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Epidemiological surveillance of Schmallenberg virus in small ruminants in southern Spain.

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Abstract

Schmallenberg virus (SBV) is an emerging *Culicoides*-borne *Orthobunyavirus* that affects ruminant species. Between 2011 and 2013 it was responsible for a large-scale epidemic in Europe. In the present study, we aimed to determine the seroprevalence, spatial distribution and risk factors associated to SBV exposure in sheep and goats in the region where the first Schmallenberg disease outbreak in Spain was reported. Blood samples from 1,796 small ruminants from 120 farms were collected in Andalusia (southern Spain) between 2015 and 2017. Antibodies against SBV were detected in 536 of 1,796 animals (29.8%; 95%CI: 27.7-32.0) using a commercial blocking ELISA. The individual seroprevalence according to species was 31.1% (280/900; 95%CI: 28.1-34.1) in sheep and 28.6% (256/896; 95%CI: 25.6-31.5) in goats. The farm prevalence was 76.7% (95%CI: 69.1-84.2). Seropositivity to SBV was confirmed in both sheep and goats in all provinces by virus neutralization test. Two significant ($p < 0.001$) spatial clusters of high seroprevalence were identified. The generalized estimating equation analysis showed that management system (extensive), temperature (> 14 ºC) and altitude (< 400 meters above sea level) were risk factors associated with SBV exposure in small ruminants. Our results highlight widespread but not homogeneous circulation of SBV in small ruminant populations in Spain.

Keywords: Schmallenberg virus; risk factors; small ruminants; spatial analysis, animal health.

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1. Introduction.

Schmallenberg disease (SBD) is a vector-borne disease transmitted among domestic and wild ruminants by biting midges of the genus *Culicoides*. The etiological agent, Schmallenberg virus (SBV) is an *Orthobunyavirus* belonging to the Simbu serogroup (family *Peribunyaviridae*) and infection is generally asymptomatic or self-limiting in adult ruminants. Clinical signs reported in cattle include acute fever, diarrhea and decreased dairy production (<50%), while diarrhea, nasal discharge and reduction in milk yield have been observed in small ruminants. The disease is also characterized by reproductive disorders in naïve pregnant ruminants infected during the period of maximum susceptibility (between days 28–50 and 76-174 of gestation in small ruminants and cattle, respectively), which include abortions, stillbirths and congenital malformations described as arthrogryposis-hydranencephaly syndrome (EFSA, 2014). Other findings such as ankylosis, torticollis, kyphosis, lordosis, scoliosis, brachygnathia, cerebellar hypoplasia or thymus hyperplasia have also been reported in domestic ruminants (Rodríguez-Prieto et al., 2016). Experimental SBV infection in these species predominantly results in a short viremic phase followed by seroconversion approximately two weeks later (Laloy et al., 2015; Wernike et al., 2013). Adult animals acquire effective immune protection after natural infection and the level of neutralizing antibodies seems to be effective to prevent viremia, and hence transmission of SBV to other susceptible animals (Martinelle et al., 2015; Rodríguez-Prieto et al., 2016).

Schmallenberg virus was first detected in a cattle herd in 2011 in northwest Germany (Hoffmann et al., 2012) and was the first report of a Simbu serogroup virus in Europe. The virus spread rapidly throughout the continent over the next two years and more than 8,000 outbreaks were reported in 22 European countries (EFSA, 2013). That large-scale epidemic caused significant economic losses in ruminant livestock, mainly associated with international trade restrictions, production losses and veterinary costs (EFSA, 2014; Stavrou, Daly, Maddison, Gough, & Tarlinton, 2017). Schmallenberg virus is showing a pattern of cyclic re-circulation in Europe. Serological surveys of small ruminants at the end of the first epidemic period (2011-2013) revealed high within-flock immunity levels in affected countries (Supporting Information Table S1). Subsequent serosurvey studies have shown a decreasing trend in SBV circulation in ruminant livestock populations in Europe (Delooz et al., 2017; Esteves et al., 2018; Stokes, Baylis & Duncan, 2016; Veldhuis, Mars, Roos, van Wuyckhuise & van Schaik, 2017; Wernike, Hoffmann,

