Research Paper

Bovine Alphaherpesvirus-1 Infection in Dairy Cattle Herds in Khorasan Razavi province, Iran: Herd-Level Prevalence in Bulk Tank Milk, Risk Factors, and Regional Insights

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ABSTRACT

Introduction: Bovine alphaherpesvirus-1 (BoHV-1) causes infectious bovine rhinotracheitis, reproductive disorders, immunosuppression, and major economic losses in dairy cattle. This cross-sectional study estimated herd-level prevalence of BoHV-1 in industrial dairy herds of Khorasan Razavi province, Iran, using bulk tank milk (BTM) samples and identified associated risk factors.

Materials and Methods: A total of 160 industrial dairy herds were sampled. BTM samples were tested for BoHV-1 antibodies using a commercial indirect ELISA.

Results: Apparent prevalence was calculated as the proportion of positive herds, and true prevalence was estimated using the Rogan-Gladen method. Herds were categorized as small (6–50 cows), medium (51–100 cows), or large (101–1050 cows). Associations between BoHV-1 positivity and herd characteristics were assessed using chi-square tests and logistic regression. The apparent herd-level prevalence was 66.9% (107/160), and the adjusted true prevalence was 70.2%. Prevalence increased significantly with herd size, from 48.8% in small herds to 79.2% in medium herds and 93.8% in large herds ($P < 0.001$). Logistic regression showed significantly higher odds of BoHV-1 positivity in medium herds (OR 9.0) and large herds (OR 19.3) compared with small herds. Although resident artificial insemination technicians were initially associated with positivity, this effect was not significant after adjustment for herd size.

Conclusion: BoHV-1 prevalence is high in industrial dairy herds of Khorasan Razavi, and larger herd size appears to be the main risk factor. Targeted vaccination, improved biosecurity, and active surveillance are recommended.

Keywords: BoHV-1, Bulk tank milk, Dairy cattle, Herd prevalence, Iran, Risk factors

1.Introduction

Bovine alphaherpesvirus-1 (BoHV-1), a member of the *Alphaherpesvirinae* subfamily within the *Herpesviridae* family, is a major global pathogen in cattle [1]. It is the causative agent of infectious bovine rhinotracheitis (IBR), manifesting as respiratory disease (e.g., nasal discharge, fever, conjunctivitis), reproductive disorders (e.g., abortion, pustular vulvovaginitis, balanoposthitis), and immunosuppression that exacerbates the bovine respiratory disease complex [2,3]. The virus establishes lifelong latency in the trigeminal ganglia, with reactivation triggered by stressors, enabling transmission through aerosols, semen, aborted fetuses/fetal membranes, or fomites [4–6]. Artificial insemination (AI) represents a key transmission risk when semen biosecurity is inadequate [6].

In Iran, the dairy sector is economically critical, contributing significantly to national milk production (approximately 10–11 million tons annually from ~8 million dairy cattle) [7]. Khorasan Razavi Province is a prominent milk-producing area, accounting for roughly 10% of the country's output with an average annual production of ~900,000 tons [8]. Intensification of production, larger herd sizes, and increased cattle movement heighten BoHV-1 transmission risks in such settings. Seroprevalence of BoHV-1 in Iran varies regionally: individual-level estimates include 58.7% (herd-level 82.9%) in Hamedan [9], 10.7% (herd-level 80%) in Zanjan [10], and 50% (herd-level 65%) in central desert areas [11]. Earlier studies in Razavi Khorasan have been suggested relatively lower exposure [12], but more recent data from Mashhad- district dairy herds indicate higher seropositivity (~64.4% individual-level in calves from some outbreaks), potentially reflecting an upward trend linked to intensification and limited control measures [13].

Globally, BoHV-1 control strategies vary: marker vaccines and staged eradication programs have succeeded in parts of Europe, whereas many developing countries, including Iran, rely on limited or no systematic programs [14-16]. Bulk tank milk (BTM) ELISA offers a practical, non-invasive method for herd-level surveillance of BoHV-1 exposure [17,18]. This study aimed to: (1) estimate herd-level BoHV-1 prevalence using BTM ELISA in dairy herds of the Khorasan Razavi province, (2) investigate associated risk factors (e.g., herd size, AI practices), and (3) propose regionally tailored control recommendations, informed by local epidemiological patterns and outbreak observations.

