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Short communication

Molecular Evidence of Epizootic Haemorrhagic Disease Virus and Bluetongue Virus Circulation in Wild Ruminants in Namibia.

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Abstract

Bluetongue virus (BTV) and epizootic haemorrhagic disease virus (EHDV) are Culicoides-borne orbiviruses affecting domestic and wild ruminants. Information on their circulation in Namibian wildlife is limited. This study investigated the molecular detection and serotype distribution of BTV and EHDV in wild ruminants from a commercial game farm in the Khomas Region, Namibia, where wildlife and livestock coexist. Between June and September 2019, spleen samples from 62 clinically healthy animals (kudu, oryx, and red hartebeest) were analysed by real-time RT-PCR using pan-BTV and pan-EHDV assays, followed by serotype-specific tests for selected BTV types. Two animals (3.23%) tested positive for EHDV. BTV RNA was detected in 24/62 animals (38.71%), with Ct values ranging from 28.3 to 38.4. BTV-3 and BTV-4 were the most frequently identified serotypes, while one sample was positive for BTV-1; six BTV-positive samples remained untyped. High Ct values and low RNA loads likely limited sequencing success. Although restricted to a single farm and a limited serotype panel, this study provides preliminary molecular evidence of BTV and EHDV circulation in Namibian wild ruminants, highlighting the need for broader epidemiological investigations at the wildlife–livestock interface.

Keywords

BTV, EHDV, RT-PCR, Namibia, Wildlife

Bluetongue virus (BTV) and epizootic haemorrhagic disease virus (EHDV) are vector-borne viruses transmitted by biting midges of the genus *Culicoides* (Verwoerd and Erasmus, 2004). Both infect domestic and wild ruminants and belong to the family *Sedoreoviridae*, genus *Orbivirus* (Mellor et al., 2009; Matthijnssens et al., 2022). To date, 36 BTV serotypes have been described, including 24 classical and 12 atypical serotypes (Ries et al., 2020; 2021), whereas seven serotypes have been identified for EHDV (Martinez et al., 2025). Both viruses are endemic in many African regions and have repeatedly been introduced into Europe (Cappai et al., 2019; Lorusso et al., 2023; Gondard et al., 2024; Martinez et al., 2025; Plebani et al., 2025; Marcacci et al., 2026). In southern Africa, particularly in the Republic of South Africa, high BTV serotype diversity has been documented (Gerdes, 2004; Coetzee et al., 2012; Van Schalkwyk et al., 2023). EHDV has also been reported in several African countries, including Sudan, South Africa, Kenya, and Zimbabwe (Mohammed and Mellor, 1990; Barnard and Meiswinkel, 1998; Toye et al., 2013; Gordon et al., 2017; Chiuya et al., 2024). In Namibia, information on EHDV circulation is lacking, and molecular data on circulating BTV serotypes are limited. A previous study based on serology identified antibodies against several BTV serotypes in cattle from the Otjozondjupa Region (Molini et al., 2018). However, molecular investigations in wild ruminants are scarce, and no studies have applied real-time RT-PCR directly to field spleen samples from game species.

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The aim of this study was to investigate the molecular detection and serotype distribution of BTV and EHDV in wild ruminants from a commercial game farm in the Khomas Region of Namibia, in order to provide preliminary data on virus circulation in a setting where wild and domestic ruminants coexist.