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Conraths, & Beer, 2015; Wernike, Holsteg, Szillat, & Beer, 2018), which could favour SBV re-emergence in favorable epidemiological scenarios (Larska, 2018; Lievaart-Peterson, Luttikholt, Peperkamp, Van den Brom, & Vellema, 2015; Poskin et al., 2016; Stavrou, Daly, Maddison, Gough, & Tarlinton, 2017; Wernike, & Beer, 2020). In this connection, the re-emergence of SBV in 2016 in different European countries was associated with an increase in the number of naïve ruminants (Delooz et al., 2017; Larska, 2018).

The first SBD outbreak in Spain was reported in March 2012 (Jiménez-Ruiz et al., 2019) in a small ruminant flock in the south of the country. A high SBV seroprevalence (up to 54.4%) was found on nearby farms and in other central and northwestern Spanish regions a year after this outbreak was detected (Supporting Information Table S1). Nevertheless, information about SBV distribution in small ruminants in Spain is still very scarce and tends to be limited to local studies focused on the early period after the emergence of the virus in Europe. In the present study, we aimed to determine the seroprevalence, spatial distribution and risk factors associated with SBV infection in sheep and goats in Andalusia (southern Spain), the region where the first SBD outbreak in Spain was reported (Jiménez-Ruiz et al., 2019).

2. Materials and methods.

2.1. Study design and sampling.

A large-scale cross-sectional study was carried out in sheep and goat farms in Andalusia (36° N-38° 60′ N, 1° 75′ W-7° 25′ W) between 2015 and 2017. The number of sampled farms was calculated assuming a farm-level prevalence of 20%, a 95% confidence interval and an accepted error of 10% (Thrusfield, 2018). A stratified sampling design was adopted based on the proportion of farms in the eight provinces of Andalusia. The census of small ruminants was retrieved from the Spanish Ministry for Agriculture, Fisheries and Food (MAPA, 2015). Within each province, farms were selected randomly. A total of 120 farms (60 sheep flocks and 60 goat flocks) were sampled in 81 municipalities. The location of each sampled farm was identified by GPS. Whenever possible, 15 animals more than nine months old were randomly selected from each farm to detect SBV exposure at a minimum expected seroprevalence of 20% with a probability of 95%. A total of 1,796 small ruminants (900 sheep and 896 goats) were finally included in the study. Blood samples were collected by puncture of the jugular vein using sterile tubes without anticoagulant (Vacutainer®, Becton-Dickinson, USA). Samples were centrifuged for 10 min at 400 *g* to obtain serum and stored at -20°C until analysis.

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Epidemiological data were collected through personal interviews with the farmers using a questionnaire (supplementary material). The questionnaire was especially designed to collect information using ‘‘close-ended’’ questions to avoid ambiguous or lengthy answers. A total of 19 explanatory variables were included in the questionnaires to obtain information on exposure levels to potential risk factors associated with SBV in the farms. The variables were grouped by topic: 1) Individual data: identification number, species (sheep or goat) and sampling date. 2) Farm data: farm code, province, municipality, location of the farm, density of ruminants at municipality level, census of ruminants per farm, management system (extensive, semi-extensive or intensive), type of production (meat, milk or mixed), distance to nearest farm, contact with livestock from other farms, presence of wild ruminants, transhumance (seasonal movement of livestock from one grazing ground to another, typically to lowlands for winter and highlands for summer) in the last three years, animal insecticide treatment, cleaning and disinfection protocols and reproductive disorders in the last two years. 3) Environmental variables: mean annual temperature (ºC), mean annual rainfall (l/m^2) and altitude (meters above sea level (masl)). Altitude was determined using the geographic coordinates of the farms. Climatic variables recorded by weather stations in the proximity of the sampling farms (average distance: 19.5 km; range: 0.2-59.0 km) were obtained from the National Meteorological Institute (Spanish Ecological Transition and Demographic Challenge Ministry) (Figure 1).

