Farm practices and risk factors which influence the high prevalence of brucellosis in small ruminant flocks in Northeast Portugal

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Multifactorial correspondence analysis, Brucellosis, Risk factor, Small ruminants, Vaccine Rev-1.

Summary
The region of Tras-os-Montes e Alto Douro in northeast Portugal displayed the highest prevalence of brucellosis in small ruminants of the country. A vaccination programme of the whole population with *Brucella melitensis* Rev-1 was carried out from 2001 to 2004 in an attempt to reduce prevalence levels and protect public health. Although individual prevalence decreased from 5.6% in 2001 to 0.4% in 2007, several flocks continued to present individual prevalence ≥ 5.0%. Given the multifactorial characteristics of brucellosis, the current study evaluated farming practices and risk factors in flocks with an individual prevalence over 5% by multifactorial correspondence analysis. Results showed that a lack of recognition of the symptoms of brucellosis and lack of Rev-1 vaccination were the main factors contributing to the high individual prevalence of brucellosis in flocks. Other factors such as the consumption of raw milk, presence of dog commingling with animals and use of communal pastures also contributed to the persistence of the disease. Family farms with low economical profit, minimal training/education of farmers, and a scarcity of veterinary support may explain the persistence of factors contributing to the high prevalence of brucellosis. The results of this study highlight several risk factors and farming practices that might have contributed to the maintenance of a high prevalence of brucellosis in flocks with high brucellosis prevalence. These results could be used to adopt new approaches to improve the efficiency of brucellosis eradication programs.

Fattori di rischio e pratiche gestionali che influenzano gli elevati tassi di prevalenza di brucellosi ovi-caprina nella regione nord est del Portogallo

Parole chiave
Analisi della corrispondenza multifattoriale, Brucellosi, Fattori di rischio, Piccoli ruminanti, Vaccino Rev-1.

Riassunto
Tras-os-Montes e Alto Douro è la regione a più alta prevalenza di brucellosi ovi-caprina del Portogallo. Nel tentativo di ridurre i livelli di prevalenza e di proteggere la salute pubblica, dal 2001 al 2004 è stato condotto un programma di vaccinazione dell'intera popolazione ovi-caprina con *Brucella melitensis* Rev-1. Sebbene la prevalenza individuale sia scesa dallo 5,6% del 2001 allo 0,4% nel 2007, diversi allevamenti hanno continuato a presentare livelli di prevalenza individuale di oltre il 5,0%. Viste le caratteristiche multifattoriali della brucellosi, questo studio ha valutato utilizzando l'analisi della corrispondenza multipla l'influenza esercitata da possibili fattori di rischio e pratiche gestionali negli allevamenti con una prevalenza ≥ 5,0%. I risultati hanno mostrato che il mancato riconoscimento dei sintomi e l'assenza di vaccinazione con Rev-1 sono stati i principali fattori che hanno contribuito all'elevata prevalenza individuale della brucellosi nelle greggi. Sulla persistenza della malattia hanno contribuito anche altri fattori come il consumo di latte crudo, la presenza di cani negli allevamenti e l'uso di pascoli comuni. La gestione familiare, lo scarso profitto economico, la scarsità di conoscenze, le scadenti capacità manageriali degli allevatori e il mancato supporto veterinario possono spiegare la persistenza degli elevati tassi di prevalenza della brucellosi ovi-caprina negli allevamenti portoghesi. I risultati potrebbero essere utilizzati per migliorare l'efficienza dei programmi di eradicazione della brucellosi.

Introduction

Brucellosis is a contagious zoonotic disease responsible for reproductive failure with important public health significance (Seleen *et al.* 2010). In small ruminants, *Brucella melitensis* is responsible for heavy economic losses resulting from clinical disease, abortion, neonatal losses, increased births intervals, reduced fertility, commercial restrictions, increased culling rates and the emergency slaughtering of infected animals (Radostits *et al.* 2000). In Portugal, as well as in other European countries, the National Veterinary Authority is responsible for the control of brucellosis in small ruminant (Portugal 2013).

