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Identification of Bovine Rotavirus Group A in Bogor, West Java

Identifikasi Bovine Rotavirus Group A di Bogor, Jawa Barat

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Abstrak

Diare merupakan penyakit yang menyebabkan angka kesakitan yang tinggi pada pedet dan kematian neonatal. Penyakit ini dapat disebabkan oleh beberapa agen penyakit yang berbeda seperti Rotavirus A (RVA), bovine kobuvirus (BKV), bovine viral diarrhea 1 dan 2 (BVDV-1 and BVDV-2), enteropathogenic *Escherichia coli* (ETEC) and *Cryptosporidium* spp.*Rotavirus* Grup A (RVA) atau *Bovine Rotavirus* merupakan salah satu agen infeksi penyebab diare pada pedet. Selanjutnya, diare neonatal pada pedet dapat berdampak pada kerugian ekonomi bagi ternak sapi perah dan sapi potong di seluruh dunia karena menyebabkan gangguan pertumbuhan, meningkatnya biaya perawatan, dan/atau kematian pada hewan sakit. Prevalensi *Bovine Rotavirus* dapat berbeda antar negara di seluruh dunia. Sirkulasi *Bovine Rotavirus* pada sapi telah dilaporkan di beberapa negara tetapi sirkulasi *Bovine Rotavirus* pada sapi di Indonesia belum diketahui. Untuk mengetahui prevalensi *Rotavirus* grup A atau *Bovine Rotavirus* (BRV), 100 sampel feses dikoleksi dari pedet dengan gejala klinis diare atau tidak diare di Kabupaten Bogor, Jawa Barat pada tahun 2021. Sampel dianalisis terhadap urutan yang mengkode protein kapsid bagian dalam VP6 (subkelompok) menggunakan *Reverse Transcriptase Polymerase Chain Reaction* (RT-PCR). Lima dari 100 sampel feses sapi (5%) terdeteksi positif BRV. Pada penelitian ini menunjukkan bahwa kelompok *Rotavirus* atau *Bovine Rotavirus* (BRV) telah bersirkulasi di antara ternak sapi di Indonesia, khususnya Kabupaten Bogor. Sampel positif *Rotavirus* grup A atau *Bovine Rotavirus* (BRV) dapat diidentifikasi dengan metode diagnosis dini (RT-PCR).

Kata kunci: *Bovine Rotavirus*; RT PCR; Bogor

Abstract

Diarrhea is the most common disease that causes high morbidity in calves and neonatal mortality. Several different infectious agents can cause this disease, including Rotavirus A (RVA), bovine kobuvirus (BKV), bovine viral diarrhea 1 and 2 (BVDV-1 and BVDV-2), enteropathogenic *Escherichia coli* (ETEC) and *Cryptosporidium* spp.Group A rotaviruses (RVA) or Bovine Rotavirus are one of the infectious agents causing diarrhea in calves. Then, Neonatal calf diarrhea can impact economic losses to dairy and beef cattle herds worldwide as a consequence of growth disorders, the value of treatment, and death of sick animals. The prevalence of Bovine Rotavirus can become different in the worldwide. The circulation of these bovine Rotavirus in calves from some regions has already been demonstrated, but the circulation of bovine Rotavirus in Indonesia is not known. The aim of this study is to investigate the prevalence of A-group Rotaviruses or Bovine Rotavirus (BRV). One hundred faecal samples were collected from calves with diarrhea or no diarrhea in Bogor district, West Java,in 2021. The samples were analyzed for sequences encoding the inner capsid protein VP6 (subgroup) using RT-PCR. Five of 100 specimens of bovine faecal (5%) were detected positive as BRV positive. In this study, A group of Rotaviruses or Bovine Rotavirus (BRV) have been circulated among

cattle herds in Indonesia, particularly in Bogor District. The positive samples of A group Rotaviruses or Bovine Rotavirus (BRV) can be identified using the early diagnosis method (RT-PCR).