2.2 Serological analyses.

Serum samples were analyzed using a commercial blocking ELISA (bELISA, 3.SBV.K3 INGEZIM Schmallenberg COMPAC 2.0®, INGENASA, Madrid, Spain) which detects specific SBV-antibodies against the N protein antigen. The bELISA was performed according to the manufacturer’s recommendations. The sensitivity and specificity values provided by the manufacturer were 99%. This bELISA has been used previously in different studies of domestic and wild ruminant species (García-Bocanegra et al., 2017; Jiménez-Ruiz et al., 2019).

A selection of 40 bELISA-positive sera, including sheep and goats from each province, were tested by virus neutralization test (VNT) using the BH80/11–4 isolate (provided by the Friedrich-Loeffler-Institut, Isle of Riems, Germany), 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 $TCID_{50\%}$) in Vero cells. Sera that showed neutralization (absence of cytopathic effect) at dilutions ≥1:4 were considered positive.

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2.3 Statistical analysis

The prevalence of antibodies against SBV was established from the proportion of seropositive samples to the total number of small ruminants examined. Confidence intervals of 95% (95%CI) for proportions were obtained by the exact binomial method. A spatial statistical scan was carried out to detect significant clusters ($p < 0.05$) of high SBV seroprevalence using a Bernoulli model (Kulldorff, Huang, Pickle, & Duczmal, 2006). The number of Monte Carlo simulations was set at 1,000 for the cluster scan statistic. The analysis was performed using SaTScan™, v9.6.

The distribution of the categorical and continuous variables was assessed with frequency distributions and histograms, respectively. For the continuous variables (number of small ruminants in the farm, census of small ruminants and small ruminants' farms in the region, mean annual rainfall and mean annual temperatures), the assumption of linearity was tested, and if the assumptions were not met, the variables were categorized taking the percentiles 33 and 66 as cut points in order to homogenize the scales of the explanatory variables. Associations between seropositivity and explanatory variables were initially screened using the Chi-square test or Fisher's test, as appropriate. Variables with a *p*-value < 0.20 in bivariate analysis were selected for inclusion in the multivariate analysis. Cramer's V coefficient was computed to assess collinearity between pairs of selected variables. Finally, a generalized estimating equation model (GEE) was used to assess the effect of the variables selected in bivariate analysis. "Farm" was included as a random factor and the number of seropositive animals was assumed to follow a binomial distribution. The model was re-run until all remaining variables presented statistically significant values ($P < 0.05$) and there was a potential relationship with the response variable. The choice of the best model was based on the quasi-likelihood under independence model criterion (QIC). Statistical analyses were performed using SPSS v25.0 software (Statistical Package for Social Sciences Inc., Chicago, IL, USA).

3. Results.

The overall individual seroprevalence of SBV was 29.8% (536/1,796; 95%CI: 27.7-32.0). Antibodies against SBV were detected in 280 of 900 sheep (31.1%; 95%CI: 28.1-34.1) and 256 of 896 goats (28.6%; 95%CI: 25.6-31.5). The VNT results confirmed specific anti-SBV antibodies in 18 of the 20 sera tested from both sheep and goats. Two samples (one sheep, one goat) could not

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be tested by VNT due to cytotoxicity and the remaining two sera (one sheep, one goat) showed negative result to this technique. At least one seropositive animal was detected on 46 sheep farms and 46 goat farms. Therefore**,** seropositivity was found on 76.7% (92/120; 95%CI: 69.1-84.2) of the farms.

Seropositivity was also detected in 65 (80.3%) of 81 municipalities sampled. Statistically significant differences were observed between provinces ($p < 0.001$) with the lowest seroprevalences at province level being found in Granada (9.0%) and Almeria (10.5%) and the highest in Huelva (66.1%) and Jaen (66.7%). In the remaining provinces, seropositivity values were homogeneous and ranged between 24.3% and 31.2% (Table 1, Figure 2). Seropositive animals of both species were confirmed by VNT in all provinces. The spatial analysis identified two statistically significant clusters in the study area. The first cluster, with a radius of 65 km, was located in the northern part of Huelva province and included eight farms (Relative Risk (RR) = 2.4; $p < 0.001$). The second one, with a radius of 53 km, was located in the northern part of Jaen province and included 11 farms (RR = 2.2; $p < 0.001$) (Figure 2).

