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Detection of Ovine Herpesvirus 2 (OvHV-2) in Sheep in Riau Province using Conventional Nested PCR

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ABSTRACT

Malignant catarrhal fever is caused by Ovine Herpesvirus type 2. It is a disease that is common in sheep but can be fatal to large ruminants such as buffalo and cattle. Data from 2022 shows that the number of sheep in Riau Province has increased. This has made people more concerned about the increasing number of MCF cases in the province. The goal of this study is to determine the prevalence of Ovine Herpesvirus type 2 (OvHV-2) in sheep in Riau Province. For this study, 159 sheep swab samples were taken from five different districts in the province of Riau. The nested PCR method was used to identify OvHV-2. We used a structured questionnaire to find out what the risk factors were, and then we used SPSS's chi-square tests to analyze the data. The study found that 44.7% of the sheep tested positive for OvHV-2. Bivariate and multivariate analyses showed that female sheep had higher rates of OvHV-2 detection (adjusted odds ratio [aOR]=2.281; P=0.024). In the meantime, fat-tailed sheep had lower rates of OvHV-2 detection than other breeds (adjusted odds ratio [aOR]=0.199; P=0.014). The overall prevalence of Ovine Herpesvirus 2 (OvHV-2), which causes malignant catarrhal fever (MCF) in sheep in five districts in Riau Province, Indonesia, is quite high. Fat-tailed sheep had lower rates of OvHV-2 detection than other breeds, and female sheep were more likely to test positive for the virus. This is the first comprehensive prevalence study of OvHV-2 in sheep in Riau Province.

Keywords: Malignant catarrhal fever, Nested PCR, Ovine Herpesvirus 2, Risk factor, Sheep.

INTRODUCTION

The global demand for small ruminant production has risen steadily over the past decade, driven by population growth, increased meat consumption, and the expansion of livestock-based economies worldwide. This increase has been accompanied by a growing recognition of the epidemiological importance of sheep as natural reservoirs of ovine herpesvirus 2 (OvHV-2), the causative agent of sheep-associated malignant catarrhal fever (SA-MCF), a frequently fatal disease in susceptible ruminants (Russell et al. 2012; Headley et al. 2020; Li and Cunha 2020). In many countries, including those in Asia, expanded sheep populations and intensified animal movement have been associated with higher risks of viral transmission to susceptible hosts such as cattle and buffalo (García-Pérez et al. 2018; Mananguit et al. 2021; Shyamsundar et al. 2023).

Globally, OvHV-2 continues to be reported in diverse production systems, with recent studies indicating molecular detection rates of 22–78.5% in sheep herds, influenced by husbandry systems and diagnostic methods employed (Li et al. 2016; García-Pérez et al. 2018; Mananguit et al. 2021; Hajikolaei et al. 2025). Most epidemiological studies between 2020 and 2025 have relied on nested PCR and real-time PCR, which remain the most sensitive tools for identifying both clinical and subclinical infections (Cunha et al. 2015; Li et al. 2017; Yang et al. 2018; Partin et al. 2021; Coradduzza et al. 2022; Headley et al. 2025). Despite this progress, OvHV-2 surveillance remains uneven across regions, and updated prevalence data are still lacking in many lower- and middle-income countries (Headley et al. 2020; Li and Cunha 2020).

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In Indonesia, SA-MCF continues to pose a major threat to livestock health, particularly for Bali cattle (*Bos javanicus*), which have been consistently reported as highly susceptible to OvHV-2 infection. Studies in East Nusa Tenggara in 2021 have detected OvHV-2 in apparently healthy sheep with a prevalence rate of approximately 54.55%, indicating silent circulation of the virus within small ruminant populations (Wiyono et al. 2021). Earlier studies proposed a tentative sensitivity hierarchy among cattle breeds (Daniels et al. 1988; Wiyono and Damayanti 1999), however, more recent reviews suggest that susceptibility differences are more pronounced at the species level rather than among cattle breeds, with buffalo, deer, and wild ruminants being highly susceptible, while *Bos taurus* and *Bos indicus* are relatively more resistant (Russell et al. 2012; Headley et al. 2020; Kumar et al. 2021; WOAH 2021).

