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Abstract:

During 2002-2003 the seroprevalence of *Mycoplasma capricolum* subsp. *capricolum* was studied among 104 small ruminant's flocks (18 sheep, 27 goat and 59 mixed flocks) in northern Jordan. At least 5 serum samples/flock were tested using iELISA test. The true flock-level seroprevalences of *Mycoplasma capricolum* subsp. *capricolum* were 56%, 39%, 28% in small ruminant (sheep and goats), sheep and goats respectively. There was no significant difference ($X^2 = 2$, d.f. =1, $p = 0.15$) between seroprevalences in sheep and goats at the flock level. A total of 29 variables including production and health management practices were tested as risk factors for seropositive flocks and analyzed using logistic regression analysis. The use of communal grazing was found to be a risk factor for *Mycoplasma capricolum* subsp. *capricolum* seropositivity with odds ratio of 5.2 and drinking the animals with spring water was a protective factor with odds ratio of 0.27. More than half of the examined flocks were seropositive to *Mycoplasma capricolum* subsp. *capricolum* indicating a role for *Mycoplasma capricolum* subsp. *capricolum* in contagious agalactiae in small ruminants. The education of farmers regarding the use of communal grazing and housing the newborn in separate barns is expected to help reducing the *Mycoplasma capricolum* subsp. *capricolum* infections in their flocks.

Keywords: *Mycoplasma capricolum subsp; capricolum; small ruminants; sheep; goat; seroprevalence; risk factors; Jordan.*

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Introduction

Mycoplasma capricolum subsp. *capricolum* (*Mcc*) is one of four mycoplasmas designated by OIE as a cause of contagious agalactia (CA) (a list B disease). Similar clinical and pathological findings also caused by *Mycoplasma* spp. that are grouped in the "*Mycoides* cluster", namely, *Mycoplasma mycoides* subsp. *capri*, *Mycoplasma putrfaciens* and *Mycoplasma agalactiae* (Nicolet, 1994). Although mycoplasmas are host specific, closely related animal species may share the same *Mycoplasma* spp. including sheep and goats (Ruffin, 2001). *M. capricolum* subsp. *capricolum* is primarily a pathogen of goats and may cause acute disease and death (DaMassa et al., 1992).

Natural infection leads to contagious agalactia syndrome, characterized by mastitis, arthritis and keratoconjunctivitis (Bolske, 1995). Experimental infection results in an acute syndrome of septicemia and severe arthritis. Although, *M. capricolum* subsp. *capricolum* is seldom isolated from pneumonic lesions (Bolske, 1995), kids do show acute respiratory distress in experimental infections (Rodriguez et al., 1998) and pneumonia may be seen at necropsy. Severe joint lesions are seen in experimental infections and accompanied by intense periarticular subcutaneous edema affecting tissues to some distance from the joint (Bolske et al., 1988). Fatal septicemic polyarthritis may occur in kids or lambs fed with contaminated milk with *Mcc* mastitis. Sporadic arthritis and abscesses may be less serious manifestations of *M. capricolum* subsp. *capricolum* (Perreau and Breard, 1979).

The diagnosis of *M. capricolum* subsp. *capricolum* is based on clinical signs, isolation and identification of the causal agent and other laboratory techniques including serological tests (Poveda and Nicholas, 1998) and molecular biology techniques (Bashiruddin et al., 1994).

The re-infection of the same herd is a major problem of sheep and goat mycoplasmoses. The main source of re-infection is the chronic carriers of mycoplasma from inside the herd and/or newly introduced animals to the herd. Some strains of mycoplasma can persist in organs such as ear and is often associated with mites (Cottew and Yeats, 1982; DaMassa, 1990).

Highly pathogenic CA caused by *M. capricolum* subsp. *capricolum* is widely distributed and, particularly in North Africa (Benkirane et al., 1993), but the frequency of occurrence is low (Bergonier et al., 1997). Such disease was observed in Australia, USA, Chile, Sweden and Oman (Nicolet, 1994). CA causes economic losses by decreased production, mortality, diagnosis and treatment costs; the losses can be high in severe outbreaks (Nicholas, 1998). The economic losses caused by CA was estimated to be 30$ million in the European countries around the Mediterranean (Nicholas, 2002)

In Jordan, four small ruminant mycoplasmas (*M. mycoides* subsp. *mycoides* LC, *M. capricolum* subsp. *capricolum*, *M. putrfaciens, M. agalactiae*) were isolated (Al-Momani et al., 2006). The prevalence of *Mcc* was not studied in Jordan. This study investigates the seroprevalence of *M. capricolum* subsp. *capricolum* among sheep and goat flocks in northern Jordan along with some risk factors associated with *M. capricolum* subsp. *capricolum* seropositivity.

