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Bluetongue Virus Serotype 1 in Wild Ruminants, France, 2008–10

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ABSTRACT: The persistence of Bluetongue virus serotype 1 (BTV-1) circulation was evaluated in red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), mouflons (*Ovis ammon*), and Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) sampled during two hunting seasons between September 2008 and February 2010 in the East Pyrenean Mountains, France. The prevalence of BTV antibody in red deer was high and not significantly different between the two hunting seasons (50.9% and 49.6%, respectively). The prevalence of BTV-1 RNA in red deer was 50.3% in 2008. Conversely, only 10.8% of samples from red deer were BTV-1 RNA–positive in 2010, and most of them showed only weak positive results. In other investigated species, the prevalence of infection was low. High elevation was associated with reduced infection rates and could explain the low prevalence observed in mouflons and Pyrenean chamois. These results support the hypothesis that, apart from red deer, wild ungulates are unlikely to be involved in the maintenance or circulation of BTV in the investigated region. Mass vaccination in livestock might have reduced BTV-1 circulation in red deer, although annual variation due to acquired immunity or fluctuations in vector abundance should also be considered.

Key words: Bluetongue serotype 1, epidemiology, mouflons, Pyrenean chamois, red deer, roe deer, survey.

In Europe, bluetongue virus (BTV) serotypes 1, 4, and 8 have been reported in red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), fallow deer (*Dama dama*), Spanish ibex (*Capra pyrenaica hispanica*), mouflon (*Ovis ammon*), and aoudad (*Ammotragus lervia*) (reviewed by Falconi et al., 2011), but, to date, data for Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) are limited (Rossi et al., 2010).

Many factors influence the persistence of BTV in wildlife and domestic ruminant populations. In livestock, the effective control of BTV infections has been achieved by mandatory vaccination and the protective immunity due to previous infections (Caporale and Giovannini, 2011). Data are scarce in wild animals. Our aim was to evaluate BTV prevalence in several wild species of ruminants, before and after its clearance in livestock.

The study took place in the French East Pyrenean Mountains (43°10′ to 42°24′N, 1°03′ to 3°00′E). This area is 0 to >3,000 m above sea level, and high densities of cattle and sheep graze up to above 2,000 m from spring to autumn. In this area, the first BTV outbreak in livestock was reported in late August 2008. By the end of 2008, >1,500 cattle, sheep, and goat farms (over approximately 3,200 registered production units) were recognized to be infected. The distribution and density of wild ungulates in the area have increased in the last decades, and their populations usually share pastures with domestic ruminants. Thus, we hypothesized that the exposure of wild animals to infected vectors was high during summer and autumn 2008. There was evidence for BTV-1 in all but one livestock farm, and mandatory vaccination using inactivated vaccines was initiated in late summer 2008 and repeated in spring 2009. Only one BTV-1–infected farm was detected in the study area by the end of 2009, and no further cases were reported in 2010. We also hypothesized that, due to the ecology of biting midges (Racloz et al., 2008), elevation and temperature strongly influence the course of bluetongue epidemics in wild ruminants.

From September to February of the 2008–09 and 2009–10 hunting seasons, 898 samples were collected from red deer (n=332), roe deer (n=185), mouflons

(n=74), and Pyrenean chamois (n=307). Blood and spleen samples were collected by hunters and stored at −20 C until analysis. Species, location, approximate age, and sex were recorded.

Serum samples were analyzed in duplicate using an anti-VP7 antibody enzyme-linked immunosorbent assay (ELISA; ID Screen Bluetongue Competition, IDVet, Montpellier, France) according to the manufacturer's instructions. This ELISA has been used in previous surveys in domestic and wild ungulates (Linden et al., 2008; Conraths et al., 2009). Spleen samples were tested for BTV RNA by two quantitative real-time reverse transcription–polymerase chain reactions (RT-qPCRs). The first detected all BTV serotypes (Taqvet BTV "All genotypes," LSI, Lissieu, France) and was applied to all available spleen samples. An RT-qPCR specific to the BTV-1 serotype (Taqvet BTV-1, LSI) was used on samples positive with the first assay. Finally, detection of BTV-8 and epizootic hemorrhagic disease virus (EHDV) RNA was implemented in samples that were positive by the group-specific PCR but negative for BTV-1 (Taqvet BTV-8 and TaqVet Epizootic Hemorrhagic Disease Virus, LSI).

Prevalence of BTV antibodies and viral RNA and exact binomial 95% confidence intervals (95% CI) were calculated for each species and for each hunting season. Between-group differences were assessed using the chi-squared test. To give a better estimate of the infection rate at the population level, serologic and virologic results were combined, and an animal was considered as BTV infected whenever it was positive for either BTV antibodies, RNA, or both. To investigate potential determinants of the infection risk, we fitted logistic regression models with BTV status as the binary response variable and sex, hunting season, month, elevation of sampling, and first-level interactions as fixed effects. Because mostly adult animals were hunted, the effect of age could not be properly investigated.