Despite advances in molecular diagnostics and epidemiological understanding, region-specific and up-to-date data on OvHV-2 prevalence in Indonesia remain limited. In addition, few studies have evaluated the diagnostic performance of different anatomical sample types for field-level detection, even though viral distribution has been shown to vary among tissues and body secretions (Taus et al. 2014; Li et al. 2017; Yang et al. 2018). Riau Province has experienced substantial growth in small ruminant populations in recent years. Provincial data indicate that the sheep population increased from 27,640 to 31,084 in 2021, with continuing growth in subsequent years, while the goat population increased from to 235,858 in 2020 to 252,432 in 2021(Indonesian Ministry of Agriculture 2022). The widespread use of free-range and mixed-grazing husbandry systems facilitates close contact between sheep and cattle, thereby increasing opportunities for OvHV-2 transmission (Li et al. 2016; García-Pérez et al. 2018). In 2021, preliminary surveillance in Riau reported that 54% of sheep blood samples tested positive for OvHV-2 using nested PCR, indicating active viral circulation in the region (Tikaso and Yuhendra 2024).

The OvHV-2 virus primer nucleic acid sequence is presented in Table 1. Despite these indications, no study in Riau has yet compared viral detection across multiple sample types, such as ocular, nasal and vaginal swabs, which have the potential to improve field diagnosis and surveillance capacity. This represents a clear knowledge gap, especially considering the high susceptibility of Bali cattle and the economic impact of MCF outbreaks (Headley et al. 2020; Li and Cunha 2020).

Therefore, this study aims to determine the prevalence of OvHV-2 in sheep in Riau Province using molecular detection from ocular, nasal and vaginal swabs, and to assess the diagnostic value of different anatomical sample types. The findings are expected to provide updated epidemiological evidence to support local veterinary authorities in strengthening surveillance strategies and improving the prevention and control of malignant catarrhal fever.

MATERIALS AND METHODS

Research methodology

This study utilised a cross-sectional approach across five districts in Riau Province (Fig. 1), Indonesia the districts comprised Pelalawan, Siak, Kampar, Rokan Hilir, and Bengkalis. The data collected was the result of sampling sheep that were kept near cattle. The total sample consisted of 159 swabs from the mucous membranes of the eyes, nose, or vagina of sheep. The gathered samples were then examined by using the nested PCR method.

The collected samples were then analyzed using the nested PCR method in accordance with WOAH recommendations. The samples were initially extracted using a Qiagen DNA kit. Subsequently, nested PCR testing was used. This method was employed to detect the OvHV-2 virus. This method adheres to the standards specified by the World Organisation for Animal Health (WOAH 2018) and employs the testing protocols developed by Baxter et al. (1993), which are shown in the Table below.

The DNA extraction procedure began with the collection of sheep samples using swabs from their eyes, nostrils and vagina. The samples were then extracted using the Qiagen QiaAmp DNA Mini Kit, following the provided instructions. The Qiagen HotStart Taq Plus Master Mix Kit was used as the master mix in this test.

The next phase involved using the DNA sample MF629 from the SA-MCF case as a positive control and nuclease-free water (NFW) as a negative control (Baxter et al. 1993). Amplification occurred in two stages. Stage 1 used primers 556 and 755, and stage 2 used primers 556 and 555 (Baxter et al. 1993; WOAH 2018). Initially, the amplification was performed with modifications following the kit's protocol, as outlined below. The initial thermal activation occurred at 95°C for 5 minutes. The protocol consisted of 35 cycles: denaturation at 94°C for 30 seconds, annealing at 64.4°C for 30 seconds, and extension at 72°C for one minute. After 35 cycles, the reaction concluded with a final extension at 72°C for 10 minutes. Amplification occurred at 95°C for five minutes in the second stage (the first heat activation). The second stage consists of 35 cycles: 30 seconds at 94°C (denaturation), 30 seconds at 64.4°C (annealing), and 1 minute at 72°C (extension). After 35 cycles, the treatment concludes with a final extension at 72°C for 10 minutes, followed by holding at 12°C until electrophoresis is performed. The amplification results are presented on a 1.5% agarose gel and recorded with a UV transilluminator.

