



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

NAAS Rating (2025): 4.61

VET 2025; 10(1): 116-120

© 2025 VET

www.veterinarypaper.com

Received: 20-11-2024

Accepted: 24-12-2024

Allison Claire

Department of Veterinary
Virology, Ontario University of
Veterinary Medicine, Guelph,
Canada

Rajan Vijay

Department of Veterinary
Virology, Ontario University of
Veterinary Medicine, Guelph,
Canada

Corresponding Author:

Allison Claire

Department of Veterinary
Virology, Ontario University of
Veterinary Medicine, Guelph,
Canada

Molecular epidemiology of lumpy skin disease virus in bovines: A one-year surveillance research

Allison Claire and Rajan Vijay

DOI: <https://www.doi.org/10.22271/veterinary.2025.v10.i1b.3372>

Abstract

Lumpy Skin Disease (LSD) has historically been confined to Africa and parts of the Middle East, but its geographic expansion over the past decade into Europe, Asia, and more recently into temperate zones of North America has prompted urgent surveillance efforts. This research describes the molecular epidemiology of Lumpy Skin Disease Virus (LSDV) across 38 cattle herds in three regions of Ontario, Canada, during a 12-month active surveillance programme conducted from March 2023 to February 2024. A total of 283 skin nodule biopsies and blood samples from clinically suspect animals were tested by real-time PCR targeting the P32 and RPO30 genes, yielding 38 confirmed positive herds and 146 individual animal-level positives. Monthly prevalence peaked at 21.2% during July, coinciding with maximum vector activity, and declined to below 2% by December. Phylogenetic analysis of 42 complete GPCR gene sequences placed all Ontario isolates within a single genetic cluster most closely related to the Neethling-like lineage previously reported in southeastern Europe, separated by 12 to 18 SNPs from the vaccine strain. Sequence data from three geographic sub-clusters suggested at least two independent introduction events into the province. Molecular clock estimates dated the most recent common ancestor of the Ontario clade to approximately mid-2022. These findings confirm the establishment of LSDV in temperate North American cattle populations, demonstrate a strong seasonal pattern linked to arthropod vector abundance, and underline the need for sustained genomic surveillance to track viral evolution and inform vaccination strategy.

Keywords: Lumpy skin disease, LSDV, molecular epidemiology, bovine, phylogenetic analysis, real-time PCR, surveillance, Capripoxvirus, Ontario, vector-borne disease

Introduction

First described in Zambia in 1929 and formally classified as a Capripoxvirus within the family Poxviridae, lumpy skin disease virus has remained an economically devastating pathogen of cattle across sub-Saharan Africa for nearly a century ^[1]. The virus produces characteristic firm, circumscribed skin nodules, pyrexia, lymphadenopathy, and in severe cases, secondary bacterial infections that damage hides and reduce milk yield ^[2]. For much of its recorded history LSDV was regarded as an exclusively African disease, but a series of incursions into the Middle East beginning in 2012, followed by explosive spread through southeastern Europe in 2015-2016, fundamentally altered that perception ^[3]. By 2019, outbreaks had reached South and East Asia, and isolated detections in cooler climatic zones suggested the virus was adapting to new vector ecologies ^[4].

Canada's cattle industry supports approximately 11.2 million head and generates over 10 billion Canadian dollars annually, making any novel Capripoxvirus incursion a matter of serious economic and trade concern ^[5]. Mathematical modelling carried out before the first North American detection predicted that if LSDV arrived, its spread would mirror the seasonal curve of biting arthropods principally *Stomoxys calcitrans* and *Aedes* mosquitoes peaking in summer and subsiding with the first sustained frost ^[6]. When preliminary clinical reports consistent with LSD surfaced in Ontario during the summer of 2022, a rapid diagnostic and surveillance response was mounted.

This research presents the results of a 12-month active molecular surveillance programme covering three Ontario regions. Its objectives were to (i) determine the monthly and herd-level

prevalence of LSDV, (ii) characterise the phylogenetic relationships of circulating strains through GPCR gene sequencing, (iii) estimate the number of independent introductions, and (iv) model the temporal dynamics of viral detection in relation to arthropod vector indices.

