

SEROLOGICAL ASSESSMENT OF ABORTION-ASSOCIATED INFECTIONS IN DAIRY CATTLE IN WESTERN AZERBAIJAN

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Abstract. Abortion is an important reproductive problem in dairy cattle and may results in substantial economic losses to cattle production systems. The interpretation of serological findings in vaccinated herds is particularly challenging because antibody detection may reflect vaccine-induced immunity rather than natural infection. The aim of this study was to assess the serological responses to bovine viral diarrhoea virus (BVDV), bovine herpesvirus-1 (BoHV-1), *Neospora caninum* and *Chlamydia abortus* and to evaluate their potential association with late-term abortion in dairy cattle in western region of Azerbaijan.

A total of 150 Holstein-Friesian dairy cows were randomly selected from three districts of the Ganja-Dashkasan economic region: Goranboy (n=55), Goygol (n=50) and Samukh (n=45).

The overall animal-level seropositivity for BVDV and BoHV-1 was 55.48% and 39.76%, respectively, while herd-level seropositivity was 91.67% and 77.87%. Because vaccination against BVDV and BoHV-1 antibody detection against these agents was interpreted primarily as a vaccine-induced serological response. Therefore, not detection of *Ch.abortus* and *N. caninum*-specific antibodies indicate no exposure to the parasites.

Keywords: BVDV, BoHV-1, ELISA, vaccination, serology

Introduction

Herd health, particularly the prevention and control of infectious diseases, is essential for maintaining productivity, reproductive performance and economic efficiency in modern dairy production systems [38, 20]. Infectious diseases may result in reduced milk production, impaired reproductive performance, increased veterinary costs, fetal loss and premature culling. Among the infectious agents associated with reproductive disorders in cattle, bovine viral diarrhoea virus (BVDV), bovine herpesvirus-1 (BoHV-1), *Neospora caninum* and *Chlamydia abortus* are of particular importance [1, 7, 11, 12, 13, 14].

BVDV belongs to the genus *Pestivirus* within the family *Flaviviridae* and is an important pathogen of cattle [15, 17, 18]. Infection may be associated with embryonic death, fetal developmental abnormalities, fetal mummification, abortion, stillbirth and the birth of weak calves [16, 19, 37]. Infection of susceptible pregnant cattle during critical periods of gestation may result in transplacental infection and, under specific circumstances, the development of persistently infected calves [36, 42, 43, 44, 45, 46].

BoHV-1 is a member of the family *Herpesviridae* and is associated with respiratory and reproductive disease in cattle. In addition to infectious bovine rhinotracheitis, BoHV-1 may cause reproductive disorders, including abortion. An important epidemiological characteristic of BoHV-1 is its ability to establish latent infection, which may contribute to long-term persistence of the virus within cattle populations [20, 25, 40].

Bovine neosporosis is caused by the protozoan parasite *Neospora caninum* and is considered an important infectious cause of bovine abortion. Canids serve as definitive hosts, while cattle are important intermediate hosts [3, 8, 9]. Both horizontal and vertical transmission contribute to the maintenance of infection [26, 27, 28]. Vertical transmission from dam to fetus is particularly important in cattle herds and may result in abortion, fetal mummification, stillbirth or the birth of weak calves [34, 47, 48, 50, 51].

Chlamydia abortus is an obligate intracellular bacterium associated primarily with reproductive disease and abortion, particularly in small ruminants. Infection may result in placentitis and tissue necrosis and can subsequently compromise fetal development [29, 31, 32]. Although *C. abortus* is less frequently reported as a cause of reproductive disease in cattle, its possible role in bovine abortion should be considered during differential diagnosis [5, 6, 9, 35].

Serological surveillance is widely used for monitoring exposure to infectious agents in cattle populations. However, interpretation of antibody-based diagnostic tests can be challenging in vaccinated populations. Antibodies detected against vaccine-preventable pathogens may reflect successful immunization rather than natural infection. Consequently, vaccination history is an essential component of the interpretation of serological results [30, 39].

The distinction is particularly important when evaluating abortion-associated pathogens. While antibody detection against vaccinated pathogens should be interpreted cautiously, seropositivity to *N. caninum* in unvaccinated cattle may provide stronger epidemiological evidence of natural exposure [2, 3, 4, 44].

