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Case-control study of risk factors for high within-flock small-ruminant brucellosis prevalence in a brucellosis low-prevalence area

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SUMMARY

A case-control study was conducted in a brucellosis low-prevalence area of NW Spain to determine factors associated with high within-flock small-ruminant brucellosis prevalence in 1998. Forty-one cases and 69 controls were selected and information from both official sources and personal interviews was retrieved for every flock. The relationship between variables obtained and flock status was assessed by unconditional multivariable logistic regression analysis. The introduction of replacement animals into the flock, the presence of older farmers, an inadequate brucellosis vaccination programme and higher flock seroprevalence in the town in 1997 were positively associated with case flocks. Thus, specific actions directed at farms presenting these characteristics should be included within official eradication programmes. In addition, for the 1999 campaign the time from sampling to culling the seropositive animals correlated positively ($r = 0{\cdot}53$; $P < 0{\cdot}01$) with the flock seroprevalence the following year, suggesting the need for a faster removal of the infected animals to increase the efficacy of the eradication campaigns.

INTRODUCTION

Small-ruminant brucellosis (SRB) by *Brucella melitensis* is an endemic disease in Mediterranean countries [1]. In addition to the important economic losses associated with the infection, *B. melitensis* is a matter of Public Health concern as it is an important cause of human brucellosis (Malta fever). Official animal–brucellosis eradication programmes were set up in those countries in order to reduce both animal and human incidence [2, 3].

Official eradication programmes (OEPs) have been based mainly on knowledge of the nature of the infection, immunization of susceptible animals and the diagnosis and culling of infected animals [4, 5]. In most countries, OEPs have led to a dramatic reduction in the prevalence of animal brucellosis, with a resulting decrease in human brucellosis. However, it remains the case that few countries have achieved complete eradication [3].

In Spain, important progress has been made since official SRB control programmes were initiated, with the estimated individual and flock seroprevalence decreasing from 6·5 and 64% respectively in 1982 to 1·33 and 11·35% respectively in 2000 (Ministry of Agriculture, Fisheries and Food, unpublished data). However, in spite of the mechanisms of transmission being well known, and effective vaccines and useful diagnostic tests being available, the decline in prevalence in Spain has been slower than expected [6].

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It has been suggested that one difficulty may lie in reliance on imperfect diagnostic tests in low-prevalence areas [4, 7, 8]. However, the same diagnostic techniques have been used in other countries where brucellosis has been virtually eradicated [3, 9, 10]. It seems clear that for low-prevalence, endemic diseases, it is particularly important to ensure that non-biological (technical and socioeconomic) aspects of any eradication programme are addressed. Knowledge of the organizational structure of the small-ruminant industry, proper farmers' attitudes and compliance with legislation are required to achieve the OEP's goals [7, 11, 12]. Therefore new epidemiological approaches that take into account these aspects may be useful for defining new tools for the control of SRB.

In a recent study [13], we showed that the simple strategy of blood sampling and testing, followed by culling of seropositive animals was not as successful as expected in a low-prevalence area. Even when multiple samples were taken from some flocks during the 1999 OEP in order to detect and remove as many seropositive animals as possible, the ability to eradicate infection did not differ significantly from the flocks tested just once in that year. It was concluded that, when working in low-prevalence areas, flock-specific characteristics must be incorporated into the epidemiological analysis of the OEP results to improve the OEP's efficacy.

In this study, we show the results of a case-control study developed in the same area to detect those farm factors that may be related to high within-flock brucellosis prevalence. In addition, data from the OEP are used to assess the likely importance of the time from sampling to culling of infected animals on the infection status of flocks in the subsequent year. The results of both studies should lead to the implementation of new activities within the official Spanish framework in the fight against SRB.

METHODS

Area of study and OEPs

The study was conducted in the province of León (NW Spain), which is divided into four regions (Table 1). For more detailed information regarding the area and the small-ruminant census in the province see ref. [13].