Keywords: Bogor District; *Bovine Rotavirus*; RT PCR

Introduction

Diarrhea has been reported as a cause of high morbidity and mortality in animals. Neonatal calf diarrhea is an important disease and inducing serious economic loss in cattle. Diarrhea is caused by several factors. The infectious agents are one of several factors causing diarrhea (Cheng, *et al.,* 2021). Viruses, bacteria, and protozoa are agents that cause outbreaks of severe neonatal diarrhea in cattle aged 5-18 days. The disease agents are usually Rotavirus A (RVA), bovine kobuvirus (BKV), bovine viral diarrhea 1 and 2 (BVDV-1 and BVDV-2), enteropathogenic *Escherichia coli* (ETEC) and *Cryptosporidium* spp. (Agnol, *et al.,* 2021).

Rotaviruses are the major viral agents that are responsible for diarrhea (Cheng, *et al.,* 2021). Rotavirus is a double-stranded RNA (dsRNA), non-enveloped, and has three protein capsids belonging to the Reoviridae family. Rotavirus is composed of 11 gene segments encoding 6 structural proteins (VP1, VP2, VP3, VP4, VP6, VP7) and 6 nonstructural proteins (NS1-NS6). Rotaviruses are divided into 9 groups (A-H) based on antigenic differences and genetic diversity of the VP6 protein (ICTV, 2021).

Rotaviruses A is the predominant occasion of bovine viral diarrhea (Uddin Ahmed, et al, 2022). The VP6 protein of group A Rotavirus is greatly immunogenic among the distinct serotypes. Antibodies to the VP6 protein conveniently appear so as to be the primary sensitive diagnostic (Svensson, *et al.,* 1987). The lengths of VP6 genome segments is 1353 or 1354 nt (Matthijnnsess, *et al.,* 2012). The VP6 protein is a conserved protein that can associate with itself and VP4, VP2 and VP7 proteins. The surface VP6 protein bear the major conserved amino acids (Mathieu, *et al.,* 2001). Sebagian besar komponen struktural kapsid *Rotavirus* disusun oleh protein VP6. The VP6 protein is an important role in determining the structure of Rotavirus virions and usually used as a target gene for detecting of Group A Rotavirus or Bovine Rotavirus genes (Cheng *et al.,* 2021).

There are several methods in diagnostic laboratories used routinely to detect the Rotavirus from fecal samples. These encompass electron microscopy, immunoelectrophoresis, ELISA, passive hemagglutination, latex agglutination assays and virus isolation (VI) (Patel, *et al.,* 2019). The one-step RT-PCR can reduce cross contamination and will be over worthwhile in developing countries, particularly countries that have restricted laboratory and skilled labour force (Esona, *et al.,* 2015).

The PCR method of the group A rotavirus typing in strains used samples obtained directly from faecal and cell culture. The RT-PCR assay possesses characteristics of sensitive, specific, and rapid for identification of bovine rotaviruses in faecal samples (Kassem, et al., 2017). The purpose of this research was to detect the A rotaviruses or Bovine Rotavirus (BRV) from faecal sample calves in Indonesia, particularly Bogor District, using the RT-PCR method with the gen target of VP6.

. Materials and methods

Materials

Field samples are required for examination of the RT-PCR protocol. The faeces samples of 100 were obtained from bovine farms in Bogor District. Faeces were collected directly from cow's rectum with the clinical symptoms of diarrhea (more frequent of fluid feces) or no diarrhea (frequent passing of shaped feces). Before sampling, an evaluation is carried out for the data history of cow's health and the clinical observations on the cow that its fecal samples will be collected. Fecal sampling is prioritized for cows that have symptoms of diarrhea and cows that have a previous history of diarrhea. Futhermore, samples were placed in a sampling bag and kept at 4^{o}C. The samples were taken to laboratory for storage at -80^{o}C until further analysis for RNA extraction. Ten to twenty percent (w/v) of the fecal sample are dissolved in phosphate buffer saline (PBS; pH 7.2) for the

RT-PCR test. The sample is then vortexed for one minute and centrifuged for three minutes at 10,000 rpm. This investigation was carried out in 2021.

