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Research Paper

EPIDEMIOLOGICAL SURVEY AND EFFECTS OF BRUCELLOSIS IN SMALL RUMINANTS IN SELECTED PASTORAL LANDS OF DALABA, GUINEA

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Abstract

Brucellosis is a well-known zoonosis which not only causes serious economic losses on the farms where it occurs, but also represents an ongoing threat to public health. The objective of this work was to collect as much information and data as possible on the epidemiological basis of the Djalonke and Dwarf Guinean breeds in order to propose effective strategies to combat the disease in accordance with the realities on the ground. To achieve this objective, retrospective study, sampling, serological study and statistical analysis were the different methodologies adopted for this purpose. The retrospective study carried out among the different executives made it possible to understand that brucellosis is a disease that is not too well known in the Dalaba prefecture. Most farmers are unaware that this disease is a zoonosis. Out of 157 animals examined, 123 blood samples were taken and 100 sera collected and tested using the EAT or Rose Bengal test were found to be positive (i.e. 36.84% in goats with a maximum confidence interval of 37.47% and minimum 36.20%, and 30.23% in sheep with a maximum confidence interval of 32.26% and minimum 28.19%). In addition, cases of abortion, placental retention, stillbirths, orchitis, epididymitis, hygroma and, rarely, arthritis have been observed. These results demonstrate a serious situation for livestock farming in general and that of small ruminants in particular, taking into account the risks associated with the disease and the economic losses it causes.

Key words: Brucellose, Reproduction, Small ruminants, Dalaba- Guinea.

INTRODUCTION

Livestock plays a crucial role in the livelihoods of the majority of Africans [1]. Small ruminants form an integral and important component of the pattern of animal production in several countries. Both goats and sheep are widespread and their importance is primarily associated with their small size, which is significant and to the advantage of mankind for three important reasons: economic, managerial and biological

[2]. They are among important domestic animals which are highly adaptable to a broad range of environmental conditions [3]. Though large ruminant dairy sector is more profitable but small ruminants have some advantages over the counterparts. They have wide adaptability to harsh environments, low production inputs and capital investment, high fertility and fecundity, low feed and management needs, less space and feeding requirements, and high feed conversion efficiency [4].

Brucellosis is an important zoonosis worldwide. The disease is caused by a small gram-negative bacterium of the genus brucella belonging to the family α2-proteobacteriacea which affects humans as well as many domestic and wild animal species [5]. It is still a cause for special concern in developing countries today; this is particularly acute in countries whose food and economies depend in part on livestock farming. It causes enormous losses on animals: reduced production (abortions, sterility, stillbirths, milk losses...) [6] and on the economy of livestock farmers (cost of vaccines, cost of products, trade in animals and the early culling of animals) [7].

Humans are usually infected through contact with fluid discharges from an infected animals or by ingestion of unsterilized dairy products mainly sheep and goat's milk and fresh soft cheese made out of unpasteurized milk. The disease in human is serious and long lasting and often results in chronic and disabling symptoms. The prevalence of brucellosis can vary according to climatic conditions, geography, species, sex and age. In addition, the applied diagnostic tests might affect the results[8,6]. The disease is one of the mandatory notifiable diseases [9] and therefore belongs to the list of priority diseases of the World Organisation for Animal Health. In supervised livestock farms in some African countries, economic losses in sedentary herds can be estimated at 150 million CFA francs per year, or 10% of the owners' income. In Swaziland, economic losses due to abortion amount to 2,900,023 Euros, while milk losses are estimated at 1,272,210 Euros and export losses at 47,384 Euros. In 2009, Tunisia and the Democratic Republic of Congo referred to economic losses in abortions, loss of labour force and drop in milk secretion, without giving a financial evaluation [10,11].

Considering the importance of brucellosis and its impact on the reproduction of animals, we took an interest in this project in order to highlight the consequences of the disease on the reproduction of small ruminants in the Prefecture of Dalaba- Guinea Conakry.