Materials and methods

Study area and animals:

Jordan lies at the crossroad of three continents (Asia, Europe and Africa) and is considered a transit area for animal transportation from the northern to the southern states of the Middle East. There are six climatic zones in Jordan, including, the cool temperate rainy climate, warm temperate rainy climate, cool steppe climate, warm steppe climate, cool desert climate and warm desert climate (Anon, 1984). Jordan has 4 distinct seasons with an average temperature of 19°C.

Small ruminants are the most abundant domestic animals in Jordan 74.3% are sheep and 23.2% goats (Anon, 1995). Sheep and goats are usually kept together mainly under free range roaming transhumance husbandry with a small number of flocks adapting semi-intensive husbandry methods. Awassi sheep and local goats are the main small ruminant breeds in Jordan. The main sources of water used for sheep and goat drinking are collected rain, springs, dams and piped.

The routinely recommended control measure for infectious disease is vaccination. Currently, vaccines are used against; enterotoxaemia, brucellosis, pasteurellosis, anthrax, pox, foot and mouth disease and pest des petits ruminant, and no vaccine is used against *M. capricolum* subsp. *capricolum*.

The study area included the five Governorates (Irbid, Mafraq, Zarka, Ajloun and Jerash) of northern Jordan. The area coordinates are, 35° 42/-38° 12/ E, 32°17/-32° 34/ N at 500-1200 m altitude above the sea level and the rainfall varied between 100 and 600 mm. The temperature in winter varies between -4 and 8°C and 29-41°C in summer. The main small ruminant farming activities were within 3 climatic zones: the warm desert, cool temperate rainy, and the cool steppe. Flock's practice grazing during spring to early summer and in fall and winter supplements of fodders and concentrates were given. Lambing season lasts from November until May.

Sample size determination and sampling:

The prevalence of *Mcc* infections in northern Jordan was not reported previously, it was assumed

to be 50%. The total number of sheep and goat flocks in northern Jordan is estimated to be 10,000 (Anon, 2001). According to Thrusfield (1995) the appropriate number of flocks to be examined is 62, because 95% level of confidence and 5% absolute precision were required. The main communal grazing areas were sampled. At least one flock/grazing area was included. Representative flock samples were selected according to estimated density in each area. The flocks were numbered in each grazing area, and then a number was drawn to be interviewed and sampled. On seven occasions farmers declined for different reasons; do not like blood to be drawn from their animals, the animal owner was not available to give the permission, absence of adult-male family members, and another number was drawn. A few flocks (39) from un-shared grazing area (either private or common grazing area with no access to other farmers) were included. Thus, 104 flocks (18 sheep, 27 goat and 59 mixed) having 12093 sheep and 4225 goats were randomly selected. The maximum number of sheep/flocks is 620 and the minimum is 20 (Quartiles are 25%= 40, 50 %= 90, 75%= 200). The maximum number of goat/flocks is 300 and the minimum is 8 (Quartiles are 25%= 15, 50%= 25, 75%= 57).

Due to limited fund, selection of individual sheep/goat for testing included the animal most likely to be infected in the flock (older or poorer condition). Thus, we sampled 5 to 10 female animals of > 2 years of age/flock/species. All single species flocks were sampled, 20 mixed flocks were sampled from both species and 39 mixed species flocks were sampled from either sheep (n = 24) or goat (n = 15) depending on the main species in the flock.

During the months of 2002-2003, each farm flock was visited once for blood and data collection. Five milliliters blood samples were obtained from the jugular vein of each ewe (n = 678) in a vacuum tube without anticoagulant. Serum samples were separated and stored in aliquots at -20° C until used.

Data collection:

A questionnaire was especially designed to collect information using closed questions. The collected information covered production and health management practices (see Table 1).

