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Short Communication

Epidemiology of reproductive pathogens in semi-intensive lamb-producing flocks in North-West Spain: A comparative serological study

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ABSTRACT

The aim of this study was to better understand the epidemiology of five major reproductive pathogens in semi-intensive lamb-producing flocks in North-West Spain. Two thousand four hundred serum samples were collected from 44 flocks, and several factors were studied to assess their influence on seropositivity.

Farms that tested seropositive for more than one pathogen were common (84.1%), but seroprevalence was high only for *Toxoplasma gondii* (38.1%), and low for the other four pathogens, namely, *Neospora caninum* (5.5%), *Coxiella burnetii* (4.0%), *Chlamydia abortus* (3.9%) and *Pestivirus* (2.3%). Farm level exposure was highest for *T. gondii* and *N. caninum* (100% and 72.7%, respectively). For both of these pathogens, and for *C. burnetii*, seropositivity was associated with age and area. Farm level exposure was lowest for *Pestivirus* and *C. abortus* (13.6% and 18.2%, respectively), and, for the latter, seropositivity was principally related to flock size.

Abortion is a major cause of economic loss for sheep farmers. Ovine enzootic abortion (OEA, *Chlamydia abortus*), toxoplasmosis (*Toxoplasma gondii*) and Border Disease (BD; a pestivirus) are among the most commonly diagnosed causes of abortion (Longbottom and Coulter, 2003; Nettleton and Willoughby, 2007; Dubey, 2009). Q fever (caused by *Coxiella burnetii*) has also been reported as a cause of significant abortion outbreaks in sheep (Rodolakis, 2006). Neosporosis (*Neospora caninum*) is not considered to be a major reproductive disease in sheep. Indeed, its epidemiological importance in sheep remains uncertain (Dubey and Schares, 2011). However, the level of exposure of sheep to *N. caninum* is not well characterized and the disease may be important under certain conditions.

Serological studies of abortion are usually focused on one pathogen or are based on investigations of clinically-evident abortion outbreaks. The aim of this study was to better understand the relative importance of five reproductive pathogens in semi-intensive lamb-producing flocks in North-West Spain by studying the seroprevalence of these pathogens together and by assessing the influence of different factors on the risk of exposure.

This study was conducted in Galicia (a region in North-West Spain), which in 2010 had 262 lamb-producing flocks with more than 100 animals. Of these farms 137 were registered with the Galician Health Association of Ovine and Caprine Breeders (ACIVO). Participation in this study was offered to all the farms of the association; 44 accepted and were included in the study (mean flock size, 187.4; median, 150.5; Q1, 85.75; Q3, 207.25). Farms were distributed throughout the whole agro-livestock area of Galicia. None of the farms had previously used vaccination against any of the five pathogens we tested.

All animals >6 months of age were eligible for sampling. The 'sample()' function in R (R Development Core Team, 2011) was used to obtain random numbers to determine which animals were sampled. The sample size was calculated for each farm using the statistical software Epidat 3.1.[1] Confidence level was set at 95% and desired absolute precision at 10%. Since seroprevalence was unknown, a theoretical prevalence of 50% was used to maximize sample size (Thrusfield, 2007). A total of 2400 serum samples were collected (mean number of samples/farm, 54.5; median, 59; Q1, 40.5; Q3, 68). The tests used for determining serological status are summarized in Table 1. Sensitivities and specificities were obtained from the literature or manufacturer, and were used to calculate true seroprevalence (Noordhuizen et al., 1997). Farms were considered positive when their true seroprevalence was >0.

The management system and bioclimatic areas are fully described in Lago et al. (2012) which also includes the questionnaire that was used to identify potential risk factors. In addition,

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[1] See http://dxsp.sergas.es/ (accessed 25 June 2013).

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Table 1
Kits used in the study to analyse the serological status of sampled sheep from semi-intensive lamb-producing flocks in Galicia.

Pathogen	Type of assay	Kit used	Se	Sp	Source of Se and Sp
Chlamydophila abortus	Indirect ELISA	ELISA ID Screen *Chlamydophila abortus* Indirect Multi-species (ID VET)	70.4%	95.6%	McCauley et al., 2007
Neospora caninum	Indirect ELISA	ID Screen *Neospora caninum* Indirect Multi-species (ID VET)	100%	98.8%	Reichel et al., 2008
Coxiella burnetii	Indirect ELISA	Chekit Q fever antibody ELISA Test Kit (Idexx)	100%	99.6%	Horigan et al., 2011
Toxoplasma gondii	Direct agglutination	Toxo-Screen DA (BioMérieux)	92.6%	95.2%	Mainar-Jaime and Barberán, 2007
	Indirect ELISA	ELISA ID Screen *Toxoplasma gondii* Indirect Multi-Species (ID VET)[a]	90.5%	95.8%	Mainar-Jaime and Barberán, 2007
Pestivirus	Competitive ELISA	ELISA BVD Ab/BVD/MD P80 Antikörper (Institute Pourquier)	97.6%	97.2%	Manufacturer
	Direct ELISA	SERELISA BVD p80 Ag Mono Indirect (Synbiotics)[b]			