2-MATERIAL AND METHODS

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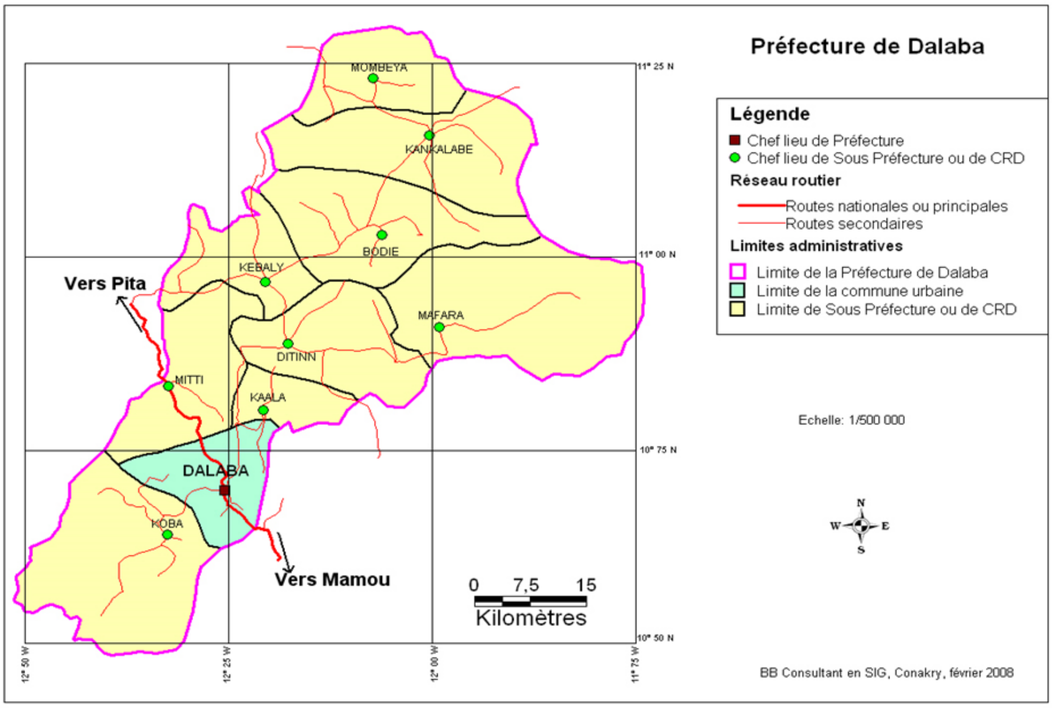
A-MATERIAL

Presentation of the prefecture of Dalaba

Dalaba is one of the three (3) prefectures of the Administrative Region of Mamou, located 327 km from the capital Conakry and 55 km from the chief town of the region. It covers an area of 4494 km2 with a population of 133,400 inhabitants with a density of 29.68 inhabitants per km^2. The prefecture of Dalaba is bounded: to the east by the prefectures of Mamou and Tougué; to the west by the prefecture of Pita; to the north by the prefecture of Labe and to the south by the prefecture of Kindia. It is located in the Fouta Djalon massifs between 9°45' and 11°35' north latitude and 10°21' and 12°16' west longitude with an average altitude of 1200 m.

The relative humidity varies between 21% and 100%. The highest humidity recorded in August and the lowest in February and March is favourable to the proliferation of pathogenic germs. This is why infestation and infection rates are often very high during both seasons (dry and wet seasons). On the other hand, it allows the good development of the breeding because it oscillates around the animal's comfort zone.

The different research localities surveyed were: Koba, Mitty, Ditinn and the urban municipality.



Scheme 1: Map of the Prefecture of Dalaba

2- METHOD

2- 1 Retrospective study

(a) Executive consultation and analysis of archives

In order to carry out the work properly, the local livestock department managers were contacted to enquire about the brucellosis situation in general and that of small ruminants in particular. This step was carried out in order to know the previous epidemiological situation of the disease.

(b) Survey of breeders

This cross-sectional survey of farmers was carried out using a pre-established questionnaire, interviews were conducted with farmers to obtain information on their knowledge of the disease, to assess the farming conditions, the type of farming, the parameters related to the productivity of animals affected by this disease and the management of sick animals.

2-2 Clinical examination

This study initially consisted of a general examination of the animals, observing them on pasture, in their movements and at rest. Then the breeding animals and those suspected during this study were examined in further detail (thermometry, palpation of the genital organs, examination of the mucous membranes) in order to detect possible signs of pathologies, in particular those linked to brucellosis (orchitis, hygroma, placental retention, infertility, etc.).

As well as females that have had abortions.