Tras-os-Montes and Alto Douro, a region of the Northeast Portugal, has the highest prevalence of brucellosis in small ruminants (Portugal 2013) as well as the highest number of cases of brucellosis outbreaks (DGS 2014). Due to the public health concern, the National Veterinary Authority implemented a mass vaccination programme for young and adult small ruminants. The programme, which ran from 2001 until 2004, aimed to decrease high prevalence rates through the use of a commercial, live, freeze-dried vaccine against brucellosis. Briefly, all sheep and goats over 3 months were vaccinated with *B. melitensis* strain Rev-1 (Ocurev, Shering and Plough, US) via conjunctival route. Animals were identified with both a tattoo in the left ear and special ear-tags that included the vaccination date. All animal data were recorded in the national animal health software (Pisa.net®). Blood samples were obtained from a jugular puncture and were collected at the same time as the vaccine was administered. Sheep and goats positive for both rose bengal test (RBT) and complement fixation test (CFT) were culled (DGV 2011, Directive 91/68/EEC). After 12 months, blood samples from young-vaccinated sheep and goats were collected and those which were seropositive were culled. In adult-vaccinated animals, a blood sample was collected after 30 months to assess vaccine response. Control flocks were sampled 0 days, 1 month, 4 months, and 12 months after vaccination. This allowed us to assess the vaccine response across the seasons. Animal replacement was only allowed with young-vaccinated sheep and/or goats in order to prevent brucellosis outbreaks. Commercial trade restriction was enforced for 21 days after the Rev-1 vaccination, and the National Veterinary Authority restricted commercial trade of positive flocks (Portugal 2000).

After the mass vaccination programme, from 2005 to 2007, Rev-1 vaccination was used in sheep and goats from 3 to 6 months as previously described. The following special measures were applied to infected flocks. They include the study of the source of infection through an epidemiological survey at the farm site, restriction of animal trade and a minimum of 4 serological tests in a 240-day period with negative results.

The implementation of the mass vaccination and test-and-slaughter programme (described above), resulted in the decrease of the prevalence of brucellosis in both small ruminants and humans. However, although individual brucellosis prevalence decreased from 5.6% in 2001 to 1.3% at the end of the mass vaccination programme and progressively to 0.4% in 2007 (DGV 2011), individual prevalence of brucellosis in Tras-os-Montes remains the highest in Portugal and some flocks still present prevalence values higher than those observed in 2001. This indicates that control of brucellosis not only depends on test-vaccination-slaughter programmes but also on other factors such as husbandry, grazing, hygiene, veterinary management, training, farmers´ education and implementation of biosecurity measures, among others (García-Díez *et al.* 2013, Mainar-Jaime *et al.* 1999). The prevalence of brucellosis may also vary according to flock size, main animal production, and flock composition (Minas 2006, Coelho *et al.* 2007). Moreover, particular characteristics of *Brucella* spp. relating to routes of infection and resistance in the environment make their control in high prevalence areas more difficult (Lithg-Pereira *et al.* 2001). The aim of this study was therefore to evaluate farming practices and risk factors relating to brucellosis in flocks with high brucellosis prevalence values.

Materials and methods

Study design

A total of 37 registered flocks with animal prevalence of brucellosis ≥ 5% in 2007 was studied. The cut-off value of ≥ 5% was identified as the selection criteria because the brucellosis prevalence rate was 5% at the beginning of the mass vaccination programme in 2001. All flocks belonged to the Trás-os-Montes e Alto Douro region (Northeast of Portugal). Information regarding farm characteristics (species, flock size, main animal production, birth date, sex, breed, blood sampling date, Rev-1 vaccination date, RBT and CFT results and culling date of positive animals) was obtained from interviews with farmers and from the national animal health database (Pisa.net®). Each farmer participated in an epidemiological survey during their personal interview. Interview questions are presented in Table I.

Data analysis

Farming practices and risk factors for brucellosis were assessed by multifactorial correspondence

Table I. *Risk factors and farming practices which may influence the prevalence of brucellosis in small ruminant flocks of Trás-os-Montes e Alto Douro region (Northeast of Portugal)*[*].