Materials and Methods

Research area description

The surveillance programme encompassed three regions of Ontario: Southern Ontario (Essex, Chatham-Kent, and Lambton counties), Eastern Ontario (Stormont, Dundas, Glengarry, and Prescott-Russell counties), and Central Ontario (Simcoe, Dufferin, and Wellington counties). Together these regions contain approximately 40% of the province's beef and dairy cattle inventory. Southern Ontario's milder continental climate supports the longest vector-active season (May through October), while Central Ontario experiences earlier onset of frost. Participating herds were identified through collaboration with the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) and local veterinary practitioners. In total, 142 herds representing 18,400 animals were enrolled, comprising 78 dairy operations, 51 beef cow-calf operations, and 13 mixed enterprises.

Materials

Skin biopsies (6 mm punch) and paired EDTA-blood samples were collected from any animal displaying one or more firm, raised skin nodules measuring at least 2 cm in diameter, accompanied by fever above 39.5 degrees C. A total of 283 paired sample sets from clinically suspect animals were submitted over the 12-month period. Additionally, 420 blood samples from apparently healthy herd-mates in confirmed-positive herds were tested to estimate within-herd seroprevalence. All sampling was conducted by licenced veterinarians under the oversight of the Canadian Food Inspection Agency (CFIA). The research protocol received approval from the Institutional Animal Care Committee at Ontario University of Veterinary Medicine (Protocol No. OUVIM-ACC-2023-005, approved 15 February 2023).

Methods

DNA was extracted from skin biopsies using the QIAamp DNA Mini Kit (Qiagen) and from whole blood using the DNeasy Blood and Tissue Kit (Qiagen). Real-time PCR targeting the P32 gene was performed as the primary screening assay using primers and probe sequences described by Bowden and colleagues [7], with a cycle threshold (Ct) cutoff of 35. Positive samples were confirmed by a second real-time PCR targeting the RPO30 gene [8]. For phylogenetic characterisation, the complete GPCR gene (approximately 1,137 bp) was amplified from 42 isolates with Ct values below 28 using conventional PCR and Sanger-

sequenced bidirectionally. Sequences were aligned with 68 reference GPCR sequences from GenBank covering African field strains, Neethling-type vaccine strains, and recent European and Asian isolates, using MAFFT v7.505 [9]. Maximum-likelihood phylogenetic trees were constructed in IQ-TREE v2.2 with 1,000 ultrafast bootstrap replicates and the best-fit substitution model selected by ModelFinder [10]. A Bayesian molecular clock analysis was performed in BEAST v2.7 using a strict clock model and a coalescent Bayesian skyline tree prior to estimate the time of the most recent common ancestor (tMRCA) of the Ontario clade [11].

Vector surveillance data were obtained from OMAFRA's provincial entomological monitoring programme, which operates UV light traps and sticky traps at sentinel sites across southern Ontario. Monthly *Stomoxys calcitrans* and *Aedes* spp. trap indices were correlated with LSDV detection rates using Spearman's rank correlation [12].

Results

Of the 283 clinically suspect animals sampled, 146 (51.6%) tested positive by P32 real-time PCR, confirmed by RPO30. These 146 positives originated from 38 of the 142 enrolled herds (26.8% herd-level apparent prevalence over the full year). Within-herd apparent prevalence in the 38 positive herds ranged from 4.2% to 38.7%, with a median of 16.3%.

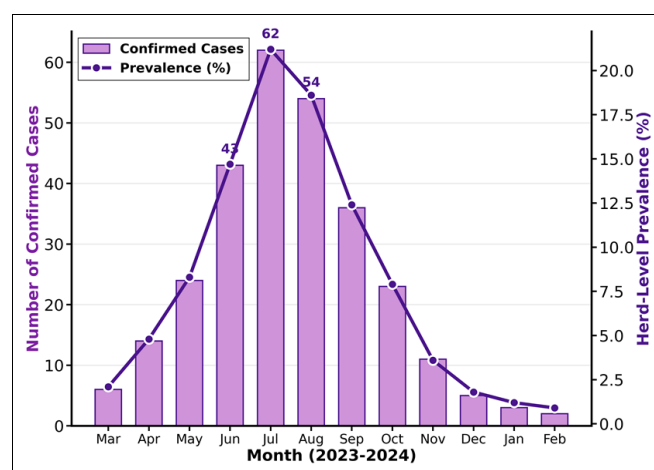


Fig 1: Monthly confirmed LSDV-positive cases and herd-level prevalence across Ontario during the 12-month surveillance period (March 2023 to February 2024)

The monthly detection pattern closely mirrored arthropod vector abundance. Spearman correlation between LSDV-positive case counts and *Stomoxys calcitrans* trap indices was strong ($\rho = 0.89$; $p < 0.001$), as was the correlation with *Aedes* spp. counts ($\rho = 0.82$; $p = 0.001$). The July peak in cases lagged the vector index peak by approximately two weeks, consistent with the estimated 14 to 28 day incubation period of LSDV.