Between June and September 2019, spleen samples were collected from 62 wild ruminants on a commercial livestock and game farm located approximately 45 km from Windhoek. The farm covers about 10,000 hectares and hosts cattle, goats, sheep, and free-ranging game species hunted for meat production. The sampled species were kudu (*Tragelaphus strepsiceros*, n = 2), oryx (*Oryx gazella*, n = 26), and red hartebeest (*Alcelaphus buselaphus caama*, n = 34). All animals were born and raised on the same farm, were older than three years, and appeared clinically healthy at slaughter. This study was conducted on a single farm and included a limited number of animals; therefore, the results reflect only this specific population and cannot be generalised to other regions or management systems. Total RNA was extracted from spleen homogenates using the MagMAX™ CORE Nucleic Acid Purification Kit (Applied Biosystems, Austin, TX, USA) on a KingFisher™ Flex Purification System (Thermo Fisher Scientific, MA, USA), according to the manufacturer's instructions. All samples were screened using the VetMAX™ EHDV Kit (Applied Biosystems, Austin, TX, USA) and the VetMAX™ BTV NS3 All Genotypes Kit (Applied Biosystems, Austin, TX, USA) (pan-EHDV and pan-BTV assays). BTV-positive samples were further tested using (i) the VetMAX™ European BTV Typing Kit (Applied Biosystems, Austin, TX, USA) (serotypes -1, -2, -4, -6, -8, -9, -11, and -16); (ii) an in-house real-time RT-PCR targeting segment 2 of BTV-3 (Lorusso et al., 2018), and (iii) published assays for BTV-25, -26, and -27 (Hoffmann et al., 2010; Maan et al., 2012; Zientara et al., 2014). At the time of the study (2019), validated molecular assays were available only for a subset of classical serotypes. Consequently, not all known BTV serotypes could be investigated. Whole-genome sequencing was attempted on selected samples but was unsuccessful, most likely due to low viral RNA loads. Detection frequencies and 95% confidence intervals were calculated using the binomial Wilson score method.

Two samples tested positive for pan-EHDV (3.23%): one kudu (Ct 38) and one red hartebeest (Ct 37). Twenty-four samples (38.71%) tested positive for pan-BTV, with Ct values ranging from 28.3 to 38.4. Only one sample was positive for BTV-1 (Ct 30). Samples positive for BTV-3 showed Ct values between 25.8 and 38.1, and those positive for BTV-4 between 32.0 and 38.0. Six pan-BTV-positive samples could not be assigned to any of the tested serotypes. BTV-3 and BTV-4 were the most frequently detected serotypes in this population. The distribution of positive results is summarised in Table I. Prevalence estimates are reported in Table II.

		Positive to		Typing			
Animal	**N° samples (F/M)**	**EHDV (F/M)**	**BTV (F/M)**	**BTV-1 (F/M)**	**BTV-3 (F/M)**	**BTV-4 (F/M)**	**Neg to typing (F/M)**
Kudu	2 (2/0)	1 (1/0)	1 (1/0)	0 (0/0)	0 (0/0)	1 (1/0)	0 (0/0)
Oryx	26 (12/14)	0 (0/0)	8 (5/3)	0 (0/0)	3 (2/1)	3 (2/1)	2 (1/1)
Red hartebeest	34 (18/16)	1 (1/0)	15 (10/5)	1 (0/1)	4 (3/1)	6 (4/2)	4 (3/1)
TOTAL	**62 (32/30)**	**2 (2/0)**	**24 (16/8)**	**1 (0/1)**	**7 (5/2)**	**10 (7/3)**	**6 (4/2)**

Table I. Number of samples collected per species and sex, with PCR results for EHDV and BTV, including the distribution of identified BTV serotypes. Note: "F" for females, "M" for "males", and "Neg" for negative.

Animal	**EHDV % (95% CI)**	**BTV % (95% CI)**	**BTV-1 % (95% CI)**	**BTV-3 % (95% CI)**	**BTV-4 % (95% CI)**	**Neg to typing % (95% CI)**
Kudu	50.00 (9.59-90.41)	50.00 (9.59-90.41)	0.00 (0.00-97.50)	0.00 (0.00-97.50)	100.00 (2.50-100.00)	0.00 (0.00-97.50)
Oryx	0.00 (0.00-12.97)	30.77 (15.88-50.09)	0.00 (0.00-36.87)	37.50 (15.18-65.04)	37.50 (15.18-65.04)	25.00 (7.15-59.07)
Red hartebeest	2.94 (0.15-14.58)	44.12 (28.48-60.53)	6.67 (1.18-27.23)	26.67 (11.84-49.22)	40.00 (21.90-61.27)	26.67 (11.84-49.22)
TOTAL	**3.23 (0.89-11.09)**	**38.71 (26.63-51.05)**	**4.17 (0.74-20.43)**	**29.17 (15.65-47.30)**	**41.67 (25.52-59.98)**	**25.00 (12.44-44.99)**

Table II: Prevalence of EHDV and BTV by species, based on PCR results, including the distribution of identified BTV serotypes. Note: "Neg" for negative.