Farmers - socio-demographic characteristics
Age
Sex
Education (no education, primary education, higher education)
Existence/knowledge of brucellosis infection in the family
Farm characterization
Farm registration number
Location
Flock characterization
Species at farm (sheep, goat, cattle, others)
Number of small ruminants in the flock
Characterization of the flock by sex, age, production (milk or meat)
Existence of contact with other flocks
Animal movement/purchase
Purchase of animals from local farms
Purchase of animals from fairs
Purchase of animals from animal sellers
Purchase from other country
Existence of official documents of animal movement (entrance/exit)
Quarantine for purchased animals
Farm management
Use of communal pastures
Existence of cleaning and disinfection (C&D) plan
Frequency of C&D operations
Manure removal (frequency and destination of manure)
Existence of pest control
Frequency of pest control verifications
Existence of fly control
Frequency of fly control verification
Verification of the of animal cleanliness
Presence of tap water or spring water
Milking technique is used in the farm
Presence of dogs at farm
Dogs eat or may eat fetal membranes, foetuseses or abortions
Reproductive management
Identification of the parturition season
Existence of a maternity pen
Technical assistance during reproduction season
Isolation of females before and after parturition from the flock
Proper removal of foetuses/abortions/fetal membranes
Existence of artificial insemination
Use of own or lent males
Brucellosis status
Characterization of brucellosis-positive animals (sex, age, breed, main production)
Official brucellosis classification of the farm
Correct identification of brucellosis-positive animals
Correct identification of brucellosis-positive animals vaccinated with Rev-1
Permanence (in days) of brucellosis-positive animals at farm before culled kids and lambs are properly vaccinated with Rev-1
Farmer drinks raw milk
Farmers eat fresh cheese made from raw milk
Existence of animal products commerce (milk, cheese)
Sale of milk
Sale of cheese made from raw milk (fresh or cured)
Identification of main clinical signs of the flock
Abortions
Metritis
Orchitis
Retention of fetal membranes
Mastitis
Existence of fertility problems
Decrease in milk/meat production yield

[*]Data obtained from farmers´ interviews and from the national animal health database (Pisa.net®)

analysis (MCA) to the discreet qualitative variables, especially nominal or ordinal ones (Matias 2006). This methodology is an exploratory multivariate technique that converts a matrix of non-negative data into a graphical display in which the rows and columns of the matrix are depicted as points. In an MCA plot of the data's attributes, the negative and positive attributes are represented as separate points (Torres and van de Velden 2007). All multivariate analysis methods work by computing successive axes of decreasing importance called successive axes. MCAs reduce the data contained in large tables and depict them in a vectorial plan. The degree of correlation among variables and/or observations is assessed by their relative proximity. Thus, the closer they are, the more correlated they are.

Ten sets of multifactorial correspondence analysis (MCA) linked to risk factors detected in the interviews were carried out using ANDAD version 7.1 developed by Instituto Superior Técnico, Centro de Valorização de Recursos Minerais, Instituto Superior Técnico of Lisboa. The main result of the correspondence analysis was a 2-dimensional plot of the associations between qualitative, explanatory, and outcome variables. It was assumed that the initial data had 2 clouds in 2 multi-dimensional vector spaces: 1 cloud for the columns (the variables studied), which were represented by 4 letters, and 1 cloud for the rows (flocks), which were represented by 2 letters and 2 numbers or 3 letters and 1 number. The MCA succeeded in constructing factorial axes that enabled the modalities to be positioned according to their coordinates on the selected factorial map. The interpretation of the quality of a MCA consists of the following: a) the contribution of an axis to the total inertia of the cloud is given by the ratio of the corresponding eigenvalue by the sum of all eigenvalues. This percentage is used to select the number of significant axis; b) this partial contributions allows us to determine which points play a major role in the orientation of the factorial axis; and c) the representation of a point in the Euclidean space is defined as the difference between the square of the distance of the point of origin and the square of the distance of the point in the profile.

