. Gene sequencing of the *meq* gene

Samples with adequate amplification of the *meq* gene were selected for the sequencing analysis. The DNA fragments of the *meq* gene were purified using QIAquick PCR purification kit (Qiagen). The fragment of the *meq* gene was sequenced in two directions by meqF26 and meqR988 primers using Big Dye terminator mix version 3.1 (Applied Biosystems) in the *Genetix Analyzer* (ABI-3130 PE Applied Biosystems). The sequencing results were analyzed by Clustal W (Bioedit). The MEQ protein sequences of the field samples were aligned against the CVI988/Rispens Intervet (Acc. No. DQ534538). Later, the MEQ sequence of the field sample was compared with other characterized strains of MDV-1. Phylogenetic tree of the *meq* gene of several MDV-1 strains from the GenBank NCBI database was constructed using Mega 6.06 software.

## 3. Results

### 3.1. Screening of field samples for *Mardivirus* serotypes

The results of *Mardivirus* serotypes screening are shown in Table 1. In the Kampung chicken, only MDV-1 were detected with quite high percentage, which was 38.8%, 35% and 20.8% in the blood, feather and dust samples, respectively. Two other serotypes were negative in the mPCR-screening test. Different outcomes were found in the Arabic chicken farms. Serotype 1 and 2 were detected only in feather samples with low percentage in about 15% and 5%, respectively. The presence of HVT as serotype 3 was not detected in either Kampung or Arabic chicken farms.

### 3.2. Molecular characteristics of the *meq* gene

Sequencing of *meq* gene of MDV-1 was successfully accomplished for 14 samples of those 73 positive samples with MDV-1 from the *Mardivirus* screening test (Figure 1). From these 14 samples, 13 belong to Kampung chicken farms namely PBL6, PBL23, PBL34, PBL60, PBL64, FFE5, FFE20, FFE26, FFE36, FFE41, FE60, FFE74 and DFE8. Because these 13 samples are identical with 100% nucleotide sequence identity, these samples are coded as SMI14-KampungCk. Meanwhile, only one sample belonging to Arabic chicken farms was successfully sequenced. Because the sample from Arabic chicken farm is only one, the code for this sample is remain the same as the original code that is SMI14.FFE117.

Apparently, the *meq* gene of SMI14-KampungCk and two spleen samples of layer from Medan (MDN13.SF.SPLN and MDN13.AF.SPLN) are identical displaying 100% nucleotide sequence identity. The gene sequence of these samples has several differences with vaccine strain CVI988/Rispens Intervet (Acc. No. DQ534538). On the other hand, SMI14.FFE117 from Arabic chicken farm has 100% nucleotide sequence identity with the strain CVI988/Rispens (Acc. No. AF493555). However, the *meq* gene of SMI14.FFE117 is absent for 183

