

A sero-survey of major infectious causes of abortion in small ruminants in Morocco

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Small ruminants,
Toxoplasmosis.

Summary

A serological survey was conducted to estimate the seroprevalence of 5 major abortive infections in 13 sheep flocks and 10 goat herds in 2 regions of Morocco. A total of 308 from aborted females (202 ewes and 106 does) and 197 sera (97 ewes and 99 does), were tested for brucellosis, chlamydiosis, Q fever, toxoplasmosis, and for 9 major serovars of *Leptospira*. An average abortion rate of 12.10% was found in ewes and 10.26% in does. The serological analyses revealed the presence of all 5 abortive infections, both in sheep and in goats. Ten (43%) herds/flocks were positive to brucellosis, 21 (91%) to chlamydiosis, 17 (74%) to toxoplasmosis, 13 (57%) to Q fever, and 5 (22%) to leptospirosis. *Leptospira* spp. serovars Copenhageni and Grypothyphosa were found in a single sheep flock, while Tarassovi and Copenhageni were detected in 4 goat herds. Of the 23 investigated herds/flocks, 22 (96%) showed mixed infections. The findings of this study confirmed the possible involvement of the 5 selected abortive infections in abortion outbreaks occurring in the investigated regions. Further investigations are needed to better understand the aetiology of infectious abortions in herds and flocks within investigated regions.

Indagine sierologica sulle più comuni cause di aborto infettivo nei piccoli ruminanti in Marocco

Parole chiave

Aborto,
Brucellosi,
Clamidosi,
Febbre Q,
Leptosirosi,
Marocco,
Piccoli ruminanti,
Toxoplasmosi.

Riassunto

Per indagare la sieroprevalenza in Marocco di cinque delle più diffuse cause di aborto infettivo nei piccoli ruminanti è stato condotto uno studio sierologico campionando 13 greggi di pecore e 10 di capre in due regioni del Paese. In totale sono stati prelevati 308 sieri (202 pecore, 106 capre) da 9 femmine con anamnesi di aborto e sono stati testati per brucellosi, clamidiosi, febbre Q e toxoplasmosi. Di questi, 197 sieri (97 pecore, 99 capre) sono stati testati anche nei confronti di nove delle principali sierovarianti di *Leptospira*. Il tasso medio di aborti è risultato essere del 12,10% nelle pecore e del 10,26% nelle capre. La sierologia ha identificato la circolazione di tutti e cinque gli agenti infettivi indagati in entrambe le specie. Dieci greggi (43%) sono risultati positivi alla brucellosi, 21 (91%) alla clamidiosi, 17 (74%) alla toxoplasmosi, 13 (57%) alla febbre Q e 5 (22%) alla leptosirosi. Per *Leptospira* sono state trovate positività verso le sierovarianti Copenhageni e Grippothyphosa in un gregge di pecore, mentre positività verso Tarassovi e Copenhageni sono state rilevate in quattro greggi di capre. Dei 23 greggi indagati, 22 (96%) hanno mostrato la presenza di infezioni miste. I risultati di questo studio confermano il possibile coinvolgimento dei cinque agenti infettivi indagati nei casi di aborto che si verificano nelle regioni oggetto di studio. Ulteriori studi sono tuttavia necessari per meglio comprendere l'eziologia degli aborti infettivi nei piccoli ruminanti in Marocco.

Introduction

Abortion in livestock is the cause of considerable economic losses for farmers and farming communities. In addition, abortion may be of great importance to public health, if it prompted by microorganisms that may cause diseases to humans. *Brucella*, *Listeria*, *Coxiella*, *Chlamydia*, *Leptospira*, and *Toxoplasma* are some of the microorganisms of public health importance causing abortion in small ruminants (Jonker 2004). These infectious agents easily spread among animals and humans in all farming systems. At the same time they are of increased importance where communal grazing is practised and farms are family holdings (Gwaze Rumosa 2009). Communal grazing is common in North Africa, with the exception of Morocco, and Middle Eastern countries (Spickler and Roth 2008). *Brucella* spp. is a frequent cause of severe human illness, thus it is the first suspected pathogen when small ruminant abortions are investigated (Blancou and Lefèvre 2006). In Morocco several investigations conducted on small ruminants have repeatedly shown that Brucellosis was rare and geographically limited to the Eastern part of the country (El Idrissi et al. 1995, Laghzaoui et al. 1996, El Jay et al. 2003). Conversely other causes of abortions, such as *Coxiella*, *Salmonella*, *Toxoplasma* or *Chlamydia*, have been frequently reported (Benkirane et al. 1990, El Idrissi et al. 1995, El Jay et al. 2003).