Table 1. Information of Field Samples

District	Quantity of Samples	Symptom of Health
Ciawi	50	diarrhea, no diarrhea
Cipelang	50	diarrhea, no diarrhea

Methods

Sequence Primer

The specific primer sequences of RT-PCR assays were determined based on the VP6 gene segment of Rotavirus A according to previous studies (Chinsangarams, *et al.,* 1993; Wang, *et al.,* 2019). The product of amplification were 1356 bp base pair (bp) (Chinsangarams, *et al.,* 1993), and 450 base pair (bp) (Wang, et al., 2019). The primers applied in this assay were seen in Table 2. These primers were synthesized at AITbiotech PTE LTD, Singapore.

Table 2. Name, sequence of the primers and size of RT-PCR product that used for rotavirus detection in this study

Name	Sequence	Product size
Chins_BRVP6F	5'-GGCTTTTAAACGAAG TCTTC-3'	1356 bp
Chins_BRVP6R	5'-GGTCACATCCTCTCA CTACG-3'	
Wang_BRVP6F	5'-TTTCCCTTATTCAGCT TCATTCACGTTGAACAG ATCGCA-3	
Wang_BRVP6R	5'-AACGCCGCTACCGCT GGTGTCATATTTGGTGG TCTCATC-3	450 bp

RNA Extraction

Faeces samples were diluted to a weight ratio of 1:9 (w/v) using sterile phosphate-buffered saline (PBS). The diluted samples were then centrifuged for three minutes at 10,000 rpm. Using a commercial Geneaid total RNA extraction kit (Geneaid Biotech Ltd., Taiwan) and the kit provider's instructions, RNA was extracted from the diluted faeces samples. A 0.5 ml microfuge tube with 70% alcohol and four µl of beta-mercaptoethanol was filled with 0.2 ml of diluted faecal samples. After adding the sample to the Geneaid reagent, the sample was rinsed, and 50 µl of free RNAse was used to remove the RNA. The RNA was then stored at -20^{o}C until further use.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

The RT-PCR procedure used the My Taq One Step RT-PCR kit (Bioline reagent Ltd) reagent according to the kit procedure on the AB9700 or AB9800 Fast Thermal Cycler Machine. RT-PCR using primers specific for VP gene of Bovine Rotavirus was developed according to previous studies (Chinsangarams, *et al.,* 1993; Wang, *et al.,* 2019). For each 25 µl reaction consists of 5 µl of extracted RNA, 12.5 µl 2x My Taq One Step Mix, 1 µl Forwad primer (10 M), 1 µl Reverse primer (10 M), 0.25 µl Reverse Transcriptase, 0.5 µl Ribosafe RNase Inhibitor, 4.75 µl DPEC H2O. The step of reverse transcriptase at 45oC for 40 minutes for 1 cycle followed by enzyme inactivation at 95^{o}C for 1 minute for one cycle. The amplification reaction was for 40 cycles, consisting of denaturation (95^{o}C, 20 seconds), annealing (60^{o}C, 20 seconds) and elongation (72^{o}C, 1 minute). The final extension at 72^{o}C for 5 minutes. The PCR product was visualized on 1.5% agarose gel stained ethidium bromide by electrophoresis (100 volts, 1 hour) of Tris Boric EDTA (TBE) solution. DNA ladder 100 bp (Invitrogen™) was used as a marker of the amplified gene fragment. The estimated sizes of amplified products were 1356 bp for primers (9), 450 bp for primers (Wang, *et al.,* 2019).

Results and discussion

To investigate of bovine RVAs in Bogor District, we analyzed samples with diarrhea and no diarrhea symptoms from 100 collected from cattle by using the RT-PCR based on the gene segment encoding VP6. Most of rotavirus protein is composed of VP6 protein. The VP6 protein is majority conserved among the serotypes of Rotavirus. The homology of the amino acid sequence of VP6 protein is >90% which sustains it can be used to develop the early diagnostic method. This study succeeded in detecting BRV in five of 100 faecal samples from cattle using primers Wang et al. (2019) (Figure 1.) but bovine RRotavirus were not identified using primers Chinsangarams et al.

