



Epidemiological surveillance of Schmallenberg virus in wild ruminants in Spain

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3 **1 Epidemiological surveillance of Schmallenberg virus in wild ruminants in Spain.**
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25 Summary

26 Schmallenberg disease (SBD) is an emerging vector-borne disease that affects
27 domestic and wild ruminants. A long-term serosurvey was conducted to assess exposure
28 to Schmallenberg virus (SBV) in all the wild ruminant species present in mainland Spain.
29 Between 2010 and 2016, sera from 1,216 animals were tested for antibodies against SBV
30 using a commercial blocking ELISA. The overall prevalence of antibodies was 27.1%
31 (95%CI: 24.7-29.7). Statistically significant differences among species were observed,
32 with significantly higher seropositivity found in fallow deer (*Dama dama*) (45.6%;
33 99/217), red deer (*Cervus elaphus*) (31.6%; 97/307) and mouflon (*Ovis aries musimon*)
34 (28.0%; 33/118) compared to Barbary sheep (*Ammotragus lervia*) (22.2%; 8/36), Iberian
35 wild goat (*Capra pyrenaica*) (19.9%; 49/246), roe deer (*Capreolus capreolus*) (17.5%;
36 34/194) and Southern chamois (*Rupicapra pyrenaica*) (10.2%; 10/98). Seropositive
37 animals were detected in 81.4% (57/70; 95%CI: 70.8-88.8) of the sampled populations.
38 To the authors' knowledge, this is the first report of SBV exposure in both Barbary sheep
39 and Iberian wild goat. SBV seroprevalence ranged from 18.8% (48/256) in bioregion
40 (BR)2 (north-central, Mediterranean) to 32.3% (31/96) in BR1 (northeastern or Atlantic,
41 Eurosiberian). Anti-SBV antibodies were not found before 2012, when the first outbreak
42 of SBD was reported in Spain. In contrast, seropositivity was detected uninterruptedly
43 during the period 2012-2016 and anti-SBV antibodies were found in yearling animals in
44 each of these years. Our results provide evidence of widespread endemic circulation of
45 SBV among wild ruminant populations in mainland Spain in recent years. Surveillance
46 in these species could be a useful tool for monitoring SBV in Europe, particularly in areas
47 where wild ruminants share habitats with livestock. Further research is warranted to
48 assess the real impact of SBV in wild ruminant populations in Spain.

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3 49 **Keywords:** vector-borne diseases; Schmallenberg virus; wild ruminants; surveillance;
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10 52 **Introduction**

