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Seroprevalence of some infectious abortion-related diseases in friesian holstein cattle on commercial dairy farm in Subang Indonesia

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Abstract

Abortion is the expulsion of a non-viable fetus between 42 and 260 days of gestation. Abortion poses a significant economic threat to the dairy industry due to losses of potential offspring, reduced milk production, and increased veterinary costs. This study investigated the seroprevalence of some infectious abortion-related diseases on a commercial dairy farm in Subang by analyzing 76 Friesian Holstein cattle serum samples. The data used antibody-ELISA to detect *Neospora caninum*, *Leptospira spp.*, *Chlamydophila abortus*, *Coxiella burnetii*, and *Mycoplasma bovis*, antigen-ELISA for BVDV, and the Rose Bengal Test for *Brucella abortus*. The findings revealed a high seroprevalence of *Mycoplasma bovis* (63.1%), indicating a substantial burden of this infection within the herd. Lower seroprevalences were observed for *Neospora caninum* (6.6%) and *Leptospira spp.* (5.2%), while *Chlamydophila abortus*, *Coxiella burnetii*, and BVDV were not detected. These results highlight the importance of *M. bovis* as a potential major contributor to abortion on this farm. The high prevalence of *M. bovis* infection suggests immediate intervention strategies, such as improved biosecurity measures and targeted vaccination programs, to mitigate further economic losses and improve overall herd health. Further research is warranted to investigate the specific impact of *M. bovis* on abortion rates and reproductive performance in this herd.

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Introduction

The reproductive health of dairy and beef cattle is essential for ensuring profitability in these industries. Abortion, which refers to the premature expulsion of a non-viable fetus, creates a significant economic burden due to the loss of offspring, decreased milk production, and increased veterinary costs (1,2). Poor reproductive performance can result from inaccuracies in heat detection, fertility disorders, and pregnancy losses (3). Pregnancy loss in dairy cows can have a significant impact on their lactation and reproductive performance, potentially disrupting the regular lactation schedule and leading to issues such as retained fetal membranes and the development of endometritis (4,5). Prolonged postpartum intervals may also occur. Additionally, abortion in cows leads to increased replacement rates of heifers and cows, resulting in culling, negatively affecting herd genetics, and reducing farm revenue. Studies worldwide show that the abortion rate in dairy farms may vary between 8% and 14% depending on the moment of gestation in which the pregnancy was diagnosed, dairy herd management, and the cow biotypes (6,7). The prevalence of abortion in cattle in various regions in West Java ranges from 2-5%. Research conducted recently showed that the prevalence of brucellosis, which mainly causes abortus in dairy cows in West Bandung Regency, is 5.1% (8). The etiology of abortion is varied, and several risk factors have been reported, including genetic, environmental, management, geographical, and infectious factors, in which infectious causes contribute up to 90% of

