Jurnal Sain Veteriner, Vol. 43. No. 2. Agustus 2025, Hal. 203-211
DOI: 10.22146/jsv.101993
ISSN 0126-0421 (Print), ISSN 2407-3733 (Online)
Tersedia online di https://jurnal.ugm.ac.id/jsv

Bovine Viral Diarrhea Virus Antigen and Immunoglobulin-G Detection in Unvaccinated Cattle

Deteksi Antigen Virus Bovine Viral Diarrhea dan Immunoglobulin-G pada Ternak Sapi yang tidak Divaksin

Raden Rara Bhintarti Suryohastari[1,*], Muharam Saepulloh[2], Ana Roudlotul Jannah[1]

[1]Department of Biology, Faculty of Sciences and Technology, State Islamic University Syarif Hidayatullah Jakarta, Banten, Indonesia.
[2]Indonesian Research Center for Veterinary Science (IRCVS), Bogor, West Java, Indonesia
*Email: bhintarti.hastari@uinjkt.ac.id

Article submitted: November 29, 2024, revised: June 3, 2025, accepted: June 25, 2025

Abstrak

Pemenuhan terhadap kebutuhan gizi masyarakat akan daging dapat didukung melalui impor ternak sapi, yang dilakukan dengan kewaspadaan tinggi terhadap *Bovine Viral Diarrhea* (BVD) sebagai penyakit menular yang disebabkan oleh *Bovine Viral Diarrhea Virus* (BVDV). Virus patogen ini dapat menimbulkan masalah pada gastrointestinal, pernafasan, dan reproduksi pada sapi di seluruh dunia. Pengendalian BVDV merupakan tindakan terpenting dalam mencegah BVD, dan salah satu strategi efektif dalam upaya ini adalah vaksinasi. Keberhasilan pelaksanaan vaksinasi bergantung pada perolehan informasi rinci tentang antigen BVDV dan antibodi di dalam serum sapi yang tidak divaksinasi, yang dapat menjelaskan profil protein yang dihasilkan dari infeksi alami. Penelitian ini bertujuan untuk mendeteksi antigen BVDV dan antibodi imunoglobulin-G (IgG), berdasarkan berat molekul pada profil protein dari berbagai bangsa sapi yang diimpor oleh Indonesia. Melalui analisis SDS-PAGE, terdeteksi pita protein 55,3 kDa pada sapi FH dan Wagyu positif BVD, dan pita 151,3 kDa pada sapi FH positif BVD. Pita-pita tersebut tidak terdeteksi pada sapi negatif BVD. Semua sapi yang belum divaksin namun positif BVD menunjukkan *optical density* (OD) ≥ 0,30 yang dapat menunjukkan respons imun terhadap paparan BVDV secara alami. Pita-pita protein merefleksikan profil proteomik spesifik yang terkait dengan infeksi aktif, bukan hasil vaksinasi. Disimpulkan bahwa pita 151,3 kDa diduga mewakili antibodi IgG, sedangkan pita 55,3 kDa kemungkinan merupakan antigen E2 dari BVDV yang dapat digunakan sebagai dasar bagi pengembangan vaksin BVD.

Kata kunci: BVD; immunoglobulin; profil protein; SDS-PAGE.

Abstract

To address the community's nutritional for meat can be supported by importing cattle while maintaining vigilance against *Bovine Viral Diarrhea* (BVD), an infectious disease caused by the *Bovine Viral Diarrhea Virus* (BVDV). This pathogen is associated with gastrointestinal, respiratory, and reproductive issues in cattle worldwide. Consequently, controlling BVDV stands as a paramount measure in preventing BVD. One effective strategy in this endeavor is vaccination. The successful implementation of vaccination relies on acquiring comprehensive information about BVDV antigens and antibodies present in the serum of unvaccinated cattle, which can provide insights into the protein profile resulting from natural infection. This study aimed to detect BVDV antigen and immunoglobulin-G (IgG) based on molecular weight in protein profiles from various cattle breeds imported into Indonesia. SDS-PAGE analysis revealed a 55.3 kDa protein band in BVD-positive FH and Wagyu cattle, and a 151.3 kDa band in BVD-positive FH cattle. These bands were absent from BVD-negative animals. All BVD-positive cattle had not been vaccinated but showed optical density (OD) values ≥

0.30, indicating natural exposure to BVDV. The presence of these protein bands suggests a specific proteomic response associated with active infection. The 151.3 kDa band is presumed to represent an IgG antibody, while the 55.3 kDa band is likely the E2 antigen of BVDV, which holds potential for BVD vaccine development.