Table 1: Nucleic Acid sequence of OvHV-2 viral primers

Primer Name	Primer Length	Primer Sequence
556	30 mer	5’-AGT CTG GGT ATA TGA ATC CAG ATG GCT CTC-3’
555	28 mer	5’-TTC TGG GGT AGT GGC GAG CGA AGG CTTC-3’
755	30 mer	5’-AAG ATA AGC ACC AGT TAT GCA TCT GAT AAA-3’

Data examination

Data obtained from laboratory analyses were entered into a database using Microsoft Excel 2019. The cattle data were analysed descriptively and underwent chi-square analysis using SPSS software.

RESULTS

The examination of sheep mucosal swab samples yielded varied results. In Kampar Regency, 15 out of 43 samples tested positive, meaning 34.9% of the samples were positive. In Pelalawan Regency, 15 out of 26 (57.7%) samples were positive. In Siak District, 19 out of 55 samples (42.2%) were positive. In Rokan Hilir District, 15 out of 35 (42.9%) samples were positive, and in Bengkalis District, 7 out of 10 (70%) samples proved to be positive.

The study used univariate analysis at the level of individual sheep to describe the results of the OvHV-2 virus detection test in sheep, including their gender, breed, and age. The results of the lab tests on 159 sheep samples can be shown as follows.

Table 2 shows that 44.7% of the sheep tested positive for the OvHV-2 virus, and the remaining 55.3% tested negative. The prevalence observed was higher than previously reported. This study used 62.9% of ewes and 37.1% of rams, consisting of thin-tailed sheep breeds (67.9%), 11.9% fat-tailed breeds, and other breeds 20.1%. The study used three age variables. The majority of the sheep were 2 years old (47.8%), followed by those aged 1 year or less (42.1%), and only 10.1% were 2 years or older. This composition shows that most of the sheep were of productive age. Bivariate analysis was performed, and the results are presented in Table 3.

Table 2: The univariate analysis

No	Variable	Category	Frequency (n)	Percentage (%)
1	Test Result	Positive	71	44.7
		Negative	88	55.3
2	Sex	Female	100	62.9
		Male	59	37.1
3	Breed	Fat-tailed	19	11.9
		Thin-tailed	108	67.9
		Others	32	20.1
4	Age	≤1 year	67	42.1
		2 years	76	47.8
		>2 years	16	10.1

Table 3: Relationship between variables and OvHV-2 virus in sheep

No	Variable	Description	Positive	Negative	Chi-Square (P)	Odds Ratio (OR)
1	Gender	Female	52 (52)	48 (48)	0.024	2.281
		Male	19 (32.2)	40 (67.8)		
2	Breed					
	Fat-tailed	Yes	3 (15.8)	16 (84.2)	0.014	0.199
		No	68 (48.6)	72 (51.4)		
	Thin-tailed	Yes	54 (50)	54 (50)	0.071	-
		No	17 (33.4)	34 (66.7)		
	Other	Yes	14 (43.7)	18 (56.3)	1.000	-
		No	57 (44.9)	70 (55.1)		
3	Age					
	≤1 year	Yes	30 (44.8)	37 (55.2)	1.000	-
		No	41 (44.6)	51 (55.4)		
	2 years	Yes	23 (42.1)	44 (57.9)	0.646	-
		No	39 (47.0)	44 (53.0)		
	>2 years	Yes	9 (56.2)	7 (43.8)	0.472	-
		No	62 (43.4)	81 (56.6)		

Values in parentheses are %.