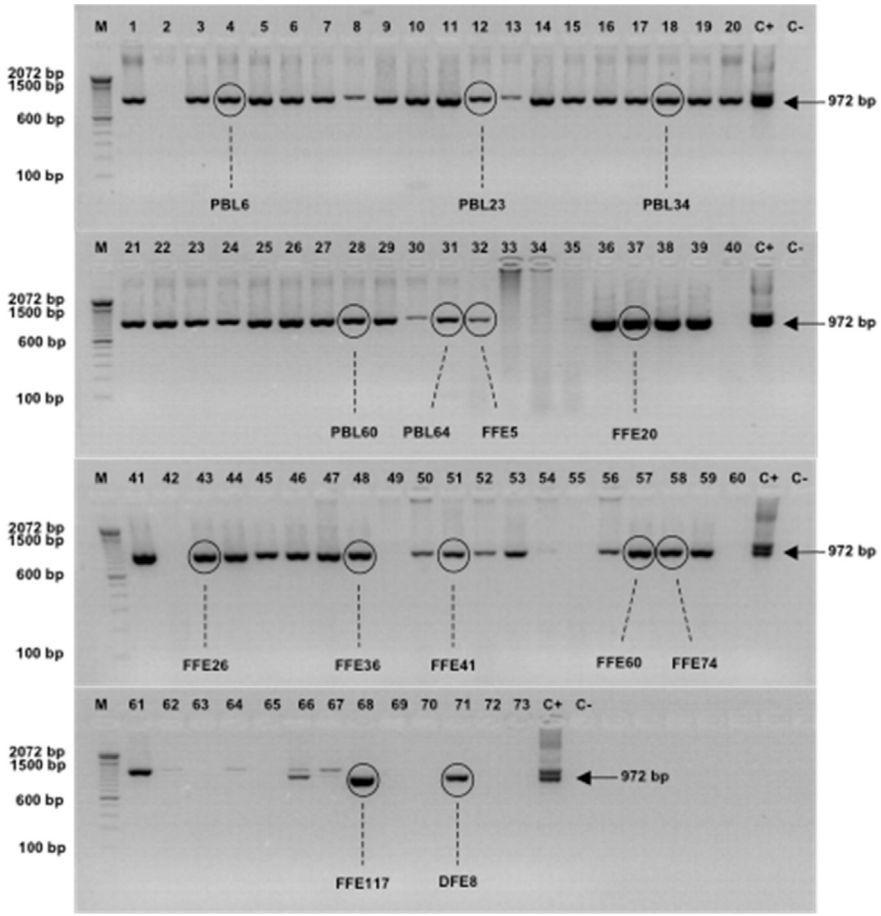


**Figure 1.** Amplification of *meq* gene using meqF26 and meqR988 of the 73 field samples. The 14 samples successfully sequenced were marked by circling the amplicons followed by sample identity. PBL refers to peripheral blood lymphocyte sample. FFE refers to feather follicle epithelium sample. DFE refers to dust of farm environment sample. M is ladder DNA 100 bp (Invitrogen). C+ and C− are positive and negative control, respectively.

nucleotide at position 576–758 in comparison with the vaccine strain CVI988/Rispens Intervet (Acc. No. DQ534538).

Multiple sequence analysis of the MEQ protein between Sukabumi (2014) and Medan (2013) samples plus the vaccine strain CVI988/Rispens Intervet (Acc. No. DQ534538) is presented in Figure 2. There are six aa differences, namely glycine at position 66 becomes arginine (G66R), serine at position 71 becomes alanine (S71A), aspartate at position 80 becomes tyrosine (D80Y), valine at position 115 becomes alanine (V115A), threonine at position 180 becomes methionine (T180M) and proline at position 277 into alanine (P277A). Unlike the vaccine strain CVI988/Rispens Intervet, the 60 aa insertion was absent from SMI14-KampungCk, MDN13.SF.SPLN and MDN13.AF.SPLN. The MEQ sequence of SMI14.FFE117 from Arabic chicken farm in Sukabumi showed high proximity with the strain CVI988/Rispens Intervet, except the 61 aa absence at positions 194–254.

Subsequently, the BLAST-n analysis of the *meq* gene of SMI14-KampungCk compared with genome database in the NCBI GenBank demonstrated the highest proximity (99%) with strain G2 from China (Acc. No. AF493556), which has a very virulent (vvMDV-1) characteristic. There is single nucleotide substitution at position 539 (C for G2 and T for SMI14-KampungCk) causing dissimilarity of encoded aa, which is threonine or and methionine for G2 and SMI14-KampungCk, respectively. Furthermore, the multiple sequence analysis of the MEQ sequence of SMI14-KampungCk against other 43 MDV-1 characterized isolates reveals several aa variations (Table 2). The SMI14-KampungCk differs with all other isolates at position 180 that is methionine, whereas the other isolates are threonine or alanine. The phylogenetic tree analysis of the *meq* gene of the SMI14-KampungCk against other 64 MDV-1 strains in Figure 3 revealed that the SMI14-KampungCk is not in the same group with other MDV-1 strains from other regions such as the United States, Europe, India, Egypt, Australia, etc. The SMI14-KampungCk has high proximity with strain G2 and LZY from China. This Indonesian strain also has close proximity with strain Tokachi-m2 from Japan and strain DL/1106 and HNLH303 from China.

