Short communication

Zoo animals as sentinels for Schmallenberg virus monitoring in Spain.

J. Caballero-Gómez[a,b], I. García-Bocanegra[a*], N. Navarro[c], R. Guerra[d], E. Martínez-Nevado[e], P. Soriano[f], D. Cano-Terriza[a]

[a] Grupo de Investigación en Sanidad Animal y Zoonosis (GISAZ), Departamento de Sanidad Animal. Universidad de Córdoba (UCO), 14014 Córdoba, Spain.

[b] Grupo de Virología Clínica y Zoonosis, Unidad de Enfermedades Infecciosas, Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Hospital Reina Sofía, Universidad de Córdoba (UCO), 14004 Córdoba, Spain.

[c] IRTA, Centre de Recerca en Sanitat Animal (CReSA, IRTA-UAB), Campus de la Universitat Autònoma de Barcelona, 08193 Barcelona, Spain.

[d] Parque Zoológico de Córdoba, 14004 Córdoba, Spain.

[e] Zoo Aquarium de Madrid, 28011 Madrid, Spain.

[f] Río Safari Elche, 03139 Alicante, Spain.

*Corresponding author: Prof. Ignacio García-Bocanegra. Department of Animal Health, University of Córdoba. Campus Universitario Rabanales, 14014 Córdoba, Spain. Tel.: +34 957218725; E-mail: nacho.garcia@uco.es

Summary

Schmallenberg virus (SBV) is a newly emerged vector-borne pathogen that affects many domestic and wild animal species. A serosurvey was carried out to assess SBV exposure in zoo animals in Spain and to determine the dynamics of seropositivity in longitudinally sampled individuals. Between 2002 and 2019, sera from 278 animals belonging to 73 different species were collected from five zoos (A-E). Thirty-one of these animals were longitudinally sampled at three of these zoo parks during the study period. Seropositivity was detected in 28 (10.1%) of 278 animals analyzed by blocking ELISA. Specific anti-SBV antibodies were confirmed in 20 (7.2%; 95%CI: 4.2-10.3) animals of six different species using virus neutralization test (VNT). The multiple logistic regression model showed that "order" (*Artiodactyla*) and "zoo provenance" (zoo B; southern Spain) were risk factors potentially associated with SBV exposure. Two (8.7%) of the 31 longitudinally-sampled individuals showed specific antibodies against SBV at all samplings whereas seroconversion was detected in one mouflon (*Ovis aries musimon*) and one Asian elephant (*Elephas maximus*) in 2016 and 2019, respectively. To the best of the author's knowledge, this is the first surveillance conducted on SBV in zoos in Spain. The results confirm SBV exposure in zoo animals in this country and indicate circulation of the virus before the first Schmallenberg disease outbreak was reported in Spain. Surveillance in zoological parks could be a complementary approach to monitoring SBV activity. Further studies are warranted to assess the impact of this virus on the health status of susceptible zoo animals.

Keywords: *Schmallenberg, Vector-borne, Emerging, Surveillance, Wildlife, Captive, Animal health*.

Introduction

Schmallenberg virus (SBV) (genus *Orthobunyavirus*; family *Peribunyaviridae*) is a newly emerged vector-borne pathogen transmitted by biting midges (Diptera: *Ceratopogonidae*, *Culicoides* spp.). After the first Schmallenberg disease outbreak was reported in cattle in Germany in 2011 (Hoffmann et al., 2012), the virus spread rapidly across Europe and into other territories (Collins et al., 2019). In adult domestic ruminants, Schmallenberg disease is either asymptomatic or characterized by mild non-specific clinical signs, including mild fever, diarrhea and a drop in milk production. In pregnant females, SBV infection can lead to early embryonic loss, abortion, stillbirths and congenital malformations in newborn animals (Endalew et al., 2019).

Although SBV mainly affects domestic ruminants, with a major impact on animal health, production and international trade, free-ranging and captive wild species have also been shown to be susceptible to SBV infection (Collins et al., 2019; Molenaar et al., 2015). Previous studies have suggested the potential role of wildlife in the maintenance of SBV in Europe, particularly at the livestock-wildlife interface (García-Bocanegra et al., 2017; Jiménez-Ruiz et al., 2020; Rossi et al., 2015). Serosurveys have also been proposed as a useful tool in wildlife for monitoring this virus (Jiménez-Ruiz et al., 2020; Mouchantat et al., 2015). In Spanish Mediterranean ecosystems, SBV circulation has been detected in free-ranging wild ungulate species, with seroprevalence values ranging between 18.2% and 29.8% (García-Bocanegra et al., 2017; Jiménez-Ruiz et al., 2020).

