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Epidemiology

Seroprevalence and associated risk factors of epizootic hemorrhagic disease virus in cattle from the northern region of Peru: first serological report

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ABSTRACT. Epizootic hemorrhagic disease virus (EHDV) is a vector-borne pathogen that affects both wild and domestic ruminants. Climate influences vector-borne diseases by driving vector migration to new areas, where they spread the virus. However, the lack of surveillance in some areas hinders accurate assessment the true disease burden. This study aimed to determine the seroprevalence of EHDV and associated risk factors in cattle from the northern region of Peru in 2022. Blood serum samples were collected from 578 cattle in the departments of Tumbes, Piura, Lambayeque, Cajamarca, and La Libertad and analyzed using cELISA. The overall EHDV seroprevalence was 17.82% (95% confidence interval 14.78–21.19). The highest proportion of seropositive animals was observed in two deparments closest to the equator, Piura and Tumbes (50%), followed by Lambayeque (39.1%) and Cajamarca (11.08%). In addition, the highest seroprevalence was found at temperatures ≤20°C (50.46%), wind speeds ≤3 m/sec (31.43%), and altitudes ≤1,260 masl (37.28%), with a significant decrease at higher elevations ($P<0.05$). After adjusting for temperature, the odds of EHDV seropositivity were significantly lower in cattle from areas located at >2,000 to ≤3,290 masl (Odds ratio [OR]=0.15) and >3,290 (OR=0.07), compared to those from the reference altitude category of ≤1,260 masl. Similarly, after adjusting for altitude, cattle from deparments with temperatures >20°C had significantly lower odds of seropositivity (OR=0.17) compared to those exposed to temperatures ≤20°C. This study reports anti-EHDV antibodies in cattle from northern Peru for the first time, highlighting associations with bioclimatic factors.

KEYWORDS: epizootic hemorrhagic disease virus, orbivirus, risk factors, seroprevalence, tropics

INTRODUCTION

The epizootic hemorrhagic disease virus (EHDV) is an arbovirus belonging to the Sedoreoviridae family that is distributed worldwide [11, 16]. This virus is transmitted by hematophagous biting midges of the genus Culicoides and affects both wild and domestic ruminants. Furthermore, it is included in the list of notifiable diseases by the World Organisation for Animal Health (WOAH) [11].

EHDV targets multiple organs due to its infection of mononuclear phagocytes and endothelial cells, in which it induces apoptosis, leading to hemorrhage and thrombosis [17]. Hyperacute infection causes sudden death due to pulmonary vascular injury and subsequent edema, associated with a cytokine storm [8]. Although infection in cattle is generally less severe than in wild ruminants, such as deer, recent outbreaks in cattle have demonstrated increased virulence, with epizootics reported in various regions globally [11]. In cattle, infection with EHDV is typically subclinical, but may occasionally present with fever, lethargy, oral lesions, lameness, decreased milk production, and other clinical signs. Morbidity is generally low, ranging from 1% to 18%, and mortality is rare; however, certain serotypes, such as EHDV-6 and EHDV-7, have been associated with more severe outbreaks in specific regions [24].

Traditionally, the distribution of EHDV has been limited to temperate and tropical climates that support vector populations. However, in recent years, the distribution and severity of arboviruses have changed considerably, largely attributed to climate change [2, 14]. In South America, updated reports on EHDV are scarce, although molecular identification of two serotypes (EHDV-1 and EHDV-6) has been documented in asymptomatic cattle in Ecuador [28].

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In Peru, there are currently no serological or molecular reports of EHDV. However, the bluetongue virus has been detected in sheep and Culicoides insignis [22], a midge species that could potentially also serve as a vector for EHDV. Thus, it is plausible that EHDV is circulating within Peruvian territory, particularly in the northern region, which encompasses a diversity of ecoregions and is geographically close to the equator. Therefore, this study aimed to determine the seroprevalence of EHDV in cattle, as well as to identify associated risk factors, in addition to its geographic distribution within the northern region of Peru.

MATERIALS AND METHODS

Sampling area

Peru is located in the central-western region of South America, encompassing an area of 1,285,216 km^2 and divided into 24 departments. For agricultural and health purposes, the country is classified into four major regions—North, South, Center, and East—according to the Servicio Nacional de Sanidad Agraria del Perú (SENASA). This study was conducted in the northern region, which includes the departments of Tumbes, Piura, Lambayeque, La Libertad, and Cajamarca.