Table (1): Description of risk factors for flock-level small ruminant *Mycoplasma capricolum* subsp. *capricolum* seropositivity in northern Jordan

Variable	Coding	Seropositivity to *Mcc*	
		No (n=57)	Yes (n=47)
A. Climatic zone			
Warm steppe	0	22	24
Hot desert	1	17	11
Cold temperate	2	18	12
B. Small ruminant species			
Sheep	0	9	9
Goat	1	16	11
Mixed	2	32	27
C. Source of animals, buying			
No			
Yes	0	23	22
	1	34	25
D. Drinking water			
Rain	0	1	2
Spring[a]	1	24	20
Pipes	2	28	23
Mixed	3	4	2
E1. Grazing locality			
Same village	0	43	36
Local area	1	12	9
Others	2	2	2
E2. Communal grazing[a]			
No	0	29	10
Yes	1	28	37
E3. Grazing and concentrates			
No			
Yes	0	39	34
	1	18	13
F1. Introduce new SR into farm			
No			
Yes	0	37	27
	1	20	20
F2. Use outsider rams			
No	0	43	31
Yes	1	14	16
F3. Sources of breeding animals			
Same village	0	42	36
Local area	1	1	3
Other	2	14	8

[a] significant $p < 0.05$

Table 1 (Continued)

Variable	Coding	Seropositivity to *Mcc*	
		No (n =57)	Yes (n =47)
G1. Young separated from dam			
No	0	25	15
Yes	1	32	32
G2. Newborn barn with and without ewe			
No	0	33	20
Yes	1	24	27
H1. Weaning age, < 2 months			
No	0	45	44
Yes	1	12	3
H2. 2-6 months			
No	0	15	4
Yes	1	42	43
I1. Udder cleaning			
No	0	38	32
Yes	1	19	15
I2. Change in milk production			
No	0	47	28
Yes	1	10	19
I3. Milking manual			
No	0	2	3
Yes	1	55	44
I4. Cleaning milking utensils			
No	0	40	27
Yes	1	17	20
J1. Neonatal death			
No	0	32	18
Yes	1	25	29
J2. Neonatal death/year			
No death	0	23	16
<5	1	15	17
5-10	2	9	11
>10	3	10	3
K1. Treat against parasite			
No	0	19	9
Yes	1	38	38
a significant p < 0.05			

Table 1 (Continued)

Variable	Coding	Seropositivity to *Mcc*	
		No (n =57)	Yes (n =47)
K2. When signs appear			
No	0	31	25
Yes	1	26	22
K3. Respiratory signs			
Absent	0	20	19
Present	1	37	28
K4. Mastitis			
Absent	0	17	15
Present	1	40	32
K5. Abortion			
No	0	33	17
Yes	1	24	30
K6. Loss of weight			
No	0	46	35
Yes	1	11	12
L1. Veterinary supervision			
No	0	33	38
Yes	1	24	9
L2. Trust vet			
No	0	8	5
Yes	1	49	42
L3. Vaccines used			
No	0	29	18
Yes	1	28	29
[a] significant $p < 0.05$			

The questionnaire was filled by direct interview with the farmers and conducted by a veterinarian who spoke the same dialect of Arabic as the farmers, so there was no problem in communication.

ELISA

The iELISA was adapted from Nicholas et al. (1996) for the detection of antibodies against *M. agalactiae* in small ruminant sera.

M. capricolum subsp. *capricolum* antigen was diluted in carbonate-bicarbonate buffer pH 9.6 (Na_2CO_3; $NaHCO_3$) 1/400 and coated on Nunk Maxi Sorp microtiter plates with 100 µl in each well. The plates were incubated overnight at 4°C, and then washed 4 times with PBST pH 7.4 to remove the unabsorbed antigens. To the washed wells 100 µl/well of the serum (1/100 dilution) were added. The plates were incubated at 37°C for 30 minutes and then washed 4 times with PBST. Then 100 µl of donkey anti-sheep IgG conjugated to alkaline phosphatase (Sigma) diluted 1/ 12000 in PBS with Marvel and Tween 20 was added to each well and incubated at 37°C for 30 minutes; then the wells were washed 4 times with PBST. The colour was developed after incubation with 100 µl of 5-Bromo-4-chloro-3-Indolyl Phosphate/ Nitro Blue Tetrazolium (BCIP/ NBT) alkaline phosphatase substrate and the color development was stopped by the addition of 50 µl of 1 M citric acid. The absorbance was determined at 405 nm by BIO-TEK INSTRUMENTS, ELX 800 ELISA plate reader. This test sensitivity and specificity are 82% and 97% respectively. A positive and negative control samples were tested in this sheep and goat ELISA system. A flock was considered seropositive when one or more samples were positive. The cutt-off value was calculated as the mean OD_{405} value of the negative controls.