2-3 Sampling

Extensive farming was the choice of this study with representative numbers. The combination of sampling methods was random. The sample size was calculated based on the formula proposed by Thrusfield (2005):

$$N_t = \frac{IC^2 \, . Pexp(1 - Pexp)}{d^2}$$

$$N_t = \frac{1.96^2 \, . Pexp(1 - Pexp)}{d^2}$$

Pexp is the expected prevalence, d is the desired precision, Nt is the sample size and IC is the confidence interval.

Thus, taking these data into account, a proposal for an expected prevalence of 18% was made. The confidence interval is 95% with a desired precision of 6%.

$$N_t = \frac{1.96^2 \, . \, 0{,}18(1 - 0{,}18)}{0{,}0036}$$

$N_t = \frac{0{,}566}{0{,}0036}$=157

The sample size is : 157

2- 4 Blood sampling and serum collection :

After restraining the animal, the jugular vein was identified on the lateral side of the neck and pressure was applied to this area with the thumb as a garrote. We inserted the needle of a 5 ml syringe into the canal of the vein, making sure that the needle was effectively in the vein, then removed the pressure with the thumb and absorbed a quantity of 1 to 2 ml of blood. The syringes containing the blood were labelled according to the animals' information and then deposited until the serum was formed for 12 to 24 hours.

Using new syringes, the sera were collected and transferred to the cryotubes and labelled according to the pre-existing numbers on the syringes, then placed in the cooler containing ice and sent to the laboratory of the Higher Institute of Science and Veterinary Medicine in Dalaba for storage and analysis. To further extend this investigation, random sampling in the different study areas was carried out.

2-5 Serological analysis

The Rose Bengal test (RBT), a serological method proposed by Nielsen (2002), was used in the study. The test was performed according to the OIE protocol. Briefly, sera were defrosted at room temperature (23°C) for 30min, and the material required for the Rose Bengal Test (RBT) was disinfected. The reaction opaline plate was cleaned with soapy water and then dried in room air. The magnifying glass was cleaned with 70°C alcohol, then set the measuring pipette to 30µl.

Using a dosing micropipette, the sera were taken from the cryotubes, arranged according to the notation on the sampling sheet. Place two drops of equal volume side by side: 30µl of serum and 30µl of Rose Bengal buffered antigen. Homogenize the antigen and serum using a mouthpiece (single use). In a rotary movement, shake the opaline plate and wait for 4 minutes, then proceed to the reading.

2- 6 Statistical analysis of the data

The data were analyzed using SPSS software (version 13.0, SPSS Inc., Chicago, Illinois, USA). The data were presented as mean ± standard deviation (SD). The statistical difference between the groups was determined by analysis and variance and t-test. P-values<0 .05 are considered statistically significant.

3- RESULTS

1 - Retrospective study

a-Executive consultation and analysis of archives

The study conducted among the officials of the Livestock Department led to the understanding that brucellosis is a disease that is not well known in Dalaba Prefecture. No previous studies have been carried out on brucellosis in small ruminants in this locality.

b- Survey of breeders

Table 2: Numbers of animals in the surveyed localities

	Localities	Number of breeders		Numbers of animals			
		Men	Women	Ovins		Caprins	
				Males	Females	Males	Females
1	Koba	57	60	173	281	468	602
2	Ditinn	73	85	294	743	566	948
3	Mitty	45	69	221	862	356	523
4	Urban Municipality	22	36	269	309	235	618
Sous-Total		**197**	**250**	**957**	**2195**	**1625**	**2691**
TOTAL		**447**		**3152**		**4316**	

Source: Department of Livestock / Conakry / Guinea

2- Clinical examination

Using a survey sheet, 157 animals were examined, 67 of which were sheep and 90 goats, in order to observe the pathological impact of this disease on the animals in the different study areas.

Table 3: Results of the clinical examination

N°	Localities	Number of animals		Number of animals showing symptoms		Symptoms observed hygroma									
						Abortions		Orchitis		Infertility		Hygroma		Placental retention	
		Sheep	Goats	Sheep	Goats	Sheep	Goats	Sheep	Goats	Sheep	Goats	Sheep	Goats	Sheep	Goats
1	Koba	10	19	2	3	1	2	0	0	1	1	0	0	0	1
2	Mitty	15	18	1	1	0	0	1	0	0	1	0	0	0	0
3	Ditinn	17	21	0	0	0	0	0	0	0	0	0	0	0	0
4	Urban Municipality	25	32	1	4	0	1	0	0	1	2	0	0	0	0
Total		**67**	**90**	**4**	**8**	1	3	1	0	2	4	0	0	0	1

3-Results of sampling and serological analysis

Blood sampling and serum collection

A total of 123 blood samples were taken. The results of this investigation are explained in the following table.