The final GEE model identified three risk factors potentially associated with SBV seropositivity in small ruminants: management system, mean annual temperature and altitude (Table 2). Extensively raised animals had significantly higher seropositivity (41.4%; 234/565) than individuals managed under semi-extensive (25.3%; 228/901) and intensive (21.7%; 65/300) production systems. SBV seropositivity in small ruminants increased significantly in areas with mean annual temperatures between 14 and 16 ºC (34.9%; 182/521) and above 16 ºC (35.2%; 322/915) relative to animals sampled in areas with mean annual temperatures below 14 ºC (8.9%; 32/360). Finally, the prevalence of antibodies against SBV was significantly higher in animals on farms situated at altitudes below 400 masl (36.3%; 272/750) compared to those found above 1,000 masl (9.3%; 14/150).

4. Discussion.

The individual seroprevalence obtained in the present study indicates high SBV exposure in small ruminant populations in southern Spain. Significant differences in seroprevalence were not found between sheep and goats, indicating similar levels of exposure to SBV in both species. Although it has been shown that sheep are clinically more susceptible to SBV infection than goats (Helmer et al., 2016), similar seroprevalence values have also been found for the two species in

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other European countries (Supporting Information Table S1). In Europe, wide variations in the prevalence of antibodies to SBV have previously been reported in small ruminants, ranging between 0.0% in the United Kingdom (2015) and 89.0% in the Netherlands (2011-2012) for sheep, and between 2.8% in Turkey (2006-2013) and 78.3% in Austria (2012-2013) for goats (Supporting Information Table S1). The seroprevalence found in our study falls within these values, although comparisons between studies should be made with caution, given differences in the numbers of animals examined, study designs and sampling periods. It should also be noted that most of previous serosurveys were carried out during the first epidemic period (2011-2013). In connection with this, the seropositivity obtained in our study in the province of Cordoba (29.0%) was markedly lower than that reported a year after the first outbreak was detected in Spain in the same species and region (42.6%) (Jiménez-Ruiz et al., 2019). Our results highlight a decrease in seroprevalences of SBV in small ruminant populations in the study area. A significant decreasing trend was also observed in wild ruminant populations in the same region, with seroprevalence values ranging from 25.5% to 16.5% between hunting seasons 2012/2013 and 2014/2015, respectively (García-Bocanegra et al., 2017). These findings could indicate endemic but decreasing SBV circulation in southern Spain. The establishment of high within-flock immunity after SBV introduction could help reduce the virus circulation. Nevertheless, the natural replacement of previously exposed animals by seronegative youngstock may increase the risk of SBV re-emergence in immunologically naïve ruminant populations (Larska, 2018; Wernike et al., 2015).

The detection of seropositive animals on 76.7% of farms analyzed, in 80.3% of municipalities sampled, and in all the provinces of Andalusia indicates that SBV is widespread in southern Spain, despite the fact that the spatial analysis showed a heterogenous distribution throughout the study region. The two spatial clusters identified were located in the provinces with the highest seroprevalence (Figure 2). Geographical variations in SBV seroprevalence have been widely documented (Esteves et al., 2016; Helmer et al., 2016; Méroc et al., 2014; Wernike et al., 2014) and are mainly associated with environmental conditions that favor the presence and abundance of competent vectors in certain regions (Cuéllar et al., 2020; Ribeiro et al., 2015). In this regard, previous entomological surveillance of *Culicoides* species showed that the western and central regions of Andalusia had the highest densities of these vectors (Cuéllar et al., 2018), which is consistent in the present study with the lowest SBV seropositivity in the easternmost provinces. Our results also agree with the spatial distribution of outbreaks of bluetongue virus (BTV), another

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Culicoides-borne virus that has been shown to be markedly higher in western and central Andalusia (Allepuz et al., 2010; RASVE, 2020). Additional studies are warranted to elucidate the factors involved in the higher SBV circulation in the risk areas identified.