This region is characterized by a tropical to subtropical climate, with monthly average temperatures ranging from 15°C to 35°C, and annual rainfall between 500 mm and 1,500 mm, depending on elevation and proximity to the coast or Andes. According to the Instituto Nacional de Estadística e Informática (INEI), cattle farming is well established in the North, Center, and South, while sheep production is concentrated in the South, and goat farming is mainly found along the coastal areas of the North and Center. Notably, there is currently no vaccination program for epizootic hemorrhagic disease virus (EHDV) control in Peru.

Sample size and sample collection

In the absence of national data on EHDV in mammals, the minimum sample size was estimated using a presumed prevalence of 50%, a 95% confidence level, a 5% margin of error, and an infinite population. The total number of animals was proportionally distributed across each department, based on data from the IV Censo Nacional Agropecuario (2012) conducted by the Instituto Nacional de Estadística e Informática of Peru [9].

Cattle of both sexes, various ages (from 6 months to 7 years), with no apparent clinical signs, were sampled. Blood was collected by coccygeal or jugular venipuncture, depending on feasibility, using a vacuum system and additive-free tubes after cleaning the puncture site. Samples were left to rest and then centrifuged to separate the serum.

Detection of antibodies against EHDV

Serum samples were tested using a competitive ELISA with the ID Screen® EHDV Competition kit (IDVet, France, city of France). Optical density (OD) readings were taken at 450 nm. The percentage of inhibition (S/N%) was calculated as: S/N%=(sample OD / negative control OD) ×100. Samples with an S/N%≤30% were considered positive; those with values between >30% and <40% were considered doubtful; and samples with an S/N%≥40% were classified as negative. Samples with doubtful results were retested to confirm their status as either positive or negative. If a doubtful result was obtained again, the sample was classified as negative.

Data acquisition

Each sampling site was georeferenced, and climatic variables (temperature, wind speed, precipitation, and elevation) were obtained from the NASA POWER Data Access Viewer (https://power.larc.nasa.gov/) using the site coordinates. We used annual mean values for all variables at each sampling site. Annual mean temperature, precipitation, and wind speed reflect the average across 24 hr and capture daily fluctuations. Elevation was taken directly from the coordinates of each site.

In addition, sex was recorded through direct observation during blood sample collection. Age was obtained verbally from the owner at the time of sample registration on the data sheet, where the sample code and animal information were documented.

Statistical analysis

EHDV seroprevalence was calculated by dividing the number of positive samples by the total number of samples analyzed, then multiplying by 100%. Confidence intervals (CI) at 95% were also calculated. In cases where seroprevalence was 0%, Hanley's rule of 3 was applied: CI (0.3/n), if n>30 [5].

Independent variables were categorized according to previous arbovirus studies. These included: age (years): ≤2 and >2 [21]; altitude (masl): ≤1,260; >1,260– <2,000; >2,000– <3,290, and >3,290 [4, 7]; accumulated rainfall (mm): ≤700 and >700 [30]; temperature (°C): ≤20 and >20 [19], and wind speed (m/sec): ≤3 and >3 [29]. To evaluate possible associations between independent variables (age, sex, temperature, wind speed, rainfall, elevation, and department) and the dependent variable (EHDV serostatus), the χ^2 test or Fisher's exact test was used.

Binary logistic regression models were applied within the same category to evaluate the odds ratio (OR) for seropositivity to EHDV. Additionally, a multivariable logistic regression analysis was performed, using forward stepwise selection based on the Akaike Information Criterion (AIC). The model was built iteratively by adding variables that reduced the AIC, and the final model with the lowest AIC value was selected. Two-way interactions between variables were evaluated during model building; however, their inclusion resulted in a slight increase in AIC, and they were not retained in the final model. The unit of analysis was the individual animal. Adjusted OR were estimated using STATA version 16, with statistical significance set at P<0.01.

Ethical statement

The study was approved by the Comité de Ética y Bienestar Animal of the Faculty of Veterinary Medicine at the National University of San Marcos (Code CEBA No. 2022-30), Lima, Peru.

RESULTS

The overall seroprevalence of EHDV in cattle from the northern region of Peru was 17.82% (95% CI: 14.78–21.19). The highest proportions of seropositive animals were observed in the departments of Piura and Tumbes, both geographically closest to the equatorial line, with a seroprevalence of 50%. This was followed by Lambayeque (39.1%) and Cajamarca (11.08%). In contrast, no seropositive cases were detected in La Libertad, the department located farthest from the equator (Fig. 1).