Table 3. Results of the RT-PCR test for Rotavirus A used primer Wang et al. (2019) and Chinsangarams et al. (1993)

No	Sample code	Cow code	Location	Results of the RT-PCR	
				Primer (Wang)	Primer (Chins)
1	BET II/1/19-1-21	Calf 321569 FH	Cage of calf	Negative	Negative
2	BET II/2/19-1-21	Calf 220199 FH	Cage of calf	Negative	Negative
3	BET II/3/19-1-21	Calf 270083 FH, diarrhea	Cage of calf	Positive	Negative
4	BET II/4/19-1-21	Calf 172082 FH	Cage of calf	Negative	Negative
5	BET II/5/19-1-21	Calf 172081 FH diarrhea	Cage of calf	Positive	Negative
6	BET II/6/19-1-21	Calf 220205 FH diarrhea	Cage of calf	Positive	Negative
7	BET II/7/19-1-21	Calf 221206 FH	Cage of calf	Negative	Negative
8	BET II/8/19-1-21	Calf 220204 FH	Cage of calf	Negative	Negative
9	BET II/9/19-1-21	Calf 221209 FH	Cage of calf	Negative	Negative
10	BET II/10/19-1-21	Calf 221208 FH	Cage of calf	Negative	Negative
11	BET II/11/19-1-21	Calf 221207 FH	Cage of calf	Negative	Negative
12	BET II/12/19-1-21	Calf ABB 21118	Cage of calf	Negative	Negative
13	BET II/13/19-1-21	Calf 821218 FH	Cage of calf	Positive	Negative
14	BET II/14/19-1-21	Calf ABB 21120	Cage of calf	Negative	Negative
15	BET II/15/19-1-21	Calf 220202 PO	Cage of A	Negative	Negative
16	BET II/16/19-1-21	Calf 220201 PO	Cage of A	Positive	Negative
17	BET II/17/19-1-21	Calf 992014 PO	Cage of A	Negative	Negative
18	BET II/18/19-1-21	Calf 220197 PO	Cage of A	Negative	Negative
19	BET II/19/19-1-21	Calf 220200 PO	Cage of A	Negative	Negative
20	BET II/20/19-1-21	Calf 220195 PO	Cage of A	Negative	Negative
21	BET II/21/20-1-21	Calf 220194 PO	Cage of A	Negative	Negative
22	BET II/22/20-1-21	Calf 220198 PO	Cage of A	Negative	Negative
23	BET II/23/20-1-21	Calf 320556 FH	Cage of A	Negative	Negative
24	BET II/24/20-1-21	Calf 320567 FH	Cage of A	Negative	Negative
25	BET II/25/20-1-21	Calf 320558 FH	Cage of A	Negative	Negative
26	BET II/26/20-1-21	Calf 320568 FH	Cage of A	Negative	Negative
27	BET II/27/20-1-21	Calf 320559 FH	Cage of A	Negative	Negative
28	BET II/28/20-1-21	Calf 220193 PO	Cage of A	Negative	Negative
29	BET II/29/20-1-21	Calf 2033 Bram	Cage of A	Negative	Negative
30	BET II/30/20-1-21	Calf 820217	Cage of A	Negative	Negative
31	BET II/31/20-1-21	Calf 172079 Angus, diarrhea	Cage of A	Negative	Negative
32	BET II/32/20-1-21	Calf 320552 FH	Cage of A	Negative	Negative
33	BET II/33/20-1-21	Calf 172077 Angus	Cage of A	Negative	Negative
34	BET II/34/20-1-21	Calf 172075 Angus, diarrhea	Cage of A	Negative	Negative
35	BET II/35/20-1-21	Calf 320555 FH, diarrhea	Cage of A	Negative	Negative
36	BET II/36/20-1-21	Calf 992013 Glasi	Cage of A	Negative	Negative
37	BET II/37/20-1-21	Calf 32554 FH	Cage of A	Negative	Negative
38	BET II/38/20-1-21	Calf 172080 Angus	Cage of A	Negative	Negative
39	BET II/39/20-1-21	316388 FH	Cage of A	Negative	Negative
40	BET II/40/20-1-21	Calf 320561 FH	Cage of B	Negative	Negative
41	BET II/41/20-1-21	Calf 320563 FH	Cage of B	Negative	Negative
42	BET II/42/20-1-21	Calf 320053 FH	Cage of B	Negative	Negative
43	BET II/43/20-1-21	Calf 320566 FH	Cage of B	Negative	Negative
44	BET II/44/20-1-21	Calf 320564 FH, diarrhea	Cage of B	Negative	Negative
45	BET II/45/20-1-21	Calf 320565 FH, diarrhea	Cage of B	Negative	Negative
46	BET II/46/20-1-21	Calf 320557 FH, diarrhea	Cage of B	Negative	Negative
47	BET II/47/20-1-21	Calf 320562 FH	Cage of B	Negative	Negative
48	BET II/48/20-1-21	Calf 220196 FH	Cage of B	Negative	Negative
49	BET II/49/20-1-21	Calf 320560 FH	Cage of B	Negative	Negative
50	BET II/50/20-1-21	316433 FH	Cage of B	Negative	Negative
51	Bnak II/1/21-1-21	2001 FH Calf 5 months	Cage of Cow	Negative	Negative
52	Bnak II/2/21-1-21	2002 FH Calf	Cage of Cow	Negative	Negative
53	Bnak II/3/21-1-21	BBX Calf	Cage of Cow	Negative	Negative
54	Bnak II/4/21-1-21	2008 Calf	Cage of Cow	Negative	Negative
55	Bnak II/5/21-1-21	2011 BBX Calf, diarrhea	Cage of Cow	Negative	Negative
56	Bnak II/6/21-1-21	20011 BBX Calf, diarrhea	Cage of Cow	Negative	Negative
57	Bnak II/7/21-1-21	19021 lims Calf	Cage of Cow	Negative	Negative
58	Bnak II/8/21-1-21	19025 FH Calf >5 months	Cage of Cow	Negative	Negative
59	Bnak II/9/21-1-21	19028 lims Calf >5 months	Cage of Cow	Negative	Negative