Among females, 50.00% (95% CI: 33.63-66.37) tested positive for BTV and 6.25% (95% CI: 1.73-20.15) for EHDV, whereas among males 26.67% (95% CI: 14.18-44.94) tested positive for BTV and none for EHDV. These figures should be interpreted descriptively, given the limited sample size and the study design.

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The relatively high Ct values observed in several samples (up to 38.4) indicate low amounts of viral RNA. Such results should be interpreted cautiously, as high Ct values may reflect low-level viral RNA detection, residual viral material, or subclinical infection, rather than active viral replication. The low RNA concentrations likely contributed to the unsuccessful sequencing attempts and prevented further characterisation of untyped strains.

Compared with previous serological evidence from Namibia (Molini et al., 2018), the present study provides molecular confirmation of BTV circulation in wild ruminants on a game farm. However, given the single-farm design, the limited number of animals, and the restricted panel of molecular assays, no conclusions can be drawn regarding regional prevalence, temporal trends, or the full spectrum of circulating serotypes. The identity of untyped strains remains undetermined, and further investigations using broader molecular panels and higher-quality samples would be required to clarify their classification.

A higher proportion of females tested positive for BTV compared to males in this dataset. However, the study was not designed to assess sex-related risk factors, and the sample size does not allow robust statistical inference. Therefore, this observation should be considered descriptive only.

In conclusion, this study provides preliminary molecular evidence of BTV and EHDV RNA detection in wild ruminants from a commercial game farm in central Namibia. The findings suggest BTV circulation in this specific setting, while EHDV detection was limited. Broader studies including multiple farms, larger sample sizes, and comprehensive molecular characterisation are needed to better understand the epidemiology of these orbiviruses in Namibian wildlife and their potential interface with domestic livestock.

Ethical approval

No ethical approval was required. All included samples were collected from carcasses in an abattoir.

Conflict of interest

The authors do not have any conflict of interest.

Author Contributions

Conceptualization: Umberto Molini; Methodology: Umberto Molini, Alessio Lorusso, and Ottavio Portanti; Formal analysis: Umberto Molini, Maria Yvonne Hemberger, Nicandro Rodi, Mariassunta Iannetta, and Ottavio Portanti; Investigation: Maria Yvonne Hemberger, and Juliet Kabajani; Writing original draft preparation: Umberto Molini, and Gloria Plebani; Writing, review and editing: Umberto Molini, Gloria Plebani, Alessio Lorusso, and Ottavio Portanti; Supervision: Umberto Molini

All authors have read and agreed to the published version of the manuscript.

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References

Barnard, B. J., Gerdes, G. H., & Meiswinkel, R. (1998). Some epidemiological and economic aspects of a bluetongue-like disease in cattle in South Africa--1995/96 and 1997. The Onderstepoort journal of veterinary research, 65(3), 145–151.

Cappai, S., Rolesu, S., Loi, F., Liciardi, M., Leone, A., Marcacci, M., Teodori, L., Mangone, I., Sghaier, S., Portanti, O., Savini, G., & Lorusso, A. (2019). Western Bluetongue virus serotype 3 in Sardinia, diagnosis and characterization.

Transboundary and emerging diseases, 66(3), 1426–1431. https://doi.org/10.1111/tbed.13156

Chiuya, T., Fèvre, E. M., Okumu, N. O., Abdi, A. M., Junglen, S., & Borgemeister, C. (2024). Exposure to Arboviruses in Cattle: Seroprevalence of Rift Valley Fever, Bluetongue, and Epizootic Hemorrhagic Disease Viruses and Risk Factors in Baringo County, Kenya. Pathogens (Basel, Switzerland), 13(8), 613. https://doi.org/10.3390/pathogens13080613

Coetzee, P., Stokstad, M., Venter, E. H., Myrmel, M., & Van Vuuren, M. (2012). Bluetongue: a historical and epidemiological perspective with the emphasis on South Africa. Virology journal, 9, 198. https://doi.org/10.1186/1743-422X-9-198

Gerdes G. H. (2004). A South African overview of the virus, vectors, surveillance and unique features of bluetongue. Veterinaria italiana, 40(3), 39–42.