Keywords: antigen; BVD; immunoglobulin; protein profile; SDS-PAGE

Introduction

One strategy to address the demand for an adequate meat supply in Indonesia involves the importation of cattle. However, it is crucial to implement vigilant cattle import policies to mitigate the risk of introducing diseases, particularly Bovine Viral Diarrhea (BVD) caused by the Bovine Viral Diarrhea Virus (BVDV) (Polak *et al.,* 2016). BVDVs exhibit two distinct biotypes: cytopathic and non-cytopathic. The cytopathic can destroy infected cells, whereas the non-cytopathic can replicate in infected cells without causing harm, allowing it to persist within the host cell. The cytopathic effects lead to cell death and non-cytopathic effects result in persistent infections (PI) (Ammari MG, 2015). Non-cytopathic BVDV infection during the first trimester of pregnancy can lead to fetal infection, causing embryonic death, teratogenic effects, or the birth of calves with PI. Calves in the PI state can continually spread BVDV through excretions and secretions throughout their lives, serving as the primary route for BVDV transmission (Lanyon *et al.,* 2014). Consequently, stringent measures and monitoring are necessary to safeguard against the potential spread of BVD and its adverse effects on the cattle population (Primawidyawan *et al.,* 2023).

The BVDV infection poses substantial economic losses and manifests in various detrimental ways, including reproductive dysfunction, diminished fertility, reduced milk production, and slowed fetal growth. Clinical symptoms in affected cows may include diarrhea, fever, lesions on the mucous membrane of the tongue, weight loss, compromised immune function, and even death (Khodakaram-Tafti & Farjanikish, 2017). The economic repercussions of BVD are evident across 15 countries, where losses per cow range from 0.50 to 687.80 US dollars (Richter *et al.,* 2017). In Germany, a staggering 60% of the 5,325 cows across 21 farms have succumbed to BVD, leading to the culling of 400 cows with persistent BVDV (Gethmann *et al.,* 2015). Meanwhile, the prevalence of BVD in Indonesia is higher in dairy than in beef cattle (Subekti *et al.,* 2021). Additionally, it is crucial to note that BVDV is strictly regulated in ruminant livestock breeding. The semen of superior males intended for breeding must be free from BVDV, to mitigate the spread of BVD and safeguard the health and productivity of livestock populations, as outlined in Regulation No.10/Permentan/PK.210/3/2016 by the Agriculture Minister of the Republic of Indonesia.

Addressing the substantial economic losses attributed to BVD requires effective control measures, with protein-based vaccination emerging as a promising strategy. Comprehensive data on BVDV antigens and antibodies in unvaccinated cattle is essential to understanding the immune responses generated through natural infection. Identifying BVDV antigens and antibodies is imperative, given the virus's ability to evade host immunity, leading to the development of PI (Gethmann *et al.,* 2015). In infected cattle, their immune system triggers a response marked by the production of immunoglobulins (Ig), with IgG being the predominant component in serum (Vidarsson *et al.,* 2014). Understanding the antigenic protein profile of BVDV can aid in the development of sub-unit vaccines, as effective prevention hinges on the specificity between the vaccine and the infecting virus strain in the animal's environment (Poetri *et al.,* 2017). Therefore, this study aimed to analyze the protein profile of BVDV antigens and IgG through *Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis* (SDS-PAGE) in the serum of unvaccinated cattle to discern the natural protein profile that emerges due to BVDV infection.

This research was conducted at the Livestock Embryo Center (BET) focusing on breeding stock. In 2011, a study revealed that 5

out of 11 cattle at BET were infected with BVDV, accounting for a 45% prevalence (Nugroho *et al.,* 2022). This finding underscores the coexistence of both BVDV-infected and uninfected cattle at BET, necessitating a comprehensive exploration of viral antigens and Ig in unvaccinated cattle. This exploration involves describing their protein profiles. The antigen profile analysis can be effectively carried out using the SDS-PAGE method (Saadh *et al.,* 2021). The data obtained on BVDV antigen and IgG protein profiles in cattle imported by Indonesia can play a pivotal role in enhancing BVD control for preventing PI and mitigating the risk of producing cows that could potentially spread the virus throughout their lifespan.