The results of the χ^2 test and Fisher's exact test showed that the origin of the cattle (department), ambient temperature, wind speed, and altitude were all significantly associated with the presence of anti-EHDV antibodies ($P<0.05$). The highest proportions of seropositive cases were recorded in cattle exposed to temperatures ≤20°C (50.46%), wind speeds ≤3 m/sec (31.43%), and altitudes ≤1,260 masl (37.28%), with a progressive decline in seroprevalence at higher altitudes (Table 1).

Binary logistic regression revealed that higher temperature, greater altitude, and increased wind speed were associated with decreased OR for EHDV seropositivity, indicating that these variables acted as protective factors (Table 2). The variable 'deparment' was not analyzed due to one category having zero cases. Likewise, the variables 'sex' and 'age' were not included, as they were not statistically significant in the χ^2 analysis.

Multivariable logistic regression analysis identified altitude and temperature as the only factors significantly associated with EHDV seropositivity in cattle (Table 2). After adjusting for temperature, cattle located at altitudes between >2,000 and ≤3,290 masl had significantly lower odds of seropositivity (OR=0.15; 95% CI: 0.08–0.29), and those at altitudes >3,290 masl had even lower odds (OR=0.07; 95% CI: 0.02–0.32), compared to the reference group located at ≤1,260 masl. Similarly, after adjusting for altitude, cattle from areas with temperatures >20°C showed significantly reduced odds of seropositivity (OR=0.17; 95% CI: 0.10–0.29), relative to cattle in areas with temperatures ≤20°C. These findings indicate that cattle from lower-altitude and cooler-temperature areas had higher odds of being EHDV seropositive (Table 3). The variable 'wind speed' was not included in the multivariable analysis because a 3 × 2 contingency table showed zero positive EHDV cases, which invalidates the regression model.

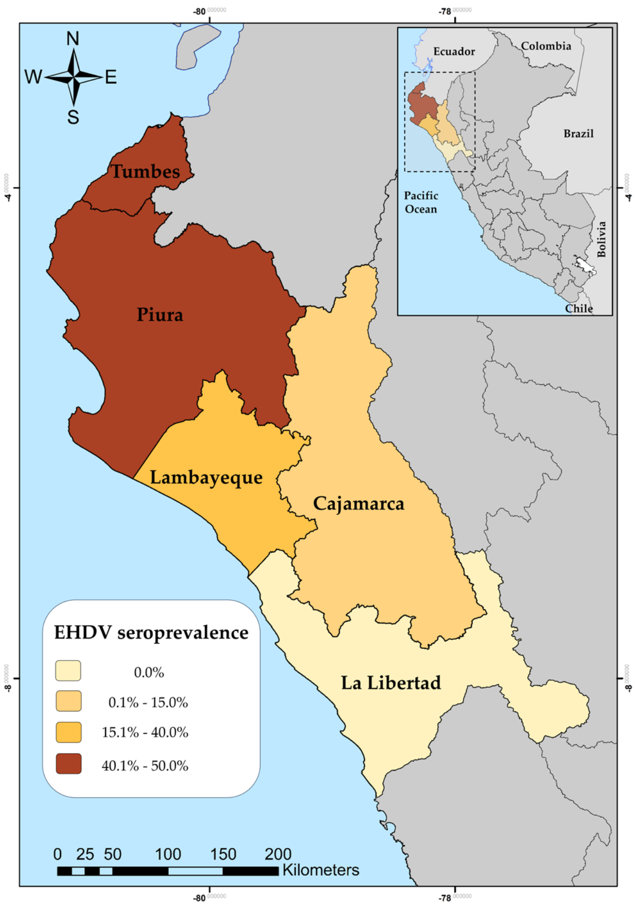

Fig. 1. Geographic distribution and seroprevalence ranges of epizootic hemorrhagic disease virus (EHDV)-positive cases in cattle from the northern region of Peru.