Statistical methods:

The true seroprevalence (Rogan and Gladen, 1978) and 95% confidence intervals were calculated for seroprevalences. Chi-square analysis was employed to test the significance between prevalences. P value of < 0.05 was considered significant. Odds ratio and its 95% confidence intervals were calculated. SPSS 11 program was used (Anon. 2001).

Data were analyzed according to the case-control design, where *M. capricolum* subsp. *capricolum* seropositive and seronegative flocks were compared in relation to exposure to potential risk factors (Thrusfield, 1995). Variables that were associated with *M. capricolum* subsp. *capricolum* sero-positive flocks at $p < 0.25$ were used in multivariable logistic regression (Hosmer and Lemeshow, 1989). It was checked whether these variables showed a correlation of more than 0.05 with each other.

In the multivariable model, variables were excluded from the model by forward procedure. The least-significant variables (based on Wald's statistic) were deleted, the model fitted, and the results then compared (both parameters estimate and difference in -2log likelihood of the model) with those of the previous run to check for confounding with a change in parameter estimates of more than 30%, the deleted variable was a confounder and included in the model again. This resulted in a model containing variables related to *M. capricolum* subsp. *capricolum* seropositivity ($p < 0.05$). Two-way interaction was tested for significance.

Results

Seroprevalences:

Seroprevalences of *M. capricolum* subsp. *capricolum* were calculated and analyzed as flock-level seroprevalence for small ruminants (both sheep and goats); sheep and goat (Table 2).

Table (2): Seroprevalence of *Mycoplasma capricolum* subsp. *capricolum* in small ruminant flocks by iELISA during January 2002 to December 2003, in northern Jordan

Species	Flock-level				Individual-level			
	No. examined	Prevalence	True seroprevalence	95% CI	No. examined	Prevalence	True seroprevalence	95% CI
Small ruminant	104	47%	56%	36,55	678	17%	18%	15,20
Sheep	62	34%	39%	29,53	376	17%	18%	14,22
Goat	62	25%	28%	43,67	302	17%	18%	13,22

The flock level seroprevalence of *M. capricolum* subsp. *capricolum* were 56%, 39%, 28% in small ruminants (sheep and goats) sheep and goats respectively. There was no significant difference (X^2 = 2, d.f.=1, p = 0.15) at the flock level seroprevalences of sheep and goats.

Multivariable analysis:

Out of twenty-nine variables six variables were associated at $P < 0.25$ with *M. capricolum* subsp. *capricolum* seropositivity. After forward selection (Table 1), only two variables remained in the final logistic regression model, namely, communal grazing and drinking water, spring (Table 3).

Table (3): Logestic regression analysis of factors associated with seropositivity to *Mycoplasma capricolum* subsp. *capricolum* in small ruminant's flocks in Jordan

Risk factors	β	SE (β)	*P*	*OR*	95% CI
Communal grazing	0.224	0.14	0.001	5.2	2, 13
Drinking water, spring	0.224	0.078	0.004	0.27	0.1, 0.66
Constant	0.136	0.476	0.11	NA	NA

Likelihood ratio of chi-square (LR $_{x2}$): -62 on 2 degrees of freedom.

Discussion

The results showed a high flock-level true seroprevalence of 56% among small ruminant flocks in northern Jordan which can be explained by the local herding practices and transhumance, the movement of animals from eastern Jordan to the west during spring and early summer seeking better pasture, cross-border movements with the neighboring Arab countries namely Syria, Iraq and Saudi Arabia, which can easily transmit the disease across boundries. There was no study of small ruminants seroprevalence of *M. capricolum* subsp. *capricolum* either on flock or individual levels.

The seroprevalence of *M. capricolum* subsp. *capricolum* in Jordan was never studied before. *Mcc* is widely distributed and highly pathogenic, particularly in North Africa (Benkirane et al., 1993), but the frequency of occurrence is low (Bergonier et al., 1997). Such a disease was observed in Australia, USA, Chile, Sweden, and Oman (Nicolet, 1994). In Jordan, small ruminants had lower true seroprevalences of both *M. agalactiae* (30%) (Al-Momani et al., 2008) and *M. mycoides* subsp. *capri* (36%) (Al-Momani et al., 2010) compared to the true seroprevalence results of *M. capricolum* subsp. *capricolum* in this study.

