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ORIGINAL ARTICLE

Seroprevalence of sheep and goat brucellosis in the northeast of Portugal

Seroprevalencia de brucelosis ovina y caprina en el nordeste de Portugal

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RESUMEN

Se realizó una encuesta para estimar la seroprevalencia de brucelosis ovina y caprina en la región de Trás-os-Montes e Alto Douro, al noreste de Portugal. En total fueron analizados 278.097 pequeños rumiantes distribuidos en 5.466 rebaños pertenecientes a 13 organizaciones de Ganaderos (OPP) fueron analizados. Cuatrocientos ochenta y siete (8,9%) rebaños tenían uno o más animales serológicamente positivos, con valores que oscilaban entre 8,2% y 9,7%. La prevalencia individual fue de 0,44% (IC 95% 0,40-0,48%). No se detectaron diferencias estadísticamente significativas asociadas al tamaño de los rebaños, las especies, la constitución del rebaño, el tipo de producción y la OPP. Basándose en los resultados de esta encuesta, un pequeño porcentaje de animales y un alto porcentaje de los rebaños en el noreste de Portugal fueron serológicamente positivos. Dada la escasez de estudios epidemiológicos sobre la brucelosis en el norte de Portugal, la información sobre la seroprevalencia obtenida en este estudio es importante a la hora de definir medidas de control de la brucelosis en la zona.

Palabras clave: Brucella melitensis, pequeños rumiantes, epidemiología, seroprevalencia.

SUMMARY

A survey to estimate the seroprevalence of ovine and caprine brucellosis was conducted in the region of Trás-os-Montes e Alto Douro, Northeast of Portugal. In total, 278,097 small ruminants and 5,466 flocks from 13 Livestock Farmers Organizations (OPP's) were analysed. Four hundred and eighty seven (8.9%) flocks had one or more serologically positive animals with values ranging between 8.2% and 9.7%. The individual seroprevalence was 0.44% (CI 95% 0.40-0.48%). There were significant differences in seroprevalence rates among herd sizes, species, constitution of herd, production's type and OPP. Based on the results of this survey, a small percentage of animals and a high percentage of flocks in the Northeast of Portugal were serologically positive. Considering the paucity of epidemiological reports on brucellosis in the Northeast of Portugal the information on seroprevalence provided in this study is necessary to define control measures for brucellosis in the area.

Key words: Brucella melitensis, small ruminants, epidemiology, seroprevalence.

INTRODUCTION

Brucella melitensis occurs naturally in sheep and goats and is highly pathogenic for humans, causing one of the most serious zoonosis in the world. The disease is responsible for considerable economical losses to the small ruminant industry (Benkirane 2006, OIE 2009[1]). Sheep and goats brucellosis is endemic in most countries of the Mediterranean basin, the Middle East and Central Asia (Omer *et al* 2000, Al-Majali *et al* 2005), Latin America, and parts of Africa (Benkirane 2006). The first report of brucellosis in Portugal is from 1873. An eradication programme was initiated in Portugal, in 1990, in small ruminants, with the financial support of the European Commission. This programme was based on test and slaughter policy, using Rose Bengal Test (RBT) and Complement Fixation Test (CFT) and the farmers received compensation for the slaughtered animals. A new program of control and eradication started in Portugal with flock vaccination during 2001 - 2004 with the live *Brucella melitensis* reversion 1 strain vaccine (Rev. 1 vaccine, conjunctival route and dose of $1x10^9$), and continued the following years with vaccination of young replacements (Neto and Vaz 2002). Traditionally, brucellosis diagnosis was based in the detection of circulating antibodies followed by bacteria isolation of the microorganisms (Cassataro *et al* 2004, O'Leary *et al* 2006). Bacteriological diagnosis has lack of sensitivity, and is not a practical and reliable means for diagnosis in large-scale programs (Cassataro *et al* 2004, Garin-Bastuji *et al* 2006). These limitations make serology the most useful epidemiological tool for laboratory diagnosis of *Brucella* infection (Erdenebaater *et al* 2004, Nielsen *et al* 2002). The RBT and the CFT are the most widely used tests for diagnosis of *Brucella melitensis* infection and are the

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only prescribed tests (OIE 2009). Usually, the RBT is used as a screening test and CFT as a confirmatory test. Both tests are based in an antigen reaction of the entire cells of *Brucella* and the antibodies produced as response of infection (OIE 2009).