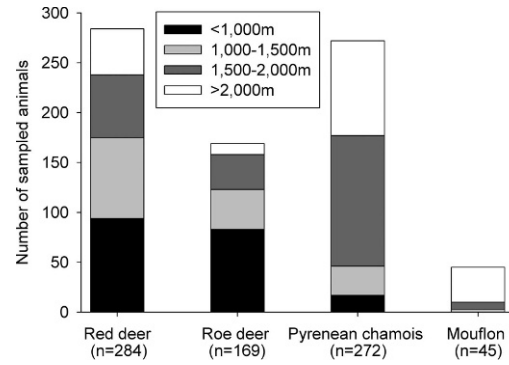

FIGURE 1. Distribution of sampled animals according to elevation (meters above sea level), East Pyrenean Mountains, France, 2008–10. The elevation of hunting was known for 770 of 898 animals sampled (85.7%). Red deer: *Cervus elaphus*; roe deer: *Capreolus capreolus*; mouflon: *Ovis ammon*; Pyrenean chamois: *Rupicapra pyrenaica pyrenaica*.

The elevation of hunting was available for 770 animals (85.74%) and reflected the ecology of the investigated species (Fig. 1). Distributions were significantly different between all species (P=0.003 to $P<10^{-4}$). Red deer and, to a lesser extent, roe deer were mostly present at low to middle elevations, while Pyrenean chamois and mouflons were, usually, sampled in higher areas. Within species, the elevation and geographic distributions of sampled animals were comparable between the two hunting seasons.

In roe deer, mouflons, and Pyrenean chamois, no or very few samples were found positive by both ELISA and RT-qPCR, yielding low prevalence estimates (Table 1). Moreover, among the four RNA-positive animals, threshold cycle (Ct) values were over 35.00 for one Pyrenean chamois and the two roe deer, with the possibility of false-positive results.

In red deer collected during the 2008–09 hunting season, anti-VP7 antibodies and BTV RNA were detected in 50.9% and 50.3% of samples, respectively. BTV-1 RNA was detected in 81 out of the 92 positive spleens (88%), with Ct values ranging between 24.80 and 38.40 (median 31.60). The BTV-1–negative spleens (n=11) were also negative for BTV-8 and EHDV.

TABLE 1. Bluetongue virus (BTV)-VP7 antibody and BTV RNA prevalence according to species and hunting season, East Pyrenean Mountains, France, 2008–10.

Species	BTV status	Hunting season			
		2008–09		2009–10	
		No. positive/no. tested (%)	95% CI[a]	No. positive/no. tested (%)	95% CI
Red deer (*Cervus elaphus*)	VP7 antibodies	83/163 (50.9)	43.0–58.8	57/115 (49.6)	40.1–59.0
	BTV RNA[b]	92/183 (50.3)	42.8–57.8	13/120 (10.8)	5.9–17.8
	Overall[c]	107/198 (54.0)	46.8–61.1	59/134 (44.0)	35.5–52.9
Roe deer (*Capreolus capreolus*)	VP7 antibodies	0/129 (0.0)	0.0–2.8		
	BTV RNA	2/173 (1.2)	0.1–4.1		
	Overall	2/184 (1.1)	0.1–3.9		
Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*)	VP7 antibodies	1/98 (1.0)	0.0–6.0	0/179 (0.0)	0.0–2.0
	BTV RNA	2/89 (2.2)	0.3–8.0	0/176 (0.0)	0.0–2.0
	Overall	2/108 (1.8)	0.2–6.5	0/199 (0.0)	0.0–1.8
Mouflon (*Ovis ammon*)	VP7 antibodies	0/44 (0.0)	0.0–8.0	0/20 (0.0)	0.0–17.0
	BTV RNA	0/43 (0.0)	0.0–8.0	0/27 (0.0)	0.0–13.0
	Overall	0/48 (0.0)	0.0–8.0	0/28 (0.0)	0.0–12.0

[a] Exact binomial 95% confidence interval (CI).

[b] Results are shown for the group-specific BTV reverse transcription–polymerase chain reactions only.

[c] Overall number of animals tested for antibodies and/or BTV RNA.

They were all from antibody-positive red deer and had shown weak positive results (mean Ct values 35.44±1.44) with the more sensitive group-specific RT-qPCR. Based on combined serologic and virologic results, the overall BTV infection prevalence for the 2008–09 hunting season was 54.0% (95% CI 46.83–61.13%).

