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Serological survey of Schmallenberg virus in red deer (Cervus elaphus), France, 2010-2012

Мониторинг распространения вируса Шмалленберг у благородного оленя (Cervus elaphus) с помощью серологического метода, Франция, 2010-2012

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РЕЗЮМЕ/SUMMARY

В последнее время вирус Шмалленберг появляется среди домашних и диких жвачных животных Европы. Для изучения распространения вируса в дикой природе мы провели серологические исследования сывороток благородного оленя, собранных в популяциях девяти различных регионов Франции с сентября 2010 по март 2012 г. Мы также показали обоснованность использования нового конкурентного метода ИФА для диагностики вируса Шмалленберг в дикой природе.

Ключевые слова: трансмиссивные инфекции, Orthobunyavirus, вирус Шмалленберг, дикая природа, серология, ИФА.

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The Schmallenberg virus is emerging in European domestic and wild ruminants. We investigated the serological status of nine red deer populations to describe virus spread from September 2010 to March 2012 over French wildlife. We also pinpointed the reliability of a new competitive ELISA method for SBV diagnosis in wildlife.

Keywords: vector-borne disease; Orthobunyavirus; SBV; wildlife; serology; ELISA.

Introduction

In summer and fall 2011 an unidentified disease was reported in Germany with decreased milk production, fever and diarrhea in dairy cattle. The virus associated with these clinical signs was identified as a new Orthobunyavirus of the Simbu serogroup named Schmallenberg virus (SBV) (1). This virus was later associated with abortions and congenital malformations in calves, lambs and kids in several European countries (2). Serological testing among wild cervids in Belgium revealed antibodies against Schmallenberg virus in roe deer (Capreolus capreolus) and red deer (Cervus elaphus) (3). Seroprevalence was already high (27% on average) in wild cervids in October 2011 in Belgium, suggesting that the virus began circulating months earlier (before August 2011). It has recently been showed that SBV already circulated in Culicoides vectors in Belgium during August and September 2011 (4). While SBV has been closely monitored among domestic ruminants in France, suggesting that clinical cases and antibodies appeared almost at the same time in 2011-2012 (5), few is known about the geographical spread on SBV virus in wildlife. To highlight this point, we conducted a serological study using sera collected in red deer over different regions in France.

The study

Blood samples from 502 shot-dead or captured red deer were collected within nine French departments (administrative units) during one or two sampling seasons (i.e., from September 2010 to January 2011 and from September 2011 to March 2012). The sera were first screened using an SBV indirect ELISA test (i-ELISA) yet validated in cattle, sheep and goats (ELISA ID Screen® Schmallenberg virus Indirect, bicupule, ID Vet, France) (6). The results were expressed as S/P values using the cut-off recommended for domestic species (S/P = (OD sample/OD positive control)*100): S/P<60%: negative; S/P>70%: positive and S/P between 60% and 70%: doubtful). Sera were also tested with a new competitive ELISA (c-ELISA) (ELISA ID Screen® Schmallenberg virus Competitive, ID Vet, France). Positive results to c-ELISA corresponded to a percentage of inhibition (PI) < 50, while doubtful if 40 > PI > 50 and negative when PI > 50. The antigen used in both c-ELISA and i-ELISA is the same N recombinant protein. A subset of samples were also submitted to a seroneutralization test according to a protocol previously described (SNT) (6).

From 502 sera, 492 could be tested using i-ELISA and 486 using the c-ELISA. The two ELISA methods exhibited 92.4% match (449/486). Since our samples taken from dead animals in non sterile conditions generated bacterian contaminations or cytotoxicity, conclusive SNT were available from 114 animals only: 64 samples with positive or doubtful i-ELISA results and 50 samples (S/P >20) with a negative i-ELISA. A large part of the sera positive or doubtful with ELISA methods were also found positive to SNT, suggesting a good specificity of both methods, even though slightly better for c-ELISA compared to i-ELISA (Table 1). Many sera negative either to i-ELISA or c-ELISA (all collected in 2011-2012) were found positive to SNT (Table 1). Even though the c-ELISA kit appeared slightly more sensitive than the i-ELISA kit, these results suggest that SNT is the most sensitive technique for detecting antibodies targeting the SBV in a recently infected population of red deer. These results are consistent with the fact that SNT and c-ELISA are able to detect IgG but also IgM antibodies when i-ELISA detects only IgG imunoglobulins that appear after the IgM adaptive response (E. Breard, pers. comm.). Considering the performance of

Table 1: Serological results observed among red deer sera tested with VNT, i-ELISA and c-ELISA.

	VNT method			
	97 sera VNT pos		17 sera VNT neg	
ELISA method	Positive or doubtful	negative	Positive or doubtful	negative
i-ELISA	57	40	7	10
c-ELISA	67	30	6	11
i-ELISA and c- ELISA	49	22	6	9

serological methods in that study, seroprevalence was finally estimated as the proportion of positive or doubtful sera using the c-ELISA kit.