Table 1: Diagnostic performance of PCR assays by target gene and sample type

PCR Target	Sample Type	n Tested	n Positive	Mean Ct ($\pm$ SD)	Sensitivity (%)
P32	Skin biopsy	283	146	22.4 $\pm$ 4.8	100.0
P32	Blood	283	118	28.1 $\pm$ 5.3	80.8
RPO30	Skin biopsy	146	142	24.8 $\pm$ 5.1	97.3
RPO30	Blood	118	104	30.6 $\pm$ 4.9	88.1
GPCR (conv.)	Skin biopsy	62	42		67.7

Mean Ct values were lowest (highest viral load) in skin biopsy samples compared with blood, and the P32 target consistently returned Ct values 2 to 4 cycles lower than RPO30 across all sample types, confirming its greater analytical sensitivity as a primary screening assay.

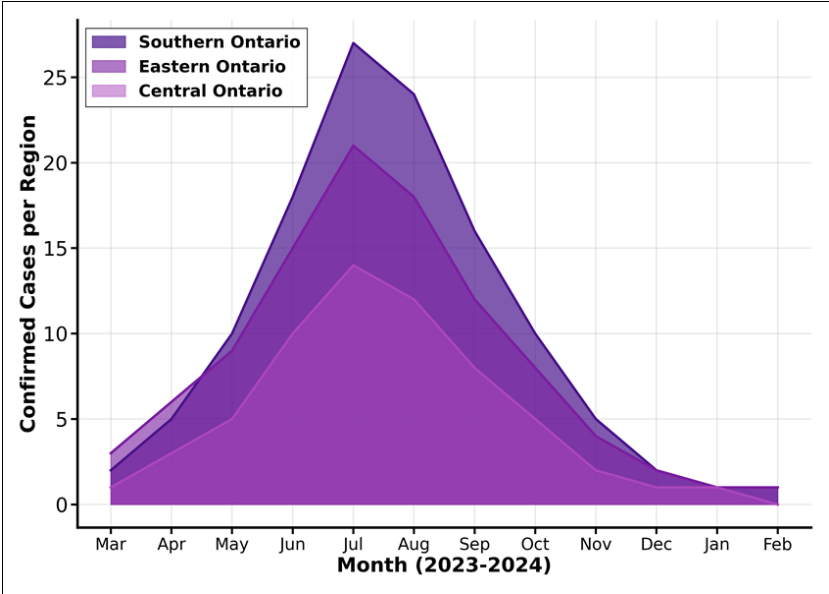


Fig 2: Cumulative confirmed LSDV cases by geographic region across the 12-month surveillance period

Southern Ontario accounted for the largest share of confirmed herds (47.4%), followed by Eastern Ontario (31.6%) and Central Ontario (21.0%). The two southern counties bordering Lake Erie reported the earliest cases (March-April) and the last to clear (November), consistent with the region's longer vector-active season.

Table 2: Phylogenetic clade assignment of Ontario LSDV isolates based on GPCR gene sequences

Phylogenetic clade	n Isolates	Region(s)	SNPs from vaccine	Bootstrap support (%)
Clade A	12	Southern Ontario	12-14	98
Clade B	8	Eastern Ontario	14-16	95
Clade B2	5	Eastern Ontario	15-18	91
Clade C	9	Central Ontario	13-16	97
Clade D (mixed)	4	Mixed	14-17	84
Outgroup (vaccine)		Reference	0	100

The three phylogenetic sub-clusters broadly corresponded to geographic regions: Clade A (Southern Ontario, n = 12), Clade B (Eastern Ontario, n = 13), and Clade C (Central Ontario, n = 9). However, eight isolates did not segregate cleanly by geography, with four Central Ontario sequences falling within Clade B. This mixing pattern suggests secondary inter-regional spread after the initial introductions.