Three factorial axes whose variables exhibited the greatest behavioural variability were considered in this study. The plot identifies clusters of associated variables. Those clusters with greater distance from the intersection have stronger associations. Explanatory variables of less than - 0.5 and more than 0.5 in the analysis were considered to show a significant association (high risk) whereas those having variables of less than - 1.0 and more than 1.0 were considered to show a very high significant association (very high risk). In this study, the original data enabled the detection of 3 axes that explained 45.8% of the total variance. The second and third

dimensions accounted to 22.4% and 12.7% of the data variance, respectively.

Results

Farmer socio-demographic composition and flock characterisation

A total of 37 farmers were interviewed. All the respondents were males. Over 85% were older than 51 years and reported primary-level education. The combined flocks included over 150 animals, comprising both sheep and goats that were used for meat production.

Multifactorial correspondence analysis

The results of the MCA of farming practices and risk factors for brucellosis are presented in Figures 1 and 2.

A significant association between the following variables was observed (Figure 1): absence of abortions, animal movement/commercial trade and clinical signs of brucellosis, pest control in farms,

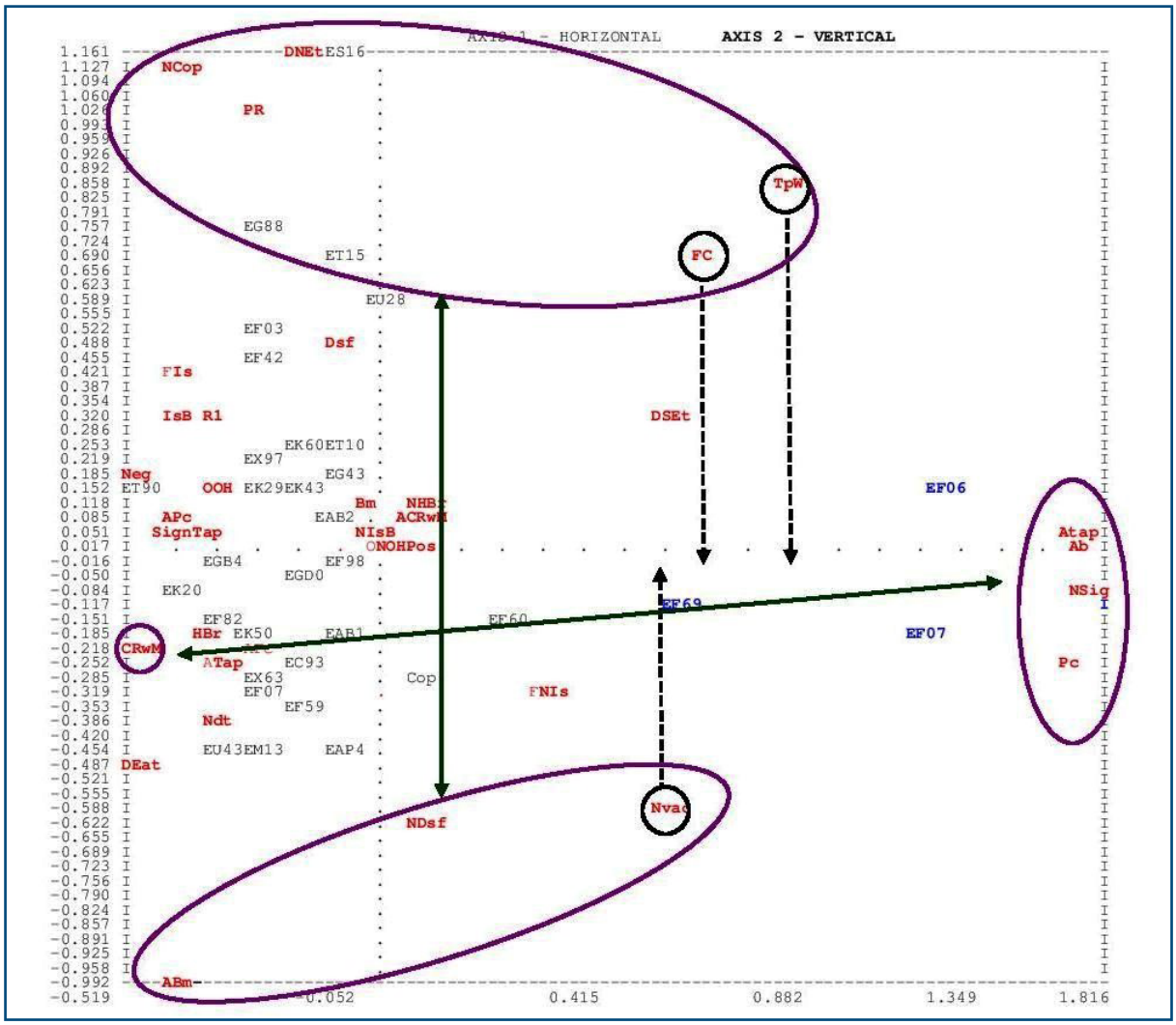

Figure 1. *Plot of multifactorial correspondence analysis related to flock's characteristics for axis 1 and axis 2.*
Flock isolated (FIs); Flock not isolated (FNIs); Owners with other herds (OOH); Owners without other herds (ONOH); Positive animals (Pos); Negative animals (Neg); Correct higienization of premises (Dsf); absence of correct higienization of premises (NDsf); Fly control (Fc); Absence of fly control (AFc); Pest control (Pc); Absence of pest control (APc); Presence of breeding management (Bm); Absence of breeding management (ABm); Animal isolation on birth period (IsB); No