Brucellosis OEPs in the province are based on national legislation. For small ruminants they consist of conjunctival vaccination (with the Rev 1 strain of *B. melitensis*) of replacement animals between 3 and 6 months old and testing for *Brucella* sp. antibodies [the Rose Bengal test (RBT) as screening and a complement fixation test (CFT) for confirmation] in all animals ⩾18 months old. Sensitivity and specificity estimates for this serial testing strategy are shown in ref. [13]. Seropositive animals should be culled within 30 days of marking them on the farm. The OEP usually begins in March of each year. In recent years, the requirement for vaccination activities has been lifted for some flocks in order they can obtain a brucella-free health certificate.

Table 1. *Distribution of the case and control flocks by natural region in a study on risk factors for high within-flock small-ruminant brucellosis seroprevalence in the León province of Spain* (*1998*)

Natural region	No. of controls	No. of cases	Ratio (control/case)
Bierzo	5	3	1·7
Montaña	8	3	2·7
Transición	16	14	1·1
Páramo-Campos	39	21	1·8
Total	68	41	1·7

Selection of cases and controls

Small-ruminant flocks with more than 25 animals (⩾18 months old) were used. The case and control flocks were selected from the OEP results for 1997 and 1998. All flocks with a within-flock seroprevalence ⩾5% in 1998 were classified as cases. A 5% within-flock seroprevalence level was used as threshold for several reasons. As the main objective of this work was to determine factors associated with high levels of within-flock brucellosis in low-prevalence areas, this threshold ensured the exclusion of false-positive (negative or low-prevalence) flocks in the 'case' group. Furthermore, these flocks may be acting as important shedders of *B. melitensis* in the province, and understanding brucellosis in these flocks might be especially helpful in improving OEP efficacy. A within-flock seroprevalence ⩾5% legally allows the use of the CFT on the whole flock without the prior application of the RBT, and this approach allowed us to reach the entire population of individual cases.

Two control flocks per case were randomly selected from the flock population that was seronegative for 1997 and 1998 in the same province. We assumed that seronegativity for two consecutive years allowed us to disregard the possibility of false-negative flocks within the control group.

Information obtained

A questionnaire was administered by personal interview to the owners of the selected flocks during the years 1999 and 2000 (available upon request). The questionnaire was designed to elicit information on flock characteristics such as size, breed, and infrastructures; on flock management, including the purchase of replacement animals in the years prior to 1998, the type of pasture, type of veterinary services, and brucellosis vaccination programmes; and also farmer characteristics such as age, educational level, and membership of farmers' organizations. The questionnaire was previously tested with farmers not included in this study [14].

In addition to the questionnaire, when available, data such as dates of animal testing and dates of culling of seropositive individuals were retrieved from the 1997, 1998 and 1999 OEPs for all case flocks.

Data management and statistical analysis

Questionnaire and OEP data were stored in a database (Microsoft Access® 1997) and further analysed using Epi-Info 6·04 [15], EGRET (Statistic and Epidemiology Research Co., Seattle, WA, USA) and BMDP (Statistical Software, Los Angeles, CA, USA) software.

To identify likely factors associated with brucella-flock infection, univariable analyses comparing case and control flocks were first performed. Thus the outcome variable was the flock status (case or control) in 1998 and the independent variables were those obtained from the questionnaire. Both χ^2 and Mann–Whitney rank-sum tests were used to check for univariable significant ($P<0{\cdot}1$) relationships between the flock status and the categorical and continuous variables respectively.

All significant variables were then included in an unconditional multivariable logistic regression analysis to determine the main factors associated with our case definition [16]. All continuous variables were categorized into quartiles. The model was constructed by a forward and backward stepwise approach based on a P value for entering $\leqslant 0{\cdot}1$ and a P value for removing $>0{\cdot}1$.

Given the heterogeneous SRB distribution in the province for 1997 and 1998 [17], natural region and flock size were forced into the multivariable model as possible confounders regardless of their significance. Two-factor interactions between the remaining variables were also tested and the goodness-of-fit of the model was checked using the Hosmer and Lemeshow statistic [16].