Table 1: Results of serum collection

N°	Localities	Number of animals registered		Samples collected							
				Sheeps				Goats			
		Ovins	Caprins	Young animals		Adult animals		Young animals		Adult animals	
				Males	Females	Males	Females	Males	Females	Males	Females
1	**Koba**	**10**	**19**	**1**	**01**	**02**	**05**	**0**	**01**	**02**	**07**
2	**Mitty**	**15**	**18**	**1**	**02**	**02**	**07**	**01**	**03**	**04**	**10**
3	**Ditinn**	**17**	**21**	**0**	**3**	**02**	**10**	**2**	**2**	**3**	**09**
4	Urban Municipality	**25**	**32**	**1**	**2**	**3**	**12**	**2**	**3**	**5**	**15**
-		**67**	**90**	**3**	**8**	**9**	**34**	**5**	**9**	**14**	**41**
Total				**11**		**43**		**14**		**55**	

Serological analysis

The sera were subjected to an analysis (Rose Bengal test) to determine the prevalence of brucellosis. The prevalence of seropositivity to brucellosis in the different groups of animals in the different districts is presented in Tables 4 to 7 and Histogram 2.

Table 4: Prevalence of brucellosis per head and per group in sheeps

Localities	n^t	n^e	n^p	Prevalence en %	Ic 95%		N^T	n^e	n^p	Prevalence en %	Ic 95%	
					min	max					min	max
Koba	10	07	03	42,85	41,19	44,50	15	08	01	12,5	8,68	16,31
Ditinn	15	10	04	40,00	39,01	41,96	12	07	03	42,85	41,45	44,24
Mitty	17	11	02	18,18	17,41	18,94	16	10	02	20,00	19,01	24,98
UM	25	15	04	26,66	24,11	29,20	25	18	03	16,66	15,89	17,42
TOTAL	**67**	**43**	**13**	**30,23**	28,19	32,26	**68**	**43**	**09**	**20,93**	20,70	21,2,

Caption: **UM**: Urban Municipality; **n^t** = number of livestock; **N^T**= total number of livestock; **n^e**= number of samples tested; **n^p**= number of positive samples; **Ic**= confidence interval; **min**= minimum and **max**= maximum

Table 5 : Prevalence of brucellosis per head and per group in goats

Localities	n^t	n^e	n^p	Prevalence en %	Ic 95%		n^T	n^e	n^p	Prevalence en %	Ic 95%	
					Min	max					min	max
Koba	19	08	05	62.5	59,95	65,04	10	06	02	33,33	32,18	34,47
Ditinn	18	14	06	42,85	42,34	43,35	09	05	01	20,00	19,01	21,96
Mitty	21	15	04	26,66	25,00	28,31	11	08	03	37,5	36,22	38,77
UM	32	20	06	30,00	29,1	31,96	20	12	05	41,66	40,00	43,31
TOTAL	**90**	**57**	**21**	**36,84**	36,20	37,47	**50**	30	**11**	**36,66**	36,27	37,04

Caption: **UM**: Urban Municipality; **n^t** = number of livestock; **N^T**= total number of livestock; **n^e**= number of samples tested; **n^p**= number of positive samples; **Ic**= confidence interval; **min**= minimum and **max**= maximum