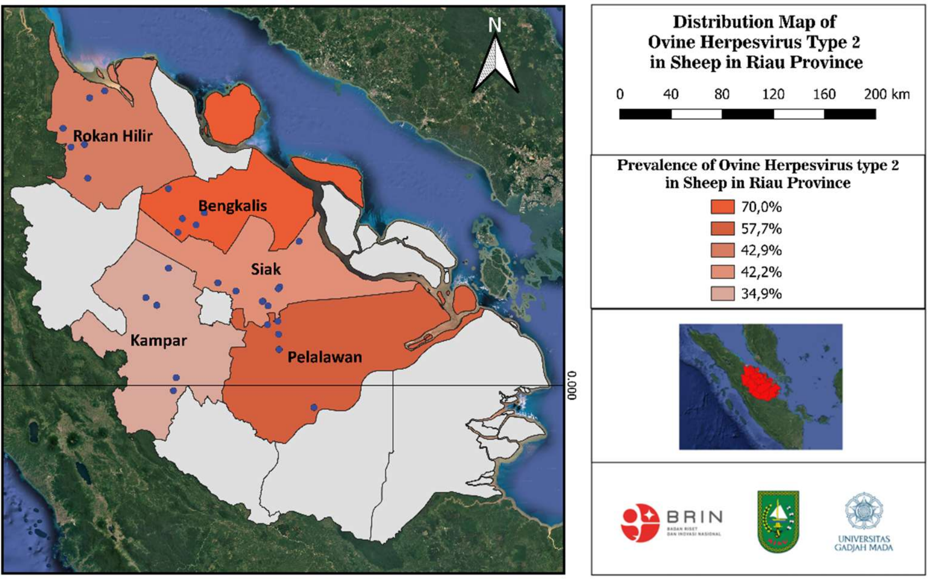

Fig. 1: Distribution map of OHV2 in sheep in Riau Province.

The research shows that female sheep are more likely to be positive for the OvHV-2 virus (52%) than male sheep (32.2%). Male sheep, on the other hand, had more negative results for the OvHV-2 virus (67.8%) than female sheep (48%). The chi-squared test's P<0.05 of 0.024 shows that there is a statistically significant link between the sex of the sheep and the presence of the OvHV-2 virus (P<0.05). The results show that female sheep are more likely than male sheep to get OvHV-2. The odds ratio (OR) of 2.281 shows that female sheep are linked to OvHV-2.

There are three types of sheep breeds based on their phenotypic characteristic: fat-tailed, thin-tailed, and others. The study found that fat-tailed sheep had a 15.8% positivity rate for the OvHV-2 virus, thin-tailed sheep had a 50% positivity rate, and sheep of breeds other than thin-tailed and fat-tailed had the highest positivity rate at 43.7% for the OvHV-2 virus. The chi-square test gave a P value of 0.014 for the fat-tailed sheep variable, which means that there is a strong link between fat-tailed sheep and the presence of the OvHV-2 virus (P<0.05). The results indicate that fat-tailed sheep exhibit a lower likelihood of OvHV-2 detection compared with other breeds; however, this association was not statistically significant. Fat-tailed sheep have a negative link with the occurrence of MCF, with an odds ratio of 0.199.

The study found that 44.8% of sheep less than 1 year old had the OvHV-2 virus, 42.1% of sheep between 1 and 2 years old had it, and 56.2% of sheep older than 2 years old had it. Although there were no significant results on the three age variables, sheep older than 2 years were more likely to be more susceptible to having the OvHV-2 virus.

