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Prevalence and risk factors of contagious agalactia in small ruminants in Mali

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Abstract
Mycoplasma agalactia is the main agent of contagious agalactia syndrome in small ruminants characterized clinically by mastitis, arthritis and kerato-conjunctivitis. The objective of this study is to determine the serological and microbiological prevalence as well as the risk factors associated with infection by Mycoplasma agalactiae in small ruminants. A serological and microbiological survey was carried out on 3660 serum samples and 1860 organ and milk samples in 252 herds of small ruminants. An individual and herd serological prevalence rate was 4.51% (165/3660) and 32.94% (83/252). Out of 1860 organ and milk samples cultured, 58 were found to be positive for Mycoplasma agalactiae in 38 herds, representing a respective microbiological prevalence rate of 3.1% and 15.07%. The risk factors significantly associated (p<0.05) with infection by Mycoplasma agalactiae were the sheep species (OR=5.03), sex (OR=3.10), breed (OR=12, 17), age (OR=4.46), calving (OR=6.71), mastitis (OR=14.39), abortion (OR=10.07), overcrowding (OR =29.72), introduction of new animals (OR=20.75), season (OR=10.07), livestock markets (OR=33.2) and transhumance (OR=19.37). The results of this study showed that the pathogen responsible for contagious agalactia circulates among small ruminants in practically all regions of Mali. These identified local strains could help develop a candidate vaccine against contagious agalactia in small ruminants.

Keywords: Contagious agalactia, mycoplasma agalactiae, risk factors, odds ratio

1. Introduction
Contagious agalactia is an infectious disease characterized by mastitis and caused by Mycoplasma agalactiae Bergonier and al., Dalanezi and al., Jafarizadeh and al., Nicholas and al., R PA, ER, S GM and al., Zendulková and al. [1-6]; abortions in sheep Hosein and al. [7] and goat infertility Pourbakhsh and al. [8]. It affects sheep and goats with a morbidity rate of 50-90% in lactating females and a mortality rate of 10-40%. The clinical picture of contagious agalactia is breast localization (decreased milk production to total agalactia), joint localization (stiffness of the leg, severe lameness), ocular localization (conjunctivitis, tearing, photophobia) and pulmonary localization (bronchopneumonia) Lambert M. [9]. Some authors mention the auricular and vaginal localization of Mycoplasma agalactiae ER and al. [10]. The incubation period varies between 7 and 56 days and many cases of infection occur in wintering during parturition and lactation Khezri and al. [11]. In Mali, there are still no vaccines against contagious agalactia and no epidemiological study has been conducted on this infection but only a few serological studies with a serological prevalence rate of 8.7% obtained in 2003 Niang M. [12]. These results are only serological indices to be confirmed by the circulation of the causative agent of contagious agalactia in small ruminants, hence the objective of this study to isolate and identify Mycoplasma agalactiae in small ruminants in Mali.

2. Materials and Methods
2.1 Study area and sites
The study took place in the administrative regions of Kayes, Koulikoro, Sikasso, Ségou and Mopti and the District of Bamako (Figure 1). All the circles of the regions were chosen and the municipalities of Bamako to represent the different eco-climatic zones of Mali except the sub-desert zone.

The intervention sites were the small ruminant farms and the slaughterhouses and slaughtering areas of the circles and regional capitals which were geolocated (Figure 2). Small ruminant farms were selected for the serum and mastitis milk samples from sheep and goats. At the level of each circle, 2 herds of small ruminants were chosen (a herd of sheep, a herd of goats). The slaughterhouses and slaughtering areas of each circle and region were chosen for the sampling of udder samples.