Materials dan Methods

The sample size was determined using the detect disease method by Cameron & Baldock assuming a disease prevalence of 5% and a test confidence level of 95% (Cameron *et al.,* 2020). Sample selection followed a purposive approach, focusing on cattle suspected of BVDV infection exhibiting clinical symptoms such as thin body condition, diarrhea, nasal discharge, and a history of positive antibodies to BVDV. The Veterinarian conducts clinical symptom investigation and blood sampling at BET. A total of 5 ml of blood was drawn from the jugular vein using a syringe. Subsequently, the blood was transferred into a vacutest tube and underwent centrifugation at 1500 rpm for 5 minutes, then the serum was carefully transferred to a microtube.

Antibodies against BVDV were detected in serum samples using the IDEXX BVDV Total Ab Test (IDEXX Laboratories, USA) according to the manufacturer's instructions. Serum (25 µL) and controls were added to antigen-coated wells, incubated for 90 minutes at room temperature, then washed. HRP-conjugated anti-bovine IgG was added, followed by a 30-minute incubation and 10-minute TMB substrate reaction. Absorbance was measured at 450 nm, and results were interpreted using the kit's cut-off values. Optical density (OD) was measured at 450 nm using a microplate spectrophotometer to assess color intensity from the ELISA's enzymatic reaction. The color, generated by a chromogenic substrate, is proportional to the target analyte concentration. The sensor detected transmitted light and converted it into absorbance values.

In the preparation of the SDS-PAGE, the resolving gel was formulated by combining 3.75 mL of 30% acrylamide, 3.75 mL of Tris HCl pH 8.8, 150 µL of 10% SDS, 7.28 mL of distilled water, 7.5 µL of TEMED, and 75 µL of 10% APS. This mixture was homogenized and poured into a glass plate. The stacking gel was made by blending 9 mL of distilled water, 3.78 mL of Tris HCL pH 6.8, 1.98 mL of 30% acrylamide, 150 µL of 0.5% SDS, 15 µL of TEMED, and 75 µL of 10% APS, then carefully poured into a glass plate, followed by the insertion of a well-printing comb, allowing the gel to solidify. For sample preparation, bovine serum was diluted 100 times using distilled water and mixed with Laemmli buffer in a 1:1 ratio. The resulting solution was heated at 95°C for 5 minutes. Electrophoresis was conducted by placing the glass plate into the Biometra model chamber and submerging the gel in the electrophoresis buffer solution. Subsequently, samples and markers were introduced into the gel wells, and electrophoresis was executed at 155 V for 1 hour, then the gel was carefully removed from the glass plate and transferred to a Petri dish containing Coomassie blue dye.

The molecular weight (MW) of protein was determined through a calibration graph using measured migration distances, or Retardation Factor (Rf) values. Each band's Rf value was calculated using the Rantam formula (Rantam, 2003), representing the ratio of the protein's distance traveled to that of a reference color from the same starting point. These Rf values were then utilized in a linear regression equation ($y = ax + b$), where y denotes MW and x represents the Rf value of the sample. The interpretation of the protein profile is based on the linear regression equation derived from the marker's protein bands.

Results and Discussion

The result of Cameron & Baldock method indicated a total population of 600 imported cattle in BET with a sampling size of 43 cows. Subsequent serological tests using the Enzyme-

linked immunosorbent assay (ELISA) revealed that out of the 43 cows, 21 were BVD-positive, while 22 were BVD-negative (Table 1). According to Saepulloh & Sendow (2015), the potential origin of BVDV in these cattle might be linked to vaccine strains administered before they entered Indonesia. Although physical tests and laboratory analysis were conducted on cattle displaying clinical symptoms of BVD, those infected with non-cytopathic BVDV might not exhibit any observable clinical signs. This hidden carrier state could contribute to the spread of the BVDV in farms. Consequently, regular serological tests are conducted at the BET facilities to proactively prevent the dissemination of BVDV.