The GEE model showed that management system, mean annual temperature and altitude were risk factors associated with SBV exposure in sheep and goats. Significantly higher seropositivity was found in extensively managed small ruminants than in those raised under semi-extensive or intensive production systems. This result is in accordance with previous observations in livestock ruminants (Valas et al., 2015; Veldhuis, Carp-van Dijken, Van Wuijckhuise, Witteveen, & van Schaik, 2014; Wernike et al., 2014, 2018), and is probably associated with a higher risk of exposure to infected *Culicoides* midges while grazing. Keeping pregnant females indoors during the critical period of early gestation has been suggested as a management measure to protect small ruminants against SBV exposure (Helmer et al., 2016). In addition, extensive management of female youngstock during the vector-active season could facilitate SBV infection and the consequent development of naturally acquired immunity before they become pregnant (Collins, Doherty, Barrett, & Mee, 2019; Veldhuis et al., 2014).

Significantly higher seropositivity was observed in animals from areas where the mean annual temperatures were above 14ºC compared to those from colder areas (Figure 1). Temperature is one of the main determinants of *Culicoides* activity and virogenesis (Carpenter et al., 2011; Purse, Carpenter, Venter, Bellis, & Mullens, 2015). In this sense, modelling of the spread of SBV during the 2011-2013 epidemic, evidenced vector activity at temperatures above 16°C (EFSA 2014). Similarly, the optimal temperature for BTV transmission in Europe lies in the range of 15º to 25ºC (Gubbins, Carpenter, Baylis, Wood, & Mellor, 2008). It has also been shown that temperatures below 13ºC are a limiting factor for *Culicoides imicola* (Kieffer, 1913) vector capacity (Leta et al., 2019; Venter, Boikanyo, & de Beer, 2019), the main vector of *Culicoides*-borne viruses in the study region (Arenas-Montes et al., 2016; Calvete et al., 2008). In this regard, Napp et al. (2016) observed a positive correlation between temperature and the case-reproduction ratio of BTV during the 2007 BTV-1 epidemic in southern Spain, which fell below 1 when temperatures dropped below 21°C.

Altitude is also a determinant factor for SBV transmission, since *Culicoides* midges are known to be highly altitude-dependent (Acevedo et al., 2010; Scolamacchia, Van Den Broek, Meiswinkel, Heesterbeek, & Elbers, 2014). The occurrence of *C. imicola* is limited at altitudes

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above 420 masl (Torina, Caracappa, Mellor, Baylis, & Purse, 2004). In our study area, the risk of being seropositive to SBV was 2.9 times higher on small ruminant farms situated at altitudes below 400 masl (Figure 1). The negative correlation between SBV presence and altitude was also reported previously for both domestic (Sibhat, Ayelet, Gebremedhin, Skjerve, & Asmare, 2018) and wild ruminant species (Rossi et al., 2017). While it has been shown that the probability of *C. imicola* occurrence is very limited at altitudes > 900 m (Conte et al., 2003), we nevertheless detected seropositivity in flocks above 1,000 masl in non-transhumant farms. Consistent with our results, SBV exposure was detected in domestic and wild ruminant species in areas of the Iberian Peninsula above 2,000 m altitude (Esteves et al., 2018; Fernández-Aguilar et al., 2014). These findings indicate that other *Culicoides* species such as those belonging to *Culicoides obsoletus* group (Meigen, 1818), [*C. obsoletus* and *Culicoides scoticus* (Downes & Kettle, 1952)], *Culicoides pulicularis* group (Linnaeus, 1758) [*C. pulicularis* and *Culicoides lupicaris* (Downes & Kettle, 1952)] and others (Alarcón-Elbal, 2015; Pérez, García-Ballester, López-Olvera & Serrano, 2012), may also play a role in SBV transmission in the study area, as has been suggested for other Spanish regions (Pagés et al., 2018).