The additional information retrieved from official sources was used to assess the influence of the time between sampling and culling of seropositive animals on the brucellosis status of the flock in the subsequent year. The hypothesis was that the more time seropositive animals spent within the flock, the higher the risk of spread of infection and, therefore, the less the efficacy of the OEP in that flock. As the time at which animals were infected was not known, the period between sampling and culling seropositive animals was considered a partial measure of the time at risk of the susceptible animals within the flock.

To test this hypothesis two approaches were carried out. First, the relationship between the number of days from bleeding to culling of seropositive animals and brucellosis seroprevalence the following year was assessed using the Pearson correlation coefficient (r). Secondly, the relationship between that period and the flock status (infected *vs.* non-infected) the next year was tested using a χ^2 analysis. In the latter case, flocks were placed into two categories: flocks from which seropositive animals were removed in a shorter time than the group median, and flocks taking longer to remove those animals. A flock was considered infected when at least two animals yielded a positive test result. These analyses were carried out for the 1997, 1998 and 1999 campaigns.

RESULTS

Flock selection

According to the OEP, 53 farmers had flocks classified as cases in the province of León in 1998. As ten of them managed their flocks together with other farmers, they were considered as only five independent flocks, and thus there was a total of 43 case flocks in the province. Two of them (3·8%) had stopped their farming activities at the time of the interview, and so a total of 41 flocks were included in this study. All of the farmers agreed to participate in the study. All flocks classified as cases had at least three seropositive animals.

Initially, 82 farmers were randomly selected from the list of farmers with negative flocks for 1997 and

Table 2. *Questionnaire results for categorical variables and their significance with regard to flock status (case/control) by univariable analysis (χ^2), in a study on risk factors for high within-flock small-ruminant brucellosis seroprevalence in the León province of Spain (1998)*

	Cases		Controls		
Variables	*n*	%	*n*	%	*P*
Farm's characteristics block					
Breed					
Local and their crosses	22	53·7	43	63·2	0·32
Foreign and their crosses	19	46·3	25	36·8	
Electricity and piped water					
Yes	34	2·9	56	82·3	0·51
No	7	17·1	8	11·7	
Farm workers					
Family exclusively	32	78	61	89·7	0·09
Family and hired workers	9	22	7	10·3	
Mixed flocks (sheep and goats)					
Yes	15	36·6	14	20·6	0·06
No	26	63·4	54	79·4	
Sharing males					
Yes	8	30·8	1	2·0	
No	18	69·2	48	98·0	< 0·01
No answer	15	—	19	—	
Type of management					
Intensive/semi-extensive	18	43·9	39	57·4	
Extensive	18	43·9	28	41·2	0·04
Transhumant (nomadic)	5	12·2	1	1·5	
Type of production					
Meat	21	51·2	34	50	0·90
Milk	20	48·8	34	50	
Use of common pastures					
Yes	34	82·9	56	82·4	0·93
No	7	17·1	12	17·6	
Veterinary services					
Absence	17	41·5	16	23·5	0·05
Regular*	24	58·5	52	76·5	
Farm-management characteristics block					
Animal quarantine or serological checking prior to the introduction of new animals into the flock					
No	24	70·6	31	64·6	
Yes	10	19·4	17	35·4	0·57
No answer	3	—	1	—	
Brucellosis flock status of the farms from which animals were purchased					
Without qualification/unknown	24	68·6	12	24·5	
Free of infection	11	31·4	37	75·5	< 0·01
No answer	6	—	19	—	
Proper brucellosis vaccination programme†					
Yes	22	53·7	55	80·9	< 0·01
No	19	46·3	13	19·1	
Purchase of replacement animals					
Yes	37	90·2	49	72·1	0·01
No	4	9·8	19	27·9	

Table 2 (*cont*.)