2. Material and Methods

2.1. Study Area and Population

This cross-sectional study was conducted in the Khorasan Razavi Province, northeastern Iran, a major dairy production region. The target population comprised industrial Holstein-Friesian dairy herds equipped with bulk milk tanks. Herd sizes ranged from 6 to 1050 lactating cows (total herd size typically 250–3000 animals, with 30–750 lactating cows per herd). Small traditional farms

(<6 cows) were excluded due to the absence of bulk tanks. Participating herds were managed under intensive systems, including 75% free-stall housing, total mixed ration (TMR) feeding (primarily alfalfa, corn silage, and concentrates), and machine milking 2–3 times daily. None of the herds were vaccinated against BoHV-1; routine vaccinations targeted against foot-and-mouth disease (FMD) and clostridial diseases. All herds used artificial insemination (AI) with computerized record-keeping.

2.2. Sample Size and Sampling

Sample size was calculated using the Thrusfield formula for estimating prevalence in finite populations [19], assuming an apparent herd-level prevalence of approximately 80–89% based on prior Iranian bulk tank milk (BTM) studies in nearby regions [20]. Parameters included 95% confidence level and 5% absolute precision, yielding an estimated minimum of 155 herds. To account for potential non-response or logistical constraints, 160 herds were randomly selected using a lottery method from 38 dairy complexes, with proportional geographic stratification across Khorasan Razavi province.

2.3. Sample Collection

From each selected herd, approximately 500 mL of BTM was collected immediately after complete milking, into sterile containers. Samples were transported on ice, centrifuged to remove fat, and stored at −20°C until analysis.

2.4. Laboratory Analysis

BTM samples were tested for BoHV-1 antibodies using a commercial indirect ELISA kit (Svanovir® BoHV-1, SVANOVA Biotech AB, Sweden; or equivalent gE/gB-specific kit), with manufacturer-reported diagnostic sensitivity (DSe) of 95.2% and specificity (DSp) of 100% for bulk milk. Optical density (OD) was measured at 450 nm, and results were expressed as percent positivity (PP): PP < 7 classified as negative, PP ≥ 7 as positive.

2.5. Statistical Analysis

Apparent herd-level prevalence was calculated as the proportion of positive herds. True herd-level prevalence was estimated using the Rogan-Gladen formula: true prevalence = (AP + Sp − 1) / (Se + Sp − 1), where AP is apparent prevalence, Se is sensitivity, and Sp is specificity [21,22]. Associations between herd-level BoHV-1 status and potential risk factors (e.g., herd size categorized as small [<250 lactating cows; reference], medium [250–500], and large [>500]) were evaluated using chi-square tests for univariable screening, followed by logistic regression analysis to estimate odds ratios (OR) and 95% confidence intervals (CI). Variables with $P < 0.20$ in univariable analysis were considered for inclusion in the multivariable model. Statistical significance was set at $P < 0.05$. AI practices; technician presence) were initially assessed using chi-square (χ^2) tests (significance at $P < 0.05$). Multivariable logistic regression was performed to estimate adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for significant factors, with herd size as the primary exposure variable and adjustment for confounders such as AI technician presence. Analyses were conducted using SPSS version 25 (IBM Corp., Armonk, NY, USA).