Se, sensitivity; Sp, specificity.
[a] Used to check doubtful cases from the direct agglutination test.
[b] Used to test all animals from farms where antibody seroprevalence was over 10%. True seroprevalence was not calculated.

for this analysis, farmers were asked about abortions in the previous 5 years. A chi-square test was used to analyse, for each pathogen, the association between seropositivity and the independent variables from the questionnaire (see Table 2). As no co-linearity of variables was detected by examination of the correlation matrix and the variance inflation factors (Faraway, 2002), those variables where $P \leq 0.1$ in the chi-square test were introduced into multivariate models using generalized estimating equations, with flock included as a random effect. Variables were removed by *P*-value order from the model, keeping those that were statistically significant ($P \leq 0.05$) and looking for the best goodness-of-fit using quasi-likelihood under the independence model criterion. All biologically plausible interactions were evaluated. Statistical analysis was performed using the functions 'cc()' from the **epicalc** package (Chongsuvivatwong, 2011) and 'geeglm()' from the 'geepack' package (Højsgaard et al., 2006) in R.

Differences were found in epidemiology between pathogens. Most farms had been exposed to *T. gondii* and *N. caninum* (100% and 72.7%, respectively; Fig. 1). For both of these pathogens, seropositivity was higher in animals >16 months of age, indicating that risk of exposure increased over time. Seropositivity was also higher in animals from the centre-coast area (Table 3), which has a greater sheep population than the mountain areas (Panadero et al., 2010), indicating that exposure was greater where the intermediate hosts were more likely to have contact with sheep. Seropositivity to *C. burnetii* showed a similar pattern.

Few farms were exposed to *C. abortus* and *Pestivirus* (13.6% and 18.2%, respectively; Fig. 1). However, some flocks showed a high seroprevalence; on such farms seropositivity was similar across animals of all ages. Flock size was associated with increased seroprevalence against *C. abortus* (Table 3), probably because a high number of animals on a farm increased the number of contacts and the diffusion of contaminated material within pens. For the current study, we used a major outer membrane protein ELISA, which Livingstone et al. (2005) reported was useful for detecting antibodies against *C. abortus* slightly prior to abortion. Using this ELISA allowed us to identify sheep which were seropositive for *C. abortus* that were <16 months of age (21.2% of seropositive animals were between 6 and 12 months of age), i.e. before their first lambing. Classically, infection with *C. abortus* has been undetectable before abortion, so the ability to detect infection in young animals could be very useful for controlling OEA.

Only one BD viraemic animal was found in this study, although there were four flocks with a seroprevalence >10%. Persistently infected animals (PIs) are the main source of infection in flocks, but

Table 2
Variables included in the initial univariate analysis of potential risk factors for seropositivity against five reproductive pathogens in sheep from semi-intensive lamb-producing flocks in Galicia.

Variable	Categories	Criteria	*n*	%
Flock size	<95 animals	Terciles	416	17.3
	95–177 animals		874	36.4
	>177 animals		1110	46.3
Age	<16 months	Average of first parturition (16 months)	291	12.2
	>16 months		2104	87.8
Sex	Male		122	5.1
	Female		2278	94.9
Birth season	Spring		326	13.6
	Summer		616	25.7
	Autumn		989	41.2
	Winter		469	19.5
Sampling season	Spring	Sampled	1548	64.1
	Summer		218	9.1
	Autumn		228	9.5
	Winter		416	17.3
Purchased Animals	<6	Terciles (two upper terciles combined)[a]	674	28.1
	>5		1726	71.9
Area of origin	Centre-coast		1989	82.9
	Mountain		411	17.1

[a] Each farm purchased at least one animal, so high purchases were compared with low ones.

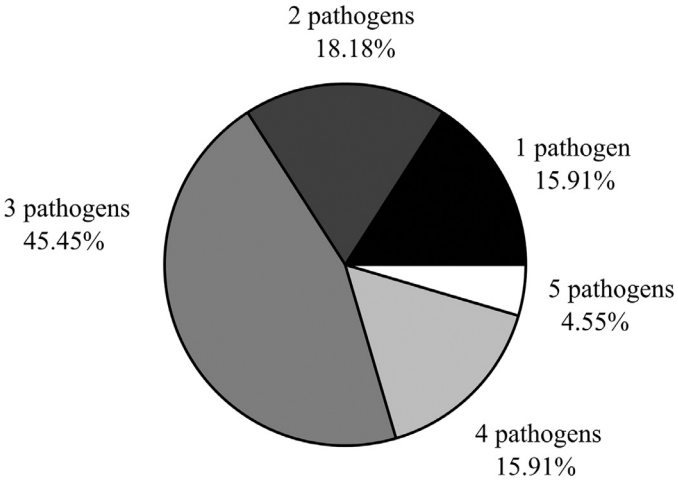

Fig. 1. Box plot of intra-flock true seroprevalence for each pathogen ($n = 44$ flocks). The percentage of positive flocks and individual seroprevalence for each pathogen is shown, with 95% confidence intervals within brackets. Whiskers extend to the most extreme data point, which is not more than 1.5 times the interquartile range from the box.