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14 53 Schmallenberg virus (SBV) is an *Orthobunyavirus* of the Simbu serogroup
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16 54 (family *Peribunyaviridae*), which is mainly transmitted among domestic and wild
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18 55 ruminant species by biting midges of the genus *Culicoides*. SBV infection can cause an
19
20 56 acute non-specific syndrome characterized by fever, reduced milk yield, diarrhea and
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22 57 reproductive disorders, such as abortions, stillbirths and congenital malformations in
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24 58 newborns. SBV was first reported in cattle on the German-Dutch border in summer 2011
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26 59 and since then, it has emerged and re-emerged in livestock in most European countries
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28 60 incurring significant productivity losses, international trade restrictions and veterinary
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30 61 costs (European Food Safety Authority, 2014; Stavrou, Daly, Maddison, Gough &
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32 62 Tarlinton, 2017). Spain reported the first outbreaks of Schmallenberg disease (SBD) in
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34 63 sheep in 2012 (Jiménez-Ruiz et al., 2019) and in cattle in 2013 (Balseiro, Royo, Gómez
35
36 64 Antona & García Marín, 2015) in southern and central regions of the country,
37
38 65 respectively. Although no further cases have been reported to date, SBV circulation has
39
40 66 been found regionally in livestock, with seroprevalence values ranging between 54.4%
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42 67 and 75.6% (Fernández-Aguilar et al., 2014; Jiménez-Ruiz et al., 2019).
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50 68 In the last decade, serosurveys in different European countries have evidenced
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52 69 widespread circulation of SBV in wild ruminant species. The high seroprevalence
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54 70 detected in some species (up to 80%) raises the question of whether wild ruminants play
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56 71 a role in the maintenance of SBV in Europe (García-Bocanegra et al., 2017). In Spain,
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58 72 there are seven wild ruminant species, with red deer (*Cervus elaphus*) and roe deer
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3 73 (*Capreolus capreolus*) being the most widely distributed. Fallow deer (*Dama dama*),
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5 74 mouflon (*Ovis aries musimon*) and Barbary sheep (*Ammotragus lervia*) are less abundant
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7
8 75 species, with locally significant populations, while Southern chamois (*Rupicapra*
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10 76 *pyrenaica*) and Iberian wild goat (*Capra pyrenaica*) are more frequent in the mountain
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12 77 ecosystems of northern and Mediterranean Spain, respectively (Palomo, Gisbert &
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14 78 Blanco, 2007). Populations of some of these species have expanded in recent decades,
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16 79 mainly because of ongoing changes in land use and more intensive game management
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18 80 practices (Acevedo et al., 2008; 2011). These epidemiological scenarios have been shown
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20 81 to increase the risk of disease transmission among sympatric species (Carrasco-García et
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22 82 al., 2016; Gortázar, Acevedo, Ruiz-Fons & Vicente, 2006). The present long-term
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24 83 serosurvey study was conducted to assess spatio-temporal trends of SBV exposure in all
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26 84 the wild ruminant species in Spain.

31 85 **Materials and methods**

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35 86 The study area comprised all mainland Spain (southwestern Europe), a country
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37 87 whose wild ruminant populations frequently share habitats with extensively reared
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39 88 domestic ruminants (Colom-Cadena et al., 2018; Kukielka et al., 2013). The Spanish
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41 89 Wildlife Disease Surveillance Scheme divides mainland Spain into five bioregions (BRs
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43 90 1 to 5; Fig. 1) based on habitat features and epidemiological criteria of the wild species
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45 91 communities (Ministerio de Agricultura, Pesca y Alimentación, 2020a). This zoning has
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47 92 previously been used to improve disease surveillance efforts in wild ruminants in Spain
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49 93 (Boadella et al., 2011; Lorca-Oró et al., 2014; Muñoz et al., 2010).

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51 94 The sample size per BR was estimated assuming a seroprevalence against SBV of
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53 95 20%, with a 95% confidence level and a desired precision of $\pm 5\%$, based on the highest
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55 96 overall prevalence of anti-SBV antibodies previously reported in wild ruminants in Spain
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3 97 (García-Bocanegra et al., 2017). Within each BR, sampling was stratified according to
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5 98 the representative distribution and population density of the wild ruminant species, and
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8 99 the number of animals was selected to ensure a 95% probability of detecting at least one
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10 100 seropositive individual for an assumed minimum prevalence of 6%. Wild ruminant
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12 101 populations were selected by simple random sampling of hunting estates or game reserves
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14 102 across the study area. Within each sampled population, between 15 and 20 animals were
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16 103 randomly selected whenever possible.

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20 104 Blood samples from 1,216 free-ranging wild ruminants, including 307 red deer,
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22 105 246 Iberian wild goats, 217 fallow deer, 194 roe deer, 118 mouflon, 98 Southern chamois
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24 106 and 36 Barbary sheep, were collected from 70 sampling populations between 2010 and
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26 107 2016. All animals were legally hunted/harvested by hunters or culled as part of population
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28 108 control programs on game reserves. Blood samples were obtained from the thoracic cavity
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30 109 or by puncture of the heart or endocranial venous sinuses (Jiménez-Ruiz et al., 2016).
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32 110 Sera were obtained after centrifugation and kept frozen at -20°C until analysis. Data on
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34 111 sampling site, BR, sampling year, sex and age (yearlings, sub-adults or adults; according
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36 112 to Sáenz de Buruaga, Lucio-Calero & Purroy-Iraizoz (2001)) were recorded for each
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38 113 animal, whenever possible.