This study has some limitations that should be taken into account. First, only animals of more than nine months old were included in the present study to avoid the effect of maternally derived antibodies. For logistical reasons, however, it was not possible to determine the ages of individuals > 9 months old and we were therefore unable to establish whether the seropositive animals had been infected in the sampling year. Further serosurveys including yearling animals older than eight months (Veldhuis et al., 2017) and molecular studies are warranted to assess the temporal circulation of SBV in the study area. Finally, in order to establish the real impact of SBV in small ruminants in southern Spain, additional laboratory analyses are required to determine the pathogens implicated in reproductive disorders detected on affected farms.

5. Conclusions

The lower seroprevalence of SBV observed in domestic ruminants in southern Spain in the study period compared with that found just after the virus was first reported in Spain indicates a decreasing trend of virus exposure, which may mean the threat of SBD re-emergence once flock immunity is weakened. Our results provide evidence of widespread but not homogeneous distribution of SBV in small ruminant populations. The greatest exposure to SBV was related to

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extensive management systems and environmental conditions. The spatial clusters identified should be prioritized in the context of future risk-based surveillance actions, not only for SBV, but also other *Culicoides*-borne diseases. In the case of decreasing flock immunity in these areas, as has been observed in other European regions, the risk of SBD outbreaks should be expected to be high. Further research is warranted to assess the real impact of SBV on the health status of small ruminant populations in the study area. There is also a need to establish comprehensive monitoring strategies in order to better understand the future dynamics of SBV in Europe.

Declaration of Competing Interests

The authors declare that they have no conflict of interest.

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Ethical statement

The collection of blood samples analyzed in the present study was part of the official Animal Health Campaigns of Regional Government of Andalusia, Spain. Therefore, no ethical approval was necessary.

Data Availability Statement

The data that support the findings of this study are available from the authors upon reasonable request.

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Table legends

Table 1. Distribution of explanatory variables associated with SBV seropositivity in small ruminants in Andalusia (southern Spain).

Table 2. Generalized estimating equation analysis of the risk factors associated with SBV seropositivity in small ruminants in Andalusia (southern Spain).

Supporting Information Table S1. Prevalence of antibodies against SBV in sheep and goats in Europe.

Figure legends

Figure 1. Map of Andalusia (southern Spain) showing the mean annual temperature (ºC) and altitude (m). Adapted from REDIAM 2020, Government of Andalusia.

Figure 2. Distribution of the sampled farms. The sampled goat and sheep farms are represented by triangles and circles, respectively. Gradations of green and gray indicate within-farm seroprevalence and seroprevalence at province level. Large pale blue circles indicate the two significant spatial clusters observed in the study region ($p < 0.001$).

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Table 1.

Variable	Categories	Positives / Total* (%)	*p* – value
Species	Sheep	280/900 (31.1)	0.240
	Goat	256/896 (28.6)	
Year	2015	74/180 (41.1)	0.001
	2016	402/1396 (28.8)	
	2017	60/220 (27.3)	
Province	Almeria	22/210 (10.5)	< 0.001
	Cadiz	34/135 (25.2)	
	Cordoba	86/296 (29.1)	
	Granada	23/255 (9.0)	
	Huelva	119/180 (66.1)	
	Jaen	90/135 (66.7)	
	Malaga	73/300 (24.3)	
	Seville	89/285 (31.2)	
Location of the farm	Eastern	135/600 (22.5)	< 0.001
	Central	159/596 (26.7)	
	Western	242/600 (40.3)	
Census of ruminants per farm	<301	242/581 (41.7)	< 0.001
	301-637	147/630 (23.3)	
	>637	147/585 (25.1)	
Density of ruminants at municipality level (animals/km^2)	<17	189/600 (31.5)	< 0.001
	17-40	136/600 (22.7)	
	>40	211/596 (35.4)	
Management system	Extensive	234/565 (41.4)	< 0.001
	Semi-extensive	228/901 (25.3)	
	Intensive	65/300 (21.7)	
Type of production	Meat	238/747 (31.9)	0.109
	Milk	134/465 (28.8)	
	Mixed	118/450 (26.2)	
Distance to nearest farm (m)	<100	282/769 (36.7)	< 0.001
	100-1000	9/26 (34.6)	