60	Bnak II/10/21-1-21	2006045 BBX Calf >5 months	Cage of Cow	Negative	Negative
61	Bnak II/11/21-1-21	2007 BBX Calf >5 months	Cage of Cow	Negative	Negative
62	Bnak II/12/21-1-21	2002 BBX Calf >5 months	Cage of Cow	Negative	Negative
63	Bnak II/13/21-1-21	2003 BBX Calf >5 months	Cage of Cow	Negative	Negative
64	Bnak II/14/21-1-21	2005044 BBX Calf >5 months	Cage of Cow	Negative	Negative
65	Bnak II/15/21-1-21	2004043 BBX cow	Cage of Cow	Negative	Negative
66	Bnak II/16/21-1-21	19026 FH cow	Cage of Cow	Negative	Negative
67	Bnak II/17/21-1-21	19017 FH cow	Cage of Cow	Negative	Negative
68	Bnak II/18/21-1-21	19019 FH cow	Cage of Cow	Negative	Negative
69	Bnak II/19/21-1-21	19022 FH cow	Cage of Cow	Negative	Negative
70	Bnak II/20/21-1-21	19023 FH cow	Cage of Cow	Negative	Negative
71	Bnak II/21/21-1-21	17003 FH cow	Cage of Cow	Negative	Negative
72	Bnak II/22/21-1-21	18008 FH cow	Cage of Cow	Negative	Negative
73	Bnak II/23/21-1-21	18002 FH cow	Cage of Cow	Negative	Negative
74	Bnak II/24/21-1-21	17005 FH cow	Cage of Cow	Negative	Negative
75	Bnak II/25/21-1-21	18009 FH cow	Cage of Cow	Negative	Negative
76	Bnak II/26/22-1-21	17014 FH cow	Cage of Cow	Negative	Negative
77	Bnak II/27/22-1-21	19005 BBX cow	Cage of Cow	Negative	Negative
78	Bnak II/28/22-1-21	19007 BBX cow	Cage of Cow	Negative	Negative
79	Bnak II/29/22-1-21	19009 BBX cow	Cage of Cow	Negative	Negative
80	Bnak II/30/22-1-21	19012 FH cow	Cage of Cow	Negative	Negative
81	Bnak II/31/22-1-21	15032 FH cow	Cage of Cow	Negative	Negative
82	Bnak II/32/22-1-21	15031 FH cow	Cage of Cow	Negative	Negative
83	Bnak II/33/22-1-21	15016 FH cow	Cage of Cow	Negative	Negative
84	Bnak II/34/22-1-21	1500 FH cow	Cage of Cow	Negative	Negative
85	Bnak II/35/22-1-21	130763 FH cow	Cage of Cow	Negative	Negative
86	Bnak II/36/22-1-21	14013 FH cow	Cage of Cow	Negative	Negative
87	Bnak II/37/22-1-21	PO 75 cow	Cage of Cow	Negative	Negative
88	Bnak II/38/22-1-21	PO 98 cow	Cage of Cow	Negative	Negative
89	Bnak II/39/22-1-21	PO 71 cow	Cage of Cow	Negative	Negative
90	Bnak II/40/22-1-21	PO 62 cow	Cage of Cow	Negative	Negative
91	Bnak II/41/22-1-21	PO 70 cow	Cage of Cow	Negative	Negative
92	Bnak II/42/22-1-21	PO 72 cow	Cage of Cow	Negative	Negative
93	Bnak II/43/22-1-21	PO 68 cow	Cage of Cow	Negative	Negative
94	Bnak II/44/22-1-21	PO 67 cow	Cage of Cow	Negative	Negative
95	Bnak II/45/22-1-21	PO 79 cow	Cage of Cow	Negative	Negative
96	Bnak II/46/22-1-21	PO 74 cow	Cage of Cow	Negative	Negative
97	Bnak II/47/22-1-21	PO 7X cow	Cage of Cow	Negative	Negative
98	Bnak II/48/22-1-21	PO XX cow	Cage of Cow	Negative	Negative
99	Bnak II/49/22-1-21	PO 64 cow	Cage of Cow	Negative	Negative
100	Bnak II/50/22-1-21	PO 66 cow	Cage of Cow	Negative	Negative