The present status of the disease in the region of Trás-os-Montes e Alto Douro is not well defined causing concern among health technicians. The aim of this study was to estimate seroprevalence of brucellosis in small ruminants considering the paucity of epidemiological reports on brucellosis in the Northeast of Portugal.

MATERIAL AND METHODS

STUDY AREA

Animals and flocks were located in Northeast of Portugal, which included 33 counties, in the region of Trás-os-Montes and Alto Douro. This region, with a total area of 12,285 km^2 is limited on the North and East side by Spain, on the South by the region of Beira Interior and on the West by the regions of Minho and Douro Litoral. The region is crossed from East to West by Douro River with a maximum altitude of 1,415 m above sea level. The monthly average temperature is over 8 ºC to 16 ºC and it presents 20 days per year of frost and 69 to 80% of humidity, a precipitation of 400 mm to 1,800 mm, and 50 to 60% of insulation per year.

FLOCKS AND ANIMALS

A cross-sectional epidemiological study was carried out between January and December 2007 to determine seroprevalence of brucellosis. The study population consisted of all animals and flocks registered in the region. Complete information about sanitary interventions (vaccination with Rev. 1), seropositivity, herd size, species, herd constitution (with one species or both), and type of production (meat or milk) was available for all the animals and herds included in this study. Animals vaccinated with Rev. 1 less than 12 months ago were not tested for brucellosis infection and were not included in the study. Flocks or animals without all this information or with contradictory results (e.g. more seropositive animals than animals' interventions) were also excluded. Some herds had one or more sanitary intervention (blood sampling) per year. Only the first intervention was counted for animals and flocks, except in the cases where the next sanitary intervention revealed positive animals, in order not to repeat the information. In total, 41 flocks and 14,091 animals were excluded from the study.

According to the number of adult animals in each flock, flocks were sorted into three different size strata: small (≤ 30 animals), medium (> 30 and ≤ 150 animals), and large (more than 150 animals). A flock was classified as a sheep or goat herd, if having more than 50.0% of the predominant species, and meat or milk flock if having more than 50.0% animals producing meat or milk. The herd was considered pure if it had only one species (sheep or goats) and mixed if it had at least two animals of different species.

Of the 5,466 flocks tested 2,985 were classified as small (54.6%), 2,078 were medium (38.0%) and 403 were large (7.4%), respectively. Most flocks had ovine species (4,220; 77.2%), and 1,246 were of caprine species (22.8%). Regarding herd constitution, 4,599 were pure (one species) (84.1%) and 867 (15.9%) were of mixed species (both species). About 4,843 of the flocks were of meat type production (88.6%) and 623 were of milk type (11.4%). Out of the 278,097 animals tested, 33,849 were from small flocks (12.2%), 159,328 were from medium flocks (57.3%) and 84,920 were from large flocks (30.5%), respectively. The majority of specimens surveyed were sheep (226,799; 81.6%) and 51,298 were goats (18.5%) and 218,191 of tested animals were pure (78.5%) and 59,906 (21.5%) were mixed species. The majority of animals tested 231,141 were of meat type production (83.1%), and 46,956 were of milk type (16.9%). The proportions of flocks and animals in each category are shown in tables 1 and 3.

Distribution of animals among the 13 Livestock Farmers Organizations (OPP) is presented in table 4. Each OPP has different criterion for classification of flocks and animals according to brucellosis prevalence in each area of influence.

SAMPLE COLLECTION

Blood samples from Portuguese small ruminants submitted to the laboratory of Health Service in Mirandela, Portugal were tested serologically by Rose Bengal plate agglutination test (RBT) and/or the complement fixation test (CFT) as described by Alton *et al* (1988). In RBT, any visible reaction of agglutination is considered to be positive. In CFT sera giving a titer equivalent to 20 ICFTU/ml or more are considered to be positive (OIE 2009). The RBT has a sensitivity of approximately 77% (Alton *et al* 1988) and a specificity between 90-100%. The CFT has a sensitivity of approximately 88% and a specificity around 100% (Garin-Bastuji *et al* 2006).