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	>1000	245/1,001 (24.5)	
Contact with livestock from other farms	Yes	172/450 (38.2)	< 0.001
	No	327/1,196 (27.3)	
Presence of wild ruminants	Yes	232/539 (43.0)	< 0.001
	No	304/1,242 (24.5)	
Transhumance in the last three years	Yes	40/105 (38.1)	0.051
	No	479/1,645 (29.1)	
Animal insecticide treatment	Yes	285/1,030 (27.7)	0.124
	No	97/301 (32.2)	
Cleaning protocol	Yes	332/1,196 (27.8)	0.009
	No	72/195 (36.9)	
Disinfection protocol	Yes	323/1,195 (27.0)	< 0.001
	No	81/196 (41.3)	
Reproductive disorders in the last two years	Yes	192/660 (29.1)	0.022
	No	26/60 (43.3)	
Mean annual temperature (ºC)	<14	32/360 (8.9)	< 0.001
	14-16	182/521 (34.9)	
	>16	322/915 (35.2)	
Mean annual rainfall (l/m^2)	<740	45/435 (10.3)	< 0.001
	740 – 1,140	264/851 (31.0)	
	>1,140	227/510 (44.5)	
Altitude (masl)	< 400	272/750 (36.3)	< 0.001
	400 – 1,000	250/896 (27.9)	
	>1,000	14/150 (9.3)	

* Missing values omitted

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Table 2.

Variable	Categories	Positives / Total (%)	*p* - value	Odds ratio	95%CI
Management system	Extensive	234/565 (41.4%)	0.013	2.9	1.3 – 6.7
	Semi-extensive	228/901 (25.3%)	0.241	1.6	0.7-3.3
	Intensive	65/300 (21.7%)	[a]	[a]	[a]
Mean annual temperature (ºC)	>16	322/915 (35.2%)	0.005	2.9	1.4 – 6.2
	14-16	182/521 (34.9%)	0.002	3.3	1.6 – 6.9
	<14	32/360 (8.9%)	[a]	[a]	[a]
Altitude (masl)	< 400	272/750 (36.3%)	0.036	2.9	1.1 – 7.7
	400 – 1,000	250/896 (27.9%)	0.099	2.0	0.9 – 4.8
	>1,000	14/150 (9.3%)	[a]	[a]	[a]

[a] Reference category.