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43 114 Sera were tested for the presence of SBV-specific antibodies against the N protein
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45 115 antigen using a multi-species blocking ELISA (bELISA; INGEZIM
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47 116 SCHMALLEMBERG Compac 2.0 13.SBV.K3[®], INGENASA, Madrid, Spain),
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49 117 according to the manufacturers' instructions. In this bELISA, the optical density (OD)
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51 118 values for each serum were converted to a blocking percentage (b%) using the formula
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53 119 $[b\% = (1 - (OD_{\text{sample}} / OD_{\text{negative control}})) \times 100]$. Sera were then classified as negative, doubtful
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55 120 or positive when b% was $\leq 50\%$, 50-55% or $\geq 55\%$, respectively. Sensitivity and
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57 121 specificity values provided by the manufacturers were 99.5% and 99.0%, respectively.

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3 122 This bELISA test also showed very good agreement (Kappa value = 0.95) with the virus
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5 123 neutralization test in sera from wild ungulate species (García-Bocanegra et al., 2017).
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8 124 The seroprevalence of SBV was determined from the proportion of positive
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10 125 bELISA samples to the total number of animals examined, with exact binomial
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12 126 confidence intervals of 95% (95%CI). Associations between serological results (as
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14 127 binomial response variable) and categorical explanatory variables (species, BR, sampling
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17 128 year, sex and age) were initially tested with a Chi-square test or Fisher's test, as
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19 129 appropriate. Those variables yielding P -values < 0.20 in bivariate analysis were selected
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21 130 for further analysis. Finally, a generalized linear mixed model (GLMM) was used to
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23 131 determine potential risk factors associated with SBV exposure. A binomial error
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25 132 distribution and a logit link function were selected, and the sampling population was
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28 133 included as a random factor. Tukey's post-hoc test was used to assess differences between
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30 134 categories. The proportion of variation (R^2) explained by the model was calculated using
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32 135 Nakagawa and Schielzeth's method (2013) where marginal R^2 (R^2m) refers to the
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34 136 proportion of variation explained only by fixed factors and conditional R^2 (R^2c) the
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36 137 proportion explained by both fixed and random factors. Values with $P < 0.05$ were
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38 138 considered statistically significant. Statistical analyses were performed using R software
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41 139 (R Core Team, 2019).
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45 140 **Results**

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48 141 A total of 330 out of 1,216 analysed sera had antibodies against SBV, giving an
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50 142 overall seroprevalence of 27.1% (95%CI: 24.7-29.7) in wild ruminants in Spain. The
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52 143 distribution of seroprevalences by species, BR, sampling year, sex and age is shown in
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54 144 Table 1. The prevalence of antibodies against SBV according to species was 45.6% in
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56 145 fallow deer, 31.6% in red deer, 28.0% in mouflon, 22.2% in Barbary sheep, 19.9% in
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59 146 Iberian wild goat, 17.5% in roe deer and 10.2% in Southern chamois. At least one
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3 147 seropositive animal was detected in 57 (81.4%) of the 70 sampled populations (Fig. 1).
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5 148 Seroprevalence values ranged between 18.8% in BR2 and 32.3% in BR1 (Table 1).
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7 149 Distribution of seroprevalence by species and further broken down by sampling year and
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9 BR is shown in Table 2. Seropositivity was found in all species in the five BRs, except
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11 150 for the roe deer sampled in BR1. Anti-SBV antibodies were not found before 2012,
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13 151 although seropositivity was detected uninterruptedly in most species between 2012 and
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15 152 2016. Seroprevalence reached 42.4% in 2013, decreased to 23.3% in 2015 and was 24.7%
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17 153 at the end of the study period, although a heterogeneous temporal trend was observed at
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19 154 species level. Seropositivity in yearling fallow deer and red deer was found every year
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21 155 between 2012 and 2016.
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27 157 Three (species, BR and year) of five explanatory variables were selected from the
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29 158 bivariate analysis. “Barbary sheep” was removed from multivariate analysis because all
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31 159 the samples (n= 36) from this species were collected in BR5. The GLMM only showed
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33 160 statistical differences between species ($F_{5,1215} = 13.155$; $P < 0.001$), not BRs ($F_{4,1215} =$
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35 161 0.373 ; $P = 0.827$) or sampling years ($F_{6,1211} = 0.120$; $P = 0.653$). Significantly higher
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37 162 seropositivity was found in fallow deer, red deer and mouflon compared to the remaining
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39 163 wild ruminant species analysed (Fig. 2). The variation in SBV seroprevalence explained
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41 164 by the model was $R^2m = 0.16$ for fixed factors and $R^2c = 0.34$ for fixed and random factors.
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46 165 **Discussion**