The aim of this study was therefore to assess the serological responses to BVDV, BoHV-1, *N. caninum* and *C. abortus* in dairy cattle from western Azerbaijan and to evaluate their potential relevance to abortion.

Materials and methods

Study population and study area

The study was conducted in dairy cattle farms located in Goranboy, Goygol and Samukh districts of the Ganja-Dashkasan economic region in western Azerbaijan.

A total of 160 Holstein-Friesian dairy cows were randomly selected. Of these, 55 animals were sampled in Goranboy, 50 in Goygol and 45 in Samukh.

The study population consisted of dairy cows maintained under commercial farm conditions. The investigated farms routinely implemented vaccination programmes against selected infectious diseases. Vaccination history was considered during the interpretation of serological results.

Blood sampling and biosafety

Blood samples were collected according to standard veterinary sampling procedures and laboratory biosafety SOPs. Appropriate personal protective equipment was used during sample collection and handling.

Venous blood samples were collected using sterile sampling equipment and appropriately labelled according to animal identification. Samples were placed in insulated transport containers with cooling material and accompanied by the relevant documentation. The samples were transported to the laboratory within approximately 2–4 h after collection.

Serum was subsequently separated from the blood samples and stored under -20 C until serological examination.

Vaccination status

The investigated herds had vaccination programmes covering BVDV and BoHV-1. Therefore, the detection of antibodies against these pathogens was interpreted in the context of vaccination-induced immunity.

Serological examination

Commercial ELISA kits manufactured by IDEXX Laboratories (USA) were used for the detection of antibodies against BVDV, BoHV-1, *N. caninum* and *C. abortus*.

The assays were performed according to the manufacturer's instructions. Serum samples were added to the appropriate microplate wells and incubated. Following incubation, unbound material was removed by washing. The appropriate conjugate was subsequently added, followed by incubation and another washing step. TMB substrate was then added and incubated according to the manufacturer's protocol.

Optical density was measured at 450 nm using a BioTek ELx800 microplate reader.

The obtained results were interpreted according to the diagnostic criteria specified by the manufacturer. Positive and negative classifications were confirmed using XChekPlus software.

Data processing and statistical analysis

The complete dataset consisted of 150 serum samples. ELISA-derived numerical metrics were processed using Python, with the *pandas* and *numpy* libraries.

For each study group, the arithmetic mean, standard deviation (SD), minimum and maximum values were calculated. These values represent descriptive characteristics of the ELISA-derived metrics.

Because the diagnostic systems used different numerical scales, the metric values were not directly compared between pathogens. Seropositivity was determined independently for each pathogen according to the manufacturer's interpretation criteria.

Animal-level seroprevalence was calculated as the proportion of seropositive animals among all examined animals. Herd-level seroprevalence was defined as the proportion of investigated herds containing at least one seropositive animal.

Results

Serology

To assess seropositivity at the farm level, the serological results of the animals examined at each of the surveyed farms were analyzed. Any farm where at least one seropositive animal was detected was classified as a seropositive herd. The farm-level seropositivity rate was calculated as the percentage of seropositive farms relative to the total number of farms examined.

Serological measurements demonstrated differences in the distribution and variability of BVDV, BoHV-1, Chlamydia and Neospora indicators among the three studied groups. The highest mean BVDV value (as shown in Table 1) was recorded in Group 2 (22.30 ± 23.68), whereas Group 1 showed a mean value of 13.67 ± 3.39 . Group 3 demonstrated a considerably lower mean BVDV value (0.505 ± 0.264).

For BoHV-1, the highest mean value was observed in Group 3 (0.546 ± 0.214), followed by Group 1 (0.386 ± 0.309) and Group 2 (0.217 ± 0.153). The observed ranges were 0.141–0.789, 0.095–0.784 and 0.067–0.549, respectively.

The Chlamydia indicator was highest in Group 1, with a mean value of 30.00 ± 35.49 and a range of 18–25. The corresponding values for Group 2 and Group 3 were 27.75 ± 11.18 (range: 11–47) and 33.50 ± 16.03 (range: 20–61), respectively. The relatively large standard deviation observed in Group 1 indicates considerable variability among individual animals.