Samples were obtained during the Annual Official Brucellosis Eradication Campaign from January to December of 2007, from all small ruminants non-vaccinated, or lambs and kids vaccinated more than 12 months ago.

In non-free brucellosis herds, all animals were first tested with RBT, and animals testing negative were then tested with CFT. Animals were classified as positive if RBT or CFT were positive (parallel testing resulting in increased sensitivity). In free or officially free brucellosis flocks, animals were classified as positive if both RBT and CFT were positive (serial testing resulting in increased specificity).

Table 1. Seroprevalence of flocks with sheep and goats brucellosis infection by herd size, species, constitution of herd and production type.
Seroprevalencia de los rebaños de ovejas y cabras infectadas con brucelosis en relación al tamaño, especies, constitución y tipo de producción.

	Flocks tested (n)	Relative distribution (%)	Seropositive	Prevalence (%)	CI 95%
Herd size			P<0.001		
≤ 30 animals	2,985	54.6	72	2.4	1.9 - 3.0
> 30 and ≤ 150 animals	2,078	38.0	323	15.5	14.0 - 17.1
> 150 animals	403	7.37	92	22.8	18.7 - 26.9
Total	5,466	100	487	8.9	8.2 - 9.7
Species			P=0.909		
Ovine	4,220	77.2	377	8.9	8.1 - 9.8
Caprine	1,246	22.8	110	8.8	7.3 - 10.4
Total	5,466	100	487	8.9	8.2 - 9.7
Constitution of herd			P<0.001		
Pure (one species)	4,599	84.1	372	8.1	7.3 - 8.9
Mixed (both species)	867	15.9	115	13.3	11.0 - 15.5
Total	5,466	100	487	8.9	8.2 - 9.7
Production type			P= 0.502		
Meat	4,843	88.6	427	8.8	8.0 - 9.6
Milk	623	11.4	60	9.6	7.3 - 12.0
Total	5,466	100	487	8.9	8.2 - 9.7

Table 2. Seroprevalence of flocks with sheep and goats brucellosis infection by OPP.
Seroprevalencia de los rebaños de ovejas y cabras infectadas con brucelosis por OPP.

	Animals tested (n)	Relative distribution (%)	Seropositive	Prevalence (%)	CI 95 %
OPP			P<0.001		
Torre de Moncorvo	316	5.8	26	8.2	5.2 - 11.3
Chaves	603	11.0	89	14.8	11.9 - 17.6
Vila Pouca de Aguiar	893	16.3	79	8.9	8.3 - 9.4
Montalegre	373	6.8	40	10.7	7.6 - 13.9
Macedo de Cavaleiros	832	15.2	61	7.3	5.6 - 9.1
Boticas	168	3.1	18	10.7	6.0 - 15.4
Vinhais	220	4.0	18	8.2	4.6 - 11.8
Moimenta da Beira	328	6.0	18	5.5	3.0 - 8.0
Tarouca	561	10.3	24	4.3	2.6 - 6.0
Miranda e Vimioso	328	6.0	14	4.3	2.1 - 6.4
Carrazeda e Vila Flor	181	3.3	27	14.9	9.7 - 20.1
Mogadouro	281	5.1	18	6.4	3.6 - 9.3
Bragança	382	7.0	55	14.4	10.9 - 17.9
Total	5,466	100	487	8.9	8.2 - 9.7

DATA ANALYSIS

Chi-square (χ^2) tests were used to compare seroprevalence values relatively to OPP's area, herd size, species, herd constitution: pure or mixed, and type of production, individually and per flocks. Analyses were performed with MS Access and SPSS 16.0 software for Windows (SPSS Inc, Chicago IL, USA) considering 0.05 as the level of significance (P). For the proportions, the 95% confidence interval (CI) was estimated using the exact binomial test.

RESULTS

A total of 5,466 flocks and 278,097 animals were analysed. One thousand two hundred and thirty six animals (0.44%, 95% CI: 0.40-0.48) were seropositive. Brucellosis seropositive animals (one or more) were detected in 487 flocks (8.9%, 95% CI: 8.2-9.7%).