## 4. Discussion

Marek's disease is an important issue among commercial poultry farms although the outbreaks have been controlled by vaccination (Biggs and Nair 2012). No sterile immunity or imperfect vaccination permits virus evolution causing breakdown of vaccination program (Gandon *et al.* 2001). Field strains of MDV-1 were detected from blood, feather and dust. Hence, this evidence indicated infection cycle of MDV-1 including source of infection in the poultry dust, viraemia period in T-cell lymphocytes and horizontal transmission via feather debris (Schat and Nair 2008).

Great concerns have arisen for the *meq* gene features because it is the principal oncogene of MDV-1 (Liu *et al.* 1998; Lupiani *et al.* 2004). Several polymorphisms, mutations and/or alterations in the *meq* gene appear to correlate with virulence of MDV-1 (Chang

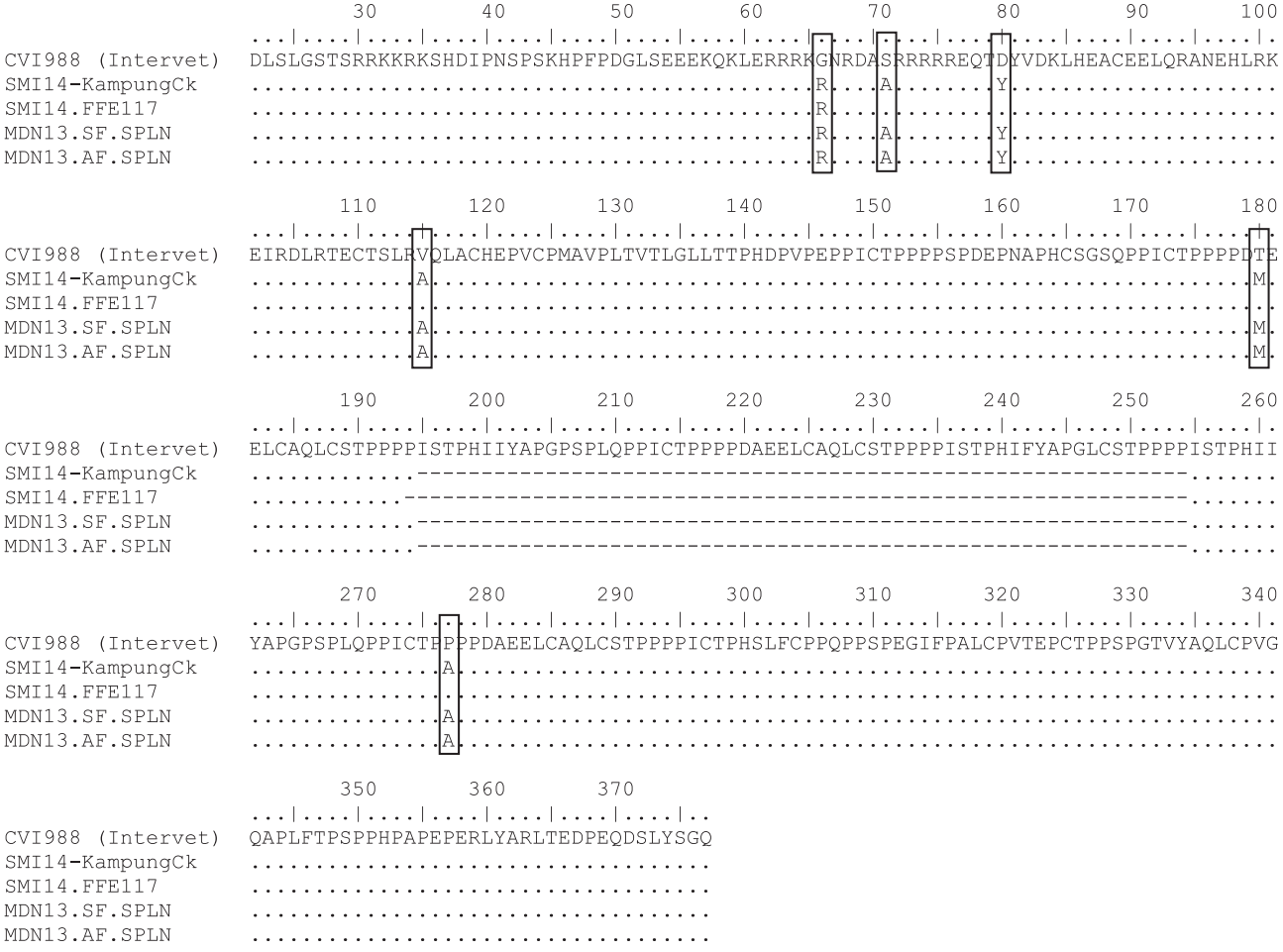


**Figure 2.** The amino acid alignment of the MEQ sequence of field samples isolated at Sukabumi 2014, two samples isolated at Medan 2013, and strain CVI988/Rispens Intervet (Acc. No. DQ534538). The CVI988/Rispens is used for numbering system. Six amino acid differences are indicated by bar marking. The 60 amino acid absence at positions 195–254 is indicated by dash.

Table 2. The amino acid differences in the MEQ protein between SMI14-KampungCk and other characterized MDV isolates