Variables	Cases *n*	Cases %	Controls *n*	Controls %	*P*
Use of the official agricultural laboratory services					
Never/once or twice	35	94·6	53	88·3	
Frequently	2	5·4	7	11·7	0·07
No answer	4	—	8	—	
Veterinarian responsible for brucellosis vaccination					
Official	27	65·9	36	53·7	
Private	9	22	23	34·3	0·21
Cooperatives or animal-health protection association	5	12·2	8	11·9	
No answer	0	—	1	—	
Whole-flock brucellosis vaccination any time					
Yes	10	26·3	19	30·6	
No	28	73·7	43	69·4	0·64
No answer	3	—	6	—	
Farmer's characteristics block					
Attendance at some livestock training courses					
Yes	12	29·3	33	48·5	0·04
No	29	70·7	35	51·5	
Educational level					
With some type of studies	27	65·9	59	86·8	0·01
No studies at all	14	34·1	9	13·2	
Farm as main job					
Yes	31	75·6	47	69·1	0·46
No	10	24·4	21	30·9	
Farmer's age					
$\leqslant$55 years	27	65·9	57	83·8	0·03
$>$55 years	14	34·1	11	16·2	
Malta fever‡					
Yes	28	68·3	40	58·8	0·37
No	13	31·7	27	39·7	
Membership of farmers' organization§					
Yes	20	48·8	34	50	0·90
No	21	51·2	34	50	

* Veterinary services from cooperatives, animal-health protection associations or private veterinarians. Official veterinarians are not included in this category as they usually are not considered practitioners.

† Yes: 100% of the own replacement animals were vaccinated every year. No: not all own replacement animals were vaccinated every year or for some years they were not vaccinated at all.

‡ Family members having suffered from Malta fever at any time.

§ Health protection association, cooperative, etc.

1998. Fourteen of them managed their flocks together with another of the selected farmers, and thus 68 independent flocks were finally considered (1·7 controls/case). All the farmers in this group were also willing to cooperate with the study.

For all those farmers working together, farmer's age and educational level were similar (they fell into the same category).

The distribution of case and control flocks is shown in Table 1.

Questionnaire results and statistical analyses

The questionnaire results for cases and controls and the variables significantly associated with flock infection status (case/control) by univariable analysis are shown in Tables 2 and 3. A total of 14 variables showed univariable significant relationships with flock infection status in 1998.

All significant variables except 'sharing males' and 'brucellosis flock status of the farms from which

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Table 3. *Questionnaire results for continuous variables and their significance with regard to flock status (case/control) by univariable analysis (Mann–Whitney rank-sum test), in a study on risk factors for high within-flock small-ruminant brucellosis seroprevalence in the León province of Spain (1998)*

Variables	Mean	1st*	Median	3rd*	Range	*P*
% of animals introduced into the flock (1993–1997)†						
Controls	5	0	0·5	3	0–60	<0·01
Cases	17	1	2	30	0–84	
Distance to town (km)						
Controls	0·6	0·05	0·2	0·5	0–7	0·33
Cases	0·9	0·1	0·5	1	0–10	
Distance to the closest cattle farm (km)						
Controls	1·3	0·1	0·5	1	0–8	0·66
Cases	1·4	0·3	1	1·7	0–9	
Flock prevalence in the town in 1997						
Controls	20	10	17	27	2·3–100	<0·01
Cases	28	13	24	33	9·4–100	
Flock size						
Controls	446	289	435	593	33–990	0·40
Cases	496	305	354	685	26–1280	
% of abortions in 1998‡						
Controls	3·1	0·8	1·8	3·3	0–15·8	0·41
Cases	3·9	0·9	2	5·1	0–29·4	
Years in farm business						
Controls	16·59	8·2	15	23	1–44	0·21
Cases	19·46	12	15	30	2–60	

* Quartiles.
† With regard to the flock size at the time of the questionnaire.
‡ As declared by the farmer (from 66 controls and 37 cases).

replacement animals were purchased' were included in the multivariable logistic regression analysis. These two variables, although highly significant, were excluded from the final analysis because the low number of answers obtained (75 and 84 respectively) precluded obtaining model convergence when other variables were entered into the model.