Table 1 summarizes the proportion of positive flocks per herd size, species, constitution of herd, type of production. Herd size and constitution of herd had signifi-

Table 3. Individual seroprevalence of sheep and goats brucellosis infection by herd size, species, constitution of herd and production type.
Seroprevalencia individual de ovejas y cabras infectadas con brucelosis por tamaño, especies, constitución y tipo de producción.

	Animals tested (n)	Relative distribution (%)	Seropositive	Prevalence (%)	CI 95 %
Herd size			P<0.001		
≤ 30 animals	33,849	12.2	126	0.37	0.31 - 0.44
> 30 and ≤ 150 animals	159,328	57.3	793	0.50	0.47 - 0.53
> 150 animals	84,920	30.5	317	0.37	0.33 - 0.41
Total	278,097	100	1,236	0.44	0.40 - 0.48
Species			P<0.001		
Ovine	226,799	81.55	852	0.38	0.35 - 0.41
Caprine	51,298	18.45	384	0.75	0.68 - 0,82
Total	278,097	100.00	1,236	0.44	0.40 - 0.48
Constitution of herd			P<0.001		
Pure (one species)	218,191	78.46	810	0.37	0.34 - 0.40
Mixed (both species)	59,906	21.54	426	0.71	0.64 - 0.78
Total	278,097	100.00	1,236	0.44	0.40 - 0.48
Production type			P<0.001		
Meat	231,141	83.12	1,068	0.46	0.43 - 0.49
Milk	46,956	16.88	168	0.36	0.31 - 0.41
Total	278,097	100.00	1,236	0.44	0.40 - 0.48

Table 4. Individual seroprevalence of sheep and goats brucellosis infection by OPP.
Seroprevalencia individual de ovejas y cabras infectadas con brucelosis por OPP.

	Animals tested (n)	Relative distribution (%)	Seropositive	Prevalence (%)	CI 95%
OPP			P<0.001		
Torre de Moncorvo	24,850	8.94	57	0.23	0.17 - 0.29
Chaves	29,242	10.51	147	0.50	0.42 - 0.58
Vila Pouca de Aguiar	27,714	9.97	317	1.14	1.02 - 1.26
Montalegre	13,239	4.76	62	0.47	0.35 - 0.65
Macedo de Cavaleiros	48,321	17.38	150	0.31	0.35 - 0.46
Boticas	6,453	2.32	51	0.79	0.57 - 1.01
Vinhais	15,374	5.53	39	0.25	0.16 - 0.32
Moimenta da Beira	10,628	3.82	54	0.51	0.37 - 0.65
Tarouca	6,710	2.41	102	1.52	1.23 - 1.81
Miranda e Vimioso	32,571	11.71	25	0.08	0.05 - 0.11
Carrazeda e Vila Flor	12,520	4.50	54	0.43	0.32 - 0.54
Mogadouro	19,304	6.94	41	0.21	0.15 - 0.27
Bragança	31,171	11.21	137	0.44	0.37 - 0.51
Total	278,097	100.00	1,236	0.44	0.40 - 0.48

cant differences (P < 0.001), but species and production type had no statistical differences (P = 0.909 and P = 0.502, respectively). Seroprevalence in flocks with 150 or more animals (22.8%) was higher than in flocks with more than 30 and less than 150 animals (15.5%) and even more than the flocks with 30 or less animals (2.4%). The seroprevalence of sheep and goats flocks was not significantly different (P = 0.909). The flock production type seroprevalence was not significant (P = 0.502) with a seroprevalence of 8.8% in meat flocks and 9.6% in milk flocks. On the other hand, with regards to constitution, mixed herds presented higher values of infection (13.3%), than flocks with only one specie (8.1%), with differences (P < 0.001).

Serologically positive sheep and goats flocks were distributed across the 13 Livestock Farmers Organizations (OPP's) areas. The frequency of seropositive flocks ranged from 4.3% in Miranda e Vimioso to 14.9% in Carrazeda e Vila Flor (table 2).