Table 1. Results of the χ^2 test and Fisher's exact test for the independent variables associated with seropositivity to epizootic hemorrhagic disease virus (EHDV)

Variable	Samples	Positive	Prevalence (%)	95% CI	*P*-value
Department					<0.001*(C)
Tumbes	6	3	50.00	11.81–88.19	
Piura	96	48	50.00	39.62–60.38	
Lambayeque	44	15	39.10	20.49–49.92	
La Libertad	98	0	0.00	0.00–3.06	
Cajamarca	334	37	11.08	7.92–14.95	
Temperature (°C)					<0.001*(C)
≤20	109	55	50.46	40.72–60.16	
>20	469	48	10.23	7.64–13.34	
Altitude (masl)					<0.001*(C)
≤1,260	169	63	37.28	29.97–45.04	
>1,260–≤2,000	91	23	25.27	16.75–35.47	
>2,000–≤3,290	247	15	6.07	3.43–9.82	
>3,290	71	2	2.81	0.34–9.80	
Windy speed (m/sec)					<0.001*(F)
≤3	315	99	31.43	26.34–36.87	
>3	263	4	1.52	0.42–3.85	
Accumulated rainfall (mm)					0.073(F)
≤700	572	100	17.48	26.34–36.87	
>700	6	3	50.00	11.81–88.18	
Sex					0.825(C)
Famale	484	87	17.96	14.66–21.69	
Male	94	16	17.02	10.05–26.16	
Age (years)					0.480(C)
≤2	185	36	19.46	14.02–25.91	
>2	393	67	17.05	13.46–21.14	
Total	578	103	17.82	14.78–21.19	

CI: confidence interval. *Statistically significant (P<0.01). χ^2 test (C) and Fisher's exact test (F).

Table 2. Results of binary logistic regression analysis of risk factors associated with seropositivity to epizootic hemorrhagic disease virus (EHDV) in cattle in the northern region of Peru

Variable/Category	Odds ratio	95% CI	*P*-value
Temperature (°C)			
≤20	Ref.	-	
>20	0.11	0.07–0.19	<0.001*
Altitude (masl)			
≤1,260	Ref.	-	
>1,260–≤2,000	0.57	0.32–1.00	0.051
>2,000–≤3,290	0.11	0.06–0.20	<0.001*
>3,290	0.05	0.01–0.21	<0.001*
Windy speed (m/sec)			
≤3	Ref.	-	
>3	0.03	0.01–0.09	<0.001*
Accumulated rainfall (mm)			
≤700	0.21	0.04–1.07	0.060
>700	Ref.	-	

CI: confidence interval. *Statistically significant (P<0.01).

Table 3. Results of multivariable logistic regression analysis of risk factors associated with seropositivity to epizootic hemorrhagic disease virus (EHDV) in cattle in the northern region of Peru

Variable	Odds ratio	95% CI	*P*-value
Altitude (masl)			
≤1,260	Ref.	-	-
>1,260–≤2,000	0.54	0.29–1.01	0.050
>2,000–≤3,290	0.15	0.08–0.29	<0.001*
>3,290	0.07	0.02–0.32	0.001*
Temperature (°C)			
≤20	Ref.	-	-
>20	0.17	0.10–0.29	<0.001*

CI: confidence interval. *Statistically significant (P<0.01).

DISCUSSION

The overall seroprevalence of EHDV in cattle from the northern region of Peru was 17.8%, representing the first serological evidence of this virus in the country. In contrast, a study conducted in Ecuador, a country neighboring Peru to the north, reported a markedly higher seroprevalence of 81.3% in cattle [28]. Although research on EHDV in South American cattle remains limited, the virus has recently been confirmed in cervids in Brazil [3]. Likewise, in Mexico, a country located at a higher latitude and farther from the equator, the presence of EHDV was documented for the first time in deer [18].

In other parts of the world, seroprevalence in cattle has varied widely, with reports from China (57.9%) [13], Pakistan (66.9%) [10], and France, where seroprevalence reached 82.6% in southern regions affected by clinical outbreaks of EHDV-8 [1]. These differences may be attributed to several factors, including circulating serotypes, vector exposure intensity, local transmission dynamics, climatic conditions, and recent outbreak activity. In the case of Peru, the relatively lower seroprevalence may reflect limited viral circulation, lower vector pressure, or potentially a recent introduction of the virus, compounded by the historical lack of surveillance that may have delayed its recognition.