The number of samples collected in each department, the proportion of positive and the date of first observation of seropositive result are indicated in Table 2. The 56 sera collected from September 2010 to February 2011 in Northeastern and Southwestern France (Bas-Rhin and Pyrenees-Atlantiques departments) were negative to both ELISA tests. From September 2011 to March 2012, 7/9 departments exhibited at least one seropositive to c-ELISA. Among these seven departments the average seroprevalence was 20.2% (95% CI [16.4%; 24.0%]) with significant variations between the seven departments exhibiting seropositive results (8.3% to 49.1%) (Chi^2=67.4, df=6, p<0.001). Seroprevalence was not influenced by individuals' age, suggesting an equal exposure of fawns born in 2011 and older individuals (Chi^2=0.16, df=2, p=0.92). It is thus likely that SBV has not spread to France before the occurrence of births, i.e., mid-May to early June 2011 for red deer in France (7). Seroprevalence was significantly varying with the period (Chi^2=25.0, df=2, p<0.001). On average, seroprevalence was higher in December 2011/January 2012 (31.1% (95% IC [24.7%; 37.4%]) compared to September/ November 2011 (7.4% (95% IC [3.0%; 11.8%]) or February/ March 2011 (14.4% (95% IC [7.2%; 21.7%]). These results suggest that SBV was actively circulating during fall 2011 until mid-November or early December. As Linden et al. for Belgium (3), we consider that the mild temperature observed in France in fall 2011 may have favored a late activity of vectors (8). The date of first occurrence of seropositive red deer and the seroprevalence observed in each department (indicated in Table 2) was not strictly dependent on the distance from the Meurthe-et-Moselle department were the 1st domestic case (congenital form) had been confirmed on January 25th 2012 (9). This result possibly arose because of uncontrolled variations in the sampling dates of red deer between the nine departments and still unknown factors associated with SBV spread. Nevertheless, most of the departments that exhibited seropositive red deer from September 2011 to March 2012 had also notified clinical cases in domestic flocks between January and March 2012. In Southwestern France (near the Pyrenees Mountains), a seropositive red deer was observed in the Hautes-Pyrenees department while congenital clinical cases of SBV in domestic livestock (congenital malformations on kids) had been notified by the 30th of March 2012 in the neighbor Pyrenees-Atlantiques department (E. Breard, pers. comm.). These results suggest thus similar spread of SBV among red deer and domestic livestock during fall 2011 at the scale of the department.

Conclusion

This study provides a first view of SBV spread among wild cervids in France between 2010 and 2012. Our data suggests a very fast spread of SBV from Northeastern to Southwestern France between October and December 2011 (~800Km). Our data also put in evidence the match of SBV spread among red deer and domestic flocks at the scale of the department, and highlights the perspective of using red deer as a sentinel of SBV spread for livestock. We also pinpointed the relevance of new competition ELISA for improving SBV surveillance in wildlife species, even though SNT remained the most reliable assay for SBV antibodies detection in red deer. Further studies including several years and a larger number of species and localities would be suitable for providing a more complete vision of virus spread and risk factors in wildlife (10).

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Biographical sketch

S. Rossi (DMV, PHD) is epidemiologist at the French game and wildlife agency (Gap, France). She is more particularly involved in the analysis of the spatiotemporal trends of wildlife

Table 2: Results to competition ELISA in 2011-2012 and indication of the first observation of a seropositive result in each department.

Department name	Average distance (in Km) to the Meurthe-et-Moselle department (*)	Number of samples with conclusive c-ELISA in 2010-2011	Number of samples with conclusive c-ELISA in 2011-2012	Proportion of positive and 95% confidence interval with c-ELISA in 2011-2012	1st observation of a positive result using c-ELISA
Moselle	46	0	26	15.4% [1.5%; 29.3%]	5th November 2011
Haute-Marne	102	0	53	49.1% [35.6%; 62.5%]	12th November 2011
Bas-Rhin	103	41	53	15.1% [5.5%; 24.7%]	25th October 2011
Côte d'Or	184	0	37	29.7% [15.0%; 44.5%]	3rd December 2011
Oise	282	0	69	37.7% [26.2%; 49.1%]	19th December 2011
Loir-et-Cher	375	0	132	8.3% [3.6%; 13.0%]	25th November 2011
Hautes-Pyrénées	789	14	12	8,3% [0.0%; 30.4%]	10th December 2011
Corsica	749	0	23	0% [0.0%; 12.2%]	/
Pyrénées Atlantiques	815	0	26	0% [0.0%; 10.9%]	/

(*) department were the 1st domestic clinical cases have been notified the 25th January 2012.

diseases and in oral mass vaccination of wildlife species such as wild boar.

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