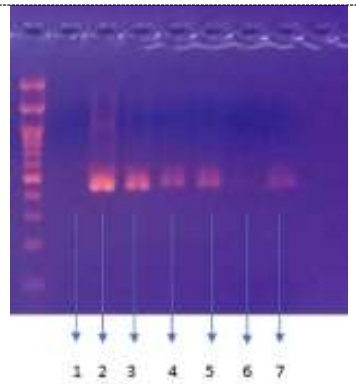

Figure 1. The samples of positive with RT-PCR method using Wang et al. (2019) primers. (1) negative control (2) BRV synthetic control, M: Marker DL2000, (3) Sample3 BET, (4) Sample5 BET, (5) Sample 6 BET, (6) Sample 13 BET, (7) Sample16 BET

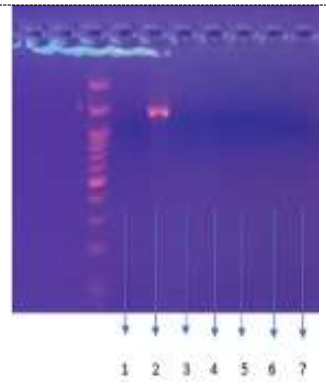

Figure 2. The samples of negative with RT-PCR method using using Chinsangarams et al. (1993) primers. (1) negative control (2) BRV synthetic control, M: Marker DL2000, (3) Sample3BET, (4) Sample5BET, (5) Sample6BET, (6) Sample13BET, (7) Sample16BET

(1993) (Figure 2.) Specification of the results of RT-PCR test are shown in Table 3.

The RNA extraction step is one of the factors that affects the outcome of Rotavirus RT-PCR testing. Several factors influence the success of the RNA extraction stage, such as the sample type and volume, the concentration of the target nucleic acid, the type of nucleic acid (DNA or RNA), and the existence of PCR inhibitors (Reck, et al., 2015). The primers used in RT-PCR determine the outcome of the process, excluding the extraction step. The incapacity to identify the Rotavirus A or Bovine Rotavirus gene in the field may arise from a mismatch in the primary sequence to the Rotavirus A or Bovine Rotavirus gene sequence in the field sample.