Table 1. Serological data of blood samples

No	Breed	BVD-positive	BVD-negative	Total
1	FH	6	0	6
2	PO	4	17	21
3	Limousin	4	0	4
4	Simmental	4	0	4
5	Angus	2	2	4
6	Brangus	0	2	2
7	Brahman	0	1	1
8	Wagyu	1	0	1
Total		21	22	43
Percentage		49%	51%	100%

The SDS-PAGE result (Figure 1) depicted distinct protein profiles in the serum of BVD-positive and BVD-negative cows. Across all samples in lanes 1-9, a prominent protein band is consistently observed at 56.2 kDa. This particular band is identified as likely being associated with albumin, based on a study by Utomo et al. (2017), albumin constitutes 92% of the protein fraction with a band size ranging from 10.46 to 68.67 kDa and a concentration of approximately 35-50%. Notably, a protein band at 55.3 kDa is discernible in lane 1 and lane 5. A distinct band of 151.3 kDa is also exclusively present in lane 5. Further characterization of these bands may provide valuable insights into the molecular composition and potential implications related to BVD. It is noteworthy that BVD-positive is identified in specific lanes: 1, 3, 4, 5, 7, and 9, corresponding to Wagyu, Simmental, Limousin, FH, Angus, and PO breeds, respectively. These findings comprehensively understand the protein variations associated with BVD status across cattle breeds. The results of the linear regression equation in this research were expressed as $y = -1.4688x + 2.3622$ with an R^2 value of 0.9649. A high R^2 value, close to 1.0, indicates a high level of accuracy in estimating the MW of the analyzed proteins, as elucidated by Suryohastari (2016). Furthermore, the MW of protein bands for various cows is detailed in Table 2.

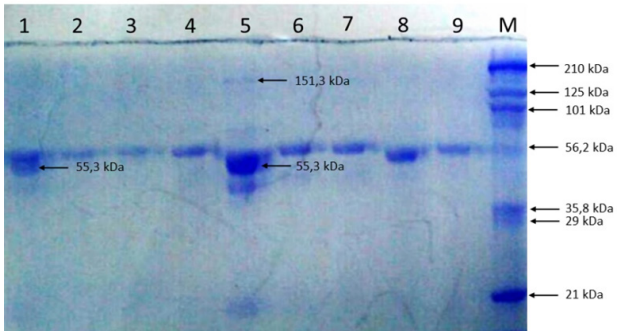

Figure 1. The SDS-PAGE results of the samples (1) Wagyu BVD-positive, (2) Brangus BVD-negative, (3) Simmental BVD-positive, (4) Limousin BVD-positive, (5) FH BVD-positive, (6) Angus BVD-negative, (7) Angus BVD-positive, (8) PO BVD-negative, (9) PO BVD-positive, and (M) Marker.

Table 2. Protein profile in serum of BVD-positive and BVD-negative cows

Lane	ID sample	Migration distance of protein bands (cm)	MW (kDa)
1	Wagyu-positive BVD	1.7	70.19
		2.04	55.35
		2.1	53.08
		4.23	11.98
2	Brangus-negative BVD	1.7	70.19
3	Simmental-positive BVD	1.7	70.19
4	Limousin-positive BVD	1.7	70.19
5	FH-positive BVD	0.6	151.39
		1.4	86.56
		2.04	55.35
		2.33	45.20
		4.18	12.41
6	Angus-negative BVD	1.7	70.19
		2.1	53.08
7	Angus-positive BVD	1.71	69.71
		2.1	53.08
8	PO-negative BVD	1.78	66.38
		2.1	53.08
9	PO-positive BVD	1.71	69.71

The protein profiles observed in FH BVD-positive exhibited the highest abundance, with a total of five discernible bands. Wagyu BVD-positive displayed four bands, while Angus BVD-positive showed two bands. Simmental BVD-positive and Limousin BVD-positive each exhibited a single band, as did PO BVD-positive. Notably, Brangus BVD-negative displayed one band, Angus BVD-negative showed two bands, and PO BVD-negative exhibited two bands. The variation in the number of protein bands observed in this study, as previously discussed by Utomo et al. (2017), that attributed to protein degradation, which factors such as pathogenic activity, enzymatic processes, and protein denaturation could contribute to these differences.