Leptospira spp. infection as a possible cause of abortion among sheep and goats is rarely investigated, because it is thought to rarely cause clinical disorders in small ruminants as compared to large ruminants, pigs and dogs (Ellis 1994). Furthermore, testing of leptospirosis is difficult because the Microscopic Agglutination Test (MAT), which is the internationally recognized method for investigating leptospirosis, requires specific equipment and the ability to grow *Leptospira* antigens.

Over the past years, livestock owners in several regions in Morocco have reported unusual abortion rates, causing significant economic losses in sheep and goats. The causes of these abortions remained undiagnosed. The present investigation attempted to explore, through a serological survey, the relative importance of the most frequently investigated small ruminant abortion causes in 2 regions of Morocco during Spring of 2013. These include *Brucella* spp., *Chlamydia abortus*, *Coxiella burnetii*, *Toxoplasma gondii* as well as various *Leptospira* serovars of widespread distribution in the Mediterranean Basin: Icterohaemorrhagiae, Copenhageni, Bratislava, Canicola, Grippotyphosa, Pomona, Tarassovi, Ballum, and Hardjo (Cerri et al. 2003).

Materials and methods

Samples selection, processing, and conservation

The investigation was performed during Spring (February – April) of 2013 in 2 regions, namely Tetouan and Chaouen, located respectively in Northern Morocco and the Middle Atlas. The 2 regions are known to have vocation for breeding of goats and sheep. At the time of the survey lambing/kidding season was coming to an end and all farms of origin had experienced abortions. Prior to sampling, a questionnaire was completed inquiring, among others, about the size and structure of the flock/herd and the history of the abortive syndrome as well as the stage of pregnancy at which abortions occur and the aspect of aborted fetuses. All 308 serum samples (202 from ewes and 106 from does) were taken from aborting animals originating from 13 sheep flocks (totalling 2,513 ewes), and 10 goat herds (totalling 1,150 does). A convenience sampling method was adopted through which up to 20 sera were taken from each flock/herd including sera from all primiparous females. Blood was obtained aseptically from a jugular vein, allowed to clump for about 1 hour at room temperature, then sera were separated and transported to the laboratory, centrifuged (15 min at 3,000 rpm) and each serum despatched in 3 aliquots and stored at -20°C until analysis.

Laboratory examination

Serum samples were tested for *Brucella* spp., *Chlamydia* spp., *Coxiella burnetii*, *Toxoplasma gondii* and for 9 serovars of *Leptospira* interrogans.

The Rose Bengal Test (Veterinary Laboratory Agency – VLA, UK) modified as to use 25 µl Antigen to react with 75 µl serum (Blasco et al. 1994) was used to test for *Brucella* antibodies. A positive reaction showed a moderate to intense agglutination.

The CHEKIT *Chlamydia* ELISA Test Kit (Idexx Laboratories, Westbrook, Maine, USA) was used for the detection of antibodies against *Chlamydia abortus*. Following the manufacturers' instructions, a sample having an S/P % value equal or over 40% was considered as positive.

The CHEKIT Q-Fever ELISA Test Kit (Idexx Laboratories, Westbrook, Maine, USA) was used for the detection of antibodies against *C. burnetii*. Following the manufacturers' instructions, a sample having an S/P % value equal or over 80% was considered positive.

Antibodies to *T. gondii* were also detected through an indirect ELISA kit (LSI, Lissieu, France). Samples with OD of 50% and above were considered positive.

The MAT was used to test for Leptospirosis and was

performed according to the OIE Manual (OIE 2008) and using as antigen 9 live serovars of *Leptospira* spp. belonging to 8 serogroups, considered as the most significant serivars for the Mediterranean region (Cerri *et al.* 2003), namely: Icterohaemorrhagiae, Copenhageni, Bratislava, Canicola, Grippotyphosa, Pomona, Tarassovi, Ballum, and Hardjo. A positive sample shows 50% or more of antigen agglutination in a titre of 1/100 or higher.

Results

Abortion features

The analysis of the questionnaire revealed that past abortion rates varied measurably from year to year (between 5% and 20%, approximately) and

the shepherds believe this would be in connection with the presence of some toxic plants in the field. However, it was reported that part of the cases consisted of stillbirths or births of weak lambs/kids with lameness and respiratory disorders that usually die within a couple of days. The causes of abortion were rarely investigated and never confirmed. There is no history of vaccination against any of the abortive agents investigated in this study. In Spring 2013 (February – April), the average abortion rate was quite similar in sheep (3.52% to 19.39%) and goats (5.16% to 28.88%) (Table I).