Rotavirus A (Bovine Rotavirus) infection mainly causes disruption of respiratory and digestive tract in calves. In cows 3–8 weeks old, the infection primarily causes diarrheal symptoms. Additionally, mixed infections with other microorganisms will make these symptoms worse (Cheng, *et al.,* 2021). Rotavirus A is the primary cause of diarrhea in newborn calves (Uddin Ahmed, *et al.,* 2022).

In this research, although bovine RVAs were usually detected from cattle diarrheic samples, bovine RVAs were identified in samples with asymptomatic of diarrhea from cattle in Bogor District during rainy season, 2021. The result of this study is convenient with prior research of bovine rotavirus infection in different region of the world. The prevalence of Rotavirus in calves was reported 5,1% in calves with diarrheic or non-diarrheic status in several countries (Barua, *et al.,* 2019). The prevalence of bovine RVA were associated with several factors, such as district, season, breed, age, geographic, nutritional, and management farm. Generally, diarrhea in calves of 3–8 weeks will be developed in fall and winter on the various livestock (Cheng, *et al.,* 2021).

However, bovine rotavirus infection are mainly risk on calves ≤ 5 weeks of age. Calves < 4-8 weeks of age have high case of rotavirus diarrhea because immune system is deficient in neonates and level of maternal antibodies is low (Uddin Ahmed, *et al.,* 2022). Implementing of health management such as the programs of vaccination and strict biosecurity on cattle farms can prevent the spread of disease infections (Agnol, *et al.,* 2021). Transmission of Rotavirus A or Bovine Rotavirus infection on cattle farms can be prevented and controlled through Rotavirus vaccination. Cows vaccinated against Rotavirus have high and long-lasting protective of antibody titers against Rotavirus in milk secretions. Furthermore, calves that received colostrum from vaccinated cows have the titers of protective antibody against Rotavirus so that infection and shedding of Rotavirus, clinical symptoms of diarrhea can be prevented (Hasan, *et al.,* 2022). Nevertheless, the mother's antibodies that are not transferred to the calf may become extremely vulnerable to rotavirus infection, which can result in diarrheal symptoms or even death. Therefore, it is critical to feed the proper colostrum to ensure the health of the calf, reduce the risk of disease infection, lessen digestive disorders, expedite the calf's recuperation from illness, and reduce the amount of antibiotics used in cattle farming (Lora, *et al.,* 2018).

After implementing a vaccination program against Rotavirus A, cattle farms are required to maintain routine surveillance of the virus's circulation within the farm (Fritzen, *et al.,* 2019). To comprehend the evolution of Rotavirus A, it is imperative to maintain a constant watch on circulating strains (Tamim, *et al.,* 2020). tracking the spread of the Rotavirus It is crucial to monitor strains on cattle farms in order to make predictions about potential effects, such as the Rotavirus A strain evolving into a more virulent form or perhaps becoming zoonotic (Fritzen, *et al.,* 2019).

In this study, the Rotavirus A were disseminated among healthy cow livestock. The environment and management farms can have impact on the occurrence of bovine Rotavirus. The group housing of management farms will enhance the contamination of bovine Rotavirus in the surrounding environment. The several calves infected bovine Rotavirus without diarrhea symptom (Bertoni, *et al.,* 2021). The individual housing of cattle herd can mitigate diarrhea risk. That can prevent direct contact between calves so that reduce the spreading of infectious Rotavirus in herds. Calves until 3 week of age should be enlarged in individual housing (Curtis, *et al.,* 2016).

Strict hygiene protocols can decrease calf mortality in cow pens. In calf breeding pens, hygiene protocols utilizing boots can lower the prevalence of pathogen infections, such as Rotavirus A. Every time a calf pen is entered, the boots that will be used must be changed. After that, to destroy any bacteria still attached to the boots, the used boots are cleaned and soaked in disinfectant (Takahashi, *et al.,* 2020).

Conclusion

Bovine Rotavirus group A (BRV) have been circulated among cattle herds in Indonesia, particularly Bogor District. The positive samples of BRV can be identified using the early diagnosis method (RT-PCR).

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