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49 166 Even though different European wild ruminant species have been shown to be
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51 167 susceptible to SBV infection, their role in the epidemiology of this virus has not been well
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53 168 studied. SBV exposure has previously been detected in wild ruminants in different
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55 169 regional studies performed in Spain, with overall prevalence values ranging from 3.4%
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57 170 (12/355) to 9.2% (160/1,744) (Fernández-Aguilar et al., 2014; García-Bocanegra et al.,
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3 171 2017). The seroprevalence obtained in the present large-scale survey (27.1%) was
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5 172 markedly higher than previously reported and indicates that SBV is widespread in wild
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7 173 ruminant populations throughout the country.
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10 174 The prevalence of antibodies against SBV in fallow deer (45.6%) was higher than
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12 175 has previously been found in this species in several European countries, where
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14 176 seroprevalence values ranged between 22.7% in Poland and 29.5% in Sweden (García-
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16 177 Bocanegra et al., 2017; Larska, Krzysiak, Kęsik-Maliszewska & Rola, 2014; Larska,
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18 178 Krzysiak, Smreczak, Polak & Zmudzinski, 2013; Malmstem et al., 2017). The prevalence
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20 179 of anti-SBV antibodies detected in red deer (31.6%) was similar to that observed in
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22 180 Poland (30.6%) (Larska et al., 2014), slightly lower than those obtained in France (38.3%)
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24 181 (Rossi et al., 2015), Italy (40.3%) (Chiari et al., 2014) and Belgium (40.5%) (Linden et
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26 182 al., 2012) and higher than reports in other European countries such as Sweden (18.2%)
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28 183 (Malmstem et al., 2017), Slovenia (19.5%) (Vengušt, Žele Vengušt, Toplak, Rihtarič &
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30 184 Kuhar, 2020) and even Spain (13.3%) (García-Bocanegra et al., 2017). In contrast, higher
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32 185 seroprevalence values were reported in both fallow and red deer (56.3% and 71.4%,
33
34 186 respectively) in the United Kingdom, although these results should be interpreted with
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36 187 caution given the limited number of samples tested (16 fallow deer and seven red deer)
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38 188 (Barlow, Green, Banham & Healy, 2013). The seropositivity observed in mouflon in our
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40 189 study (28.0%) was also higher than has been found previously in northern (0.0%) and
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42 190 southern (16.4%) Spain (Fernández-Aguilar et al., 2014; García-Bocanegra et al., 2017),
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44 191 although more variable rates for antibodies against SBV were reported in Europe in this
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46 192 species, with seroprevalence values ranging from 1.4% in Poland (Larska et al., 2014) to
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48 193 75.0% in Germany (Mouchantat et al., 2015). Nonetheless, comparisons between studies
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50 194 should be made with caution, given the differences in sampling periods, numbers of
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52 195 samples examined, study designs and serological methods employed.
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3 196 Exposure to SBV in roe deer and Southern chamois populations was also
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5 197 confirmed in the present study, with seropositivity rates (17.5% and 10.2%, respectively)
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7 198 lower than those observed in European countries, including Spain (Chiari et al., 2014;
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9 199 Díaz et al., 2015; Fernández-Aguilar et al., 2014; Linden et al., 2012; Malmsten et al.,
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11 200 2017; Morrondo et al., 2017; Rossi et al., 2015; Tavernier et al., 2015; Vengušt et al.,
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13 201 2020). To the best of the authors' knowledge, this is the first report of SBV exposure in
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15 202 Barbary sheep and Iberian wild goat, which increases the number of species susceptible
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17 203 to SBV infection. This is the first study on SBV in Barbary sheep, a wild ruminant from
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19 204 North Africa that was introduced into Europe for big game purposes during the twentieth
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21 205 century and is currently present in isolated populations in Spain, Italy and Croatia (Mori,
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23 206 Mazza, Saggiomo, Sommese & Esattore, 2017). With respect to the Iberian wild goat, an
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25 207 endemic species of the Iberian Peninsula, SBV antibodies were not detected in animals
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27 208 sampled between 2007 and 2010 (García-Bocanegra et al., 2017), before the first SBD
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29 209 outbreak was reported in Europe (EFSA, 2014). Antibodies against SBV were however
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31 210 detected in Alpine ibex (*Capra ibex*) (33.2%; 67/202), a phylogenetically related