In lane 5 of the FH BVD-positive, a band of 151.3 kDa was suggested to be IgG, inference from prior research by Tomascova et al. (2019), which indicated that IgG in Bovine Serum Albumin (BSA) typically exhibits a MW of 150 kDa. Notably, BVD-negative from Brangus, Angus, and PO breeds did not display IgG bands. This finding implies that these cattle are not infected with BVDV, as elucidated by Marshall et al. (2018) that Ig is produced by the body in response to antigen exposure or infection with pathogens. In the absence of contact between BVDV and the cattle, Ig production does not occur. Furthermore, IgG bands were exclusively observed in FH BVD-positive and were absent in BVD-positive from Wagyu, Simmental, Limousin, Angus, and PO breeds. This discrepancy could be attributed to the serum half-life of IgG, which spans 27 days (Murphy *et al.,* 2014). Notably, the successful execution of SDS-PAGE to delineate the protein profile in this study was achieved after more than a month of analysis. The absence of visible IgG protein bands in BVD-positive cattle samples may also stem from differences in cattle breeds, as reported in a previous study (Otomaru *et al.,* 2015) that variations in globulin levels among different breeds of cows.

The detection of IgG profiles in BVD-positive, manifested as protein bands, indicates the Optical Density (OD) value associated with the sample. The OD value of Ig reflects the immunoglobulin levels detected in the serum of BVD-positive. According to Grant et al. (2015), BVD-positive serum exhibits a sample *value related to a positive value* (S/P) ≥ 0.30, while BVD-negative serum demonstrates an S/P value < 0.20 when analyzed using an ELISA reader. The Ig levels, as reflected by the OD value on the ELISA reader, serve as a determinant for high or low S/P values. Notably, a higher OD value in the sample corresponds to an elevated serum S/P value. The OD values observed in BVD-positive in this study are presented in Table 3.

Table 3. The OD values of BVD-positive

No	Lane	Breed	OD
1	1	Wagyu	1.140
2	3	Simmental	0.989
3	4	Limousin $^{+}$	1.681
4	5	FH	1.906
5	7	Angus	1.238
6	9	PO	0.997

In FH BVD-positive exhibiting the presence of IgG protein bands, an OD value of 1.906 was recorded. Comparatively, BVD-positive from Wagyu, Simmental, Limousin, Angus, and PO breeds yielded OD values of 1,140, 0.989, 1,681, 1,238, and 0.997, respectively, all indicating lower values than FH BVD-positive. The disparity in OD values among BVD-positive across different cattle breeds is a crucial factor influencing the visibility of the IgG protein band. Moreover, Utomo et al. (2017) elucidate that the clarity of the visible band is directly correlated with the OD of the IgG sample. Specifically, higher OD values result in a more distinct and visible band. This phenomenon is attributed to variations in serum protein levels, where thicker bands signify elevated protein concentrations, in line with the previous study (Suryohastari, 2016) the band thickness intensity serves as a visual indicator of the protein concentration in the serum. The absence of the IgG band (151.3 kDa) in certain BVD-positive may be attributed to variances in cattle breeds, as elucidated by Irfan et al. (2014) that variations in cattle breeds contribute to disparities in total protein, albumin, and globulin concentrations. Specifically, the discrepancy in globulin concentration within the

serum could potentially account for the lack of observable IgG protein bands in BVD-positive from Wagyu, Simmental, Limousin, Angus, and PO breeds. Protein profile analysis provides valuable insight into the various types of BVDV affecting cattle. Highlighted by Poetri et al. (2017) that different regions exhibit variations in virus strains. Additionally, in line with previous reports (Wan et al., 2020), the characteristics of this protein are susceptible to change over time. Hence, BVDV strains entering Indonesia may undergo alterations in protein traits, affecting their ability to infect hosts. Noted by Retno et al. (2022), the initial detection of BVD cases was in 1989 in Sulawesi, with the disease subsequently spreading to other Indonesian islands.