The importance of zoos for the surveillance of emerging diseases has been widely documented (Caballero-Gómez et al., 2019; McNamara, 2007; Robinette et al., 2017). The wide variety of animal species, as well as the exhaustive management protocols and veterinary check-ups implemented in zoos, mean that zoo animals are easily accessible and could be considered useful sentinel species for monitoring vector-borne pathogens (Caballero-Gómez et al., 2020;

Sánchez-Romano et al., 2019). Antibodies against SBV have been detected in native and exotic wild species kept in captivity in Europe (Laloy et al., 2016; Molenaar et al., 2015; Mouchantat et al., 2015; Sánchez-Romano et al., 2019), although the information on the role of zoo animals in the epidemiology of this virus is still very scarce worldwide. The aims of this study were: (1) to assess SBV exposure in captive zoo animals in Spain, and (2) to determine the dynamics of seropositivity in individuals that were sampled longitudinally during the study period.

Material and Methods

Between 2002 and 2019, a total of 278 zoo animals belonging to 73 different species were sampled at five urban zoos (A-E) in Spain (Fig. 1). Samples were obtained from serum banks or from animals undergoing surgery, medical check-ups or participating in health programs during the study period. Sera were obtained after centrifugation and kept frozen at -20 ºC until analysis. Epidemiological data including age, sex, zoo, order and sampling date were collected for each animal, whenever possible. Sample collection was divided into three consecutive periods (2002-2010, 2011-2015 and 2016-2019) based on the first report of SBV circulation in Spain (García-Bocanegra et al., 2017) and the re-emergence of this virus in Europe (Larska, 2018). Additionally, 31 of the 278 sampled animals at three of the zoos analyzed (zoos B, C and E) were surveyed longitudinally. Longitudinally-sampled animals were not translocated during the study period. Reproductive disorders compatible with SBV infection were not observed in any of the five zoos analyzed.

SBV seropositivity was determined using a commercial multi-species blocking ELISA (bELISA; INgezim Schmallenberg Compac 2.0®, INGENASA, Madrid, Spain), carried out in accordance with the manufacturer's instructions. This bELISA detects antibodies against the N protein of SBV. Sensitivity and specificity values provided by the manufacturers were 99.5% and 99.0%, respectively. This bELISA has been used in previous surveys in wild ungulate species and showed very good agreement (Kappa value = 0.95) with the virus neutralization test (García-Bocanegra et al., 2017; Jiménez-Ruiz et al., 2020). The presence of anti-SBV antibodies in bELISA-positive animals was then confirmed by the virus neutralization test (VNT), as previously described (Loeffen et al., 2012). Titers were expressed as the reciprocal of the highest dilution that neutralized 100 tissue culture infective doses (100 $TCID_{50\%}$) of SBV (BH80/11-4 isolate; kindly provided by M. Beer, Friedrich-Loeffler-Institute, Isle of Riems, Germany) in Vero cells. Sera that showed neutralization (absence of cytopathic effect) at dilutions ≥ 1:4 were considered positive.

In the present study, only samples positive to both bELISA and VNT were considered positive for SBV exposure. Seroprevalence to SBV was calculated as the ratio of seropositive animals to the total number of animals examined, using two-sided exact binomial confidence intervals (95%CI). Associations between seroprevalence of SBV and explanatory variables were analyzed using the Pearson's chi-square test or Fisher's exact test as appropriate. Explanatory variables "order" and "zoo provenance" were converted into dichotomous dummy variables. Variables with $p < 0.10$ in the bivariate analysis were included for further analysis. Then, collinearity between pairs of variables was computed by Cramer's V coefficient. Finally, multiple logistic regression analysis to assess risk factors potentially associated with SBV exposure in zoo animals. The fit of the models was assessed using a goodness-of-fit test (Hosmer and Lemeshow, 2000). Values with $p < 0.05$ were considered statistically significant. Statistical analyses were performed using SPSS 25.0 software (Statistical Package for Social Sciences, Inc., Chicago, IL, USA).