The variable 'percentage of replacement animals introduced into the flock (1993–1997)' was grouped into three strata based on the second and third quartiles (⩽0·96, >0·96 to ⩽9·48, >9·48%); the variables 'flock prevalence in the town in 1997' and 'flock size' were also categorized into three strata based on the first and third quartiles (⩽18·18, >18·18 to ⩽43·05, >43·05%; and ⩽289 head, >289 to ⩽638 head, >638 head respectively). The variable 'educational level' was divided into two strata: no studies at all, and having any type of studies (including attendance at livestock training courses).

The results from the multivariable model are shown in Table 4. The percentage of replacement animals introduced into the flock between 1993 and 1997 and farmer's age were significantly related to flock status in 1998. There was also a significant interaction between 'proper brucellosis vaccination programme' and 'flock prevalence in the town in 1997'.

The odds of a flock presenting high brucellosis seroprevalence increased as the percentage of replacement animals introduced into the flock between 1993 and 1997 increased. Thus, a flock having more than 10% of its animals purchased from other farms during that period had a tenfold higher odds of being a case than a flock with <1% purchased animals. A farmer older than 55 years had a threefold higher

Table 4. *Variables associated with high within-flock brucellosis seroprevalence by multivariable logistic regression analysis* in a study on small-ruminant brucellosis in the León province of Spain* (*1998*)

	Logistic regression parameters				
Variables	*b*	S.E. (*b*)	*P*	OR	95% CI (OR)
% animals introduced into the flock (1993–1997)					
⩽0·96	—	—	—	1	—
>0·96 to ⩽9·48	2·0	0·73	<0·01	7·4	1·8–30·8
>9·48	2·35	0·72	<0·01	10·4	2·5–43
Farmer's age					
⩽55 years	—	—	—	1	—
>55 years	1·17	0·66	0·08	3·2	0·9–11·9
Flock prevalence in the town in 1997					
⩽18·18	—	—	—	1	—
>18·18 to ⩽43·05	2·99	1·29	0·20	na	na
>43·05	3·93	1·34	<0·01	na	na
Flock size†					
⩽289 head	—	—	—	1	—
>289 to ⩽638 head	0·54	0·76	0·48	1·7	0·4–7·7
>638 head	1·57	0·88	0·08	4·8	0·9–26·9
Natural region†					
Montaña	—	—	—	1	—
Transición	0·19	1·19	0·87	1·2	0·1–12·3
Páramo-Campos	$0·6\times10^{-1}$	1·03	0·95	1·1	0·1–8·1
Bierzo	0·69	1·30	0·59	2	0·2–25·7
Proper brucellosis vaccination programme					
Yes	—	—	—	1	—
No	4·23	1·52	<0·01	na	na
Interaction terms					
Proper vaccination × flock prevalence in town in 1997 ⩽18·18%	—	—	—	1	—
Not proper vaccination × flock prevalence in town in 1997 >18·18% to ⩽43·05%	−3·98	1·79	0·02	$0·2\times10^{-1}$	$0·6\times10^{-3}$–0·6
Not proper vaccination × flock prevalence in town in 1997 >43·05%	−2·18	1·94	0·26	0·1	$0·3\times10^{-2}$–5·1
Intercept	−6·046	1·72	—	—	—

* Hosmer and Lemeshow statistic = 6·70, D.F. = 8, *P* = 0·57.
† Variables forced into the model.
na, Not applicable.

chance (OR 3·2) of having a case flock compared to a younger farmer.

Because of the presence of a significant interaction between 'proper brucellosis vaccination programme' and 'flock prevalence in the town in 1997' these variables had to be interpreted together. The impact of brucellosis vaccination on flock status thus seemed to depend on the flock prevalence in the town. Flock seroprevalence in the town in 1997 was considered in three strata (low, medium and high prevalence), and for low-prevalence towns (⩽18·18%) the odds of being a case flock were much higher (OR 71·6) for those flocks with poor brucellosis vaccination programmes than those that vaccinated properly. However this ratio decreased significantly when flocks were situated in high-prevalence towns (OR 8·13) (Table 5).