The SDS-PAGE analysis revealed a protein band of 55.3 kDa in the BVD-positive from Wagyu and FH (lanes 1 and 5). In a previous investigation (Purchio *et al.,* 1984), three distinctive BVD-specific protein bands were identified with MWs of 115 kDa, 80 kDa, and 55 kDa. Hence, it is reasonable to suspect that the 55.3 kDa corresponds to the BVDV antigen. This assumption is supported by Callens et al. (2016) which identified a BVDV antigen with a MW of 55 kDa as the major band. Also reported in a previous study (Wuryastuti *et al.,* 2016), the presence of BVDV antigen in the blood of infected cattle, emphasizing the virus's detectability in serum samples. The 55.3 kDa protein band is therefore presumed to be the primary protein band associated with the BVDV antigen. Furthermore, clarified by Sinlae et al. (2015) that a major band is characterized by a higher concentration compared to other bands, making it more prominently visible in SDS-PAGE results. This reinforces the significance of the 55.3 kDa as the major band representing the BVDV antigen. The presence of 55.3 kDa in BVD-positive might be attributed to the viral load in infected cows, correlating with elevated Ig concentrations. In this investigation, the Ig concentration in FH BVD-positive exhibited an OD value of 1.906, surpassing the OD value observed in BVDV-positive from other breeds. Wagyu BVD-positive exhibited a 55.3 kDa, accompanied by a lower IgG OD value of 1.140. Drawing from a prior investigation (Ahmann *et al.,* 2021) established that variations in cattle breeds contribute to differences in Ig concentrations. As reported by Otomaru et al. (2015), a globulin protein concentration of 3.89 g/dL in Wagyu, while Irfan *et al.,* (2014) noted a globulin concentration of 5.70 g/dL in Limousin. Additionally, Ndlovu et al. (2009) found that Angus had a globulin concentration of 4.32 g/dL. These breed-specific variations may influence the IgG concentration in Wagyu BVD-positive, resulting in a lower OD value compared to Limousin and Angus BVD-positive. This breed-dependent disparity could be a contributing factor to the observed phenomenon, wherein despite a significant presence of 55.3 kDa in Wagyu BVD-infected, the OD value of IgG does not surpass that of Limousin and Angus. And not all BVD-positive cattle (Table 3) show a 55.3 kDa protein band that may reflect resolved infection, low viral protein levels, strain variation, or breed-specific host responses.

Furthermore, the presence of 55 kDa might be associated with findings from previous research (Wang *et al.,* 2015), wherein it was identified as the E2 glycoprotein—an essential structural protein. This protein holds paramount importance due to its capacity to engage with cell surface receptors, thereby influencing cell tropism. Moreover, it plays a pivotal role in inducing neutralizing antibodies and eliciting cytotoxic T lymphocyte responses. Further Loy et al. (2013) supported this notion by demonstrating that specific monoclonal antibodies targeting the E2 glycoprotein can neutralize both cytopathic and non-cytopathic BVDVs. Consequently, these antibodies serve as valuable references for the development of BVD sub-unit vaccines. The SDS-PAGE result of 55 kDa provides critical insight, signaling potential exposure of cattle in the area to the BVDV strain containing the E2 glycoprotein, in line with Al-Kubati et al. (2021) that the BVDV-E2 protein plays an important role in viral infection and pathogenesis. This finding can serve as a foundational reference for the development of E2 sub-unit vaccines.

Conclusions

Protein bands corresponding to the IgG antibody 151.3 kDa and the BVDV antigen 55.3

kDa with OD values ≥ 0.30 were detected in unvaccinated BVD-positive (FH and Wagyu) but not in BVDV-negative cattle. This indicates a natural immune response to BVDV infection and may serve as a foundational reference for BVDV vaccine development.

Acknowledgments

The authors extend gratitude to the Head of the Integrated Research Laboratory, State Islamic University Syarif Hidayatullah Jakarta for generously granting permission to utilize the research facilities.

References

Ahmann, J., Steinhoff-Wagner, J., and Büscher, W. (2021). Determining Immunoglobulin Content of Bovine Colostrum and Factors Affecting the Outcome: A review. *Animals.* 11 (12): 3587.

Al-Kubati, A. A. G., Hussen, J., Kandeel, M., Al-Mubarak, A. I. A., and Hemida, M. G. (2021). Recent Advances on the Bovine Viral Diarrhea Virus Molecular Pathogenesis, Immune Response, and Vaccines Development. *Frontiers in Veterinary Science*. 8: 665128.

Ammari MG, P. L. (2015). All is Not Butter that Comes from the Cow: The Bovine Viral Diarrhea. Understanding the Pathogenesis of Cytopathic and Non-Cytopathic Infection. *Journal of Ancient Diseases & Preventive Remedies.* 03 (02): 1-6.

Callens, N., Brügger, B., Bonnafous, P., Drobecq, H., Gerl, M. J., Krey, T., Roman-Sosa, G., Rümenapf, T., Lambert, O., Dubuisson, J., and Rouillé, Y. (2016). Morphology and Molecular Composition of Purified Bovine Viral Diarrhea Virus Envelope. *PLoS Pathogens.* 12 (3): 1005476.