Goats are more commonly affected than sheep, and clinical signs of fever, septicaemia, mastitis, and severe arthritis may be followed rapidly by death (Bolske et al., 1988). In this study we found that both species are equally susceptible to *M. capricolum* subsp. *capricolum*. In Jordan many small ruminant flocks (93%) are mixed flocks (Abo-Shehada et al., 2002) and this may reflect as equal exposure to the infection of *M. capricolum* subsp. *capricolum* in these mixed flocks. Infected mixed flocks will facilitate equal exposure pressure on both sheep and goats and subsequent transmission of *M. capricolum* subsp. *capricolum*.

In this study, out of twenty-nine health management and production variables only two, namely, the use of communal grazing was found as risk factor for *M. capricolum* subsp. *capricolum* seropositivity and using spring water for animals drinking was found as a protective risk factor for *M. capricolum* subsp. *capricolum* seropositivity.

Using communal grazing by different flocks (OR= 5.2, 95% CI: 2, 13) increased the risk for *M. capricolum* subsp. *capricolum* seropositivity. Such practice will contaminate pasture with all pathogens carried by any of the grazing animals. Subsequently, the pathogen will be picked up by susceptible animals that get infected and increase the number of seropositive animals in all flocks frequent the communal pasture.

Using spring water for drinking (OR= 0.27, 95% CI: 0.11, 0.66) was protective risk factor leading to decrease seropositive animals in the flock. This indicates that the other sources of drinking water (rain, pipes, mixed) may be exposed to contaminants leading to increase the seropositivity of *M. capricolum* subsp. *capricolum* in these flocks.

The education of farmers regarding the use of communal grazing and water sources is expected to help reducing the *M. capricolum* subsp. *capricolum* infections in their flocks.

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References

1. Abo-Shehada, M.N., Arab, B., Mukbel, R., Williams, D. & Torgerson, P.R., (2002). Age and seasonal variations in the prevalence of Oestrus ovis larvae among sheep in northern Jordan. *Preventive Veterinary Medicine*, 47(3): 205-212, https://doi.org/10.1016/s0167-5877(00)00160-4.
2. Al-Momani, W., Halablab, M.A., Abo-Shehada, M.N., Miles, K., McAuliffe, L. Nicholas, & R.A.J. (2006). Isolation and characterization of small ruminant mycoplasmas in Jordan using denaturing gradient gel electrophoresis (DGGE) and specific PCR. *Small Ruminant Research*, 65:106-112