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33 211 *Caprinae* species that inhabits similar mountain ecosystems in other European countries
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35 212 (Rossi et al., 2015).
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43 213 Fallow deer, red deer and mouflon had significantly higher SBV exposure than
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45 214 the remaining species analysed, which is in accordance with previous observations in the
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47 215 south of the country (García-Bocanegra et al., 2017). The gregarious behavior of these
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49 216 species, their population densities, habitat preferences, as well as the abundance and
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51 217 feeding patterns of competent vectors may explain differences between species. In
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53 218 connection with this, the abundance of wild host species, particularly red deer, was shown
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55 219 to be the most important factor involved in the abundance of *Culicoides* species in Spain
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57 220 (Acevedo et al., 2010). In this country, red deer, fallow deer and mouflon are among the
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3 221 most important wild ruminant species in terms of abundance, and are the most intensively
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5 222 managed big game species (Garrido, Ferreres & Gortazar, 2019; MAPA, 2020b),
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7 223 although the distribution of the latter is much more localized and scattered than in red
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9 224 deer. These wild ruminant species have also been identified among the preferred hosts
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11 225 for *Culicoides* feeding in Mediterranean ecosystems (Talavera et al., 2015; Talavera,
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13 226 Muñoz-Muñoz, Verdún, Pujol & Pagès, 2018). Our results suggest that wild ruminant
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15 227 communities, particularly when red deer, fallow deer and mouflon are present, may play
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17 228 a role in the maintenance of SBV in Spain, as was previously demonstrated for bluetongue
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19 229 virus (García-Bocanegra et al., 2011), an *Orbivirus* also mainly transmitted by *Culicoides*
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21 230 biting midges.
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27 231 Our results confirm widespread but not homogeneous circulation of SBV in
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29 232 mainland Spain (Table 2; Fig. 1). The differences between BRs, even when not
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31 233 statistically significant, may be explained by the presence/abundance of competent
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33 234 vectors. The lowest seroprevalence found in BR2 (18.8%) is consistent with their lesser
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35 235 abundance in that region, which has been associated with environmental and climatic
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37 236 factors (Calvete et al., 2008; Cuéllar et al., 2018; Pagès et al., 2018). In contrast, the
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39 237 Atlantic climate of BR1 has been shown to increase the abundance of the *C. obsoletus*
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41 238 group, while the seasonal precipitation and warmer temperatures in BRs 3-5 are
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43 239 predisposing factors for the presence of *C. imicola* in those regions (Calvete et al., 2008).
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45 240 It should be noted however that local biotic and abiotic conditions may be determinant
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47 241 factors in the epidemiology of SBV in particular scenarios within each BR.
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53 242 The first seropositive animals were detected in 2012 (Table 1), according to the
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55 243 first reported SBV outbreak in livestock in Spain (Jiménez-Ruiz et al., 2019).
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57 244 Seropositivity increased to 42.4% in 2013, then gradually decreased to 24.7% in 2016. A
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59 245 similar temporal trend was observed in wild ruminants in the south of this country
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3 246 (declining from 25.5% in 2012/2013 to 16.5% in 2014/2015) (García-Bocanegra et al.,
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5 247 2017). The seroprevalence found in 2013 was also consistent with the high seropositivity
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7 248 (54.4%) reported in domestic ruminants sampled that year in the same area where the first
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9 249 SBV outbreak was reported in Spain (Jiménez-Ruiz et al., 2019). Interestingly, in the
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11 250 present long-term study, anti-SBV antibodies were detected continuously in most of the
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13 251 tested species and in yearling animals between 2012 and 2016 (Table 2), providing
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15 252 evidence of stable, endemic circulation of SBV in wild ruminant populations in the last
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17 253 few years. These findings indicate that it is of interest to include wild ruminants,
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19 254 particularly yearling individuals, in early warning systems in emerging vector-borne
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21 255 disease monitoring programs (Boadella, Díez-Delgado, Gutiérrez-Guzmán, Höfle &
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23 256 Gortázar, 2012; García-Bocanegra et al., 2016; 2017).