isolation of animals on birth period (NIsB); Proper removal of fetal membranes, abortus, etc. (PR) Improper removal of fetal membranes, Existence of abortions (UPR); Absence of abortions (Ab); Tap water (TpW); Absence of tap water (ATpW); Utilization of communal pastures (Cop); Absence of utilization of communal pastures (NCop); Rev-1 vaccination (R1); Absence of Rev-1 vaccination (NR1); Trade of animal and/or its products (Tap); Absence of trade of animal and/or its products (ATap); Consumption of raw milk/fresh cheese (CRwM); No consumption of raw milk/fresh cheese (ACRwM); Dogs eat fetal membranes and/or abortions (DEat); Dogs sometimes eat fetal membranes and/or abortions (DSEt); Dogs never eat fetal membranes and/or abortions (DNEt); Presence of clinical signs of brucellosis (Sig); Absence of clinical signs of brucellosis (NSig); Human brucellosis (HBr); Absence of human brucellosis (NHBr).

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absence of animals vaccinated with Rev-1, fly control programme, and the use of tap water.

The variables with the greatest partial contributions for the variability were, in decreasing order: absence of abortions (3.15), absence of animal movement/ commercial trade (3.15), absence of clinical signs of brucellosis (2.11), pest control at the farm level (1.82), presence of tap water (0.82), fly control (0.66), absence of animals vaccinated with Rev-1 (0.57), absence of dogs in flocks that eat foetuses and foetal membranes (0.57) for the positive dimension. Contrarily, the variable "farmers drink raw milk and/or eat fresh cheese made from raw milk" (- 0.52) was presented in the negative dimension (Figure 2). At dimension 2, the following variables: correct removal of abortions (foetuses and foetal membranes), absence of dogs that eat foetuses and foetal membranes, not sharing pasture areas, use of tap water, implementation of fly control programme, proper cleaning and disinfection of farm premises, absence of Rev-1 vaccination and existence of reproduction/husbandry programme. In decreasing