| Strain | Patho type* | Origin | NCBI GenBank Acc. No. | The MEQ amino acid sequence of SMI14-KampungCk†,‡ | | | | | | | | | | | | | | | | | | | | | | | | | | Insertion 60 aa |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 48 | 66 | 71 | 77 | 80 | 88 | 93 | 101 | 115 | 119 | 139 | 153 | 176 | 180 | 186 | 194 | 211 | 217 | 261 | 263 | 277 | 281 | 282 | 283 | 301 | |
| | | | | F | R | A | E | Y | A | Q | K | A | C | T | P | P | M | Q | P | L | A | V | E | L | V | G | A | Y | |
| CVI988 | att | Holland | DQ534538 | F | G | S | E | D | A | Q | K | V | C | T | P | P | T | Q | P | L | P | V | E | L | V | G | A | Y | + |
| CU-2 | m | USA | AY362708 | F | R | S | E | D | A | Q | K | V | C | T | P | P | T | Q | – | L | P | V | E | L | V | G | A | Y | + |
| 3004 | m | China | EU032468 | F | R | S | E | D | A | Q | K | V | C | T | P | P | T | Q | – | L | P | V | E | L | V | G | A | Y | + |
| 814 | m | Russia | AF493551 | F | R | S | E | D | A | Q | K | A | C | T | P | P | T | Q | – | L | P | V | E | L | V | G | A | Y | – |
| GA | v | USA | AF147806 | F | R | A | K | D | A | Q | K | V | C | T | P | P | T | Q | P | L | P | V | E | L | V | G | A | Y | – |
| BC-1 | v | USA | AY362707 | F | R | S | A | D | A | Q | K | A | C | T | P | P | T | Q | – | L | P | V | E | L | V | G | A | Y | + |
| 567 | v | USA | AY362709 | S | R | A | E | Y | A | Q | K | V | R | T | P | P | T | Q | P | L | A | V | E | L | V | G | A | Y | – |
| 571 | v | USA | AY632710 | F | R | A | E | Y | A | Q | K | A | C | T | P | H | T | Q | P | L | P | V | E | L | V | V | A | Y | – |
| 573 | v | USA | AY362711 | F | R | A | E | Y | A | Q | K | A | C | T | P | H | T | Q | P | L | P | V | E | L | V | V | A | Y | – |
| 617A | v | USA | AY362712 | F | R | A | E | Y | A | Q | K | V | R | T | P | P | T | Q | P | L | A | V | E | L | V | G | A | Y | – |
| 637 | v | USA | AY362713 | F | R | A | E | Y | A | Q | K | V | R | T | P | P | T | Q | P | L | A | V | E | L | V | G | A | Y | – |
| 04CRE | v | Australia | EF523773 | F | R | S | A | D | A | Q | K | A | C | T | P | P | T | Q | – | L | A | V | E | L | V | G | A | C | + |
| MPF57 | v | Australia | EF523774 | F | R | S | A | A | A | Q | K | A | C | T | P | P | T | Q | – | L | A | V | E | L | V | G | A | Y | + |
| Md5 | vv | USA | AF243438 | F | R | A | K | D | A | Q | K | V | C | T | P | P | T | Q | P | L | A | V | E | L | V | G | V | Y | – |
| RB-1B | vv | USA | HM488349 | F | R | A | K | D | A | Q | K | V | C | T | P | P | T | Q | P | L | P | V | E | L | V | G | A | Y | – |
| C12_130 | vv | USA | FJ436096 | F | R | A | E | D | T | R | K | V | C | A | Q | P | T | Q | P | L | P | V | E | L | V | G | A | Y | – |
| 549 | vv | USA | AY362714 | F | R | A | K | Y | A | Q | K | V | R | T | Q | A | A | Q | P | L | A | V | E | L | V | G | A | Y | – |
| 595 | vv | USA | AY362715 | F | R | A | K | D | A | Q | K | V | R | T | Q | A | A | Q | P | L | A | V | E | L | V | G | A | Y | – |
| 634P | vv | USA | AY362716 | F | R | A | K | D | A | Q | K | V | R | T | P | A | A | Q | P | L | A | V | E | F | V | G | A | Y | – |
| 0095 | vv | China | AF493552 | F | R | A | E | Y | A | Q | K | A | C | T | P | R | T | Q | P | L | A | L | E | L | V | G | A | Y | – |