DISCUSSION

Malignant catarrhal fever (MCF) caused by Ovine herpesvirus 2 (OvHV-2) remains an important infectious disease affecting susceptible ruminants worldwide. The virus is maintained in sheep as the natural reservoir, where infection is typically subclinical, and transmission occurs mainly through nasal secretions (Pesavento et al. 2019; Riaz et al. 2021). In cattle, buffalo, and other highly susceptible species, OvHV-2 infection results in severe lymphoproliferative lesions, vasculitis, and high case fatality rates (Li et al. 2014; WOAH 2021). Recent epidemiological reports continue to emphasize the widespread distribution of OvHV-2 in small ruminant populations and the increasing importance of molecular detection for early surveillance (Headley et al. 2020; Kumar et al. 2021).

In the present study, the overall prevalence of OvHV-2 in sheep from five districts of Riau Province was 44.7%, indicating active viral circulation in local small ruminant populations. This prevalence is comparable to recent findings from Indonesia and other Asian countries, where molecular detection rates ranged between 22-78.5% in apparently healthy sheep (Mananguit et al. 2021; Hajikolaei et al. 2025). The high prevalence recorded in some districts, such as Bengkalis (70%), may reflect differences in flock density, management practices, and frequency of mixed grazing with cattle, which are known risk factors for SA-MCF transmission.

Sex-based differences in OvHV-2 positivity

Female sheep in this study were significantly more likely to test positive (52%) than male sheep (32.2%), and multivariate analysis confirmed sex as an independent risk factor (aOR=2.281; P=0.024). This finding aligns with previous reports showing increased viral shedding in ewes during late pregnancy, lambing, and postpartum periods due to physiological stress and hormonal changes (Lankester et al. 2015; Wiyono and Damayanti 2018). However, some earlier studies suggested that pregnant ewes may contribute minimally to viral transmission (Li et al. 2004), indicating that the relationship between reproductive status and OvHV-2 shedding may vary depending on management conditions, breed characteristics, or sampling time. The present results support the hypothesis that reproductive stress increases susceptibility to infection or enhances viral shedding, thereby elevating detection rates in female sheep.

Breed differences and possible immunological explanations

Fat-tailed sheep had substantially lower OvHV-2 positivity (15.8%) compared with thin-tailed sheep (50%) and other breeds (43.7%). Although breed was statistically significant in the chi-square test (P=0.014), the logistic regression results suggest that fat-tailed sheep may have a reduced risk of infection (aOR=0.199). Similar observations have been reported in recent research showing that fat-tailed breeds possess more stable lipid metabolism, enhanced immunological homeostasis, and improved adaptation to environmental stressors (Xu et al. 2023). These traits may contribute to lower susceptibility or reduced viral replication. However, because this study did not measure immune parameters directly, further investigation is needed to determine whether breed-related biological differences or environmental factors account for this variation.

Age as a risk factor

Although age was not significantly associated with OvHV-2 detection, sheep older than two years showed a higher positivity rate (56.2%). Earlier studies indicate that young lambs usually become infected between 4–9 months of age and may shed large amounts of virus during this period (Li et al. 1999; Kim et al. 2003). The higher positivity observed in adult sheep in our study suggests either cumulative lifetime exposure or persistent latent infection detectable by PCR. This is consistent with studies showing that adult sheep remain lifelong carriers and intermittently shed the virus depending on physiological status and environmental stress (Pesavento et al. 2019).

Influence of management and environmental factors

Differences among districts, including flock density, husbandry practices, level of free-range grazing, and proximity to cattle farms, have been reported to influence OvHV-2 transmission dynamics. Extensive mixed grazing systems increase interspecies contact and have been associated with a higher risk of malignant catarrhal fever outbreaks in susceptible cattle (Li et al. 2016; Headley et al. 2020). Environmental stressors such as heat, nutritional deficiencies, and parasitic burden are known to impair immune competence, potentially increasing viral susceptibility and detection rates (Russell et al. 2012; O'Toole et al. 2014). Although these management and environmental factors were not quantitatively assessed in the present study, their potential confounding effects are acknowledged and should be incorporated into future epidemiological investigations.