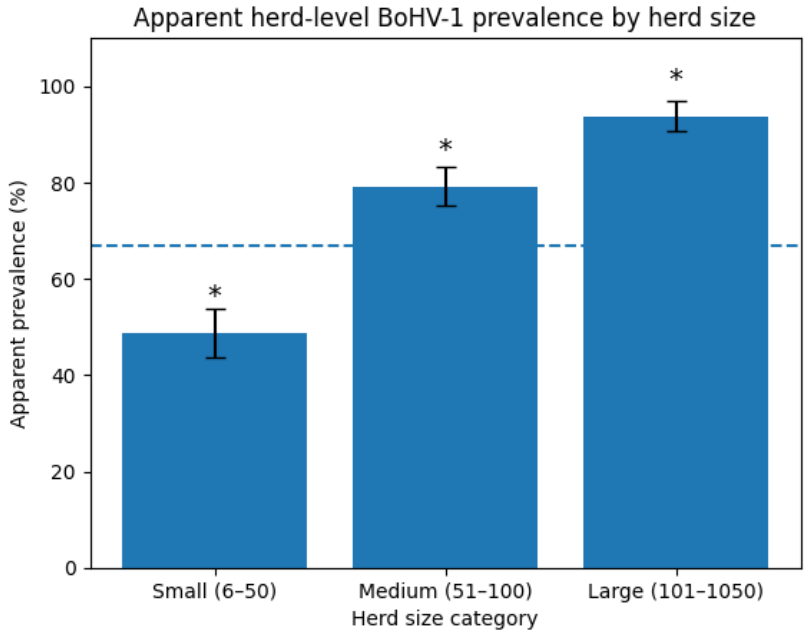
3. Results

Herd-Level Prevalence of BoHV-1 Of the 160 dairy herds sampled, 107 tested positive for BoHV-1 antibodies in bulk tank milk (BTM) by indirect ELISA, yielding an apparent herd-level prevalence of 66.9% (95% CI: 59.2–74.0%). Applying the Rogan-Gladen correction with the kit's reported diagnostic sensitivity (DSe) of 95.2% and specificity (DSp) of 100%, the estimated true herd-level prevalence was 70.2%.

Prevalence increased markedly with herd size (Table 1 and Figure 1). The association between herd size category and BoHV-1 positivity was highly significant (χ^2 test, $P < 0.001$).

Table 1. Apparent herd-level prevalence of BoHV-1 by herd size category

Herd size category (lactating cows)	No. positive / No. tested	Apparent prevalence (%)	95% CI (%)
Small (6–50)	39 / 80	48.8	37.6–59.9
Medium (51–100)	38 / 48	79.2	65.0–89.5
Large (101–1050)	30 / 32	93.8	79.2–99.2
Overall	107 / 160	66.9	59.2–74.0



- **Figure 1.** Apparent herd-level prevalence of BoHV-1 antibodies in bulk tank milk by herd size in Khorasan Razavi province dairy herds (n=160). Prevalence increases significantly with herd size ($P < 0.001$).

Risk Factor Analysis

Herd size was strongly associated with BoHV-1 positivity in univariable analysis (χ^2 $P < 0.001$). In multivariable logistic regression (adjusting for presence of a resident AI technician), medium-sized herds had significantly higher odds of positivity compared to small herds (adjusted OR = 9.0, 95% CI: 3.8–21.3, $P < 0.001$). Large herds showed even greater odds (adjusted OR = 19.3, 95% CI: 4.2–88.6, $P < 0.001$).

Presence of a resident AI technician was associated with positivity in univariable analysis (51/56 herds with technician positive vs. 5/53 without; χ^2 $P = 0.001$). However, this association became non-significant after adjustment for herd size (adjusted OR = 1.5, 95% CI: 0.5–4.3, $P = 0.457$), indicating confounding by herd size (Table 2).

Table 2. Univariable and multivariable associations between herd-level BoHV-1 positivity and key risk factors

Risk factor	Univariable P-value	Adjusted OR (95% CI)	Multivariable P-value
Herd size (reference: Small)	< 0.001	—	—
- Medium vs. Small	—	9.0 (3.8–21.3)	< 0.001
- Large vs. Small	—	19.3 (4.2–88.6)	< 0.001
Resident AI technician (yes vs. no)	0.001	1.5 (0.5–4.3)	0.457

Note: Multivariable logistic regression adjusted for herd size and AI technician presence. OR = odds ratio; CI = confidence interval.