| G2 | vv | China | AF493556 | F | R | A | E | Y | A | Q | K | A | C | T | P | P | T | Q | P | L | A | V | E | L | V | G | A | Y | – |
| GX070060 | vv | China | EU427303 | F | R | A | E | Y | A | Q | K | A | C | A | P | R | T | Q | P | L | A | V | E | L | V | G | A | Y | – |
| LMS | vv | China | JQ314003 | F | R | A | E | Y | A | Q | K | A | C | A | P | R | T | Q | P | L | A | V | E | L | V | G | A | Y | – |
| MS57 | vv | China | HQ638145 | F | R | A | E | C | A | Q | K | A | C | A | P | R | T | Q | P | L | A | V | E | L | V | G | A | Y | – |
| WS03 | vv | China | HQ638152 | F | R | A | E | Y | A | Q | K | A | C | A | P | R | T | Q | P | L | A | V | E | L | V | G | A | Y | – |
| YA | vv | China | HQ638156 | F | R | A | E | Y | A | Q | K | A | C | A | P | R | T | Q | P | L | A | V | E | L | V | G | A | Y | – |
| ATE | vv | Hungary | AY571784 | F | R | A | E | Y | T | R | R | V | C | A | P | P | T | Q | P | L | P | V | E | L | V | G | A | Y | – |
| tn-n2 | vv | India | HM749325 | F | R | A | K | D | A | Q | K | V | C | T | P | P | T | Q | L | P | P | V | G | L | V | G | A | Y | – |
| FT158 | vv | Australia | EF523771 | F | R | S | A | A | A | Q | K | A | C | T | P | P | T | Q | – | L | A | V | E | L | A | G | A | Y | + |
| 02LAR | vv | Australia | EF523772 | F | R | S | A | D | A | Q | K | A | C | T | P | P | T | H | – | L | A | V | E | L | V | G | A | Y | + |
| Woodland1 | vv | Australia | EF523775 | F | R | S | A | A | A | Q | K | A | C | T | P | P | T | Q | – | L | A | V | E | L | V | G | A | Y | + |
| L | vv+ | USA | AY362717 | F | R | A | K | D | A | Q | K | V | R | T | Q | A | A | Q | P | L | A | V | E | L | V | G | A | Y | – |
| N | vv+ | USA | AY362718 | F | R | A | K | D | A | Q | K | V | R | T | Q | A | A | Q | P | L | A | V | E | P | V | G | A | Y | – |
| New | vv+ | USA | AY362719 | F | R | A | K | D | A | Q | K | V | R | T | Q | A | T | Q | P | L | A | V | E | L | V | G | V | Y | – |
| RL | vv+ | USA | AY362720 | F | R | A | K | D | A | Q | K | V | R | T | Q | A | A | Q | P | L | A | V | E | L | V | G | A | Y | – |
| TK | vv+ | USA | AY362721 | F | R | A | K | D | A | Q | K | V | R | T | Q | A | A | Q | P | L | A | V | E | L | V | G | A | Y | – |
| U | vv+ | USA | AY362722 | F | R | A | K | D | A | Q | K | V | C | T | P | P | T | Q | P | L | A | V | E | P | V | G | A | Y | – |
| W | vv+ | USA | AY362723 | F | R | A | K | D | A | Q | K | V | R | T | Q | A | A | Q | P | L | A | V | E | L | V | G | V | Y | – |
| X | vv+ | USA | AY362724 | F | R | A | K | D | A | Q | K | V | R | T | Q | A | A | Q | P | L | A | V | E | L | V | G | A | Y | – |
| 684A | vv+ | USA | AY362725 | F | R | A | K | D | A | Q | K | V | R | T | Q | A | A | Q | P | L | A | V | E | P | V | G | A | Y | – |
| 660A | vv+ | USA | AY362726 | F | R | A | K | D | A | Q | K | V | R | T | Q | A | A | Q | P | L | A | V | E | P | V | G | A | Y | – |
| 686 | vv+ | USA | AY362727 | F | R | A | K | D | A | Q | K | V | R | T | Q | A | A | Q | P | L | A | V | E | P | V | G | A | Y | – |
| 584a | vv+ | USA | DQ534532 | F | R | A | K | D | A | Q | K | V | R | T | Q | A | T | Q | P | L | A | V | E | L | V | G | V | Y | – |