Gondard, M., Postic, L., Garin, E., Turpaud, M., Vorimore, F., Ngwa-Mbot, D., Tran, M. L., Hoffmann, B., Warembourg, C., Savini, G., Lorusso, A., Marcacci, M., Felten, A., Roux, A. L., Blanchard, Y., Zientara, S., Vitour, D., Sailleau, C., & Bréard, E. (2024). Exceptional Bluetongue virus (BTV) and Epizootic hemorrhagic disease virus (EHDV) circulation in France in 2023. Virus research, 350, 199489. https://doi.org/10.1016/j.virusres.2024.199489

Gordon, S. J. G., Bolwell, C., Rogers, C. W., Musuka, G., Kelly, P., Guthrie, A., Mellor, P. S., & Hamblin, C. (2017). A serosurvey of bluetongue and epizootic haemorrhagic disease in a convenience sample of sheep and cattle herds in Zimbabwe. The Onderstepoort journal of veterinary research, 84(1), e1–e5. https://doi.org/10.4102/ojvr.v84i1.1505

Hofmann, M. A., Renzullo, S., Planzer, J., Mader, M., Chaignat, V., & Thuer, B. (2010). Detection of Toggenburg Orbivirus by a segment 2-specific quantitative RT-PCR. Journal of virological methods, 165(2), 325–329. https://doi.org/10.1016/j.jviromet.2010.02.027

Lorusso, A., Cappai, S., Loi, F., Pinna, L., Ruiu, A., Puggioni, G., Guercio, A., Purpari, G., Vicari, D., Sghaier, S., Zientara, S., Spedicato, M., Hammami, S., Ben Hassine, T., Portanti, O., Breard, E., Sailleu, C., Ancora, M., Di Sabatino, D., Morelli, D., … & Savini, G. (2023). Epizootic Hemorrhagic Disease Virus Serotype 8, Italy, 2022. Emerging infectious diseases, 29(5), 1063–1065. https://doi.org/10.3201/eid2905.221773

Lorusso, A., Sghaier, S., Di Domenico, M., Barbria, M. E., Zaccaria, G., Megdich, A., Portanti, O., Seliman, I. B., Spedicato, M., Pizzurro, F., Carmine, I., Teodori, L., Mahjoub, M., Mangone, I., Leone, A., Hammami, S., Marcacci, M., & Savini, G. (2018). Analysis of bluetongue serotype 3 spread in Tunisia and discovery of a novel strain related to the bluetongue virus isolated from a commercial sheep pox vaccine. Infection, genetics and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases, 59, 63–71. https://doi.org/10.1016/j.meegid.2018.01.025

Maan, N. S., Maan, S., Belaganahalli, M. N., Ostlund, E. N., Johnson, D. J., Nomikou, K., & Mertens, P. P. (2012). Identification and differentiation of the twenty six bluetongue virus serotypes by RT-PCR amplification of the serotype-specific genome segment 2. PloS one, 7(2), e32601. https://doi.org/10.1371/journal.pone.0032601

Marcacci, M., Cappai, S., Palombieri, A., Puggioni, G., Plebani, G., Irelli, R., Rodi, N., Rocchigiani, A. M., Gatta, G., Teodori, L., Leone, A., Manunta, D., Portanti, O., Casaccia, C., Curini, V., Di Sabatino, D., Scroggs, S. L., Spedicato, M., & Lorusso, A. (2026). Emergence of Bluetongue virus serotype 5 in Sardinia- Italy, 2025. Veterinaria Italiana, 62(1). https://doi.org/10.12834/VetIt.3925.39028.1

Martínez, R., De Los Ángeles Risalde, M., Cano-Terriza, D., Lorusso, A., & Spedicato, M. (2025). From Africa to Europe: the rise of epizootic haemorrhagic disease virus serotype 8. Veterinaria italiana, 61(4), 10.12834/VetIt.3793.35560.1. https://doi.org/10.12834/VetIt.3793.35560.1