Temperature and altitude were both associated with anti-EHDV seropositivity and were identified as significant risk factors, with higher seroprevalence found in areas with temperatures ≤20°C and altitudes ≤1,260 masl. These two variables are interdependent, as lower altitudes tend to correspond with higher temperatures, conditions favorable to the development of vectors. Indeed, temperatures ranging from 16 to 20°C have been associated with prolonged developmental times in *Culicoides subadults* [27]. Similar results have been observed under other environmental settings, where greater *Culicoides* abundance was reported at lower altitudes and mean temperatures ranging from 11 to 18°C [20].

Rainfall was not associated with cattle seropositivity, possibly because precipitation levels were relatively homogeneous across all sampling sites. While rainfall is known to provide breeding sites and promote favorable conditions for vector development [20, 26], some studies have reported no significant influence of precipitation. For instance, Quaglia *et al*. [23] observed that *Culicoides* remained active year-round, with their presence more closely linked to stream flow and water temperature than to rainfall itself. Sex and age were also not significantly associated with EHDV seropositivity, as similar prevalence rates were observed across categories (P >0.05). These results are consistent with a previous study from Turkey [25].

Studies conducted in France reported high seroprevalence among cattle over 24 months of age, with no notable differences by age or sex in areas of intense viral circulation [1]. In contrast, a slightly higher seroprevalence was observed in females in Libya, although this was not statistically significant [15]. Research in southern Europe indicated that cows older than five years were at a higher risk of developing severe clinical signs of EHDV [6], while in Pakistan, seroprevalence was reported to be up to six times higher in older animals compared to younger ones [10]. These discrepancies may reflect differences in the stage of viral circulation, the serotypes involved, the sensitivity of diagnostic tests, or region-specific livestock management practices. Taken together, these findings highlight the need to interpret seroepidemiological patterns through locally informed and integrative approaches.

Although the geographic origin (department) was not identified as a risk factor with the presence of anti-EHDV antibodies, we observed high seroprevalence in departments located closer to the equatorial line. In Ecuador, high levels of EHDV serotype circulation have been reported in cattle [28], suggesting that this territory may serve as a potential source of viral dissemination, supported by a higher abundance of infected vectors facilitating transmission. This pattern appears to be influenced by bioclimatic factors such as temperature, altitude, and wind speed, given that the studied regions share similar environmental characteristics [12]. The absence of seropositive animals in La Libertad may be explained by its coastal location and greater distance from the equator, where coastal winds could limit the presence of competent vectors.

The emergence of EHDV in previously unreported regions has been associated in the literature with factors such as climate change, which may facilitate the expansion of vector populations into newly suitable habitats [2, 14]. However, our study does not provide direct evidence linking the detection of the virus in Peru to such environmental changes.

In the Peruvian context, the presence of EHDV is more likely related to the historical lack of surveillance and research on arboviruses in both wild and domestic animals. Although Peru lies within the tropical zone, it exhibits significant climatic heterogeneity. The northern region where this study was conducted includes tropical areas that may favor the presence of *Culicoides* midges, known vectors of other arboviruses such as bluetongue virus [11, 22]. This suggests that EHDV circulation in the region may not be recent but rather previously undetected, underscoring the urgent need for comprehensive studies to assess its prevalence, serotype diversity, host range, and potential connections with neighboring countries.

The findings of this study underscore the importance of maintaining and strengthening national serological surveillance systems, particularly in regions with ecological conditions favorable to arbovirus circulation. Early detection of EHDV would allow for the implementation of more effective control measures, thereby mitigating its impact on animal health and livestock economies.

In conclusion, this study reports, for the first time, the presence of antibodies against EHDV in cattle from the northern region of Peru, with a seroprevalence of 17.82% (ranging from 0% to 50%, depending on the department), confirming the active circulation of the virus in Peruvian territory. Seroprevalence was significantly associated with temperature, altitude, wind speed, and department of origin. Moreover, areas with altitudes ≤1,260 masl and temperatures ≤20°C were identified as significant risk factors for EHDV seropositivity, as these conditions favor the presence and activity of *Culicoides* vectors, thereby facilitating viral transmission in susceptible hosts.

CONFLICTS OF INTEREST. The authors have no conflicts of interest to declare.