Histogram 1: Comparative Analysis of Prevalences

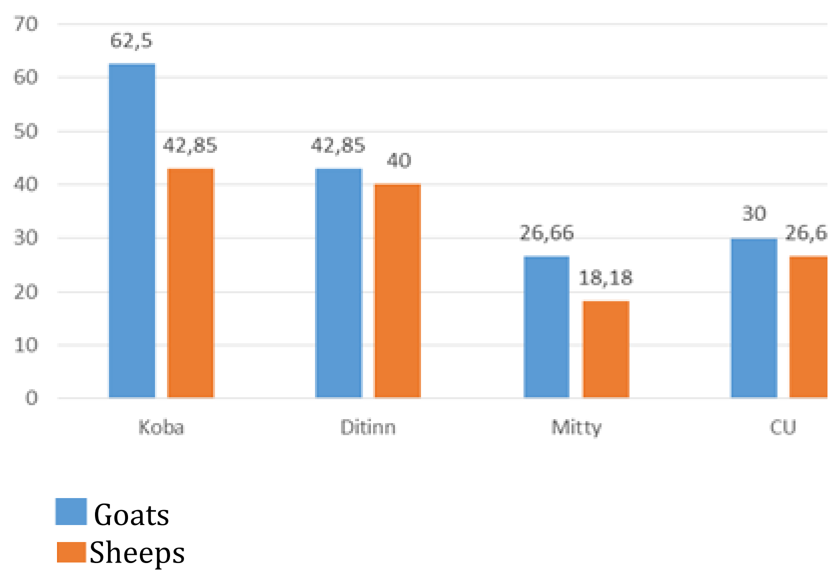


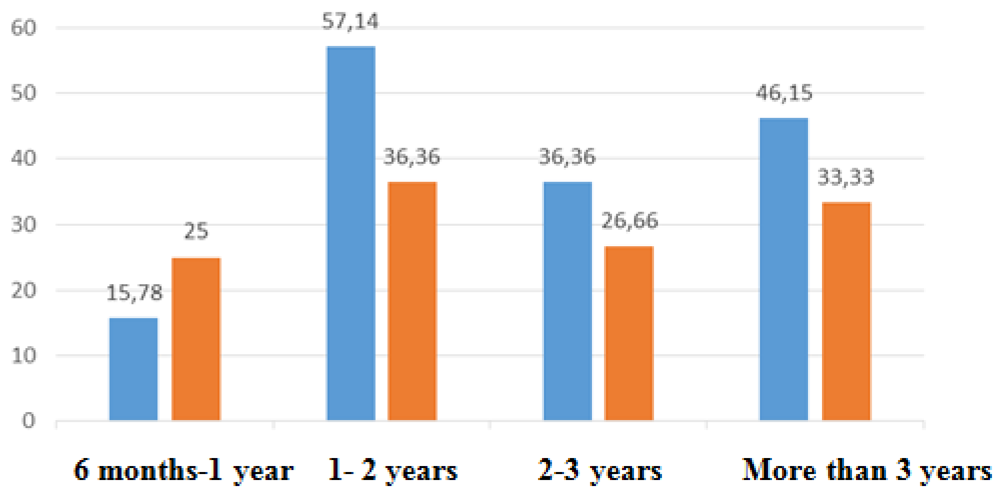
Table 6: Prevalence by age groups of sheeps

N°	Tranche d'âge	n^t	n^e	n^p	prévalence	IC 95		P-value
						min	max	
1	6mois – 1an	09	08	02	25	19,01	26,96	< 0,0001
2	1 – 2ans	17	11	04	36,36	34,83	37,88	
3	2 ans – 3 ans	25	15	04	26,66	25,13	28,18	
4	3 ans et plus	10	09	03	33,33	32,18	34,47	
5	**Total**	**61**	**43**	**13**	**30,23**	**28,57**	**31,88**	

Table 7: Prevalence by age groups of goats

N°	Age range	n^t	n^e	n^p	Prevalence	IC 95		P-value
						min	max	
1	**6 months – 1 year**	25	19	03	15,78	15,01	16,54	< 0,0001
2	**1 – 2 years**	26	14	08	57,14	56,63	57,64	
3	**2 – 3 years**	17	11	04	36,36	35,59	37,12	
4	**more than 3 years**	28	13	06	46,15	45,64	46,65	
5	Total	**96**	**57**	**21**	**36,84**	**35,82**	**37,85**	

Histogram 2: Comparative Analysis of Prevalence Rates by Age group and Species (Sheep and Goats)



DISCUSSION

In terms of familiarity with the disease, 85% of the farmers surveyed were unaware of brucellosis. However, only 2% declared that they knew the name of the disease in their dialect (Haïkou or Bakkalé in Pular). This observation represents an alarming situation for small ruminant farming, when considering the risks related to the pathogenicity, the degree of contagiousness and the zoonosis status of the disease.