* The pathotype of 43 MDV-1 strains ranging from att (attenuated), m (mild), v (virulent), vv (very virulent) to vv+ (very virulent plus);

† The numbering system of the *meq* gene based on MDV-1 strain GA (AF147806);

‡ The amino acid variations between MDV strains are shown in the gray boxes.

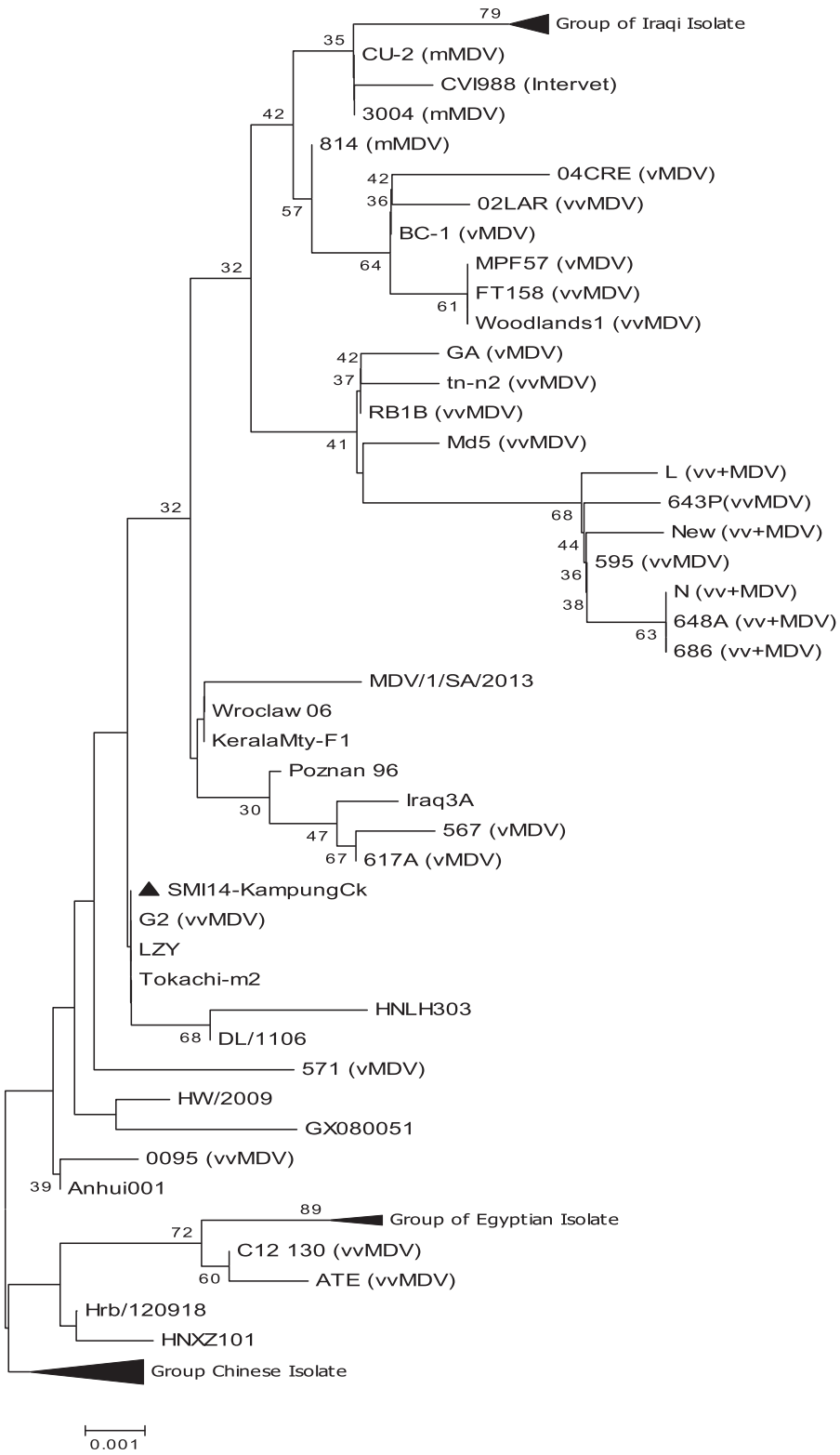


**Figure 3.** Phylogenetic tree analysis of 65 MDV-1 strains based on the *meq* gene using MEGA 6.06 using neighbor-joining analysis (1000 bootstrap replicates) with Tamura 2 parameter. The SMI14-KampungCk is indicated with a triangle marking.

*et al.* 2002; Shamblin *et al.* 2004). In comparison with the vaccine strain CVI988/Rispens Intervet (Acc. No. DQ534538), the MEQ characteristics of the Sukabumi field strain (SMI14-KampungCk) showed six aa differences, including G66R, S71A, D80Y, V115A, T180M and P277A. There are no aa property changes for three mutations (V115A, T180M and P277A) that still belong to hydrophobic nonpolar with neutral charge. Three other aa differences result in different amino properties, which are G66R from glycine (aliphatic, neutral) becoming arginine (hydrophilic, polar, positive charge), S71A from serine (polar, neutral) into alanine

(hydrophobic, non-polar, neutral charge), and T180M from aspartate (acidic, negative charge) into tyrosine (polar, neutral). These property dissimilarities could be significant for the biological characteristic for the viruses (Hartl and Jones 2008).

The MEQ sequence of SMI14-KampungCk displays aa residues of glutaminic acid at position 77 (E77), tyrosine at position 80 (Y80) and alanine at position 115 (A115) in the N-terminal basic region leucine zipper (bZIP) domain. It also has aa residue for alanine at position 217 (A217) in the C terminal transactivation domain. These respective aa substitutions have been suspected for contributing to increase the MEQ protein transactivation (Murata *et al.* 2011; Murata *et al.* 2013). The MEQ sequence of SMI14-KampungCk also lacks the 60 aa insertion that has been usually recognized in the lower virulence strains (Shamblin *et al.* 2004; Yu *et al.* 2013). However, two studies reported that the 60 aa insertion was also found in several virulent strains (Renz *et al.* 2012; Wozniakowski *et al.* 2011).