All variables of individual seroprevalence had significant differences ($P < 0.001$). The animals of medium flocks (> 30 and ≤ 150 animals) presented higher seroprevalences (0.50%) than the animals in smaller or larger flocks (both with 0.37%). The seroprevalence of animals showed a higher seroprevalence in goats (0.75%) than in sheep (0.38%). The individual seroprevalence values among animals from herds with only one specie (sheep or goat) (0.37%), and herds with both species (0.71%) were significantly different ($P < 0.001$). The seroprevalence in animals which producing meat was significantly higher than the ones producing milk ($P = 0.002$), 0.46 % and 0.36 %, respectively (table 3). The individual serological survey (table 4) distributed across all OPP's of the Northeast of Portugal (13) showed significant differences ($P < 0.001$). The lowest individual value of seroprevalence in flocks (0.08%) was found in Miranda e Vimioso, and the highest value was found in Tarouca (1.5%).

DISCUSSION

Seroprevalence knowledge is one of the cornerstone of surveillance and monitoring programmes, because it is decisive on whether to implement control measures or not, and provides data for the evaluation of the efficacy of these measures and it is the basis for modification (Mousing *et al* 1997).

This is the first epidemiological study that describes seroprevalence of brucellosis in Northeast of Portugal and there are few recent epidemiological studies conducted in Europe (Lithg-Pereira *et al* 2004, Coelho *et al* 2007). The aim of this study was to characterize the sheep and goat brucellosis prevalence by herd size, species, constitution of the herd and type of production, using used only animals and flocks with this information properly registered. In Portugal, between 1990 and 2004, the prevalence of individual animals decreased from 3.5% to 0.8%. In flocks, prevalence decreased from 12.2% to 2.8% (Vaz 2005). The individual seroprevalence in Trás-os-Montes e Alto Douro in 2007 was smaller than the described values although the seroprevalence in flocks was higher.

The results of this study are in agreement with previous studies, where brucellosis was associated with large herd size (Kabagambe *et al* 2001, Al-Majali 2005, Coelho *et al* 2007). Larger herds were more likely to have at least one positive goat than smaller herds and were usually associated with mass management practices that are typically more difficult to control and allow for closer contact between animals and their environment, which increases the potential for exposure to infectious excretions (Al-Majali 2005). Our results could also be related to a higher density of animals per flock. Stocking density allows greater contact between animals. This creates a higher bacterial load in the environment, and hence the chances of disease transmission will be increased. Other explanation might be due to the fact that grazing in communal pastures may facilitate the contact between infected and not infected flocks (Kabagambe *et al* 2001, Al-Talafhah *et al* 2003). This situation probably occurs more frequently in larger flocks, because of their size. The small herds are generally more isolated (Lithg-Pereira 2001) and generally use tethering or a zero-grazing system (Kabagambe *et al* 2001), therefore the animals do not have this contact.

Another interesting result of our study is that individual seroprevalence was significantly higher in goats than in sheep. Our results are consistent with others reported by Sobhani-Motlagh *et al* (2005), who found that goats are more susceptible to the infection than sheep. However, these results are in contrast with Reviriego *et al* (2000). A plausible explanation for this finding is difficult because ovine behaviour that get together in parturition or at night (long-term close contact), increases potential of disease transmission, and goats do not have this behaviour (European Commission 2001).

Results of this study showed a significantly higher individual and flock seroprevalence in mixed herds. Kabagambe *et al* (2001) and Ocholi *et al* (2004) found similar results. Our results support the hypothesis that keeping sheep in contact with goats is a risk factor for brucellosis.

Although numerous authors have reported that brucellosis is more prevalent in milk than in meat herds (Omer *et al* 2000, Lithg-Pereira 2001), in this study a higher seroprevalence was found in meat animals. These results are difficult to explain and could be related with a better sanitary management of milk herds in the region because the milk is used to make traditional high quality cheese, which is made with certified milk. Other explanation could be that, in meat herds, animals are more often introduced into the herd, and that practice increases the risk of introducing infected animals (Omer *et al* 2000).

The estimation prevalence recorded in the 13 OPP's indicated that *Brucella* infection was widespread in small ruminants throughout the region.

Information on the prevalence of sheep and goats brucellosis infection is necessary to define control measures for zoonotic brucellosis (Godfroid *et al* 2005). The results of this study are useful for policy makers. A coherent control program should combined mass vaccination, with serological tests and a slaughter strategy.

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