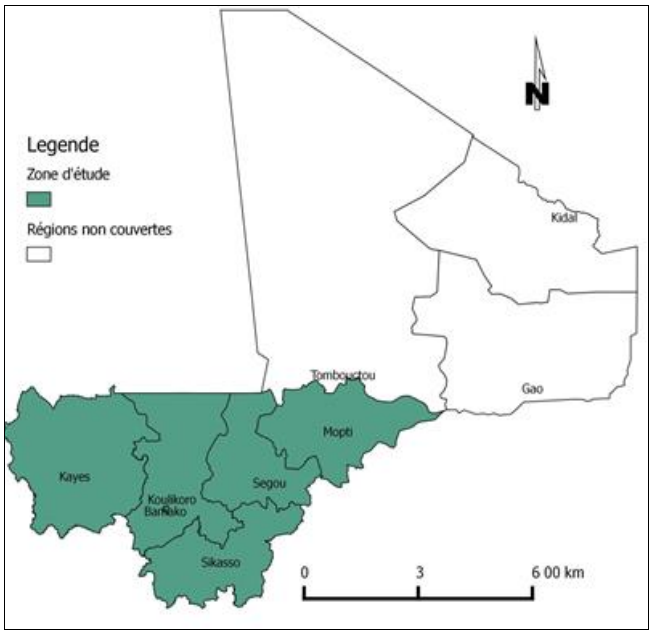


Fig 1: Study area (Mali)

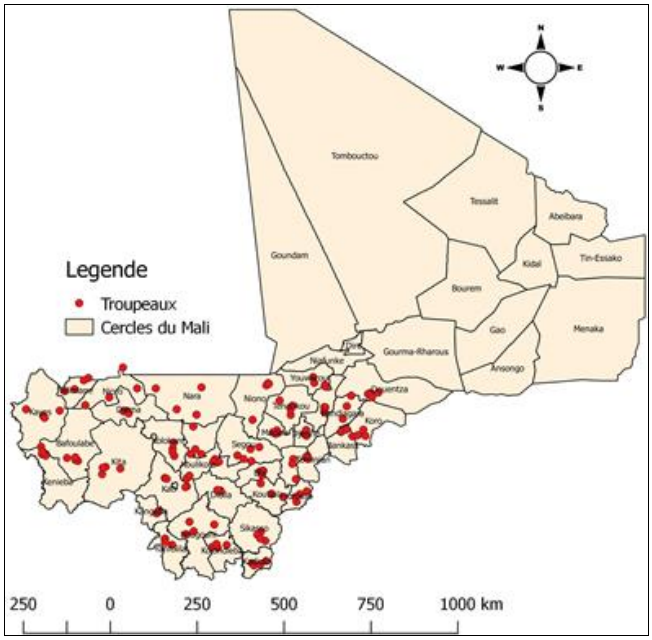


Fig 2: Sampling sites

Figure 1 represents the project study area in 5 administrative regions of Mali. Figure 2 represents the geolocation of the farms for which samples were collected.

2.2 Type of Survey

A cross-sectional survey by single pass was carried out for the geolocation of the sampling areas, the collection of epidemiological information from the farms and the sampling. Survey sheets have been drawn up and sent to each small ruminant farm to collect information relating to the zoo-sanitary situation of the animals (history of the herd in terms of pneumonia and mastitis), their spatio-temporal movements etc.

2.3 Sampling

A simple random sampling was used in the choice of small ruminants for the sampling of serum samples, on the other hand for the sampling of mastitis milk samples as well as samples of injured lungs, sampling by reasoned choice was used. A total of 3660 serum samples from small ruminants (sheep, goats), 1860 milk samples were collected in all circles of the regions of Kayes, Koulikoro, Sikasso, Ségou and Mopti and in the District of Bamako (Table 1). This sample size was calculated with an expected serological prevalence of 14% (size ±75 heads) per circle in the regions of Mali and an expected microbiological prevalence of 7% (size ±80 heads) per circle.

$$n = \frac{z^2\, p(1-p)}{d^2}$$

n = required sample size; z = margin coefficient corresponding to a confidence level of 95%; p = expected prevalence in the area; d = 5% margin of error

Table 1: Sampling by region and circle

Regions	Number of herds	Number Slaughter houses	Number of Serums	Samples milk
Kayes	42	7	700	350
Koulikoro	42	7	700	350
Sikasso	42	7	700	350
Segou	42	7	700	350
Mopti	48	8	800	400
Bamako	36	2	60	60
Total	252	38	3660	1860

2.4 Sample collection

At the level of the farms, blood (5 ml) was taken in dry tubes without anticoagulant then centrifuged to collect the serum which was aliquoted and then stored under cold conditions before the analysis (serological test). Mastitis milk samples (purulent, bloody, clear milk) were taken in 5 ml quantities in sterile bags then put under cold conditions before being analyzed by culture on special Mycoplasma agalactiae media. At the slaughterhouse level, 5g of udder samples were taken for microbiological analyzes for the search for Mycoplasma agalactiae.