The distribution of abortion cases seems to be similar in both primiparous and multiparous ewes, showing rates of 10.43% and 11.14% of the total number of females, respectively (Table II).

Seroprevalence of the five investigated infections

The seroprevalence of the 5 investigated abortive infections by sampled animals and herd/flock are shown in Table III and IV and Figures 1 and 2.

The highest number of positive samples (n=55) was to *C. abortus* for ewes and to *C. burnetii* (n=29) for does. *Brucella* spp. was third among does and fourth among ewes. Forty-two ewe samples (20,8%) showed high antibody titers to *T. gondii*, this was higher than the results concerning does, only 9 (8,5%) positive samples were recorded.

For leptospirosis, only 98 and 99 sera were examined from sheep and goats, respectively. Only 2 ewes from the same flock (O9) were found positive to serovar Copenhageni and 1 of them was also positive to serovar Grippotyphosa. Copenhageni was also detected in 1 doe in 1 herd (C3), while Tarassovi was present in 4 animals originating from 3 herds (C1, C2, C6). The MAT titers of all positive sera did not exceed 1:400.

Distribution of abortive infections by pregnancy rank

The distribution of the various infections according to the pregnancy rank (primiparous vs multiparous) is shown in Table V. There is no statistically significant

Table I. Abortion rates in sheep (data collected in Tizitine, Ain Leuh and Azrou districts, Middle Atlas) and goats (data collected in Chaouen and Tetouan districts, Northern Morocco) during Spring 2013 (February – April).

Region (species)	District	Flock/herd	Number females	Aborting (%)	Sampled (%)
Middle Atlas (sheep)	Tizitine	01	165	32 (19.39%)	20 (12.12%)
		02	278	26 (09.35%)	20 (07.19%)
		03	33	06 (18.18%)	06 (18.18%)
		04	284	10 (03.52%)	10 (03.52%)
		05	212	26 (12.26%)	20 (09.43%)
		06	185	12 (06.48%)	12 (06.48%)
	Ain Leuh	07	232	20 (08.62%)	18 (07.75%)
		08	210	22 (10.47%)	15 (07.14%)
		09	212	18 (08.49%)	18 (08.49%)
		010	186	19 (10.21%)	19 (10,21%)
		011	36	04 (11.11%)	04 (11.11%)
	Azrou	012	230	39 (16.95%)	20 (08.69%)
		013	250	36 (14.40%)	20 (08.00%)
Total		13	2513	270 (10.74%)	202 (08.03%)
North (goats)	Chaouen	C1	143	11 (07.69%)	11 (07.69%)
		C2	120	22 (18.33%)	16 (13.33%)
		C3	188	16 (08.51%)	11 (05.85%)
		C4	70	11 (15.71%)	11 (15.71%)
	Tetouan	C5	123	09 (07.31%)	09 (07.31%)
		C6	95	10 (10.52%)	10 (10.52%)
		C7	213	11 (05.16%)	11 (05.16%)
	Tetouan	C8	74	05 (06.75%)	05 (06.75%)
		C9	90	26 (28.88%)	16 (17.77%)
		C10	34	06 (17.64%)	06 (17.64%)
Total		10	1150	127 (11.04%)	106 (09.21%)
Total		23	3663	397 (10.83%)	308 (08.40%)

Table II. Breakdown of abortion cases by pregnancy rank in sheep and goats during Spring 2013 (February – April) in Tetouan and Chaouen districts (Northern of Morocco) and in the Middle Atlas.

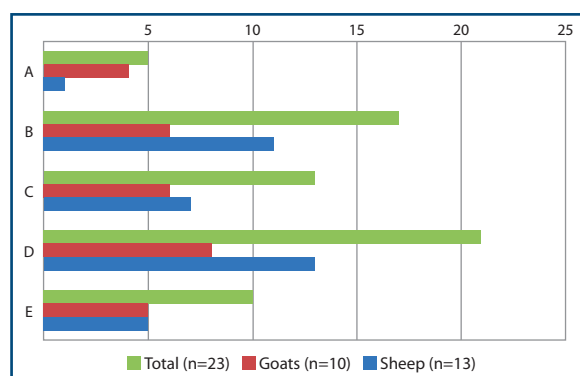
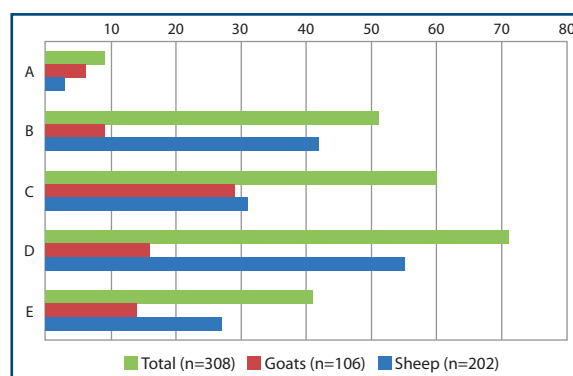
Pregnancy Rank	Number of females	Number of abortions
Primiparous	1572	164 (10,43%)
Multiparous	2091	233 (11.14%)
Total	3663	397 (10,83%)