Campaign results

A total of 33, 40 and 28 flocks were included in this part of the study for the 1997, 1998 and 1999 campaigns respectively. Flocks were selected based on the availability of accurate data from official sources and the presence of positive animals during the respective previous years (1996, 1997 and 1998).

Table 5. *Interaction results for the variables 'Proper brucellosis vaccination programme' and 'Flock prevalence in the town' in a study on risk factors for high within-flock small-ruminant brucellosis seroprevalence in the León province of Spain (1998)*

Flock-prevalence level in the town*	Proper brucellosis vaccination programme†	OR	95% CI (OR)
High	Yes	1	—
	No	8·1	0·6–104·8
Medium	Yes	1	—
	No	1·3	0·3–5·9
Low	Yes	1	—
	No	71·6	3·6–1423

* High, >43·05% of the flocks in town were positive. Medium, between 18·18% and ⩽43·05% of the flocks in town were positive. Low, <18·18% of flocks in town were positive. (A flock was considered positive when at least two animals were seropositive.)
† Yes, 100% of the own replacement animals were vaccinated every year. No, <100% of the own replacement animals were vaccinated every year or in some years there was no vaccination at all.

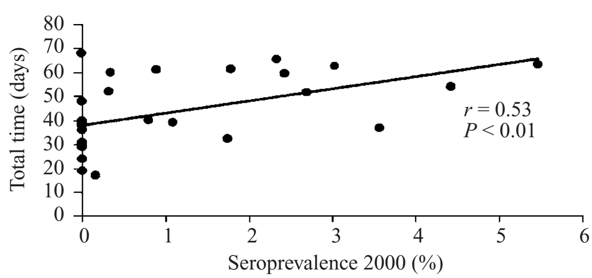

Fig. Graphical relationship between days from bleeding to culling of the seropositive animals in 1999 (total time) and within-flock seroprevalence in 2000 for case flocks.

The mean time between sampling and culling of seropositive animals was similar for the three years studied (48 days in 1997, 43 in 1998 and 43 in 1999; $P>0{\cdot}1$). The correlation analysis showed a positive and moderately high r between this period for the 1999 campaign and the within-flock seroprevalence in 2000 ($r=0{\cdot}53$; $P=0{\cdot}003$) (Fig.). This correlation was not observed for the years 1997 and 1998. The time between sampling and culling of seropositive animals explained approximately 28% of the variation in seroprevalence in 1999.

The χ^2 analysis also showed a significant relationship between the sampling–culling interval and

Table 6. *Relationship between time from bleeding to culling (total time) of the seropositive animals in 1999 and the flock status the following year for case flocks*

Total time*	Flock status 2000 Infected	Non-infected	Total
>39·5 days	10	4	14
⩽39·5 days	3	11	14
Total	13	15	28
	OR 9·2 (1·3–74·5); $P=0{\cdot}009$		

* Categories based on the group median.

brucellosis status (infected *vs.* non-infected flocks) in 1999–2000. A flock that kept its seropositive animals for more than 39·5 days (median of the group) had approximately a ninefold higher odds of remaining infected than a flock that removed its seropositive animals in less time (Table 6). No significant differences were found between the starting mean within-flock seroprevalence for these two groups (6·9% *vs.* 4·9%; $P=0{\cdot}5$).

DISCUSSION

A previous study of SRB infection based exclusively on official data, reported that brucellosis was found throughout the province of León, but that a small proportion of farms had a particularly high seroprevalence [17]. It was also suggested that some flock variables (size and previous infection status) were associated with brucellosis [13]. In this study, by means of a case-control study based on the use of personal interviews with farmers, we obtained information not usually retrieved through official sources, and used this to investigate the likely factors responsible for the maintenance of high within-flock prevalence of brucellosis in a low-prevalence area. Eliminating brucellosis from those flocks should enable better SRB control.