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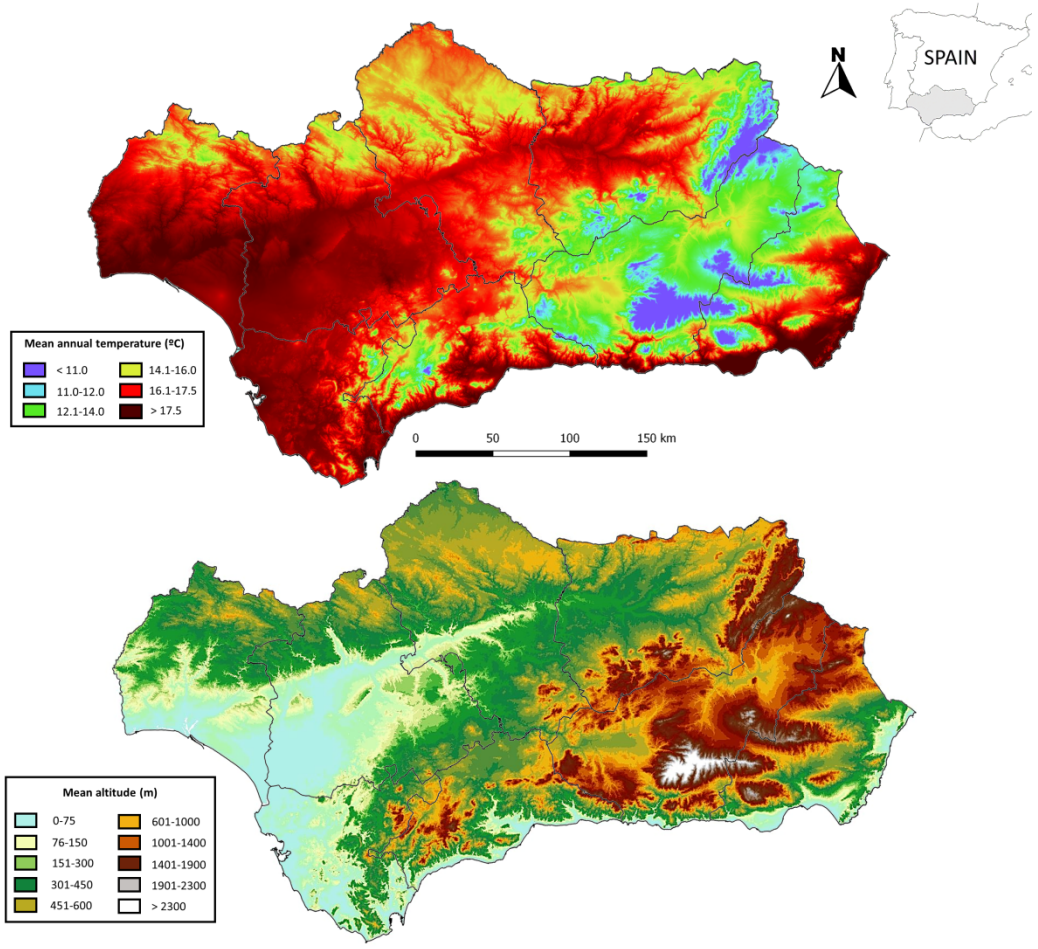

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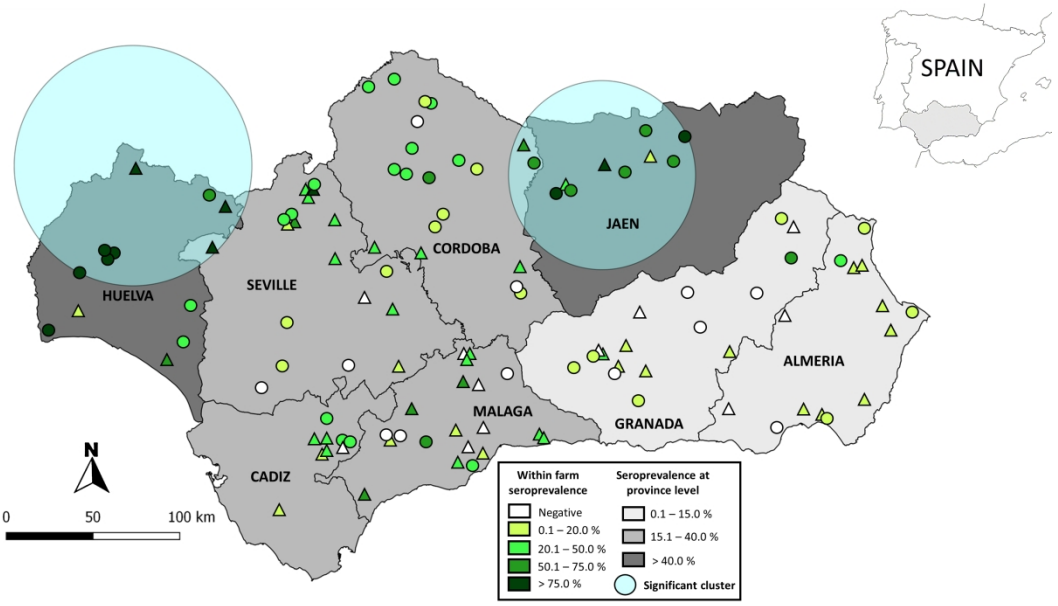

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