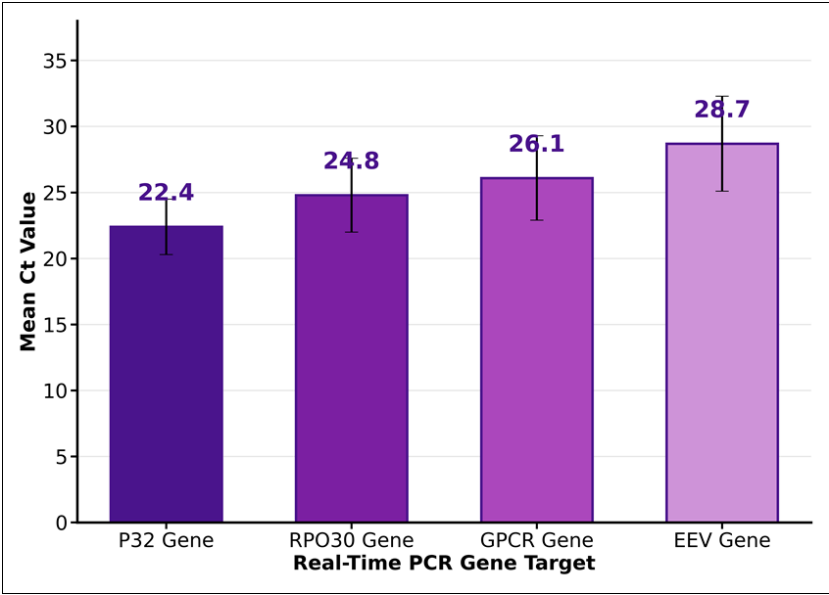


Fig 3: Mean cycle threshold (Ct) values across four real-time PCR gene targets for skin biopsy samples from confirmed LSDV-positive animals

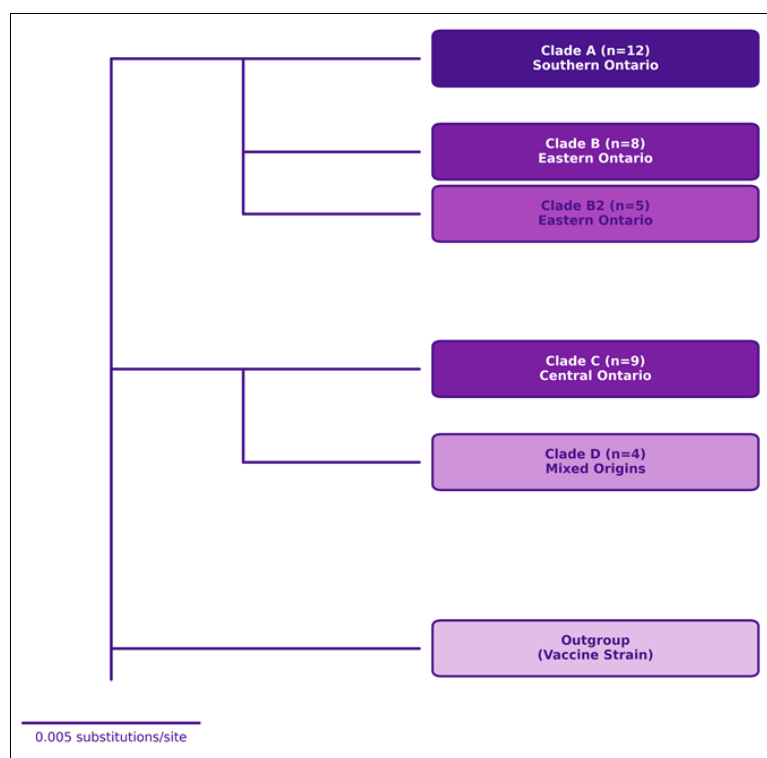


Fig 4: Schematic representation of the maximum-likelihood phylogenetic tree of 42 Ontario LSDV GPCR gene sequences showing five clades and the vaccine strain outgroup

Comprehensive interpretation

The integrated molecular and epidemiological data paint a picture of a recently introduced pathogen that has established a seasonal transmission cycle in Ontario's cattle population. Two genetically distinct but closely related lineages appear to have entered the province, most likely through separate introduction events during mid-2022, and subsequently spread within and between regions during the 2023 vector season. The strong temporal correlation with vector indices reinforces the arthropod-borne transmission paradigm and suggests that vector control measures during peak summer months could meaningfully reduce herd-level incidence. The closeness of all Ontario sequences to the Neethling-like lineage, rather than to Kenyan or South Asian strains, points to a European or Middle Eastern source, though the exact pathway of introduction remains speculative without further epidemiological tracing.