Results

Seropositivity to SBV was found in 28 (10.1%) of the 278 zoo animals analyzed by bELISA: twenty of these were positive by VNT, seven tested negative, and one could not be analyzed due to the limited volume of serum available. Hence, the overall individual SBV seroprevalence was 7.2% (20/277; 95%CI: 4.2-10.3) (Table 1; Supplementary Material, Table

S1). Anti-SBV antibodies were found in two of the six orders tested (order *Artiodactyla* (13.1%) and order *Proboscidea* (10.0%)), and in six (8.2%) of the 73 species analyzed (Table 1; Supplementary Material, Table S1). Seropositive animals were observed in three (60.0%) of the five sampled zoos (Table 2; Fig. 1) and every year between 2012 and 2019. Seropositivity to SBV were detected in two yearlings (≤ 12 months old) (one aoudad (*Ammotragus lervia*) and one mouflon (*Ovis aries musimon*)) sampled in 2014 and 2016 in zoo B (Table 1). The distribution of seropositive animals according to the explanatory variables is shown in Table 2. The variable "sampling period" was excluded from logistic regression analysis due to collinearity with the variables "order" and "zoo provenance", while "sex" was collinear with "order". The final model identified order *Artiodactyla* (19/145, 13.1%; $p = 0.005$, OR =19.3, 95%CI: 2.5-150.9) and zoo B (17/79, 21.5%; $p < 0.001$, OR = 17.5, 95%CI: 4.8-63.4) as risk factors potentially associated with SBV exposure in zoo animals in Spain. Of the 31 animals longitudinally sampled, 25 were seronegative in all samplings, while four always showed positive results by bELISA (Table 3). Of these, specific anti-SBV antibodies were confirmed by VNT in two individuals (one aoudad and one bongo (*Tragelaphus euryceros*)). In addition, seroconversions were detected in one Asian elephant (*Elephas maximus*) between 2018 and 2019 and one mouflon between January and December 2016, both housed in zoo B.

Discussion

To the best of the author's knowledge, this is the first surveillance on SBV carried out in zoo animals in Spain. Our results confirm SBV circulation in zoo parks in this country, which could be of animal health and conservation concern. The overall seroprevalence (7.2%) is lower than those previously found in captive species in Europe, which ranged between 20.2% (419/2077) and 55.6% (30/54) (Laloy et al., 2016; Molenaar et al., 2015; Mouchantat et al., 2015; Sánchez-Romano et al., 2019; Steinrigl et al., 2014). However, comparisons between studies should be made with caution given the differences in study periods, numbers of animals and species analyzed, study design, epidemiological contexts and diagnostic methods employed.

The seroprevalence obtained in our study in artiodactyl species sampled after 2011 (17.0%; 19/112) when the first SBV outbreak was detected (Hoffmann et al., 2012) is within the range of values (14.6%-27.1% (160/1099-330/1216)) previously obtained in free-ranging wild artiodactyls in Spain (García-Bocanegra et al., 2017; Jiménez-Ruiz et al., 2020). In this regard, multivariate analysis showed that the risk of seropositivity to SBV was 19.3 times higher in zoo animals belonging to the order *Artiodactyla* than in any of the other orders analyzed. This, together with the seropositivity values observed in the aoudad (46.2%), red deer (58.3%) and mouflon (37.5%), which were even higher than those previously found in free-ranging animals of the same species in the study area, indicate the potential role of captive artiodactyls in the maintenance of SBV in Spain (García-Bocanegra et al., 2017; Jiménez-Ruiz et al., 2020).

Specific anti-SBV antibodies were also found in the endangered Asian elephant (IUCN, 2020), which is consistent with the report by Molenaar et al. (2015) and confirms the susceptibility of this species to SBV infection. Interestingly, most bELISA-positive animals that tested negative by VNT belong to the orders *Perissodactyla* (white rhinoceros (*Ceratotherium simum*)) and *Proboscidea* (African elephant (*Loxodonta africana*)). The same result was obtained in one of the three brown bears (*Ursus arctos*) tested. These findings could be associated with cross-reactivity with other SBV-related viruses of the Simbu serogroup, as previously suggested (Molenaar et al., 2015). Nevertheless, these hypotheses need to be evaluated in future studies. The absence of seropositive animals in the orders *Carnivora* and *Diprotodontia* is in agreement with previous studies and suggests that these species are not reservoirs for SBV (reviewed EFSA, 2014; Garigliany et al., 2013; Molenaar et al., 2015; Mouchantat et al., 2015).