2.5 Laboratory analyzes

2.5.1 Serology

Serological screening was done using the Contagious Agalactia competition ELISA technique for the detection of antibodies to Mycoplasma agalactiae with the IDEXX antibody test kit.

Validity criteria

Mean OD CP ≥ 0.350

Average OD CP / Average OD CN ≥ 3.50

$$\frac{E}{P}\% = 100 \text{ x } \frac{\text{DO échantillon} - \text{DO CN}}{\text{DO CP} - \text{DO CN}}$$

OD = optical density; CP = positive control; CN = negative control

Results interpretation
If E/P% ≤ 50% ◊ Negative,
If E/P% > 50% - < 60% ◊ Doubtful
If E/P% ≥ 60% ◊ Positive

2.5.2 Isolation and identification
Microbiological screening was done on samples of mastitis milk which were cultured on selective media for Mycoplasma agalactiae, the causative agent of contagious agalactia in sheep and goats. These media were composed of: PPLO (BD/Lot 6244621) 21g/700 ml, Peptone special (Bacto/Lot 122434JD) 45%, Yeast extract (Fluka/Lot 0001439171) 11%, Sodium chloride (Aldrich/Lot 7647-145) 11%, HEPES (Sigma/Lot 37F-56515) 20%, Sodium pyruvate (Sigma/Lot 115K07251) 11%, Ampicillin (Sigma/Lot BCBR6229V) 2%, Glycerol (Sigma/Lot BCBR9967V) 2%, Fresh yeast extract (LCV-ELF/Lot19005) 45%, Horse Serum (Gibco/Lot 1750660) 45%.

The following isolation protocol was used

- Dilutions made at 1/10th on eight (8) tubes (10-1-10-8) of milk in liquid medium (broth)
- A few drops of each sample spread on the solid medium
- Incubate the broths (with slow agitation) and the inoculated Petri dishes at 37°C in a humid atmosphere at 5% CO_2.
- Broths and petri dishes are examined daily for signs of bacterial growth (broth) and the characteristic morphology of "fried egg" Mycoplasma colonies.
- Purification cloning followed by isolate harvesting was carried out on liquid medium for identification

The identification of Mycoplasma agalactiae isolates was made by the growth inhibition test with discs impregnated with Mycoplasma agalactiae reference serum and by PCR with specific primers using the Bioingentech VetPCR M. agactaiae Detection Kit (Ref: VET-0006-96D).

2.6. Statistical analysis
2.6.1 Prevalence rate
The apparent prevalence (pa) is defined as the ratio between the cases positive to the tests (serology, microbiology, PCR) and the total number analyzed without considering the characteristics of the tests (sensitivity, specificity). This prevalence can be assessed both at individual level (individual prevalence) and at herd level.

$$\text{Prévalence \%} = 100 \text{ x } \frac{\text{Nbre échantillons positifs}}{\text{Nbre échantillons testés}}$$

2.6.2 Risk factor analysis
The risk factors associated with the different prevalence rates and infection rates (serological, bacteriological) are determined by a multiple regression analysis using Stata Corp 12 software. A level of significance of p=0.05 was retained to assess a probable relationship between the studied factors and the binary response variable. The risk factor estimation parameter is the odds ratio (OR).

3. Results Obtained
3.1 Contagious agalactia seroprevalence
Out of 3660 serum samples taken from 252 herds of small ruminants, 165 samples from 83 herds were positive for Mycoplasma agalactiae, i.e. an overall individual prevalence rate of 4.51% (CI: 3.88 - 5.23) (of which 25.00% in Bamako, 6.00% in Mopti) and herd by 32.94% (CI: 27.43-38.96) (including 40.00% in Kayes and 25% in Bamako) (Table 2).