Cameron, A. R., Meyer, A., Faverjon, C., and Mackenzie, C. (2020). Quantification of The Sensitivity of Early Detection Surveillance. *Transboundary and Emerging Diseases.* 67 (6): 2532-2543.

Gethmann, J., Homeier, T., Holsteg, M., Schirrmeier, H., Saßerath, M., Hoffmann, B., Beer, M., and Conraths, F. J. (2015). BVD-2 Outbreak Leads to High Losses in Cattle Farms in Western Germany. *Heliyon.* 1 (1): e00019

Grant, D. M., Dagleish, M. P., Bachofen, C., Boag, B., Deane, D., Percival, A., Zadoks, R. N., and Russell, G. C. (2015). Assessment of the Rabbit as a Wildlife Reservoir of Bovine Viral Diarrhea Virus: Serological Analysis and Generation of Trans-Placentally Infected Offspring. *Frontiers in Microbiology.* 6 (9): 1000.

Irfan, I. Z., Esfandiari, A., and Choliq, C. (2014). Profil Protein Total, Albumin, Globulin dan Rasio Albumin Globulin Sapi Pejantan. *Jurnal Ilmu Ternak dan Veteriner.* 19 (2).

Khodakaram-Tafti, A., and Farjanikish, G. H. (2017). Persistent Bovine Viral Diarrhea Virus (BVDV) Infection in Cattle Herds. *Iranian Journal of Veterinary Research.* 18 (3): 154-163.

Lanyon, S. R., Hill, F. I., Reichel, M. P., and Brownlie, J. (2014). Bovine Viral Diarrhoea: Pathogenesis and Diagnosis. *Veterinary Journal*. 199 (2): 201-209.

Loy, J. D., Gander, J., Mogler, M., Veen, R. Vander, Ridpath, J., Harris, D. H., and Kamrud, K. (2013). Development and Evaluation of a Replicon Particle Vaccine Expressing the E2 Glycoprotein of Bovine Viral Diarrhea Virus (BVDV) in Cattle. *Virology Journal*. 10 (1): 1-5.

Marshall, J. S., Warrington, R., Watson, W., and Kim, H. L. (2018). An Introduction to Immunology and Immunopathology. *Allergy, Asthma and Clinical Immunology*. 14 (49): 6-14.

Murphy, J. M., Hagey, J. V., and Chigerwe, M. (2014). Comparison of Serum Immunoglobulin G Half-Life in Dairy Calves Fed Colostrum, Colostrum Replacer or Administered with Intravenous Bovine Plasma. *Veterinary Immunology and Immunopathology.* 158 (3–4): 233-237.

Ndlovu, T., Chimonyo, M., Okoh, A. I., Muchenje, V., Dzama, K., Dube, S., and Raats, J. G. (2009). A Comparison of

Nutritionally-Related Blood Metabolites among Nguni, Bonsmara and Angus Steers Raised on Sweetveld. *Veterinary Journal.* 179 (2): 273-281.

Nugroho, W., Silitonga, R. J. P., Reichel, M. P., Irianingsih, S. H., and Wicaksono, M. S. (2022). The Epidemiology and Control of Bovine Viral Diarrhoea Virus in Tropical Indonesian Cattle. *Pathogens.* 11 (2): 215.

Otomaru, K., Shiga, H., Kanome, J., and Yanagita, K. (2015). Blood Biochemical Values in Japanese Black Breeding Cows in Kagoshima Prefecture, Japan. *Journal of Veterinary Medical Science.* 77 (8): 1021-1023.

Poetri, O. N., Van Boven, M., Koch, G., Stegeman, A., Claassen, I., Wayan Wisaksana, I., and Bouma, A. (2017). Different Cross Protection Scopes of Two Avian Influenza H5N1 Vaccines Against Infection of Layer Chickens with a Heterologous Highly Pathogenic Virus. *Research in Veterinary Science* 114: 143-152.

Polak, M. P., Antos, A., Rola, J., and Zmudziński, J. F. (2016). Viral Shedders in A Herd Vaccinated Against Infection With Bovine Viral Diarrhoea Virus (BVDV) without Prior Testing for the Presence of Persistently Infected Animals. *Journal of Veterinary Research (Poland).* 60 (4): 379-384.