The most important negative effect of brucellosis on livestock appears to be abortion, followed by stillbirths, infertility, lower milk production and longer intervals between births. Thus this disease must be considered as a major pathology with a dual cause: on the one hand by the frequency and severity of human cases contracted from the animal and its production [12,13], and on the other hand by its economic consequences in livestock farming: production losses (abortions, infertility, stillbirths, milk losses...) and barriers to commercial exchange of animals.

A high prevalence of brucellosis in cattle occurs when more than 35% of the herds and more than 10% of individual animals are infected [14].The prevalence of brucellosis using the Rose Bengal Test (RBT) has been established [15]. Serological tests alone tend to underestimate the true prevalence of brucellosis and thus results of culture are always higher than those obtained from serology [16-18]. The RBT is a highly sensitive test for the detection of immunoglobulins IgG1, IgM and possibly IgG2 against Brucella in the sera of cattle [19]. The sera were analysed to determine the prevalence of brucellosis by species, area and head. The different cases of positivity observed in the localities can be attributed not only to the favourable conditions for the development and conservation of brucellas in this area, but also to the conditions of extensive breeding characterised by total divagation of the animals. The presence of weekly markets through which many animals from several localities pass in an unregulated manner explains this situation. The management of livestock farming is also a risk factor: transhumance, high density of animals and mixing of animals of different origins.

Over the different age groups (sheep and goats) evaluated, brucellosis is largely observed in adults, affecting both males and females. Juveniles that have not reached sexual maturity may be infected but show no clinical signs, although generally a weak transient serological response is detectable when contamination occurs after the establishment of their own immune system. Similar to the mechanisms of transmission of *B. abortus* in cattle, *B. melitensis* can infect the newborn from the female. Only a small proportion of the young are infected in utero or through the birth canal; the majority contract the bacterium when they ingest colostrum and then contaminated milk. However, young domestic sheep and goats appear to recover from the infection by self-healing. They are still likely to develop a new infection once they reach sexual maturity, if no effective immunity has been established.

In animals, Brucella preferentially accumulates in the genitals. Thus, brucellosis is usually spread during reproduction and during abortion or parturition; high concentrations of bacteria are found in the products of abortions and fetal fluids from an infected animal. Bacteria can survive for several months outside the animal's body, in the outdoor environment, especially in cold and wet conditions. These bacteria in the environment remain a source of infection for other animals that become infected through close contact (respiratory or conjunctival tract or even through ingestion).The bacteria can also colonize the udder and may also contaminate milk [20].

In males, the infection is usually not apparent. This is a serious situation because it exposes females of reproductive age to many infections, including brucellosis. It is generally accepted that goats and rams play a role in the epidemiology of brucellosis. However, they act as passive mechanical vectors during reproduction. In addition, orchitis and epididymitis or reduced fertility are common sequelae of infection and semen can be contaminated with Brucella in a significant proportion of males, with consequences in terms of risk of transmission (natural or via artificial insemination). Young females also play a role in the epidemiology of brucellosis because the first products of their pregnancies could be characterised by a series of abortions and constitute a public health risk. Affected females remain carriers of the germ and are often sterile. However, females often do not abort and give birth to young stunted animals resulting in the most widespread form of the infection. Nevertheless, vaccination is one of the most effective measures to reduce the prevalence of disease and has largely contributed to the success of many control programs control measures are based on strict hygiene and vaccination programs. It is regarded as a measure for reducing the prevalence of the disease to a level where eradication by test and slaughter can be implemented. Also, vaccination may be the most economical means of controlling the brucellosis [21,22]. Of the vaccines used, Rev-1 vaccine is generally preferred and used to protect small ruminants against brucellosis and its effects [23].

CONCLUSION

The present investigation based on the effect of brucellosis on the reproduction of small ruminants revealed the existence of brucellosis in the Prefecture of Dalaba. This investigation revealed that no studies on brucellosis in small ruminants have been investigated in this area. The geo-climatic characteristics (humidity, temperature) as well as the extensive farming method facilitate the maintenance of the epizootic chain. The evolution of the disease in small ruminants is enzootic and no prophylactic measures have been taken to prevent disease progression. Most farmers are unaware of brucellosis as a livestock disease and especially as a disease transmissible to humans. Consequently, they are unaware of the contamination resulting from the handling of aborted foetuses. Appropriate solutions aimed at improving the performance of the epidemiological surveillance network and reducing the prevalence of brucellosis in small ruminants are necessary to prevent the spread of the disease.

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