Seropositive animals were observed in three of the five sampled zoos. Significantly, higher seroprevalence was detected at zoo B, which is situated in the same area where the first SBV outbreak was reported in Spain (Jiménez-Ruiz et al., 2019). This zoo is also located very close to the city center, which confirms SBV circulation in urban areas, as has been previously documented (Laloy et al., 2016). The differences between zoo parks may be associated with the animal species analyzed, environmental factors and/or the presence and abundance of competent vectors at these zoos. In this regard, the densities of *Culicoides*, particularly *Culicoides imicola,* have been shown to be higher in southwestern Spain (Arenas-Montes et al., 2016; Calvete et al., 2008). Different *Culicoides* species have been confirmed at different zoos in the United Kingdom (England et al., 2020; Vilar et al., 2011), although entomological surveillance in zoo parks in Spain is needed to determine the competent vector species present in these epidemiological scenarios.

Specific antibodies against SBV were not detected before 2011 (Hoffmann et al., 2012). In Spain, SBV circulation was first detected in domestic and wild ruminants in the summer and autumn of that year, respectively (Astorga et al., 2014; García-Bocanegra et al., 2017). In our study, the first seropositive zoo animal was an adult aoudad from zoo B sampled in February 2012, one month before the first SBV outbreak was reported in livestock in Spain (Jiménez-Ruiz et al., 2019). Two yearling animals (one aoudad and one mouflon) from the same zoo park showed seropositivity in 2014 and 2016, respectively (Table 1). These findings, together with the seroconversions detected in the same zoo in one mouflon in 2016 and one Asian elephant in 2018-2019, indicates endemic circulation of SBV there over the last few years.

In conclusion, the seroprevalence detected in the present study is evidence of SBV exposure in zoo animals in Spain over the last decade. The results also confirm that urban zoo parks may be suitable epidemiological scenarios for SBV circulation. Surveillance in zoo species, particularly artiodactyls, could be a complementary approach to monitoring SBV activity. Further studies are warranted to assess the impact of this virus on the health status of susceptible zoo animals.

Acknowledgements

We are grateful to Cordoba Zoo Park, Bioparc Valencia, Bioparc Fuengirola, Zoo-Aquarium Madrid and Río Safari Elche for providing the valuable samples. We gratefully acknowledge the assistance of Dr Joan Pujols with the laboratory analyses. J. Caballero-Gómez is supported by an FPU grant of the Spanish Ministry of Science, Innovation and Universities (FPU17/01319).

Conflict of interest

None of the authors of this study has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

Ethical approval

The collection of samples was performed by personal of the zoos. All samples were collected from serum banks or from animals subjected to health programs, medical check-ups or surgical interventions during the study period. No ethical approval was necessary.

Orcid

Javier Caballero-Gómez: https://orcid.org/0000-0002-6241-3439.

Ignacio García-Bocanegra: https://orcid.org/0000-0003-3388-2604.

David Cano-Terriza: https://orcid.org/0000-0001-5657-2567.

References

Arenas-Montes, A., Paniagua, J., Arenas, A., Lorca-Oró, C., Carbonero, A., Cano-Terriza, D., García-Bocanegra, I., 2016. Spatial–temporal trends and factors associated with the bluetongue virus seropositivity in large game hunting areas from southern Spain. Transb. Emerg. Dis. 63, e339-e346. https://doi.org/10.1111/tbed.12309.

Astorga, R. J., Reguillo, L., Hernández, M., Cardoso-Toset, F., Tarradas, C., Maldonado, A., Gómez-Laguna, J. (2014). Serosurvey on Schmallenberg virus and selected ovine reproductive pathogens in culled ewes from Southern Spain. Transb. Emerg. Dis. 61, 4-11. https://doi.org/10.1111/tbed.12188.

Caballero-Gómez, J., Rivero-Juarez, A., Cano-Terriza, D., Risalde, M.A., Lopez-Lopez, P., Frias, M., Jiménez-Ruiz, S., Rivero, A., García-Bocanegra, I., 2019. Survey for Hepatitis E virus infection in non-human primates in zoos in Spain. Transb. Emerg. Dis. 66, 1771-1775. https://doi.org/10.1111/tbed.13196.

Caballero-Gómez, J., Cano-Terriza, D., Lecollinet, S., Carbonell, M.D., Martínez-Valverde, R., Martínez-Nevado, E., García-Párraga, D., Lowenski, S., García-Bocanegra, I., 2020. Evidence of exposure to zoonotic flaviviruses in zoo mammals in Spain and their potential role as sentinel species. Vet. Micro. 247, 108763. https://doi.org/10.1016/j.vetmic.2020.108763.