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REFERENCES

1. Anthonioz C, Abadie Y, Reversat E, Lafargue A, Delalande M, Renaudineau T, Delobel L, Verdeille N, Ngwa-Mbot D, Gache K, Garin E, Corbiere F. 2025. Heterogenous within-herd seroprevalence against epizootic hemorrhagic disease virus type 8 (EHDV-8) after massive virus circulation in cattle in France, 2023. *Front Vet Sci* **12**: 1562883. [CrossRef]
2. Baylis M, Caminade C, Turner J, Jones AE. 2017. The role of climate change in a developing threat: the case of bluetongue in Europe. *Rev Sci Tech* **36**: 467–478. [CrossRef]
3. Caixeta EA, Pinheiro MA, Lucchesi VS, Oliveira AGG, Galinari GCF, Tinoco HP, Coelho CM, Lobato ZIP. 2024. The study of bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV) circulation and vectors at the Municipal Parks and Zoobotanical Foundation of Belo Horizonte, Minas Gerais, Brazil (FPMZB-BH). *Viruses* **16**: 293. [CrossRef]
4. Duan YL, Bellis G, Li L, Li HC, Miao HS, Kou ML, Liao DF, Wang Z, Gao L, Li JZ. 2019. Potential vectors of bluetongue virus in high altitude areas of Yunnan Province, China. *Parasit Vectors* **12**: 464. [CrossRef]
5. Eypasch E, Lefering R, Kum CK, Troidl H. 1995. Probability of adverse events that have not yet occurred: a statistical reminder. *BMJ* **311**: 619–620. [CrossRef]
6. González-Recio O, Fernández A, Jiménez Montero JA. 2025. Epidemiological and genetic factors affecting severe epizootic hemorrhagic disease in Spanish Holstein cattle during the Southern Europe outbreak of 2023. *J Dairy Sci* **108**: 3850–3857. [CrossRef]
7. Green AL, Dargatz DA, Schmidtmann ET, Herrero MV, Seitzinger AH, Ostlund EN, Wagner BA, Moser KM, Wineland NE, Walton TE. 2005. Risk factors associated with herd-level exposure of cattle in Nebraska, North Dakota, and South Dakota to bluetongue virus. *Am J Vet Res* **66**: 853–860. [CrossRef]
8. Howerth EW. 2015. Cytokine release and endothelial dysfunction: a perfect storm in orbivirus pathogenesis. *Vet Ital* **51**: 275–281.
9. INEI. 2013. IV Censo Nacional Agropecuario 2012. In: Instituto Nacional de Estadística e Informática. https://censos.inei.gob.pe/cenagro/tabulados/?id=CensosNacionales. Accessed 24 Jan 2025.
10. Ishaq M, Jamal SM, Teodori L, Leone A, Bonfini B, Spedicato M, Savini G. 2025. Serological evidence of epizootic hemorrhagic disease and serotypes of epizootic hemorrhagic disease virus in Pakistan. *Acta Trop* **267**: 107675. [CrossRef]
11. Jiménez-Cabello L, Utrilla-Trigo S, Lorenzo G, Ortego J, Calvo-Pinilla E. 2023. Epizootic hemorrhagic disease virus: current knowledge and emerging perspectives. *Microorganisms* **11**: 1339. [CrossRef]
12. Jury MR, Alfaro-Garcia LE. 2024. Peruvian North Coast climate variability and regional ocean–atmosphere forcing. *Coasts* **4**: 508–534. [CrossRef]
13. Lv MN, Zhu JB, Liao SQ, Yang ZX, Lin XH, Qi NS, Chen QL, Wu CY, Li J, Cai HM, Zhang JF, Hu JJ, Xiao WW, Zhang X, Sun MF. 2023. Seroprevalence of epizootic hemorrhagic disease virus in Guangdong cattle farms during 2013–2017, China. *Viruses* **15**: 1263. [CrossRef]
14. Maclachlan NJ, Zientara S, Wilson WC, Richt JA, Savini G. 2019. Bluetongue and epizootic hemorrhagic disease viruses: recent developments with these globally re-emerging arboviral infections of ruminants. *Curr Opin Virol* **34**: 56–62. [CrossRef]
15. Mahmoud A, Danzetta ML, di Sabatino D, Spedicato M, Alkhatal Z, Dayhum A, Tolari F, Forzan M, Mazzei M, Savini G. 2021. First seroprevalence investigation of epizootic haemorrhagic disease virus in Libya. *Open Vet J* **11**: 301–308. [CrossRef]