Univariable analyses demonstrated several differences between case and control flocks. Some of the variables significantly related to brucellosis infection were already well-known risk factors (e.g. sharing males, the purchase of animals, nomadism, lack of brucellosis vaccination, etc.) [4, 8, 18–20]. Other variables such as the farmer's educational background and age, and the use of veterinary services, have been largely ignored [21, 22]. In this study we demonstrated that high within-flock brucellosis seroprevalence in 1998 was related to the percentage of animals introduced into the flock between 1993 and 1997, the farmer's age, the absence of a proper brucellosis vaccination programme and the local flock prevalence in 1997, with a significant interaction between the last two variables.

The importance of knowing the brucellosis status of the flocks from which the replacement animals are purchased is clear, and Spanish law forbids animal trade among brucellosis-positive flocks. However, our study suggests that this type of livestock trade may be continuing as the percentage of animals introduced into the flock from 1993 to 1997 was one of the major risk factors for high within-flock SRB in 1998. Although this relationship may be the consequence of a higher rate of replacement in case flocks, the observation of a relationship between the brucellosis status of the farms under study and the 'brucellosis flock status of the farms from which replacement animals were purchased' (Table 2) strongly supported this idea. However the latter variable could not be included in the final model because small numbers precluded determining the impact of this variable on the brucellosis-flock status after adjusting for other variables. Animal trade has been identified as a major risk factor for SRB in similar studies in other parts of the world [23].

This situation could be favoured by several factors detected by the analysis and probably associated with a traditional way of managing small-ruminant flocks [22, 24]. Traditionalism was indicated by the fact that the variable 'sharing males' still acted as an important risk factor (Table 2). The low educational level of farmers with case flocks might also hinder their understanding of the importance of a disease that apparently did not impact severely in their farms in spite of the high seroprevalence detected (there were no significant differences in the percentage of abortions in 1998 for cases and controls) (Table 3). That older farmers (>55 years old), had higher odds of having case flocks (Table 4), may simply reflect both traditionalism and poor education. It is interesting to note that in 1995 more than 35% of the farmers in the province were older than 55 years [25].

The overall analysis of the interaction between 'proper brucellosis vaccination programme' and 'flock prevalence in the town in 1997' suggested the need for ensuring adequate levels of immunization of the replacement animals. If ORs were used to estimate vaccine efficacy [26], the proper vaccination of the flocks exerted a powerful protective effect in both low- and high-prevalence areas, although it was higher in low-prevalence areas (98·6% *vs.* 87·6% respectively). This difference was probably associated with a lower level of exposure to the infection in low-prevalence areas, thus overestimating the efficacy of the vaccine [26]. In high-prevalence areas, in which animals are frequently challenged, additional protection measures should be implemented in order to decrease the level of exposure (for example, avoiding the use of common pastures or the purchase of new animals in the area).

As 82% of the small-ruminant flocks used common pastures, and only 43·4% of towns in the province were considered free of SRB, it seems sensible to maintain vaccination in the current OEP for León.

The time of the sampling–culling interval for seropositive animals is a simple, if partial, measure of the time at risk for susceptible animals, and a positive relationship was observed between these two variables for the 1999 campaign (Fig. and Table 6). A more sophisticated and complete measure might be needed to detect any similar relationship in the other years studied. No differences were observed between the sampling–culling interval in the three campaigns. However, the initial mean within-flock brucellosis seroprevalence did vary significantly (15·3% in 1999 compared to 6·8 and 8·01% for the 1997 and 1998 campaigns respectively). This suggests that the importance of the time from sampling to culling may depend, in part, on the level of initial within-flock seropositivity. Higher within-flock seroprevalence would imply the need for a faster removal of the infected animals. However, relatively few flocks were included in this analysis and the correlation between time to cull and seroprevalence in the subsequent year was low and largely dependent on those flocks with zero seroprevalence (Fig.).

In conclusion, in addition to expected factors, high within-flock SRB seemed to be related to traditional management conditions, and older farmers. Specific measures to address these issues should be included within the OEP. Special attention should also be given to vaccination, even in low- or zero-prevalence areas. The within-flock seroprevalence detected through regular testing may be useful as an indicator of the speed with which seropositive animals should be removed from the flock. Flocks with higher levels of infection should be attended to before those with a lower prevalence.

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