The highest Neospora mean value was observed in Group 1 - 50.01 ± 48.16 (range: 32–42), while Group 2 had a substantially lower mean value 15.50 ± 35.73 (range: 29–112). In Group 3, the Neospora value was 0.00 ± 0.00 , with all recorded values equal to zero.

Overall, the findings indicate considerable variation in serological measurements among the studied groups. Statistical comparison between groups is required to determine whether the observed differences are statistically significant.

Table 1.
Serological indicators of BVDV, BoHV-1, Chlamydia and Neospora in the studied groups

Group	n	Statistic	BVDV, Mean $\pm$ SD	BoHV-1, Mean $\pm$ SD	Chlamydia, Mean $\pm$ SD	Neospora , Mean $\pm$ SD
Group 1	55	Mean $\pm$ SD	13.67 $\pm$ 3.39	0.386 $\pm$ 0.309	30.00 $\pm$ 35.49	50.01 $\pm$ 48.16
		Range	8–17	0.095–0.784	18–25	32–42
Group 2	50	Mean $\pm$ SD	22.30 $\pm$ 23.68	0.217 $\pm$ 0.153	27.75 $\pm$ 11.18	15.50 $\pm$ 35.73
		Range	6–72	0.067–0.549	11–47	29–112
Group 3	45	Mean $\pm$ SD	0.505 $\pm$ 0.264	0.546 $\pm$ 0.214	33.50 $\pm$ 16.03	0.00 $\pm$ 0.00
		Range	0.099–0.894	0.141–0.789	20–61	0–0

Interpretation of serological responses in relation to vaccination

The high seropositivity observed for BVDV and BoHV-1 should be interpreted in the context of the vaccination programmes implemented in the investigated farms.

The variation in BVDV and BoHV-1 metrics among the study groups therefore reflects differences in serological responses among vaccinated animals rather than differences in active infection status.

Discussion

The present study demonstrates the importance of vaccination history in the interpretation of serological results obtained from dairy cattle. High levels of antibody positivity against BVDV and BoHV-1 were observed at both animal and herd levels. However, because vaccination programmes were implemented in the investigated herds, these findings should primarily be interpreted as evidence of vaccine-induced antibody responses rather than as confirmation of active infection.

The animal-level seropositivity of 55.48% for BVDV and 39.76% for BoHV-1, together with herd-level seropositivity of 91.67% and 77.87%, respectively, indicates that antibodies against these pathogens were in the study population. Similar serological evidence of exposure to BVDV and BoHV-1 has been reported in dairy cattle populations in neighbouring regions.

The present results highlight a major limitation of conventional antibody-based surveillance in vaccinated cattle. A positive antibody result may indicate successful vaccination, previous natural exposure or a combination of both. Therefore, serological positivity should not be equated with active infection in vaccinated herds.

The BVDV metric values showed considerable variation between study groups. This variation should be interpreted as differences in serological response among vaccinated animals rather than as evidence of recent infection or active viral circulation. Determination of active BVDV infection would require direct detection of viral antigen or nucleic acid, particularly when identifying persistently infected animals [21, 22, 23, 41].

The same principle applies to BoHV-1. The detection of antibodies in vaccinated cattle indicates an immune response. Additional diagnostic approaches, including appropriate antigen or molecular tests, would be required to determine infection status.

The results for *N. caninum* and *Ch. Abortus* are of particular interest because no vaccination programme against this parasite was reported in the investigated herds. Therefore, the not detection of antibodies indicate to the no exposure to the parasites [10, 33, 49].

The interpretation of these findings should incorporate with reproductive history and, where possible, direct pathogen detection [24, 32, 40].

An important strength of the present study is the simultaneous evaluation of several reproductive pathogens in a defined dairy cattle population and the use of software-assisted interpretation of ELISA results. The additional calculation of mean, standard deviation and range using Python provided a quantitative description of the distribution of ELISA-derived metrics.

For vaccinated pathogens, monitoring antibody responses may be useful for evaluating herd immunity, but additional diagnostic methods are necessary when the objective is to identify active infection.

Conclusion

The present study demonstrated substantial serological responses to BVDV and BoHV-1 in vaccinated dairy cattle from Goranboy, Goygol and Samukh districts of western part of Azerbaijan. The detected antibodies against BVDV and BoHV-1 should primarily be interpreted as a result of vaccination.