Table III. Seropositivity of sheep and goats flocks/herds to 5 abortive infections. The serological survey was carried out on 23 flocks/herds of Northern Morocco and Middle Atlas during Spring 2013 (February – April).

Animal Species	Brucellosis	Chlamydiosis	Q fever	Toxoplasmosis	Leptospirosis
Sheep (n=13)	05 (38%)	13 (100%)	07 (54%)	11 (85%)	01 (08%)
Goats (n=10)	05 (50%)	08 (80%)	06 (60%)	06 (60%)	04 (40%)
Total (n=23)	10 (43%)	21 (91%)	13 (57%)	17 (74%)	05 (22%)

Table IV. Seropositivity of sheep and goats to 5 abortive infections. Animals were sampled during Spring 2013 (February – April) in the Northern Morocco and Middle Atlas.

Animal Species	Brucellosis	Chlamydiosis	Q fever	Toxoplasmosis	Leptospirosis
Sheep (n=202)	27 (13.4%)	55 (27.2%)	31 (15.3%)	42 (20.8%)	01/98 (1%)
Goats (n=106)	14 (13.2%)	16 (15.1%)	29 (27.3%)	09 (8.5%)	05/99 (5%)
Total (n=308)	41 (13.3%)	71 (23.1%)	60 (19.5%)	51 (16.5%)	06 (3%)

**Figure 1.** Seropositivity to the 5 investigated abortive infections by herd/flock during Spring 2013 (February – April) in Northern Morocco and Middle Atlas. (A=Leptospirosis; B=Toxoplasmosis; C=Q Fever; D=Chlamydiosis; E=Brucellosis).**Figure 2.** Individual seropositivity to the 5 investigated abortive infections during Spring 2013 (February – April) in Northern Morocco and Middle Atlas. (A=Leptospirosis; B=Toxoplasmosis; C=Q Fever; D=Chlamydiosis; E=Brucellosis). For *Leptospira interrogans* only 197 sera were investigated.**Table V.** Distribution of the 5 abortive infections in relation with pregnancy rank. Data collected during a serological survey on sheep and goats performed during Spring 2013 (February – April) in Tetouan and Chaouen districts (Northern Morocco) and in the Middle Atlas.

	Nb females	Brucellosis	Chlamydiosis	Q fever	Toxoplasmosis	Leptospirosis
Primi- parous	87	9 (10.3%)	18 (20.7%)	14 (16.1%)	11 (12.6%)	0/41
Multi- parous	221	32 (14.5%)	53 (24%)	46 (20.8%)	40 (18.1%)	6/156 (3.8%)
Total	308	41 (13.3%)	71 (23%)	60 (19.5%)	51 (16.6%)	6 (3%)

difference in the comparative prevalence of the 3 major abortive infections (brucellosis, chlamydiosis, and Q fever) between primiparous and multiparous ewes and does. The chi square calculation shows, in turn, highly significant differences of the prevalence of both *Leptospira* and *Toxoplasma* infections, in favour of older individuals.

Twenty-two farms out of 23 were serologically positive to 2 or more of the investigated possible causes: 6 (4 sheep flocks and 2 goat herds) tested positive to 2 causes, 4 (2 sheep flocks and 2 goat

herds) tested positive to 3 causes, and 11 farms had animals positive to 4 of the 5 investigated abortion causes. All sheep flocks and 80% of goat herds were positive to *C. abortus*.