Table 2: Serological prevalence rate of contagious agalactia by region

Regions	Individual level		Herd level	
	Prevalence individual (%)	Confidence interval 95%	Prevalence Troop (%)	Confidence interval 95%
Kayes	3.71 (26/700)	2.55 - 5.39	40.48 (17/42)	27.04 - 55.51
Koulikoro	3.29 (23/700)	2.20 - 4.88	30.95 (13/42)	19.07 - 46.03
Sikasso	4.14 (29/700)	2.90 - 5.89	35.71 (15/42)	22.99 - 50.83
Segou	3.43 (24/700)	2.31 - 5.05	30.95 (13/42)	19.07 - 46.03
Mopti	6.00 (48/800)	4.56 - 7.87	33.33 (16/48)	21.68 - 47.46
Bamako	25.00 (15/60)	15.78 - 37.23	25.00 (9/36)	13.75 - 41.07
Total	4.51 (165/3660)	3.88 - 5.23	32.94 (83/252	27.43 - 38.96

At the circle level, the highest individual prevalences recorded in the Kayes region were 8% in Kita, 5% in the circles of Kayes, Nioro and Yelimane. As for herd prevalence, the highest were recorded in the circles of Nioro and Yelimane (66.67%) and Kayes and Kita (50%). In the Koulikoro region, the circles of Dioïla, Nara and Kangaba recorded the highest prevalence of 6% and 5% respectively. At the herd level, 50% were infected in the circles of Kati, Kangaba and Kolokani. In the region of Ségou, the circles of Koutiala, Yanfolila and Yorosso recorded prevalences of 8, 7 and 6% against those of herds of 50%. In the region of Ségou, the prevalences of 9, 8 and 5% were obtained in Bla, Tominia and Baroueli for respectively 66.67 and 50% of the infected herds. In the Mopti region, 10% individual prevalence was obtained in the circles of Douentza and Mopti, 8% in Koro and Tenenkou and 7% in Youwarou. At herd level, 50% prevalence was recorded in the same circles except Youwarou (33.33%) (Figure 3 - 4).