During the 2009–10 hunting season, 49.6% of red deer tested positive for anti-VP7 antibodies. The BTV antibody prevalence was not significantly different between the two hunting seasons ($P=0.93$). Conversely, the proportion of BTV RNA–positive samples in red deer was significantly lower in 2009–10 ($P<10^{-4}$): Only 10.8% (13/120) tested positive for BTV and BTV-1 RT-qPCRs (Ct values between 33.27 and 37.94, only two values below 35.00). All these BTV RNA–positive red deer were also antibody-positive.

Finally, the influence of potential confounders on the binary BTV status could only be investigated in red deer. After controlling for the effect of gender ($P=0.40$), month of hunting ($P=0.92$), and hunting season ($P=0.06$), a strong, mostly log-linear effect of elevation was evidenced (global $P<10^{-4}$). The infection rate was significantly lower at high elevations than at low elevations (Table 2).

The epidemiologic significance of the weak positive results in the BTV RT-qPCRs (Ct values over 35.00) is still a matter of debate (MacLachlan et al., 1994), as they raise the question of false-positive samples. In earlier studies in red deer, small amounts of BTV-1 and BTV-8 RNA (Ct values above 34.00) have been retrieved 3 mo after experimental challenge, but no viable virus could be detected 78 days after inoculation (Lopez-Olvera et al., 2010). In our study, because virus isolation was not performed, the presence of virus in low RNA-positive animals could not be further investigated. However, because in most cases these animals were antibody-positive, this does not detract from the validity of our conclusions regarding the overall BTV infection prevalence.

As in livestock, BTV transmission in wild ruminants in Europe depends almost exclusively on *Culicoïdes* vectors (Falconi et al., 2011). *Culicoïdes* activity and

TABLE 2. Number of bluetongue virus (BTV)-VP7 antibody–positive and/or BTV RNA–positive samples in red deer (*Cervus elaphus*) according to elevation and hunting season, East Pyrenean Mountains, France, 2008–10. Percentages are given in parentheses.

	Elevation of hunting (m above sea level)				
Hunting season	<1000	1000–1500	1500–2000	≥2000	Total
2008–09[a]	46/55[b] (84)a	36/59 (61)b	9/34 (26)c	6/35 (17)c	97/183 (53.0)
2009–10[a]	28/39 (72)a	13/22 (59)a	6/29 (21)b	0/11 (0)b	47/101 (46.5)

[a] Within a row, percentages not sharing the same letter are significantly different ($P<0.01$).

[b] Number positive/number tested.

abundance, together with the capacity of the virus to replicate, are greatly reduced at temperatures below 10–15 C (Wittmann et al., 2002), and so transmission is seasonal in livestock. Elevation also influences infection risk in domestic ruminants (de Koeijer et al., 2011). We found that high elevations were associated with reduced infection rates in red deer. Even if we have no certainty about the precise territorial distribution of these free-ranging animals, results were robust to changes in elevation category thresholds. The absence of positive results in mouflons, which are known to be susceptible to BTV-1 (Fernandez-Pacheco et al., 2008) and BTV-4 (Rodriguez-Sanchez et al., 2010), could be related to the probably low vector density at high elevations. The same assumption may hold for Pyrenean chamois. These results therefore suggest that both mouflons and Pyrenean chamois are unlikely to be involved in the maintenance or circulation of BTV in the investigated region. The very low prevalence found in roe deer is in agreement with other studies in Spain, Belgium, and France (Falconi et al., 2011; Garcia-Bocanegra et al., 2011). Since roe deer were sampled at lower elevations than red deer, different levels of exposure to BTV vectors are unlikely. Rather, this suggests that roe deer is not a relevant species for the dissemination of BTV-1.

The prevalence of BTV antibody in red deer remained stable between the two hunting seasons. After natural infection or vaccination, neutralizing antibodies are known to persist for several years in red deer and domestic animals (Stallknecht et al., 1991; Oura et al., 2012). The fact that mostly adult animals were hunted in our study therefore precludes any interpretation of the variation in antibody prevalence.

We found a strong decrease in the proportion of BTV PCR-positive red deer samples between 2008 and 2010. Only weak positive results were found in the last hunting season. These results suggest that BTV-1 circulation was barely maintained in the study area in 2009–10. This is in agreement with a recent large-scale survey in southern Spain, which suggests that BTV-1 persisted at very low levels in wild ungulates in the absence of outbreak in livestock (Garcia-Bocanegra et al., 2011). This could indicate that efficient control measures applied in livestock in 2008 and 2009 in France have also affected BTV-1 circulation in wild ruminants. However annual variations in EHDV circulation are well documented in deer in North America, although not clearly understood (Stallknecht and Howerth, 2004). A reduction in the availability of susceptible hosts due to acquired immunity or fluctuations in vector abundance could therefore explain our findings. More years of surveillance are needed to fully assess the effects of control measures in livestock on the maintenance of BTV circulation in wild ruminants.