Discussion

The present serological study showed that all 5 investigated abortion causes are widely distributed both in sheep and goats, in the 2 regions where the

study was conducted. Infection rates of chlamydiosis, Q fever, and Toxoplasmosis are similar to those previously reported in various parts of the country and at different periods (Benkirane *et al.* 1990, El Idrissi *et al.* 1995, El Jai *et al.* 2003). Furthermore, *Chlamydia abortus* and *C. burnetii* have been concurrently associated with abortions of small ruminants as it was the case in different regions in Morocco and elsewhere (El Jay *et al.*, 2003, Rekiki *et al.* 2005, Bisias *et al.* 2010). The high percentage of positivity to both *Chlamydia* and *Coxiella* infections found at both flock/herd and animal level, along with the occurrence of late abortion and the aspect of the aborted fetuses, would suggest that these 2 pathogens are actively circulating in both regions (Rodolakis 1997). With regard to the kit for *C. abortus*, it is important to note that CHEKIT is based on an inactivated antigen thus can show some possible cross-reactions against the LPS of other *Chlamydiae* (Vretou *et al.* 2007).

The situation is different for Brucellosis, which was previously considered nearly absent, with the exception of the Eastern Moroccan region (Laghzaoui, 1996), and now proves to be rather widespread both in the North and in the Middle Atlas. Vaccination campaigns were stopped in the Eastern region of Morocco in 2003¹ and this would explain the spread of the infection to the rest of the country through transhumance and commercial movements of small ruminants.

A higher number of ewe samples, 42 (20.8%), had high antibody titres to *T. gondii* as compared to doe samples, 9 (8.5%). Whether this is related to a variation of the geographical distribution of infected cats, the usual source of infection between regions (North vs Middle Atlas), or to the type of husbandry (extensive in ewes, semi-intensive in goats) remains to be assessed. The overall proportion of seropositive animals to *T. gondii* observed in this study was lower than the one reported from other parts of the world using different testing techniques (Filho *et al.* 2008, Reis *et al.*, 2007, Diakoua *et al.* 2013).

The role of *Leptospira interrogans* in the epidemiology of abortion in small ruminants is poorly investigated in Morocco and this is only the second study following an investigation conducted in Meknes and Middle Atlas almost 3 decades ago (Chaarani 1987). Of interest was the predominance of serovar Tarassovi among goat samples, although not showing high antibody titres. In contrast, serovar Hardjo, known to be associated with abortions in cattle and possibly in small ruminants (Ellis 1994), was not found in this study. While serovar Gripothyphosa was associated with Copenhageni in the same ewe. Such situation was never documented and its significance is unclear.

It was not possible, in any case, to establish with certainty a causal relationship between the observed abortions in a given flock/herd and the cause/s that was/were serologically detected. Although as mentioned above, the high prevalence of *Chlamydia* and *Coxiella* infections would indicate a possible role played by these 2 agents in causing abortion in the flocks/herds tested. *Chlamydia* and *Coxiella* could be separately or jointly involved in these cases, possibly together with other causes: *Brucella*, *Leptospira* or *Toxoplasma*.

Probably all these infections have been present for a long time in both regions and will remain endemic as long as no control measures are enforced and transhumance remains a common practice, enabling the exchange of infectious agents among farms and districts/regions (Rodolakis 1997).

These results are supported by the fact that no significant differences were found neither in the comparative overall abortion rates nor with regard to the seroprevalences for Brucellosis, Chlamydiosis, and Q fever occurring among primiparous and multiparous ewes and does. In an endemic situation, the great part of young animals do enter in contact with the agents during the first year of life and the increasing seroprevalence by age-class becomes less evident (Rodolakis 1997, Benkirane 2006).

With regard to Q fever, it is known that goats may shed *C. burnetii* for up to 2 pregnancies (Hatchette *et al.* 2003), and can abort twice following infection (Berri *et al.* 2007), while ewes do abort only once (Berri *et al.* 2007) and do not shed the organism in vaginal mucus at subsequent lambing.

Mixed infections prevailed in more than 90% flocks/herds. This situation is not unique but rather common in many parts of the world (Rekiki *et al.* 2005, Bisias 2010). The absence of predominance of a particular infection, especially among primiparous females, and the use of serological tests unable to discriminate between recent and old infections. In addition, the fact that infections were not repeated in time and so it was not possible to evidence changes in the kinetics of antibodies makes difficult to establish causal relationship in most cases.

Thus, it can be concluded that serodiagnosis of infectious abortions, although commonly used in veterinary medicine, is difficult to interpret. However, to confirm a strong serological suspicion, it is essential to use a direct diagnosis such as bacteriological isolation and identification or Polymerase Chain Reaction. Furthermore, veterinarians must be aware of abortive history within the herds/flocks under their supervision to be able to detect any new introduction of a given infection in herds through the systematic resort to a diagnostic laboratory for the search of abortive infections.

¹ Office National de Sécurité Sanitaire des Produits Alimentaire Royaume du Maroc (ONSSA) website: <http://onssa.gov.ma/fr/index.php>.

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