Diagnostic significance of nested PCR and its limitations

Nested PCR remains a highly sensitive method for detecting OvHV-2 DNA in subclinical carriers and is widely used for epidemiological surveillance (Kumar et al. 2021; WOAH 2021). In this study, nested PCR successfully detected viral DNA across three sample types (ocular, nasal, and vaginal swabs), demonstrating its utility in field conditions. However, nested PCR has several limitations, including an increased risk of cross-contamination and false-positive results due to carry-over amplicons, as well as limited suitability for large-scale surveillance compared to real-time PCR assays (Kwok and Higuchi 1989; Mackay et al. 2002; Yang et al. 2018). In addition, nested PCR does not provide quantitative data, restricting its ability to assess viral load dynamics and shedding intensity. Moreover, this method cannot reliably distinguish between latent infection and active viral shedding, and its detection sensitivity may vary depending on the sampling site, which should be further evaluated in future studies.

Despite these limitations, nested PCR provided robust evidence of OvHV-2 circulation and remains one of the most practical diagnostic tools for field epidemiology in resource-limited settings.

Overall, the findings of this study demonstrate a high prevalence of OvHV-2 among sheep in Riau Province, with significant associations observed for sex and breed. Female and thin-tailed sheep represented higher-risk groups, suggesting that targeted surveillance or management interventions may be warranted. The study contributes new epidemiological data for Indonesia and highlights the ongoing need for improved molecular surveillance, breed-specific investigations, and a better understanding of environmental and management determinants of OvHV-2 infection. Strengthening biosecurity practices in mixed grazing systems will be important for reducing the risk of SA-MCF transmission to susceptible cattle, particularly Bali cattle.

Conclusion

This study confirmed the presence of Ovine Herpesvirus 2 (OvHV-2), the causative agent of malignant catarrhal fever (MCF), in sheep across five districts in Riau Province, Indonesia. The overall prevalence was 44.7%. The most significant risk factor identified was ewes, which had higher OvHV-2 detection test results (adjusted odds ratio [aOR]=2.281; P=0.024). Conversely, fat-tailed sheep had lower OvHV-2 detection test results compared to other breeds (adjusted odds ratio [aOR]=0.199; P=0.014). These findings highlight the urgent need for targeted biosecurity interventions in mixed farming systems in Riau Province.

Recommendations

Due to the prevalence of Ovine Herpesvirus-2 (OvHV-2), which poses a significant threat to large ruminants such as cattle and buffalo in Riau Province, this study recommends distinct management techniques for cattle and sheep. Local veterinarians must educate farmers on biosecurity measures to protect their livestock. This will reduce the frequency of MCF cases. Further studies are required to examine the economic impact of MCF epidemics.

DECLARATIONS

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Conflict of Interest: The author(s) declare that they have no competing interests.

Data Availability: All data supporting the findings of this study are available within the manuscript.

Ethical Approval: This study, conducted in 2024, received ethical approval from the National Research and Innovation Agency under approval number 048/KE.02/SK/03/2024. The visitation schedule and participant availability were arranged from August to December of that year.

Author's Contribution: Author Contributions: Annisa Yohanes was responsible for the investigation, data curation, formal analysis, visualization, methodology, and writing the original draft. Dyah Ayu Widiasih, Agus Wiyono, Rini Damayanti, and Harimurti Nuradji contributed to the study's conceptualization. Agus Wiyono and Muharam Saepulloh developed the methodology. Agus Wiyono also handled validation and project administration, which was additionally managed by Rini Damayanti, who also provided supervision. Funding was acquired by Harimurti Nuradji and Muharam Saepulloh. Faralinda Sari and Dewi Anggreini provided resources, conducted investigation, and managed data curation. Dyah Ayu Widiasih, Agus Wiyono, and Rini Damayanti were involved in writing – review & editing. All authors have read and agreed to the final manuscript.

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