abortion cases (9,10). Infectious agents responsible for abortions in cattle encompass a variety of pathogens, including viruses, bacteria, protozoa, and fungi, each posing unique challenges to reproductive health. Among viral agents, Bovine Viral Diarrhoea Virus (BVDV), Infectious Bovine Rhinotracheitis Virus (IBRV), and Rift Valley Fever Virus (RVFV) are notable for their impact on cattle fertility. BVDV is associated with a range of reproductive issues, from infertility to fetal mummification and late-term abortions, primarily through vertical transmission, which leads to persistent fetal infections and elevated fetal mortality rates (11). The seroprevalences of BVDV infections were relatively high (79,1%) in the tested dairy cattle in Kenya (12). In addition to viral threats, bacterial pathogens such as *Brucella abortus, Campylobacter fetus, Chlamydophila abortus, Salmonella spp., Leptospira spp., M. bovis,* and *E. coli* are significant contributors to bovine abortions. *Brucella abortus* localizes in the placenta and fetal tissues, leading to inflammation, fetal death, and typically late-gestation abortions (13). Research conducted in Iraq showed that 70% of abortions occurred between 6 and 7 months of gestation (14). Infections with *Campylobacter fetus* disrupt the reproductive tract, causing endometritis and subsequent early embryonic death, infertility, and occasional abortions occurring at 4–7 months of gestation (15,16). Similarly, *Chlamydophila abortus,* now widely recognized as *Chlamydia abortus*, targets the placenta, leading to tissue damage, inflammation, and abortion (enzootic abortion of ewes). The highest seroprevalence of *C. abortus* has been observed in large-scale farming operations, followed by household farms and farming cooperatives. Improper management practices have been identified as a significant risk factor for Chlamydia infection in cattle (8). *Leptospira spp*. is a Gram-negative bacterium known to induce abortion in cattle at various stages of gestation. Pathogenic strains such as *Leptospira interrogans*, particularly Serovars hardjo and Pomona, are commonly associated with these abortions (17). While *Mycoplasma bovis* and *Escherichia coli* are linked to reproductive losses, they typically cause issues like repeat breeding and early embryonic death, rather than later-term abortions (18). The protozoal agent *Neospora caninum* significantly affects bovine reproduction by causing sporadic, endemic, and epidemic abortions, especially during the fifth and sixth months of pregnancy, posing a significant threat to cattle herds. Epidemiological studies have shown that Neospora infection can be present in 12% to 42% of cattle, with abortion rates sometimes reaching up to 87% (19). Abortion can occur in pregnant cattle of any age. Fungal pathogens, such as *Aspergillus spp*., also contribute to reproductive losses through mycotic abortions, which typically occur in the latter half of gestation (20).

These diverse pathogens highlight the complex nature of infectious abortion in cattle and underscore the need for comprehensive diagnostic approaches. Given the variety of pathogens implicated in bovine abortion and the potential for widespread impact within a herd, accurate diagnosis and effective control measures are essential. This study aims to assess the seroprevalence of these important infectious abortion-related diseases in a commercial dairy herd to inform targeted intervention strategies.

Materials and methods

Ethical approval

This study was conducted with the approval of The Research Ethics Committee Universitas Padjadjaran Bandung, under protocol number 2407010940.

Study design

This study was conducted on a commercial dairy farm in Subang, West Java, Indonesia, using a cross-sectional study design to determine the seroprevalence of various infectious abortion-related diseases at a specific time (21). The study used secondary data collected from serological tests conducted between March and May 2023, aimed at identifying diseases that could lead to abortions. This data was extracted from the dairy farm's management database software. Using the Slovin formula, the sample size was determined to include 76 female cows aged between 3 and 5 years who had experienced abortions during this period. These samples were then categorized based on the trimester of gestation during which the abortion occurred. The serological tests were performed using the ELISA and Rose Bengal Test (RBT) methods. In the ELISA assay, an antigen or antibody is immobilized on a microplate to capture the target molecule from the sample. A secondary antibody conjugated with an enzyme is added, specifically binding to the target and forming a detectable complex. Upon adding a substrate, the enzyme reaction produces a measurable signal, typically seen as a colour change, corresponding to the target molecule's presence and concentration (22). ELISA kits from US and UK companies were used for detecting the presence of antigens and antibodies associated with Bovine Viral Diarrhea Virus (BVDV) (IDEXX kit), as well as antibodies against *Neospora caninum* (IDEXX kit), *Leptospira spp.* (ABEXXA kit), *Chlamydophila abortus* (IDvet kit), *Coxiella burnetii* (IDEXX kit), and *Mycoplasma bovis* (IDvet kit), while the RBT method was used for detecting antibodies against *Brucella abortus.* The brucellosis kit used in this test is branded as IDvet (France). A stained Brucella antigen is mixed with a serum sample; if antibodies are present, agglutination occurs, resulting in visible clumping, which indicates a positive result (23). Our study assessed seroprevalence based on the proportion of positive serological results within the sample—specifically targeting antibodies against the previously mentioned pathogen. Additionally, the study conducted univariate analysis using descriptive statistical methods to characterize each test outcome's frequency distribution and percentage distribution (24).