Another virulence marker is related to the disruption of the number of proline-rich repeat region motif [PPPP to P(Q/A)PP] in the MEQ protein. The proline-rich repeat region at the C terminal is related to the *WT-1* tumor suppressor gene (Jones *et al.* 1992). The highly virulent viruses have the most number of interruption (Shamblin *et al.* 2004). Later, Rentz *et al.* (2012) examined proline-rich repeat region in several characterized MDV-1 strains. They recognized attenuated strain CVI988/Rispens has the highest number by eight motifs. Apparently, the numbers of the proline motifs gradually lessen along with increased level of virulence. Because the SMI14-KampungCk has six motifs, the prediction for its virulence will be ranging from virulent to very virulent.

If the molecular features of the SMI14-KampungCk are closely aligned to virulent MDV-1, why did the infection of Kampung chickens with this virus not result in an outbreak of clinical disease? Also, there is no official report about outbreak in Kampung chicken so far. Several explanations may account to this paradigm. First explanation could be that the SMI14-KampungCk is a nonpathogenic strain. However, this probability seem unlikely because identical virus caused outbreak in the layer farms, North Sumatera in 2013. Second explanation could be that Kampung chicken may have some extent of genetic resistance against MDV-1 infection. In the genetically resistant bird, MDV-1 infection remains in the latent state without any clinical symptoms (Witter *et al.* 1971). However, the protection against Marek's disease cannot be relied only on the genetic resistance because the highly virulent viruses can overcome this barrier. Therefore, the optimal control will be achieved by combining it with other factors such as vaccination and biosecurity (Buscaglia *et al.* 2004; Schat and Nair 2008).

The result of this study confirmed the circulation of field strain of MDV-1 with predicted virulent or very virulent pathotype by molecular analyses both in the Kampung chicken farm in Sukabumi 2014 and in the layer farms in Medan 2013. The molecular characteristics of *meq* gene of these field strain MDV-1 viruses also demonstrated high proximity (99%) with very virulent (vvMDV-1) isolate G2 from Chinese. As a consequence, vaccination program for Marek's disease using gold standard vaccine strain CVI988/Rispens should be applied properly to protect the commercial chicken farm industry (Biggs and Nair 2012; Witter 1997). The utilization of the MDV vaccine based on HVT that is still available commercially may not provide enough protection against the field strain MDV-1 viruses (Witter 1997).

The molecular approach for determining the pathogenicity character of MDV-1 has become more popular nowadays since it is more feasible, straightforward and affordable. The most studies have been oriented on the *meq* gene as the main oncogenic gene, where the other genes are suspected to contribute as auxiliaries functions. Because the genome of MDV-1 is large in about of 160,000–180,000 bp (Spatz *et al.* 2007), the pathogenic trait should be polygenic so the future studies should be expanded for other genes as well. However, the molecular characterization approach still could only provide predictions of pathogenic trait of MDV-1. Although the evolution into more pathogenic pathotypes has been acknowledged, the molecular basis for this phenomenon remain obscure (Schat and Nair 2008). Therefore, the pathotyping scheme such as Avian Disease and Oncology Laboratory system still becomes important method to determining pathogenicity level of MDV-1 (Witter *et al.* 2005). Nevertheless, hopefully in the future the molecular approach can achieve idyllic circumstances to replace the *in vivo* test for characterizing pathogenic level of MDV-1.

## Acknowledgements

This research was funded by the IRCVS project research number 18016.020.011B and 1806.020.001.011E (2013–2014). The authors acknowledge the supports of agricultural service of Sukabumi district staffs in the field activity. Particular thanks for Nana Suryana, Teguh Suyatno and Ace Endang Supriatna for the laboratory and technical supports.

## Conflict of interest statement

Authors declare no conflict of interest.

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