Matthijnssens, J., Attoui, H., Bányai, K., Brussaard, C. P. D., Danthi, P., Del Vas, M., Dermody, T. S., Duncan, R., Fāng [illegible], Q., Johne, R., Mertens, P. P. C., Mohd Jaafar, F., Patton, J. T., Sasaya [illegible], T., Suzuki [illegible], N., & Wei [illegible], T. (2022). ICTV Virus Taxonomy Profile: Sedoreoviridae 2022. The Journal of general virology, 103(10), 001782. https://doi.org/10.1099/jgv.0.001782

Mohammed, M. E., & Mellor, P. S. (1990). Further studies on bluetongue and bluetongue-related orbiviruses in the

Sudan. Epidemiology and infection, 105(3), 619–632. https://doi.org/10.1017/s0950268800048263

Molini, U., Capobianco Dondona, A., Hilbert, R., & Monaco, F. (2018). Antibodies against Schmallenberg virus detected in cattle in the Otjozondjupa region, Namibia. Journal of the South African Veterinary Association, 89(0), e1–e2. https://doi.org/10.4102/jsava.v89i0.1666

Plebani, G., Palombieri, A., Sghaier, S., Gatta, G., Ben Hassine, T., Curini, V., Thabet, S., Parolini, F., Hammami, S., Ancora, M., Spedicato, M., Di Sabatino, D., Marcacci, M., Scroggs, S. L. P., & Lorusso, A. (2025). Evolutionary Dynamics of Bluetongue virus serotypes 3, 4, and 8 circulating in Italy, 2024-2025. Veterinaria italiana, 61(4), 10.12834/VetIt.3915.38031.1. https://doi.org/10.12834/VetIt.3915.38031.1

Ries, C., Sharav, T., Tseren-Ochir, E. O., Beer, M., & Hoffmann, B. (2020). Putative Novel Serotypes '33' and '35' in Clinically Healthy Small Ruminants in Mongolia Expand the Group of Atypical BTV. Viruses, 13(1), 42. https://doi.org/10.3390/v13010042

Ries, C., Vögtlin, A., Hüssy, D., Jandt, T., Gobet, H., Hilbe, M., Burgener, C., Schweizer, L., Häfliger-Speiser, S., Beer, M., & Hoffmann, B. (2021). Putative Novel Atypical BTV Serotype '36' Identified in Small Ruminants in Switzerland. Viruses, 13(5), 721. https://doi.org/10.3390/v13050721

Toye, P. G., Batten, C. A., Kiara, H., Henstock, M. R., Edwards, L., Thumbi, S., Poole, E. J., Handel, I. G., Bronsvoort, B. M., Hanotte, O., Coetzer, J. A., Woolhouse, M. E., & Oura, C. A. (2013). Bluetongue and epizootic haemorrhagic disease virus in local breeds of cattle in Kenya. Research in veterinary science, 94(3), 769–773. https://doi.org/10.1016/j.rvsc.2012.11.001

Van Schalkwyk, A., Coetzee, P., Ebersohn, K., Von Teichman, B., & Venter, E. (2023). Widespread Reassortment Contributes to Antigenic Shift in Bluetongue Viruses from South Africa. Viruses, 15(7), 1611. https://doi.org/10.3390/v15071611

Verwoerd, D.W. & Erasmus, B.J. 2004. Bluetongue, in: Coetzer J.A.W., Tustin R.C. (Eds.), Infectious diseases of livestock, 2nd ed., Oxford University Press Southern Africa, Cape Town, pp. 1201–1220.

Zientara, S., Sailleau, C., Viarouge, C., Höper, D., Beer, M., Jenckel, M., Hoffmann, B., Romey, A., Bakkali-Kassimi, L., Fablet, A., Vitour, D., & Bréard, E. (2014). Novel bluetongue virus in goats, Corsica, France, 2014. Emerging infectious diseases, 20(12), 2123–2125. https://doi.org/10.3201/eid2012.140924