Study period and location

The study used data from serological tests for various infectious diseases associated with abortion in the affected population between March and May 2023. This study was conducted at a commercial dairy farm in Subang, West Java province of Indonesia. The cows were kept indoors (free stall and dryland systems). The farm has a day-to-day veterinary service, a regular vaccination program, and synchronized breeding through artificial insemination. Serological tests were conducted at the dairy farm laboratory.

Results

The majority (41 cows) occurred during the first trimester of pregnancy, highlighting early gestation as a period of heightened vulnerability to pregnancy loss. A smaller number (29 cows) were aborted during the second trimester, and a significantly lower number (6 cows) experienced abortions in the third trimester. This distribution suggests that the risk of abortion is highest during early gestation.

The results of the Rose Bengal Test (RBT) and Enzyme-Linked Immunosorbent Assay (ELISA) testing for various pathogens associated with abortion in cattle are detailed in Table 1. Out of the 76 samples tested, 57 were seropositive, resulting in an overall seroprevalence rate of 74.9% for abortion cases within the study period. A breakdown of the infections reveals distinct differences in prevalence among the detected pathogens. Specifically, *Neospora caninum* infection accounted for 6.6% of the seropositive cases, indicating its relatively low but notable role in abortion incidence in this population. *Leptospira spp*., another infection associated with reproductive disorders, was present in 5.2% of the samples, reflecting a similarly low seroprevalence. However, *Mycoplasma bov*is stood out significantly, with a high seroprevalence of 63.1%, suggesting it is the primary pathogen linked to abortion in this study. These findings highlight *Mycoplasma bovis* as a major infectious agent contributing to abortion, far surpassing the rates of *Neospora caninum* and *Leptospira spp.*, and underscore the need for targeted control measures against *Mycoplasma bovis* to reduce abortion rates in cattle.

Table 1: Result of RBT and ELISA testing in many different infectious causals of abortions

Infectious Agent	Total Positive	Seroprevalence (%)	Positive Results		
			1st Trimester	2nd Trimester	3rd Trimester
Brucella sp (RBT)	-	0	-	-	-
Chlamydophila abortus	-	0	-	-	-
Neospora caninum	5	6.6	2	3	-
Leptospira spp.	4	5.2	3	1	-
BVDV	-	0	-	-	-
Coxiella burnetii	-	0	-	-	-
Mycoplasma bovis	48	63.1	25	20	3
Total	57	74.9	30	24	3

Positive results distribution of abortion data in farm (n = 76).

Discussions

The study's results indicate that abortions in cows are more common during the first trimester of pregnancy. Pregnancy losses within the first 41 days of gestation in dairy cattle are referred to as embryonic losses, while those after 41 days are termed fetal losses. The higher incidence of abortions in the first trimester aligns with prior research findings. Research reported that embryonic losses range between 25% and 40%, whereas fetal losses range from 8% to 10%, potentially exceeding 14% in specific herds (25). Other research suggested that most pregnancy losses occur even earlier, within the first 30 days of gestation (26).

The study's detection of a 6.6% seroprevalence rate for *Neospora caninum* among aborted cows underscores its significant impact on cattle reproductive health, with pronounced effects during the first and second trimesters. These findings are in line with the results of a meta-analysis study (27), which showed that *Neospora caninum* was the most prevalent infectious agent found in cases of bovine abortion. Earlier studies have identified *N. caninum* as one of the main causative agents of abortion in cattle, presumably due to the high rate of vertical transmission of this protozoan parasite (28). Abortions in cattle induced by *Neospora caninum* can occur as early as three months into gestation, though they are most frequently observed between five and six months (29).