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29 257 In summary, our results highlight the high, widespread and heterogenous spatio-
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31 258 temporal distribution of SBV in wild ruminant species in mainland Spain. The
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33 259 uninterrupted detection of seropositive animals, including individual yearlings, indicates
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35 260 endemic circulation of this virus in wild ruminant populations in the last few years. Our
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37 261 results suggest that wild ruminant species, particularly fallow deer, red deer and mouflon,
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39 262 have a potential role as natural reservoirs of SBV in Spain; however, local interpretations
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41 263 should be made in accordance with the prevailing host community and other biotic and
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43 264 abiotic factors. Surveillance in these species could be a complementary way of monitoring
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45 265 SBV in Europe and preventing SBV circulation in sympatric livestock species. Further
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47 266 research is needed to assess the impact of SBV in wild ruminant populations in Spain.

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8
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11 274 participated in the field sampling.
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15 275 **Conflict of interest statement**

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19 276 The authors have declared that no competing interests exist.
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22 277 **Ethical approval**

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25 278 No animals were killed specifically for this study. Serum samples used in this
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27 279 study were collected from animals legally hunted in complete agreement with Spanish
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29 280 and European regulations. No ethical approval by an Institutional Animal Care and Use
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31 281 Committee was deemed necessary. Protocols, amendments and other resources were used
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33 282 according to the guidelines approved by each Autonomous government following the
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35 283 R.D.1201/2005 of the Spanish Ministry of Presidency.
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40 284 **Data Availability Statement**

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43 285 The data that support the findings of this study are available from the
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3 468 **Figure legends**
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6 469 **Figure 1.** Spatial distribution of Schmallenberg virus exposure in the wild ruminant species in
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8 470 mainland Spain (bioregions 1-5). The species drawings represent the locations of the sampling
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10 471 populations, as well as the species sampled at each sampling site.
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16 473 **Figure 2.** Schmallenberg virus (SBV) seroprevalence in wild ruminant species in Spain showing
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18 474 the variation among the observed results (bELISA) and the differences evidenced by the
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20 475 generalized linear mixed model (GLMM) after controlling for the effects of bioregion and
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Table 1. Distribution of the prevalence of antibodies against Schmallenberg virus in wild ruminant populations in Spain by category and results of bivariate analysis.