16. Matthijnssens J, Attoui H, Bányai K, Brussaard CPD, Danthi P, Del Vas M, Dermody TS, Duncan R, Fāng Q, Johne R, Mertens PPC, Jaafar FM, Pstton JT, Sasaya T, Suzuki N, Wei T. 2022. ICTV virus taxonomy profile: *Sedoreoviridae* 2022. *J Gen Virol* **103**: 001782. [CrossRef]
17. McLaughlin BE, DeMaula CD, Wilson WC, Boyce WM, MacLachlan NJ. 2003. Replication of bluetongue virus and epizootic hemorrhagic disease virus in pulmonary artery endothelial cells obtained from cattle, sheep, and deer. *Am J Vet Res* **64**: 860–865. [CrossRef]
18. Merino JO, la Cruz NID, Galvan G, De León AP, Burnes J. 2019. First molecular and serological detection of Epizootic Hemorrhagic Disease virus in white tailed deer (*Odocoileus virginianus*) from Tamaulipas, Mexico. *Arq Bras Med Vet Zootec* **71**: 77–85. [CrossRef]
19. Möhlmann TWR, Keeling MJ, Wennergren U, Favia G, Santman-Berends I, Takken W, Koenraadt CJM, Brand SPC. 2021. Biting midge dynamics and bluetongue transmission: a multiscale model linking catch data with climate and disease outbreaks. *Sci Rep* **11**: 1892. [CrossRef]
20. Mukhopadhyay E, Hazra S, Saha GK, Banerjee D. 2017. Altitudinal variation and bio-climatic variables influencing the potential distribution of *Culicoides orientalis* Macfie, 1932, suspected vector of Bluetongue virus across the North Eastern Himalayan belt of Sikkim. *Acta Trop* **176**: 402–411. [CrossRef]
21. Munmun TK, Islam S, Zamil S, Rahman Md A, Abedin J, Ahad A, Islam A. 2022. Seroprevalence and risk factors of bluetongue virus in sheep of Chattogram, Bangladesh. *Vet World* **15**: 1589–1594. [CrossRef]
22. Navarro D, Rojas M, Jurado J, Manchego A, Ramírez M, Castillo AK, Rivera H. 2019. Molecular detection of Bluetongue virus in *Culicoides insignis* and sheep of Pucallpa, Peru. *Rev Investig Vet Peru* **30**: 465–476. [CrossRef]
23. Quaglia AI, Blosser EM, McGregor BL, Runkel AE, Sloyer KE, Erram D, Wisely SM, Burkett-Cadena ND. 2020. Tracking community timing: pattern and determinants of seasonality in *Culicoides* (Diptera: Ceratopogonidae) in Northern Florida. *Viruses* **12**: 931. [CrossRef]
24. Savini G, Afonso A, Mellor P, Aradaib I, Yadin H, Sanaa M, Wilson W, Monaco F, Domingo M. 2011. Epizootic heamorragic disease. *Res Vet Sci* **91**: 1–17. [CrossRef]
25. Şevik M. 2022. Seroprevalence of the epizootic hemorrhagic disease virus in cattle in the Aegean region of Turkey. Proceedings of the ISPEC 9th International Conference on Agriculture, Animal Sciences and Rural Development, Burdur, Turkey, March 9–20, 2022.
26. Thepparat A, Kamata N, Siriyasatien P, Prempree W, Dasuntad K, Chittsamart B, Sanguansub S. 2024. Seasonal abundance and diversity of culicoides biting midges in livestock sheds in Kanchanaburi Province, Thailand. *Insects* **15**: 701. [CrossRef]
27. Van den Eynde C, Sohier C, Matthijs S, De Regge N. 2021. Temperature and food sources influence subadult development and blood-feeding response of *Culicoides obsoletus* (sensu lato) under laboratory conditions. *Parasit Vectors* **14**: 300. [CrossRef]
28. Verdezoto J, Breard E, Viarouge C, Quenault H, Lucas P, Sailleau C, Zientara S, Augot D, Zapata S. 2018. Novel serotype of bluetongue virus in South America and first report of epizootic haemorrhagic disease virus in Ecuador. *Transbound Emerg Dis* **65**: 244–247. [CrossRef]
29. Wittmann EJ, Baylis M. 2000. Climate change: effects on culicoides -transmitted viruses and implications for the UK. *Vet J* **160**: 107–117. [CrossRef]
30. Wittmann EJ, Mellor PS, Baylis M. 2002. Effect of temperature on the transmission of orbiviruses by the biting midge, *Culicoides sonorensis*. *Med Vet Entomol* **16**: 147–156. [CrossRef]