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LITERATURE CITED

Caporale, V., and A. Giovannini. 2011. Bluetongue control strategy, including recourse to vaccine: A critical review. Revue Scientifique et Technique (International Office of Epizootics) 29: 573–591.

Conraths, F. J., J. M. Gethmann, C. Staubach, T. C. Mettenleiter, M. Beer, and B. Hoffmann. 2009. Epidemiology of bluetongue virus serotype 8, Germany. Emerging Infectious Diseases 15: 433–435.

De Koeijer, A. A., G. J. Boender, G. Nodelijk, C. Staubach, E. Meroc, and A. R. Elbers. 2011. Quantitative analysis of transmission parameters for bluetongue virus serotype 8 in Western Europe in 2006. Veterinary Research 42: 53.

Falconi, C., J. R. Lopez-Olvera, and C. Gortazar. 2011. BTV infection in wild ruminants, with emphasis on red deer: A review. Veterinary Microbiology 151: 209–219.

Fernandez-Pacheco, P., J. Fernandez-Pinero, M. Aguero, and M. A. Jimenez-Clavero. 2008. Bluetongue virus serotype 1 in wild mouflons in Spain. Veterinary Record 162: 659–660.

Garcia-Bocanegra, I., A. Arenas-Montes, C. Lorca-Oro, J. Pujols, M. A. Gonzalez, S. Napp, F. Gomez-Guillamon, I. Zorrilla, E. S. Miguel, and A. Arenas. 2011. Role of wild ruminants in the epidemiology of bluetongue virus serotypes 1, 4 and 8 in Spain. Veterinary Research 42: 88.

Linden, A., B. Mousset, F. Gregoire, D. Hanrez, F. Vandenbussche, E. Vandemeulebroucke, T. Vanbinst, B. Verheyden, and K. De Clerck. 2008. Bluetongue virus antibodies in wild red deer in southern Belgium. Veterinary Record 162: 459.

Lopez-Olvera, J. R., C. Falconi, P. Fernandez-Pacheco, J. Fernandez-Pinero, M. A. Sanchez, A. Palma, I. Herruzo, J. Vicente, M. A. Jimenez-Clavero, M. Arias, J. M. Sanchez-Vizcaino, and C. Gortazar. 2010. Experimental infection of European red deer (*Cervus elaphus*) with bluetongue virus serotypes 1 and 8. Veterinary Microbiology 145: 148–152.

Maclachlan, N. J., R. A. Nunamaker, J. B. Katz, M. M. Sawyer, G. Y. Akita, B. I. Osburn, and W. J. Tabachnick. 1994. Detection of bluetongue virus in the blood of inoculated calves: Comparison of virus isolation, PCR assay, and in vitro feeding of *Culicoides variipennis*. Archives of Virology 136: 1–8.

Oura, C. A., L. Edwards, and C. A. Batten. 2012. Evaluation of the humoral immune response in adult dairy cattle three years after vaccination with a bluetongue serotype 8 inactivated vaccine. Vaccine 30: 112–115.

Racloz, V., G. Venter, C. Griot, and K. D. Stark. 2008. Estimating the temporal and spatial risk of bluetongue related to the incursion of infected vectors into Switzerland. BMC Veterinary Research 4: 42.

Rodriguez-Sanchez, B., P. J. Sanchez-Cordon, V. Molina, M. A. Risalde, A. C. De Diego, J. C. Gomez-Villamandos, and J. M. Sanchez-Vizcaino. 2010. Detection of bluetongue serotype 4 in mouflons (*Ovis aries musimon*) from Spain. Veterinary Microbiology 141: 164–167.

Rossi, S., P. Gibert, E. Bréard, M. Moinet, J. Hars, D. Maillard, M. Wanner, F. Klein, O. Mastain, P. Mathevet, and F. Bost. 2010. Circulation et impact des virus de la fièvre catarrhale ovine (FCO) chez les ruminants sauvages en France. Bulletin Epidémiologique Santé Animale Alimentation–Anses-DGAL 35: 28–32.

Stallknecht, D. E., and E. W. Howerth. 2004. Epidemiology of bluetongue and epizootic haemorrhagic disease in wildlife: Surveillance methods. Veterinaria Italiana 40: 203–207.

———, M. L. Kellogg, J. L. Blue, and J. E. Pearson. 1991. Antibodies to bluetongue and epizootic hemorrhagic disease viruses in a barrier island white-tailed deer population. Journal of Wildlife Diseases 27: 668–674.

Wittmann, E. J., P. S. Mello, and M. Baylis. 2002. Effect of temperature on the transmission of orbiviruses by the biting midge, *Culicoides sonorensis*. Medical and Veterinary Entomology 16: 147–156.

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