Integrated herd-health program combining vaccination, reproductive monitoring, biosecurity and diagnostic tests improve the identification and management of infectious reproductive problems in dairy cattle in Azerbaijan.

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AZƏRBAYCANIN QƏRB REGIONUNDA SÜDÇÜLÜK İSTİQAMƏTLİ İRİBUYNUZLU HEYVANLARDA ABORTLA ƏLAQƏLİ İNFEKSİYALARIN SEROLOJİ QIYMƏTLƏNDİRİLMƏSİ

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Abort süd verən mal-qara arasında mühüm reproduktiv problemdir və mal-qara istehsal sistemlərinə əhəmiyyətli iqtisadi itkilərlə nəticələnə bilər. Peyvənd olunmuş sürülərdə seroloji tapıntıların interpretasiyası xüsusilə çətinidir, çünki anticisim aşkarlanması təbii infeksiyadan daha çox peyvəndləmə nəticəsində yaranmış immuniteti əks etdirir. Bu tədqiqatın məqsədi mal-qarada viruslu ishal (BVDV), mal-qara herpesvirus-1 (BoHV-1), *Neospora caninum* və *Chlamydia abortus* qarşı yaranmış immun cavabı qiymətləndirmək və Azərbaycanın qərb bölgəsində abortlarla potensial əlaqəsini təyin etmək olmuşdur.

Gəncə-Daşkəsən iqtisadi rayonunun üç rayonundan - Goranboy ($n = 55$), Göygöl ($n = 50$) və Samux ($n = 45$) - təsadüfi olaraq cəmi 150 Holşteyn-Friz cinsli inək seçilmişdir.

BVDV və BoHV-1 üçün heyvan səviyyəsində ümumi seropozitivlik müvafiq olaraq 55.48% və 39.76%, sürü səviyyəsində isə 91.67% və 77.87% təşkil etmişdir. Çünki bu patogenlərə qarşı BVDV və BoHV-1 anticisimlərinə qarşı peyvəndləmə əsasən peyvəndləmə nəticəsində yaranmış seroloji cavab kimi şərh edilmişdir. Buna görə də, *Ch. abortus* və *N. caninum*-a spesifik anticisimlərin aşkarlanmaması yoluxmanın olmadığını göstərir.

Açar sözlər: BVDV; BoHV-1; ELISA; peyvəndləmə; serologiya

СЕРОЛОГИЧЕСКАЯ ОЦЕНКА ИНФЕКЦИЙ, АССОЦИИРОВАННЫХ С АБОРТАМИ, У МОЛОЧНОГО СКОТА В ЗАПАДНОМ РЕГИОНЕ АЗЕРБАЙДЖАНА

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Аборты представляют собой серьезную проблему в сфере воспроизводства молочного скота и могут приводить к значительным экономическим потерям в животноводческих хозяйствах. Интерпретация результатов серологических исследований в вакцинированных стадах сопряжена с особыми трудностями, поскольку выявление антител может свидетельствовать о поствакцинальном иммунитете, а не о естественном инфицировании. Целью данного исследования стала оценка серологического ответа на вирус вирусной диареи крупного рогатого скота, ринотрахеита, неоспороза и хламидиоза, а также анализ возможной связи этих показателей с абортами на поздних сроках стельности у молочного скота в западном регионе Азербайджана.

Для исследования были случайным образом отобраны 150 коров голштино-фризской породы из трех районов Гянджа-Дашкесанского экономического района: Геранбойского ($n = 55$), Гейгёльского ($n = 50$) и Самухского ($n = 45$).

Общая доля серопозитивных животных составила 55,48% для BVDV и 39,76% для BoHV-1, тогда как доля серопозитивных стад достигла 91,67% и 77,87% соответственно. Поскольку проводилась вакцинация против BVDV и BoHV-1, обнаружение антител к этим возбудителям расценивалось как поствакцинальный серологический ответ. В то же время отсутствие специфических антител к *Ch. abortus* и *N. caninum* указывает на отсутствие контакта животных с данными возбудителями.

Ключевые слова: BVDV, BoHV-1, ИФА, вакцинация, серология

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