Primawidyawan, A., Setiyaningsih, S., Wulansari, R., Subangkit, M., and Priosoeryanto, B. P. (2023). Detection and Characterization of Bovine Viral Diarrhea Virus in Beef Cattle Imported From Australia to West Java, Indonesia. *Veterinary World.* 16 (7): 1468-1476.

Purchio, A. F., Larson, R., and Collett, M. S. (1984). Characterization of Bovine Viral Diarrhea Virus Proteins. *Journal of Virology*. 50 (2): 666-669.

Rantam, F. A. (2003). Metode Imunologi. In *Airlangga University Press*.

Retno, N., Wuryastuty, H., Wasito, R., and Irianingsih, S. H. (2022). First Study on Genetic Variability of Bovine Viral Diarrhea Virus Isolated from Sapera Dairy Goats with Reproductive Disorders in Yogyakarta, Indonesia. *Veterinary World.* 15 (4): 1015-1021.

Richter, V., Lebl, K., Baumgartner, W., Obritzhauser, W., Käsbohrer, A., and Pinior, B. (2017). A Systematic Worldwide Review of the Direct Monetary Losses in Cattle Due to Bovine Viral Diarrhoea Virus Infection. *Veterinary Journal.* 220: 80-87.

Saadh, M. J., Tanash, S. A., Almaaytah, A. M., Sa'Adeh, I. J., Aldalaen, S. M., and Al-Hamaideh, K. D. (2021). Immunodiagnosis of Cattle Fascioliasis Using a 27 kDa Fasciola gigantica Antigen. *Veterinary World.* 14 (8): 2097-2101.

Saepulloh, M. and Sendow, I. (2015). Identification and Characterization of Bovine Viral Diarrhea Virus from Indonesian Cattle. *Jurnal Veteriner.* 16 (1): 1-7.

Sinlae, R. N., Suwiti, N. K., and Suardana, I. W. (2015). Karakteristik Protein dan Asam Amino Daging Sapi Bali dan Wagyu pada Penyimpanan Suhu Dingin 4 ° C. *Buletin Veteriner Udayana*, *7*(2).

Subekti, D. T., Fatmawati, M., Khoiriyah, A., Pramesthi, A., Fong, S., Desem, M. I., Azmi, Z., Kusumaningtyas, E., Endrawati, D., and Purwanto, E. S. (2021). Seroprevalence of Seven Reproductive Diseases in Beef and Dairy Cows from Three Provinces in Indonesia. *Veterinary Medicine International.* 1-9.

Suryohastari, R. B. (2016). Analisis Protein Defensin dari Biji Jinten Hitam (Nigella sativa L.) pada Mencit (Mus musculus) yang Diberi Biji Jinten Hitam melalui Teknik SDS-PAGE. *Al-Kauniyah: Jurnal Biologi.* 9 (1): 26-36.

Tomascova, A., Lehotsky, J., Kalenska, D., Baranovicova, E., Kaplan, P., and Tatarkova, Z. (2019). A Comparison of Albumin Removal Procedures for

Proteomic Analysis of Blood Plasma. *General Physiology and Biophysics*. 38 (4): 305-314.

Utomo, W. T., Suarsana, I. N., and Suartini, I. G. A. A. (2017). Karakteristik Protein Plasma Sapi Bali. *Jurnal Veteriner.* 18 (2).

Vidarsson, G., Dekkers, G., and Rispens, T. (2014). IgG Subclasses and Allotypes: from Structure to Effector Functions. *Frontiers in Immunology.* 5 (10): 1-17.

Wan, Q., Song, D., Li, H., and He, M. (2020). Stress Proteins: The Biological Functions in Virus Infection, Present and Challenges for Target-Based Antiviral Drug Development. *Signal Transduction and Targeted Therapy*. 5 (1).

Wang, F. I., Deng, M. C., Huang, Y. L., and Chang, C. Y. (2015). Structures and Functions of Pestivirus Glycoproteins: Not Simply Surface Matters. *Viruses.* 7 (7). 3506-3529.

Wuryastuti, H., Wasito, R., and Putro, P. P. (2016). Detection of Bovine Viral Diarrhea Virus From Blood Samples Collected Using Flinders Technology AssociatesTM Cards. *Jurnal Veteriner*. 17 (2): 176-182.