4. Discussion

The results showed that apparent herd-level prevalence of BoHV-1 in Khorasan Razavi province dairy herds (66.9%; 95% CI: 59.2–74.0%) and corrected true prevalence (70.2%) indicate substantial exposure in this intensive production area. These estimates align with herd-level seroprevalences reported in other Iranian regions, including ~83% in Hamedan [9], ~80% in Zanjan [10], 65% in central desert areas [11], and 78% in Fars Province [23]. National meta-analyses suggest pooled animal-level seroprevalence around 40% (95% CI: 32–48%) and herd-level often exceeding 60–90% in dairy systems [24]. Variability across provinces likely stems from differences in herd management intensity, cattle movement/trade, biosecurity practices, and sampling methods (individual serum vs. bulk tank milk).

Bulk tank milk (BTM) ELISA provides a reliable, non-invasive indicator of herd-level BoHV-1 exposure in unvaccinated populations, as validated for high diagnostic performance in such settings [15,17 and 18]. However, antibody dilution from seronegative cows in mixed herds limits its utility for estimating within-herd or individual prevalence [17]. Consequently, the present

findings robustly reflect herd infection status but preclude direct extrapolation to animal-level or age-specific patterns.

Herd size emerged as the primary risk factor, with significantly higher odds in medium (adjusted OR 9.0) and large (adjusted OR 19.3) herds compared to small ones. This association, consistently reported in Iranian [9,11,20 and 22] and international studies, likely reflects increased opportunities for viral transmission in larger operations via greater animal density, shared equipment, stress from overcrowding, and more frequent introductions of replacements or AI services. The univariable link between resident AI technicians and positivity (P = 0.001) attenuated to non-significance after herd-size adjustment (P = 0.457), suggesting confounding: larger herds more often employ on-site technicians, potentially amplifying transmission risks if semen biosecurity is inadequate [6]. Semen from latently infected bulls remains a plausible route, underscoring the need for certified BoHV-1-free semen in AI programs.

Clinical manifestations of IBR (e.g., respiratory signs, reproductive losses) contribute to economic impacts in endemic settings, exacerbated by immunosuppression and synergies with other respiratory pathogens. While sporadic outbreaks occur in Iranian dairy herds, systematic longitudinal data on active infections (e.g., via PCR) are limited regionally.

Control strategies in Iran, where routine BoHV-1 vaccination is uncommon, contrast with successful marker-vaccine-based eradication programs in parts of Europe [14,15 and 25]. Regionally adapted approaches for intensive Holstein systems in Khorasan Razavi province should prioritize: (1) adoption of gE-deleted (DIVA-compatible) vaccines to enable differentiation of vaccinated from infected animals if vaccination is implemented; (2) enhanced biosecurity, including testing incoming semen, disinfection of milking/AI equipment, controlled visitor access, and quarantine for replacements; (3) stress reduction through optimized housing, nutrition, and handling; and (4) ongoing herd-level surveillance using BTM ELISA to monitor trends and guide interventions.

Limitations

This study relied on BTM ELISA, which precludes individual-level, age-, or parity-stratified inferences and may underestimate low-prevalence herds due to antibody dilution. No data were collected on non-lactating animals, genomic strain typing, concurrent pathogens, or clinical/reproductive outcomes. Cross-sectional design limits causal inference and temporal trends. Future research should incorporate individual serology/PCR for active infection detection, longitudinal monitoring, and economic impact assessments.

Conclusion

BoHV-1 exposure is widespread in Mashhad dairy herds (true herd-level prevalence ~70%), with strong positive association to herd size reflecting intensification risks. These findings, consistent with patterns across Iran, highlight the need for integrated control measures—including targeted biosecurity, potential vaccination, and sustained surveillance—to mitigate economic losses from respiratory, reproductive, and immunosuppressive effects in high-density production systems.

Authors' Contribution

Study concept and design: M. GR.

Acquisition of data: D. S.

Analysis and interpretation of data: M. GR., H. A.

Drafting of the manuscript: M. GR., H. A., and D. S.

Critical revision of the manuscript for important intellectual content: M. GR.

Statistical analysis: M. GR.

Administrative, technical, and material support: M. GR., H.A.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Data Availability

The data that support the findings of this study are available upon request from the corresponding author.

AI/GAI Disclosure Statement

No generative AI tools were used in this manuscript or study.

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