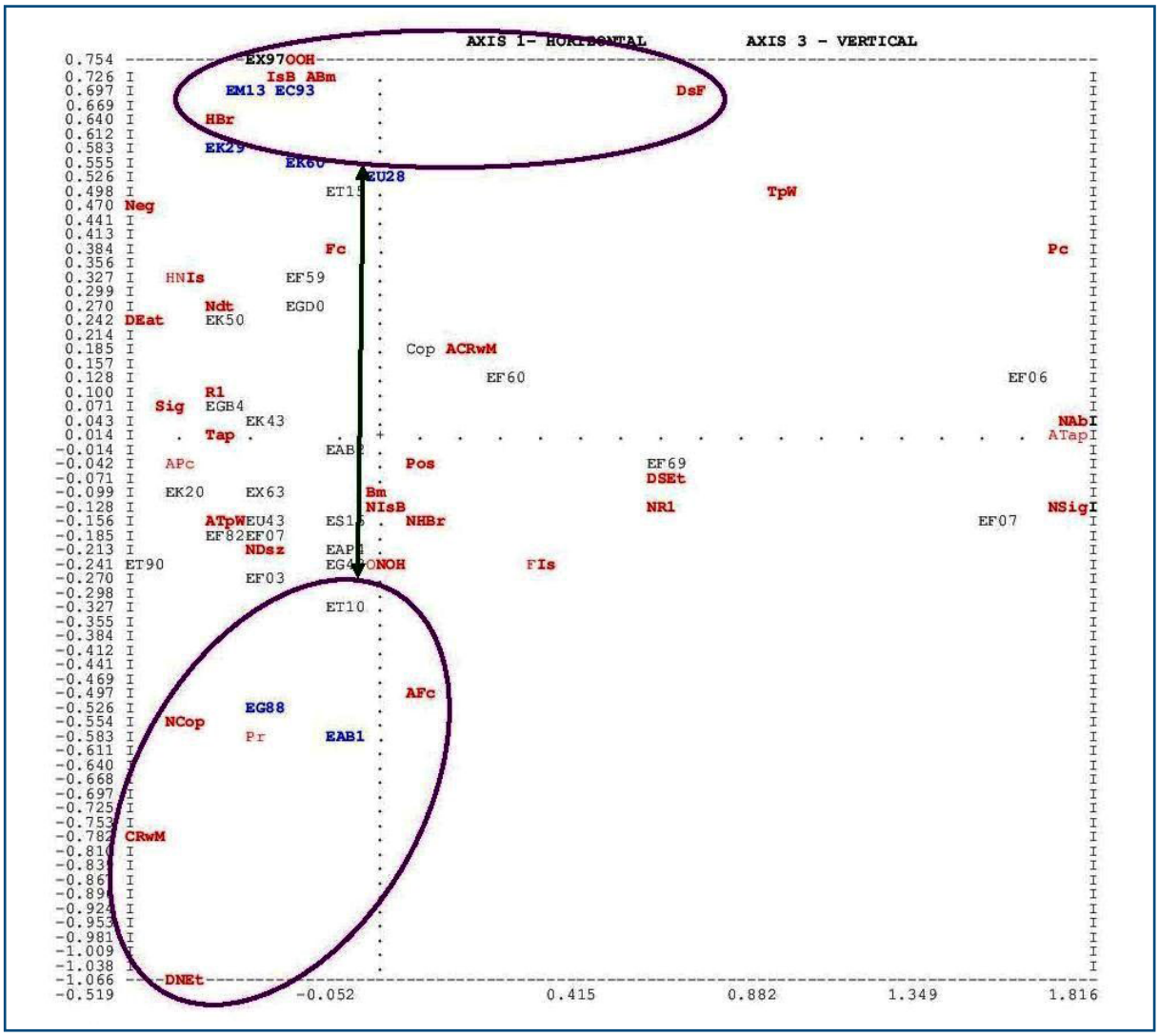

Figure 2. *Plot of multifactorial correspondence analysis related to flock's characteristics for axis 1 and axis 3.*
Flock isolated (FIs); Herd not isolated (HNIs); Owners with other herds (OOH); Owners without other herds (ONOH); Positive animals (Pos); Negative animals (Neg); Correct higienization of premises (Dsf); absence of correct higienization of premises (NDsf); Fly control (Fc); Absence of fly control (AFc); Pest control (Pc); Absence of pest control (APc); Presence of breeding management (Bm); Absence of breeding management (ABm); Animal isolation on birth period (IsB); No isolation of animals on birth period (NIsB); Proper remove of fetal membranes, abortus, etc. (PR) Inproper remove of fetal membranes, abortus, etc. (UPR); Absence of abortion (Ab); Tap water (TpW); Absence of tap water (ATpW); Utilization of communal pastures (Cop); Absence of utilization of communal pastures (NCop); Rev-1 vaccination (R1); Absence of Rev-1 vaccination (NR1); Trade of animal and/or its products (Tap); Absence of trade of aninal and/or its products (ATap); Consumption of raw milk/fresh cheese (CRwM); No consumption of raw milk/fresh cheese (ACRwM); Dogs eat fetal membranes, and/or abortions (DEat); Dogs sometimes eat fetal membranes and/ or abortions (DSEt); Dogs never eat fetal membranes and/or abortions. (DNEt); Presence of clinical signs of brucellosis (Sig); Absence of clinical signs of brucellosis (NSig); Human brucellosis (HBr); Absence of human brucellosis (NHBr).