This clustering of abortions likely arises from the parasite's ability to infect the placenta early in gestation, leading to inflammatory responses and compromised placental function, which in turn impairs fetal development and survival (30). Such disruptions often manifest as fetal resorption, mummification, or stillbirth, with some live-born calves presenting neuromuscular defects or persistent infections. The regional differences in *Neospora* prevalence, such as the 15% rate reported in Kenyan herds (31), highlight the influence of environmental, management, and genetic factors on infection rates (32). Transmission dynamics are

further complicated by the parasite's dual pathways: vertical transmission from seropositive dams to offspring and horizontal transmission via oocysts shed by definitive hosts, such as canids (33,34). Transmission via infected semen from seropositive bulls was suggested when *N. caninum* DNA was found in bull semen (35). Risk factors associated with *N. caninum* infection are elder cattle, manual milking, poor hygiene, history of abortion, and close contact with dogs (36). A study elaborates on the exogenous and endogenous mechanisms through which infections can be sustained or reactivated, posing ongoing risks, particularly in dairy farms where reused breeding stock may facilitate reinfection (37). Effective control measures must, therefore, target both environmental exposure and vertical transmission, incorporating biosecurity protocols, selective breeding for resistance, and routine serological monitoring to mitigate Neospora-associated reproductive and economic losses (38).

The ELISA test results demonstrated a 5.2% seroprevalence of *Leptospira spp*. among the sampled population of aborted cows, with a significant clustering of cases during the first trimester, suggesting an increased risk of infection and reproductive loss during early gestation. *Leptospira* infections initiate through mucosal penetration and proceed via hematogenous dissemination, with the bacteria localizing in target organs, including the reproductive system. This localization can cause inflammation, endothelial disruption, and placental dysfunction, ultimately impairing fetal development and survival, as noted in recent studies (39,40). Examination of vaccination history revealed that one cow was unvaccinated, two were vaccinated in 2020, and one was vaccinated in 2022 with Ultravac 7 in 1, a formulation targeting *Leptospira borgpetersenii* serovar Hardjo and *Leptospira interrogans* serovar Pomona. The detection of antibodies in the 2022-vaccinated cow raises questions regarding whether the seropositivity reflects a vaccine-induced immune response or exposure to a natural infection, highlighting the need to differentiate antibody sources through serological titers (41). Annual booster vaccinations are adviced to maintain adequate immunity and mitigate reproductive losses (42). A comprehensive approach, integrating vaccination with enhanced biosecurity measures and routine serological monitoring, is essential for minimizing infection risks and improving reproductive outcomes in cattle populations.

Mycoplasma bovis emerged as the predominant pathogen, with 63.1% seropositivity detected among 76 aborted cows (S/P% ≥ 60). Abortions spanned all gestational stages, with a higher concentration observed during the first trimester, underscoring the pathogen's extensive impact on reproductive viability. The high prevalence can be linked to the bacterium's sophisticated immune evasion strategies, such as antigenic variation, which complicates the development of effective vaccines and leads to notable resistance to common antibiotics (43,44). Transmission within herds is exacerbated by the bacterium's high contagion rate, particularly via respiratory secretions and infected milk, contributing to rapid spread and infection persistence (1,45). Longitudinal studies on *M. bovis* antibody dynamics emphasize significant fluctuations over time, highlighting the critical need for ongoing serological monitoring to manage infection risks (46). Persistent infections frequently result in recurrent abortions and other systemic complications, indicating that both vertical and horizontal transmission pathways play pivotal roles in herd health (2,39). The distribution of abortions throughout gestation demonstrates the pathogen's capacity to disrupt pregnancy at varying stages, necessitating comprehensive control measures, including stringent biosecurity practices, routine diagnostics, and strategic herd management interventions to minimize reproductive losses and enhance cattle health.