3. Al-Momani, W., Nicholas, R.A.J. & Abo-Shehada, M.N., (2008). Seroprevalence of and risk factors for Mycoplasma agalactiae among small ruminants in northern Jordan. *Preventive Veterinary Medicine*, 83(1): 1-10, https://doi.org/10.1016/j.prevetmed.2007.08.003.
4. Al-Momani, W., Nicholas, R.A.J. & Abo-Shehada, M.N., (2010). Sero-prevalence and risk factors of *Mycoplasma mycoides* subsp. *capri* LC among small ruminants in northern Jordan. *Tropical Animal Health and Production*, In Press
5. Anonymous. (1984). *National Atlas of Jordan. Climate and Agroclimatology*. Jordan National Geographic Center, pp. 135
6. Anonymous., (1995). *The Annual Report*. Ministry of Agriculture, Jordan
7. Anonymous., 2001. *The Annual Report*. Ministry of Agriculture, Jordan
8. Bashiruddin, J.B., Taylor, T.K. & Gould, A.R., (1994). A PCR based test for the specific identification of Mycoplasma mycoides subsp. mycoides SC. *Journal of Veterinary Diagnostic Investigation*, 6: 428-434
9. Benkirane, A., Amghar, S. & Kirchhoff, H., (1993). Analysis of membrane proteins of *Mycoplasma capricolum* strains by SDS-PAGE and immunoblotting. *Journal of Veterinary Medicine*, 40(1-10): 119–124, https://doi.org/10.1111/j.1439-0450.1993.tb00118.x.
10. Bergonier, D., Bertholet, X. & Poumarat, F., (1997). Contagious agalactia of small ruminants: current knowledge concerning epidemiology, diagnosis, and control. *Revue Scientifique et Technique- Office International des Epizooties*, 16(3): 848–873, https://doi.org/10.20506/rst.16.3.1062.
11. Bolske, G., Msami, H., Humleslo, N.E., Erno, H. & Jonsson, L., (1988). *Mycoplasma capricolum* in an outbreak of polyarthritis and pneumonia in goats. *Acta Veterinaria Scandinavia*, 29(3-4): 331–338, https://doi.org/10.1186/bf03548626.
12. Bolske, G., (1995). *Respiratory mycoplasmoses in goats: Especially regarding contagious caprine pleurpneumonia. Department of Veterinary Microbiology*, Swedish university of Agricultural Sciences. Ph.D thesis Uppsala, Sweden
13. Cottew, G.S. & Yeats, F.R., (1982). Mycoplasma and mites in the ears of clinically normal goats. *Australian Veterinary Journal*, 59(3): 77-81, https://doi.org/10.1111/j.1751-0813.1982.tb02731.x.
14. DaMassa, A.J., (1990). The ear canal as a culture site for demonstration of mycoplasmas in clinically normal goats. *Australian Veterinary Journal*, 67(7): 267-269, https://doi.org/10.1111/j.1751-0813.1990.tb07786.x.
15. DaMassa, A.J., Wakenell, P.S. & Brooks, D.L, (1992). Mycoplasmas of goats and sheep. Review Article. *Journal of Veterinary Diagnostic Investigation*, 4(1): 101-113, https://doi.org/10.1177/104063879200400126.
16. Hosmer, D. & Lemeshow, S. (1989). *Applied logistic regression*. Wiley, New York, pp 307.
17. Nicholas, R.A.J., Santini, F.G., Clark, K.M., palmer, N.M.A., De Santis, P. & Bashiruddin, J.B. (1996). A comparison of serological tests and gross lung pathology for detecting contagious bovine pleuropneumonia in two groups of Italian cattle. *Veterinary Record*, 139(4): 89-93, https://doi.org/10.1136/vr.139.4.89.
18. Nicholas, R.A.J. (1998). *Mycoplasmas of small ruminants and their relevance to Macedonia*. Proceeding of the meeting (6th International conference on sheep and Goat Production and 4th symposium on the reproduction of domestic animals), Ohrid, Macedonia.
19. Nicholas, R.A.J. (2002). Improvements in the diagnosis and control of diseases of small ruminants caused by mycoplasmas. *Small Ruminant Research*, 45(2): 145-149, https://doi.org/10.1016/s0921-4488(02)00095-0.
20. Nicolet J. (1994). Mycoplasma infections in cattle, sheep, and goats: Methods for diagnosis and prophylaxis. *Office International des Epizooties*
21. Perreau, P. & Breard, A. (1979). La mycoplasmose caprine a M. capricolum. *Comparative Immunology Microbiology and Infectious Diseases*, 2(1): 87-97, https://doi.org/10.1016/0147-9571(79)90062-6.
22. Poveda, J.B. & Nicholas, R.A.J. (1998). *Serological identification by growth inhibition and metabolic inhibition tests*. In: Miles R.J and Nicholas R.A.J. Eds. Mycoplasma Protocols. Totawa, USA: Humana Press, pp.105-113.
23. Rodriguez, J.L., Gutierrez, C., Brooks, D.L., Damassa, A.J., Oros, J. & Fernandez, A., (1998). A pathological and immunohistochemical study of goat kids undergoing septicemic disease caused by Mycoplasma capricolum subsp. capricolum, Mycoplasma capri and Mycoplasma mycoides subsp. mycoides LC. *Journal of Veterinary Medicine*, 45(1-10): 141-149, https://doi.org/10.1111/j.1439-0450.1998.tb00777.x.
24. Rogan, W.J. & Gladen, B. (1978). Estimating prevalence from the results of a screening test. *American Journal Epidemiology*, 107(1): 71-76, https://doi.org/10.1093/oxfordjournals.aje.a112510.
25. Ruffin, D.C., (2001). Mycoplasma infections in small ruminants. *Veterinary Clinical of North America for Food and Animal Practice Review*, 17(2): 315-332, https://doi.org/10.1016/s0749-0720(15)30031-1.
26. Thrusfield, M., (1995). *Veterinary Epidemiology*. Blackwell Science, London, p. 182.