Calvete, C., Estrada, R., Miranda, M.A., Borras, D., Calvo, J.H., Lucientes, J., 2008. Modelling the distributions and spatial coincidence of bluetongue vectors *Culicoides imicola* and the *Culicoides obsoletus* group throughout the Iberian Peninsula. Med. Vet. Entomol. 22, 124-134. https://doi.org/10.1111/j.1365-2915.2008.00728.x.

Collins, Á.B., Doherty, M.L., Barrett, D.J., Mee, J.F., 2019. Schmallenberg virus: a systematic international literature review (2011-2019) from an Irish perspective. Irish Vet. J. 72, 9. https://doi.org/10.1186/s13620-019-0147-3.

Endalew, A.D., Faburay, B., Wilson, W.C., Richt, J.A. 2019. Schmallenberg Disease-A Newly Emerged Culicoides-Borne Viral Disease of Ruminants. Viruses 11, 1065. https://doi.org/10.3390/v11111065.

England, M.E., Pearce-Kelly, P., Brugman, V.A., King, S., Gubbins, S., Sach, F., Sanders C.J., Masters, N.J., Denison, E., Carpenter, S., 2020. *Culicoides* species composition and molecular identification of host blood meals at two zoos in the UK. Parasite Vector 13, 1-13. https://doi.org/10.1186/s13071-020-04018-0.

European Food Safety Authority (EFSA), 2014. Schmallenberg virus: State of art. EFSA Journal 12, 3681. https://doi.org/10.2903/j.efsa.2014.3681.

García-Bocanegra, I., Cano-Terriza, D., Vidal, G., Rosell, R., Paniagua, J., Jiménez-Ruiz, S., Expósito, C., Rivero-Juarez, A., Arenas, A., Pujols, J., 2017. Monitoring of Schmallenberg virus in Spanish wild artiodactyls, 2006–2015. PloS One 12. https://dx.doi.org/10.1371/journal.pone.0182212.

Garigliany, M.M., Desmecht, D., Bayrou, C., Peeters, D., 2013. No serologic evidence for emerging Schmallenberg virus infection in dogs (*Canis domesticus*). Vector-Borne and Zoonotic Diseases 13, 830-833. https://doi.org/10.1089/vbz.2012.1251.

Hoffmann, B., Scheuch, M., Höper, D., Jungblut, R., Holsteg, M., Schirrmeier, H., Eschbaumer, M., Goller, K.V., Wernike, K., Fischer, M., Breithaupt, A., Mettenleiter, T.C., Beer, M., 2012. Novel orthobunyavirus in cattle, Europe, 2011. Emerg. Infect. Dis. 18, 469. https://dx.doi.org/10.3201/eid1803.111905.

Hosmer, D.W., Lemeshow, S., 2000. Applied Logistic Regression, Second Ed. Wiley-Interscience Press, New York, USA, pp. 143–188.

IUCN, 2020. International Union for Conservation of Nature's. Retrieved from: https://www.iucnredlist.org/ (Accessed 23rd June 2020).

Jiménez-Ruiz, S., Paniagua, J., Isla, J., Martínez-Padilla, A.B., Risalde, M.A., Caballero-Gómez, J., Cano-Terriza, D., Pujols, J., Arenas, A. García-Bocanegra, I., 2019. Description of the first Schmallenberg disease outbreak in Spain and subsequent virus spreading in domestic ruminants. Comp. Immunol. Microbiol. 65, 189-193. https://doi.org/10.1016/j.cimid.2019.06.002.

Jiménez-Ruiz, S., Risalde, M.A., Acevedo, P., Arnal, M.C., Gómez-Guillamón, F., Prieto, P., Gens, M.J., Cano-Terriza, D., Fernández de Luco, D., Vicente, J., García-Bocanegra, I., 2020. Serosurveillance of Schmallenberg virus in wild ruminants in Spain. Transb. Emerg. Dis. https://doi.org/10.1111/tbed.13680.

Laloy, E., Braud, C., Bréard, E., Kaandorp, J., Bourgeois, A., Kohl, M., Meyes, G., Sailleau, C., Viarouge, C., Zientara, S., Chai, N., 2016. Schmallenberg virus in zoo ruminants, France and the Netherlands. Emerg. Infect. Dis. 22, 2201-2203. https://dx.doi.org/10.3201/eid2212.150983.