Among the serologically tested samples, 19 were found to be seronegative. This outcome may be partly attributed to the specificity of the laboratory diagnostic kit, which targets only certain pathogen agents, potentially overlooking other causative factors. Additionally, the presence of non-infectious causes of abortion, such as nutritional deficiencies, genetic disorders, environmental stressors, or toxic exposures, should be considered, as they can also significantly impact reproductive outcomes and contribute to pregnancy loss. Infectious causes of abortion, in addition to those already tested, include Infectious Bovine Rhinotracheitis-Infectious Pustular Vulvovaginitis (IBR-IPV) virus, *Arcanobacterium (Actinomyces) pyogenes*, *Listeria monocytogenes*, *E. coli*, and *Pasteurella haemolytica*. Besides infectious causes that can be diagnosed through testing, non-infectious causes are also likely contributors to abortion in dairy farms. Common non-infectious causes of abortion include genetic abnormalities, heat stress, toxic agents, ergot alkaloids, hormone deficiencies, and nutritional deficiencies. These various possibilities can be evaluated and further investigated in correlation with individuals experiencing abortion (47).

Furthermore, the infectious causes of abortion occurring in this commercial dairy farm warrant further investigation, especially considering their potential impact on late-term abortions. The economic implications of these early losses are substantial and underscore the need for effective preventative strategies. The variation in reported incidence rates may be attributed to differences in study methodologies, herd management practices, and geographic locations (48,49).

Conclusion

This study provides valuable insights into the distribution of bovine abortions across gestation and identifies *Mycoplasma bovis* as a major contributor to infectious abortion within the studied population. However, in addition

to these infectious diseases, abortion can be caused by other infectious disease events and non-infectious causes (trauma, heat stress, genetic disorders, etc.) that could not be identified in this study. The findings emphasize the need for targeted diagnostic and preventative measures, particularly against *M. bovis*, and can inform the development of more effective vaccination strategies. By highlighting the key pathogens associated with abortion and their prevalence, this research contributes to improved reproductive management practices and lays the groundwork for future investigations into the complex factors influencing bovine reproductive health

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Conflict of interest

There is no conflict of interest.

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الانتشار المصلي لبعض الأمراض المعدية المرتبطة بالإجهاض في أبقار هولشتاين الفريزية في مزرعة الألبان التجارية في سوبانج إندونيسيا

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الخلاصة

الإجهاض هو طرح الجنين غير الحي خلال فترة تتراوح بين ٤٢ إلى ٢٦٠ يومًا من الحمل. يشكل الإجهاض تهديدًا اقتصاديًا كبيرًا لصناعة الألبان بسبب فقدان النسل المحتمل، وانخفاض إنتاج الحليب، وزيادة التكاليف البيطرية. هدفت هذه الدراسة إلى التحقيق في انتشار بعض الأمراض المعدية المرتبطة بالإجهاض في مزرعة ألبان تجارية في سوبانج من خلال تحليل ٧٦ عينة مصل من ماشية فريزيان هولشتاين. استخدمت بيانات اختبار الأجسام المضادة للكشف عن Neospora caninum، وLeptospira spp.، وChlamydophila abortus، وCoxiella burnetii، وMycoplasma bovis، واختبار المستضد لفيروس الإسهال البقري الفيروسي، واختبار روز البنغال Brucella abortus. وكشفت النتائج عن ارتفاع معدل انتشار Mycoplasma bovis (٦٣،١%)، مما يشير إلى وجود تأثير كبير لهذه العدوى داخل القطيع. ولوحظ انخفاض معدلات الانتشار المصلي لـ Neospora caninum (٦،٦%) وLeptospira spp. (٥،٢%)، في حين لم يتم الكشف عن Chlamydophila abortus، Coxiella burnetii، وفيروس الإسهال البقري الفيروسي. تسلط هذه النتائج الضوء على أهمية M. bovis كمساهم رئيسي محتمل في الإجهاض في هذه المزرعة. ويشير ارتفاع معدل انتشار عدوى M. bovis إلى ضرورة اتباع استراتيجيات تدخل فورية، مثل تحسين تدابير الأمن البيولوجي وبرامج التطعيم المستهدفة، للتخفيف من الخسائر الاقتصادية الإضافية وتحسين صحة القطيع بشكل عام. هناك ما يبرر إجراء المزيد من الأبحاث لدراسة التأثير المحدد لـ M. bovis على معدلات الإجهاض والأداء الإنجابي في هذا القطيع.