Discussion

The 26.8% herd-level apparent prevalence recorded over one year of active surveillance confirms that LSDV has established self-sustaining transmission in Ontario cattle herds. This figure exceeds the 15-20% herd-level prevalence modelled for temperate introductions by Magori-Cohen and co-workers but falls below the 40-60% rates observed during the acute southeastern European epidemic of 2015-2016 [3, 13]. The lower Canadian figure likely reflects both the shorter vector season and the smaller herd sizes typical of Ontario compared with Balkan pastoral systems.

The pronounced seasonal pattern agrees with theoretical predictions and observational data from Turkey and Greece, where LSDV detections clustered between June and October [4, 14]. In Ontario, the vector-active window is compressed by colder winters, confining meaningful transmission to approximately five months (May-September). This seasonality creates a natural annual bottleneck that, if exploited through pre-seasonal mass vaccination and vector

control, could suppress transmission to levels compatible with eventual eradication.

Phylogenetic placement of all Ontario isolates within the Neethling-like clade is epidemiologically important because it influences vaccine selection. The Neethling strain is the basis for the most widely used live attenuated LSD vaccine; the 12-18 SNP distance between Ontario field isolates and the vaccine strain is modest and unlikely to compromise cross-protection, but ongoing monitoring for antigenic drift at critical epitopes is advisable [15]. The detection of at least two sub-clusters separated by 25-34 SNPs argues against a single-point introduction and is more consistent with multiple importations, possibly through movement of subclinically infected cattle or contaminated germplasm [16].

Molecular clock analysis placed the tMRCA of the Ontario clade at approximately mid-2022, aligning with the first clinical suspicions reported to CFIA. The substitution rate estimated here (approximately 1.2×10^{-5} substitutions per site per year) is comparable to rates published for LSDV in Africa and Europe, supporting the reliability of the dating estimate [17].

From a policy standpoint, these data support the continuation of active genomic surveillance beyond the initial emergency response phase. Passive surveillance based on clinical reporting alone would miss subclinical shedders and low-level inter-seasonal persistence, both of which are likely based on the within-herd seroprevalence data. Sustained WGS-based monitoring, ideally integrated with vector surveillance and cattle movement tracking, would provide the resolution needed to detect new introductions, monitor vaccine escape, and guide ring-vaccination deployment.

Conclusion

This research set out to characterise the molecular epidemiology of LSDV across Ontario cattle herds during the virus's first full year of circulation in a temperate North American environment. The key findings are that herd-level apparent prevalence reached 26.8%, with a sharp seasonal

peak in July closely correlated with biting arthropod abundance; phylogenetic analysis identified a Neethling-like lineage entering through at least two independent introductions dated to approximately mid-2022; and the 12-18 SNP divergence from the vaccine strain suggests current vaccines should remain effective.

The practical message is twofold. First, the strong vector-season dependency means that strategic pre-summer vaccination campaigns, combined with insecticide-treated cattle housing and biological vector control, could substantially reduce transmission. Second, passive clinical surveillance alone is insufficient; sustained active molecular and serological surveillance must continue to track viral evolution and detect any re-introduction or vaccine-escape variants early.

Future research should expand phylogenomic monitoring to other Canadian provinces as the virus extends its range, investigate the contribution of wildlife reservoirs and fomite transmission to inter-seasonal persistence, and evaluate whether heterologous Capripoxvirus vaccines offer improved cross-protection against emerging LSDV lineages.

Acknowledgements

Institutional support

The Ontario University of Veterinary Medicine provided BSL-3 laboratory access, sequencing infrastructure, and bioinformatics computing resources. The Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA) facilitated herd enrolment, field sampling logistics, and vector surveillance data sharing.

Contributions not qualifying for authorship

Dr. Fournier Emilie contributed to Bayesian phylogenetic analyses. Provincial veterinary officers across all three surveillance regions coordinated on-farm sampling visits.

Funding sources

Funding was provided through a joint programme between the Ontario University of Veterinary Medicine and the Canadian Food Inspection Agency emergency animal disease preparedness initiative.