Variable	Category	No. positives/overall [†]	Seroprevalence (95%CI)	<i>P</i> -value
Species	Barbary sheep	8/36	22.2 (11.7-38.1)	< 0.001*
	Fallow deer	99/217	45.6 (39.1-52.3)	
	Iberian wild goat	49/246	19.9 (15.4-25.4)	
	Mouflon	33/118	28.0 (20.7-36.7)	
	Red deer	97/307	31.6 (26.7-37.0)	
	Roe deer	34/194	17.5 (12.8-23.5)	
	Southern chamois	10/98	10.2 (5.6-17.8)	
Bioregion	BR1	31/96	32.3 (23.8-42.2)	0.015*
	BR2	48/256	18.8 (14.4-24.0)	
	BR3	108/362	29.8 (25.4-34.7)	
	BR4	75/255	29.4 (24.2-35.3)	
	BR5	68/247	27.5 (22.3-33.4)	
Year	2010	0/20	0.0 (0.0-16.1)	< 0.001*
	2011	0/44	0.0 (0.0-8.0)	
	2012	60/189	31.8 (25.5-38.7)	
	2013	42/99	42.4 (33.2-52.3)	
	2014	66/175	37.7 (30.9-45.1)	
	2015	119/511	23.3 (19.8-27.1)	
	2016	43/174	24.7 (18.9-31.6)	
Sex	Male	56/197	28.4 (22.6-35.1)	0.280
	Female	53/157	33.8 (26.8-41.5)	
Age	Yearling	34/94	36.2 (27.2-46.3)	0.530
	Sub-adult	15/51	29.4 (18.7-43.0)	
	Adult	51/171	29.8 (23.5-37.1)	

[†]Missing values omitted. *Selected variables for the multivariate analysis.

Table 2. Spatio-temporal distribution of Schmallenberg virus (SBV) circulation in wild ruminant species in Spain. Data are presented as no. of positives/overall (%).

	B. sheep	Fallow deer	I. W. goat	Mouflon	Red deer	Roe deer	S. chamois	
Bioregion	BR1	-	8/20 (40.0)	-	-	14/20 (70.0)	0/20 (0.0)	9/36 (25.0)
	BR2	-	-	15/60 (25.0)	-	10/60 (16.7)	22/74 (29.7)	1/62 (1.6)
	BR3	-	45/83 (54.2)	2/70 (2.9)	18/71 (25.4)	37/85 (43.5)	6/53 (11.3)	-
	BR4	-	26/60 (43.3)	23/67 (34.3)	-	20/81 (24.7)	6/47 (12.8)	-
	BR5	8/36 (22.2)	20/54 (37.0)	9/49 (18.4)	15/47 (31.9)	16/61 (26.2)	-	-
Year[†]	2010	-	-	-	-	0/20 (0.0)	-	-
	2011	0/10 (0.0)	-	-	-	0/23 (0.0)	0/11 (0.0)	-
	2012	1/12 (8.3)	32/44 (72.7) [‡]	0/22 (0.0)	5/24 (20.8)	12/21 (57.1) [‡]	1/30 (3.3)	9/36 (25.0)
	2013	3/4 (75.0)	24/52 (46.2) [‡]	0/7 (0.0)	4/11 (36.4)	9/20 (45.0) [‡]	2/5 (40.0)	-
	2014	3/4 (75.0)	16/45 (35.6) [‡]	10/36 (27.8)	3/12 (25.0) [‡]	27/53 (50.9) [‡]	7/22 (31.8)	0/3 (0.0)
	2015	1/5 (20.0)	18/59 (30.5) [‡]	26/103 (25.2)	16/58 (27.6) [‡]	41/141 (29.1) [‡]	16/86 (18.6)	1/59 (1.7)
	2016	0/1 (0.0)	9/15 (60.0) [‡]	13/78 (16.7)	5/13 (38.5)	8/27 (29.6) [‡]	8/40 (20.0)	-

[†]Missing values omitted. [‡]Seropositivity to SBV detected in yearling animals.