Larska, M., 2018. Schmallenberg virus: a cyclical problem. Vet Rec. 183, 688-689. https://dx.doi.org/10.1136/vr.k5109.

Loeffen, W., Quak, S., de Boer-Luijtze, E., Hulst, M., van der Poel, W., Bouwstra, R., Maas, R., 2012. Development of a virus neutralisation test to detect antibodies against Schmallenberg virus and serological results in suspect and infected herds. Acta Vet. Scand. 54, 44. https://doi.org/10.1186/1751-0147-54-44.

McNamara, T., 2007. The role of zoos in biosurveillance. International Zoo Yearbook 41, 12-15. https://doi.org/10.1111/j.1748-1090.2007.00019.x.

Molenaar, F.M., La Rocca, S.A., Khatri, M., Lopez, J., Steinbach, F., Dastjerdi, A., 2015. Exposure of Asian elephants and other exotic ungulates to Schmallenberg virus. PLoS One 10. https://dx.doi.org/10.1371/journal.pone.0135532.

Mouchantat, S., Wernike, K., Lutz, W., Hoffmann, B., Ulrich, R. G., Börner, K., Wittstatt, U., Beer, M., 2015. A broad spectrum screening of Schmallenberg virus antibodies in wildlife animals in Germany. Vet. Res. 46, 1-5. https://doi.org/10.1186/s13567-015-0232-x.

Robinette, C., Saffran, L., Ruple, A., Deem, S.L., 2017. Zoos and public health: a partnership on the One Health frontier. One Health, 3, 1-4. https://doi.org/10.1016/j.onehlt.2016.11.003.

Rossi, S., Viarouge, C., Faure, E., Gilot-Fromont, E., Gache, K., Gibert, P., Verheyden, H., Hars, J., Klein, F., Maillard, D., Gauthier, D., Game, Y., Pozet, F., Sailleau, C., Garnier, A., Zientara, S., Bréard, E., 2015. Exposure of wildlife to the Schmallenberg virus in France (2011–2014): Higher, faster, stronger (than bluetongue)!. Transb. Emerg. Dis. 64, 354-363. https://doi.org/10.1111/tbed.12371.

Sánchez-Romano, J., Grund, L., Obiegala, A., Nymo, I. H., Ancin-Murguzur, F. J., Li, H., Król, N., Pfeffer, M., Tryland, M., 2019. A Multi-Pathogen Screening of Captive Reindeer

(*Rangifer tarandus*) in Germany Based on Serological and Molecular Assays. Front. Vet. Sci. 6, 461 https://dx.doi.org/10.3389/fvets.2019.00461.

Steinrigl, A., Schiefer, P., Schleicher, C., Peinhopf, W., Wodak, E., Bagó, Z., Schmoll, F., 2014. Rapid spread and association of Schmallenberg virus with ruminant abortions and foetal death in Austria in 2012/2013. Prev. Vet. Med. 116, 350-359. https://doi.org/10.1016/j.prevetmed.2014.03.006.

Vilar, M.J., Guis, H., Krzywinski, J., Sanderson, S., Baylis, M., 2011. *Culicoides* vectors of bluetongue virus in Chester Zoo. Vet. Rec. 168, 242-242. http://dx.doi.org/10.1136/vr.c6684.

Tables

Table 1. Results of virus neutralization test (VNT) for the detection of antibodies against Schmallenberg virus in bELISA-positive zoo animals in Spain.

Table 2. Specific seropositivity to Schmallenberg virus in zoo animals in Spain and results of the bivariate analysis.

Table 3. Schmallenberg virus exposure in longitudinally-sampled animals. Green and red dots indicate seronegative and seropositive animals respectively, by both bELISA and VNT. Orange dots indicate animals positive by bELISA and negative by VNT. In seropositive animals, VNT antibody titers to SBV are shown in brackets. When two samplings were carried out in the same year, the difference between the two in months is indicated in superscript.

Table S1. Antibodies against Schmallenberg virus in 73 mammal species sampled in zoos in Spain.

Figure captions

Fig. 1. Distribution of the zoos (A-E) sampled in Spain. The number of positive (non-green) and negative (green) animals analyzed by bELISA at each zoo park is represented in a pie chart. Non-green colors indicate the presence of specific neutralizing antibodies against SBV by VNT at each zoo (red: presence; yellow: absence; gray: not analyzed). Years when seropositive animals were detected are listed above each species.