order, the following characteristics were observed in the positive dimension 2 (Figure 2): Dogs eat foetal and abortions products (2.51), not sharing pasture areas (1.10), correct removal of foetuses and foetal membranes following abortion (0.98), use of tap water (0.81) and implementation of fly control programme (0.66).

In contrast, the following characteristics were observed in negative dimension 2: existence of reproduction/husbandry programme (- 1.03), absence of cleaning and disinfection programme of farm premises (- 0.67), absence of Rev-1 vaccination (- 0.61) and presence of dogs that eats foetuses and foetal membranes (- 0.51).

The third dimension (Figure 2) contributed 10.7% of data variance. A significant association between farmers who keep dams separate during the parturition period, existence of reproduction/husbandry program, farmers who had another(s) herd(s) and implementation of fly control program was observed in the negative dimension 2.

For the positive dimension 3, the modalities that made the highest partial contributions to variability, in decreasing order, were: dams that are kept away during parturition (1.81), existence of reproduction/husbandry program (1.56), farmers who had others herds (1.32), cleaning and disinfection program of farm premises (0.68) and farmers who had brucellosis (0.61). In contrast, the following variables were observed in negative dimension 3 (Figure 2): absence of dogs that eat foetuses and abortions (- 1.09), absence of farmers who drink raw milk or eat fresh cheese made from raw milk (- 0.82), farmers who remove abortions (foetuses and fetal membranes) (- 0.62) not sharing communal pastures (- 0.59) and deficient cleaning and disinfection of farm premises (- 0.52).

Discussion

Eradication of brucellosis in small ruminants is a necessary task to avoid heavy economic losses, reproductive issues (mainly abortions and/or neonatal losses) and the emergency slaughtering of positive animals. Moreover, control of brucellosis is also necessary in order to avoid zoonotic outbreaks, protect public health and guarantee the animal trade, especially in areas with high prevalence values (Minas 2006). Since brucellosis is a multifactorial infectious disease, the characteristics and management of flocks are potential risk factors. The application of a multifactorial correspondence analysis showed, in a graphic form, the association of risk factors and farm practices related to high brucellosis prevalence values. However, the disadvantage of this approach is that it considers all risk factors to have equal importance.

Results indicated that flocks with a high prevalence of brucellosis are associated with absence of abortions and absence of clinical signs of brucellosis. In previous studies, Coelho and colleagues (Coelho *et al.* 2007) observed that prevalence of brucellosis was higher in large flocks, with mainly over 150 animals. Since flocks in the study area were extensive and required large pasture areas, the presence of abortions and/or other clinical signs could have been difficult to be observed by farmers. In addition, the association between the lack of abortions and high prevalence of brucellosis may be associated to the lack of communitation of abortions by farmers to the local veterinary authorities, despite this being mandatory (Portugal 2006).

If farmers notice the existence of abortions to the National Veterinary Authority, the flock is subjected to several sanitary measures, including restrictions on commercial trade and new blood sample testing. In addition, the flock is restricted to the farm area. Animals belonged to large flocks are mainly fed in large pastures (mainly communal pastures). Thus, in case of positive results (or even in case of abortion notification), flocks cannot leave the farm to prevent the spread of the disease through pastures. However, sometimes there is no ability to feed them on farms since feed is based on pasture.