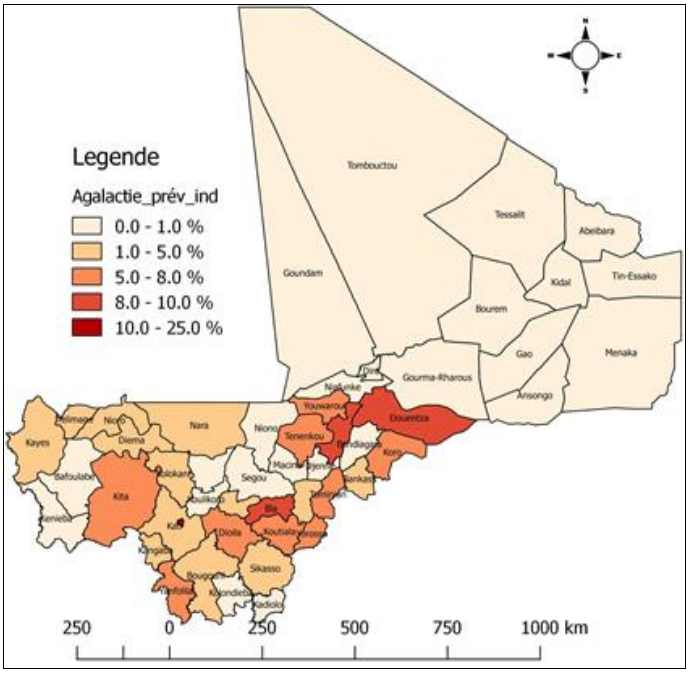


Fig 3: Individual prevalence of agalactia

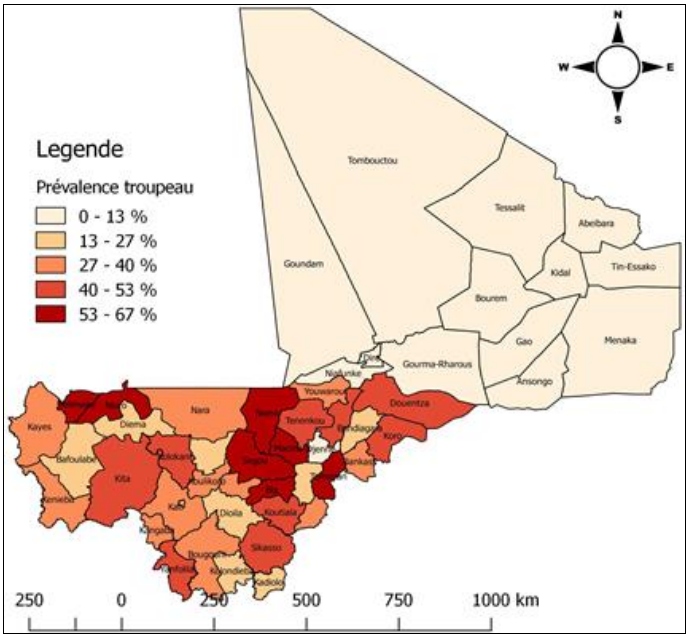


Fig 4: Herd prevalence of agalactia

Figure 3 represents the individual prevalence of contagious agalactia, and Figure 4 represents that of the herd

3.2 Isolation and identification of *Mycoplasma agalactiae* (Ma) strains

The culturing of 1860 samples of organs and milk with mastitis made it possible to isolate and identify 58 strains of Mycoplasma agalactiae (Ma) representing an individual prevalence rate of 3.11% and 15.07% of herds have had at least positive cases. Of the 58 identified strains of Mycoplasma agalactiae, 14 strains were from the Koulikoro region, 11 from Sikasso and Bamako, 10 strains from Mopti and 6 strains from the Kayes and Ségou regions (Table 3).

Table 3: Microbiological prevalence rate of Mycoplasma agalactiae by region

Regions	Individual level		Herd level	
	Prevalence individual (%)	Confidence interval 95%	Prevalence herd (%)	Confidence interval 95%
Kayes	1.71(6/350)	0.79-3.69	14.29(6/42)	6.72-27.84
Koulikoro	4.00(14/350)	2.40-6.60	14.29(6/42)	6.72-27.84
Sikasso	3.14(11/350)	1.76-5.54	11.90(5/42)	5.19-25.00
Segou	1.71(6/350)	0.79-3.69	11.90(5/42)	5.19-25.00
Mopti	2.50(10/350)	1.56-5.18	12.50(6/48)	5.86-24.70
Bamako	18.33(11/60)	10.56-29.92	27.78(10/36)	15.85-43.99
Total	3.11 (58/1860)	2.42-4.01	15.07 (38/252)	11.19-20.02

In the Kayes region, 2 strains were isolated in the circle of Diéma and one strain in each circle except Kita where no case was confirmed. In Koulikoro, 4 strains were isolated in the Kati circle, 3 in Kolokani and 2 in the other circles (Banamba, Nara, Kangaba) except Koulikoro with no confirmed cases. In the Sikasso region, out of 11 strains isolated, 3 were isolated in the circles of Sikasso, Yanfolila and Kadiolo and 2 strains in Koutiala. In the region of Ségou, 2 strains were isolated in Bla and San and one strain in Ségou and Tominia. No strain was isolated in the circles of Macina, Niono and Baroueli. In the Mopti region, out of 10 strains isolated, 2 strains were isolated in the circles of Bankass, Douentza, Tenenkou, Youwarou and one strain in Mopti and Bandiagara (Table 4).