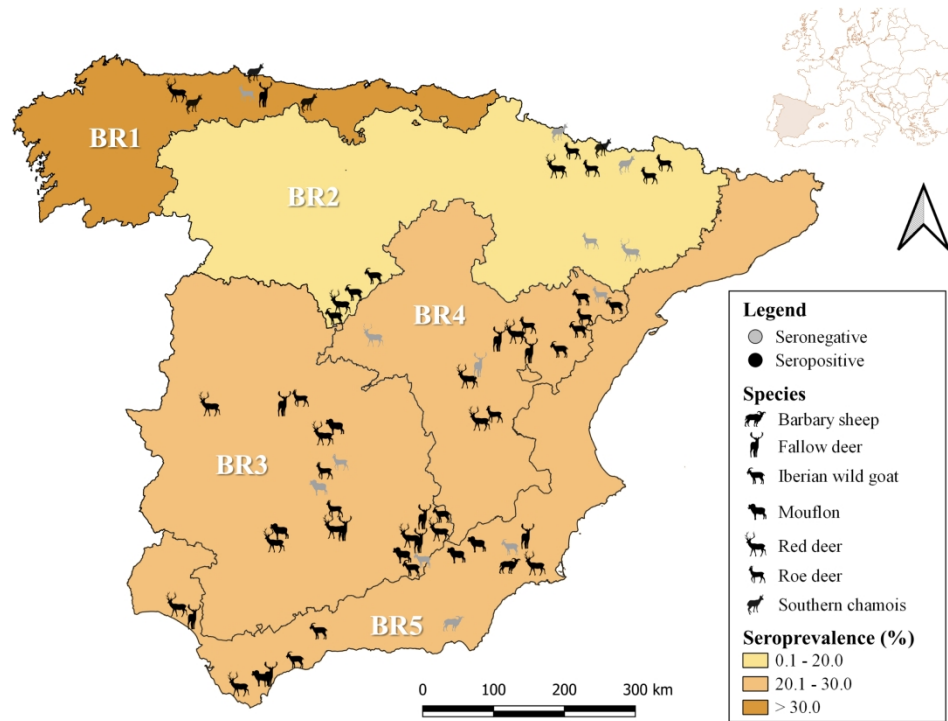


Figure 1. Spatial distribution of Schmallenberg virus exposure in the wild ruminant species in mainland Spain (bioregions 1-5).

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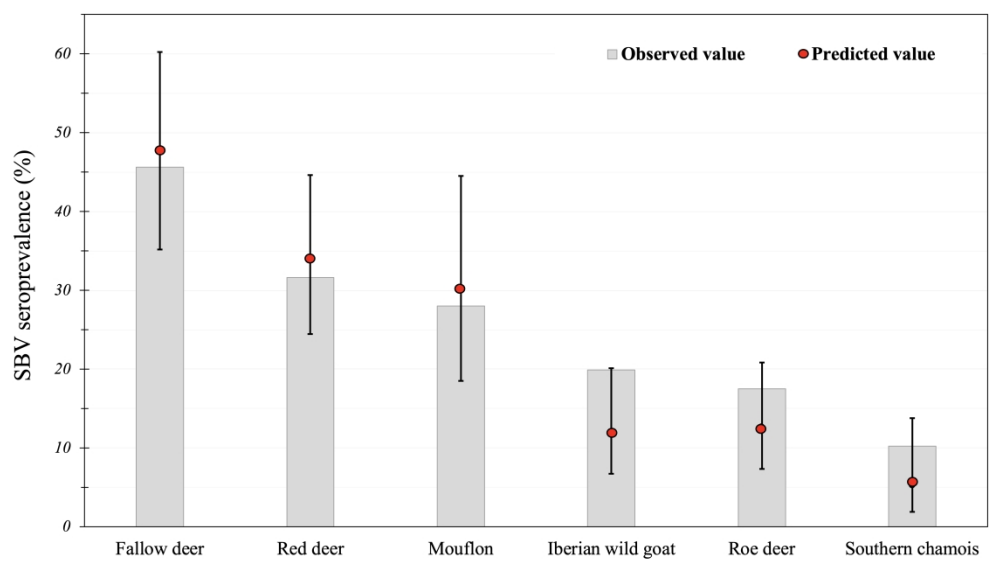


Figure 2. Schmallenberg virus (SBV) seroprevalence in wild ruminant species in Spain showing the variation among the observed results (bELISA) and the differences evidenced by the generalized linear mixed model (GLMM) after controlling for the effects of bioregion and sampling year.