The trade of live animals (mainly kids and lambs) represents the main economical income for many farmers. Thus, farmers may therefore not report abortions in order to avoid any economic losses associated to the restriction measures. This could explain the association between the absence of (reported) abortions and the high prevalence of brucellosis.

The low levels of training/education of farmers regarding brucellosis may be considered as a barrier in eradication programmes (Litgh-Pereira 2001). Lack of knowledge about policies related to animals, veterinary management or farm biosecurity could pose a risk for both animals and humans (García-Díez *et al.* 2013). This lack of knowledge about the disease and its zoonotic characteristics may explain the relationship observed between flocks with high prevalence of brucellosis and farmers who declared that they drink raw milk or eat cheese made from raw milk (Sofian *et al.* 2008). Most family farms, such as those in the study, are characterized by low economical profit. This fact suggests a lack of veterinary support and may explain the lack of knowledge regarding the association of clinical signs such as abortions, metritis, orchitis or infertility with brucellosis infection (Mainar-Jaime *et al.* 1999). The lack of notification of abortions may not only be associated with avoiding restrictive measures, but also with a lack of knowledge about legislation and clinical symptoms related to brucellosis by farmers.

Rev-1 vaccination is an essential measure to control brucellosis in areas of high prevalence (Blasco 2006). Although kids and lambs between 3 to 6 months old must be vaccinated with Rev-1, the relationship between the high prevalence of brucellosis and absence of Rev-1 vaccination may be compatible with lamb and kid management strategies whereby (unvaccinated) animals originally destined for slaughter are retained in the flock (Minas *et al.* 2004), increasing the risk of new infections and maintaining the disease within the flock (Blasco 1997, Blasco 2002).

The implementation of a biosecurity plans at the farm level is fundamental to controlling brucellosis (Ganter 2008). Measures such as the control of animal movement, cleaning and disinfection, pest control, reproductive management or preventive veterinary programmes among others are required.

Since *B. melitensis* has little resistance to most disinfectant agents (CFSPH 2009), the correct cleaning and disinfection (C&D) of farm premises (Reviriego *et al.* 2000) is fundamental to limit its survival. Thus, the absence of correct hygienic practices explains the relationship between the high prevalence of brucellosis in flocks without proper cleaning and disinfection procedures (Mainar-Jaime *et al.* 1999).

Infected animals with brucellosis are characterised by the excretion of *Brucella* spp. trough vaginal discharges, mainly during calving, that contaminates the environment. The absence of C&D procedures and proper manure management creates an environment in which insects thrive. These flies could act as mechanical carriers for the dissemination of *Brucella* spp. across the farm environment through, for example, feeders and/or taps facilitating oral infection.

The presence of dogs has been described as a risk for brucellosis infection in farm animals (Aguiar *et al.* 2007). Dogs represent a potential epidemiological threat in areas in which brucellosis is endemic since they can act as mechanical disseminators by feeding on aborted foetuses, dragging them along and spreading the bacteria. *Brucella* can also produce disease in dogs via the ingestion of infected reproductive tissues (Baek *et al.* 2003). Dogs infected with *Brucella* spp. can spread organisms into the environment through urine, vaginal secretions, aborted foetuses or faeces. Since elimination of infected animals may not necessarily eradicate the disease, they should instead be included in all sanitary measures carried in the flock (i.e. blood sampling).

The application of good farm practices (GFP) that also include the existence of a reproductive/husbandry programme was observed to be a protective factor in those farmers that suffered brucellosis. The presence of GFP may be associated with access to knowledge of treatments recommended by medical practitioners to treat zoonotic infection and sanitary recommendations that were made by the National Veterinary Authority during the compulsory epidemiological survey (Gunn *et al.* 2008).

Since the control of brucellosis is multifactorial, the characteristics of the flock (i.e. species, size) as well as its management (i.e. feeding, reproductive management) should be considered when determining the potential risk factors. The current study demonstrated that high prevalence values of brucellosis were mainly related to flock size, the absence of clinical signs of brucellosis, including abortions, and the absence of the Rev-1 vaccination. Familiar farms, the absence of veterinary technical support, and the low levels of training/education of farmers regarding brucellosis may explain the influence of these factors.

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