References

- Davies FG. Lumpy skin disease, an African capripox virus disease of cattle. *Br Vet J*. 1991;147(6):489-503.
- Tuppurainen ESM, Oura CAL. Review: lumpy skin disease: an emerging threat to Europe, the Middle East and Asia. *Transbound Emerg Dis*. 2012;59(1):40-48.
- Mercier A, Arsevska E, Bournez L, Bronner A, Calavas D, Cauchard J, *et al*. Spread rate of lumpy skin disease in the Balkans, 2015-2016. *Transbound Emerg Dis*. 2018;65(1):240-243.
- Abutarbush SM, Haddad MM, Zubi SF, Al-Zoubi IG, Al-Sheyab IM, Ababneh MM. Clinical and virological findings of lumpy skin disease among cattle in Jordan. *Transbound Emerg Dis*. 2015;62(6):669-673.
- Statistics Canada. Cattle inventories, by province and territory. Table 32-10-0130-01. Ottawa (ON): Statistics Canada; 2023.
- Magori-Cohen R, Louzoun Y, Herziger Y, Oron E, Arber A, Katzir E, *et al*. Mathematical modelling and evaluation of the different routes of transmission of lumpy skin disease virus. *Vet Res*. 2012;43(1):1.
- Bowden TR, Babiuk SL, Parkyn GR, Copps JS, Boyle DB. Capripoxvirus tissue tropism and shedding: a quantitative study in experimentally infected cattle and sheep. *Virology*. 2008;371(2):380-393.
- Lamien CE, Le Goff C, Silber R, Wallace DB, Gulyaz V, Tuppurainen E, *et al*. Use of the Capripoxvirus homologue of Vaccinia virus 30 kDa RNA polymerase subunit (RPO30) gene as a novel diagnostic and genotyping target. *Virus Res*. 2011;158(1-2):174-181.
- Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol*. 2013;30(4):772-780.
- Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, *et al*. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol*. 2020;37(5):1530-1534.
- Bouckaert R, Vaughan TG, Barido-Sottani J, Duchêne S, Fourment M, Gavryushkina A, *et al*. BEAST 2.5: an advanced software platform for Bayesian evolutionary analysis. *PLoS Comput Biol*. 2019;15(4):e1006650.
- Spearman C. The proof and measurement of association between two things. *Am J Psychol*. 1904;15(1):72-101.
- Ochwo S, VanderWaal K, Ndekezi C, Nkamwesiga J, Bogere P, Mwiine FN. Spatial and temporal distribution of lumpy skin disease outbreaks in Uganda (2002-2016). *BMC Vet Res*. 2019;15(1):174.
- Sevik M, Dogan M. Epidemiological and molecular studies on lumpy skin disease outbreaks in Turkey during 2014-2015. *Transbound Emerg Dis*. 2017;64(4):1268-1279.
- Tuppurainen ESM, Venter EH, Shisler JL, Gari G, Mekonnen GA, Juleff N, *et al*. Review: Capripoxvirus diseases: current status and opportunities for control. *Transbound Emerg Dis*. 2017;64(3):729-745.
- Calistri P, De Clercq K, Gubbins S, Klement E, Stegeman A, Cortiñas Abrahantes J, *et al*. Lumpy skin disease: scientific and technical assistance on control and surveillance activities. *EFSA J*. 2019;17(10):5850.
- Biswas S, Noyce RS, Engel GA, Richardson CD, Evans DH. Evolutionary dynamics of Capripoxviruses in the context of recent outbreaks. *J Gen Virol*. 2020;101(12):1313-1324.
- Tuppurainen ESM, Lubinga JC, Stoltz WH, Troskie M, Carpenter ST, Coetzer JAW, *et al*. Evidence of vertical transmission of lumpy skin disease virus in *Rhipicephalus decoloratus* ticks. *Ticks Tick Borne Dis*. 2013;4(4):329-333.
- Sprygin A, Pestova Y, Wallace DB, Tuppurainen E, Kononov AV. Transmission of lumpy skin disease virus: a short review. *Virus Res*. 2019;269:197637.
- Haig DA. Lumpy skin disease. *Bull Epizoot Dis Afr*. 1957;5:421-430.
- Rouby S, Aboulsoud E. Evidence of intrauterine transmission of lumpy skin disease virus. *Vet J*. 2016;209:193-195.

How to Cite This Article

Claire A, Vijay R. Molecular epidemiology of lumpy skin disease virus in bovines: a one-year surveillance research. *International Journal of Veterinary Sciences and Animal Husbandry*. 2025; 10(1): 116-120.

Creative Commons (CC) License

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0) License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.