Table 4: Microbiological prevalence rate by sampling site

Regions	Circles	Individual level			Herd level		
		Number of milk	Milk +	Prev (%)	Number of herd	Herds +	Prev (%)
Kayes	Kayes	50	1	2.00	6	1	16.67
	Kita	50	0	0.00	6	0	0.00
	Nioro	50	1	2.00	6	1	16.67
	Yelimane	50	1	2.00	6	1	16.67
	Bafoulabe	50	0	0.00	6	0	0.00
	Diema	50	2	4.00	6	2	33.33
	Kenieba	50	1	2.00	6	1	16.67
	S/Total 1	350	6	1.71	42	6	14.29
Koulikoro	Bangamba	50	2	4.00	6	1	16.67
	Katy	50	4	8.00	6	1	16.67
	nara	50	2	4.00	6	1	16.67
	Dioila	50	1	2.00	6	1	16.67
	Koulikoro	50	0	0.00	6	0	0.00
	Kangaba	50	2	4.00	6	1	16.67
	Kolokani	50	3	6.00	6	1	16.67
	S/Total 2	350	14	4.00	42	6	14.29
Sikasso	Bougouni	50	0	0.00	6	0	0.00
	Kolondieba	50	0	0.00	6	0	0.00
	Sikasso	50	3	6.00	6	1	16.67
	Yanfolila	50	3	6.00	6	1	16.67
	Kadiolo	50	3	6.00	6	1	16.67
	Yorosso	50	0	0.00	6	0	0.00
	Koutiala	50	2	4.00	6	2	33.33
	S/Total 3	350	11	3.14	42	5	11.90
Segou	Blah	50	2	4.00	6	1	16.67
	macina	50	0	0.00	6	0	0.00
	Niono	50	0	0.00	6	0	0.00
	Segou	50	1	2.00	6	1	16.67
	tominia	50	1	2.00	6	1	16.67
	Baroueli	50	0	0.00	6	0	0.00
	San	50	2	4.00	6	2	33.33
	S/Total 4	350	6	1.71	42	5	11.90
Mopti	Koro	50	0	0.00	6	0	0.00
	Bankass	50	2	4.00	6	1	16.67
	Bandiagara	50	1	2.00	6	1	16.67
	Djenne	50	0	0.00	6	0	0.00
	Douentza	50	2	4.00	6	1	16.67
	Tenenkou	50	2	4.00	6	1	16.67
	Youwarou	50	2	4.00	6	1	16.67
	Mopti	50	1	2.00	6	1	16.67
	S/Total 5	400	10	2.50	48	6	12.50
Bamako	S/total 6	60	11	18.33	36	10	27.78
Total		1860	58	31.40	252	38	92.66

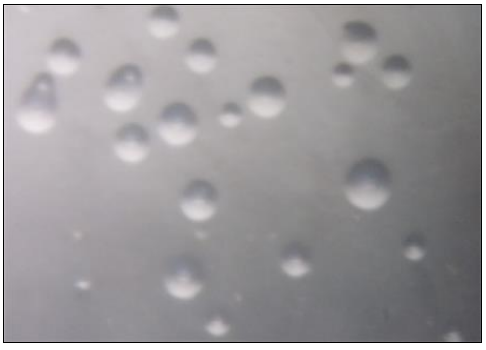
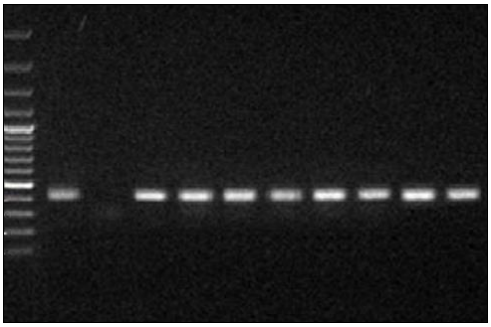


Fig 5: Colony of *Mycoplasma agalactiae*



Picture 1: Agarose gel electrophoresis of products on Mycoplasma agalactiae clones by conventional PCR amplification

3.3 Risk factors and indicators associated with the seroprevalence of contagious agalactia

The risk factors and indicators significantly associated with the seroprevalence of contagious agalactia were animal and environmental. The risk factors and indicators significantly linked to the animal were the sheep species (OR=5.03), the females (OR=3.10), the mixed breed (OR=12.17), the rank of calving (OR=6.71), cases of mastitis (OR=14.39), cases of abortions (OR=10.07); on the other hand those related to the environment were the rainy season (OR=10.07), the eco-climatic zone (OR=3), transhumance (19.37) and livestock markets (OR=33.2) (Table 5).

Table 5: Risk factors and indicators associated with the seroprevalence of contagious agalactia

Variables	Terms	Odds ratio (OR)	*p-value*	Confidence Interval (CI)
Species	Sheep vs Goats	5.03	*0.01*	1.442809 - 17.53607
Sex	Females vs Males	3.10	*0.02*	1.201011 - 8.00536
Breeds	Mixed vs Local	12.17	*0.01*	1.865606 - 79.49836
Age	Adults vs Youth	4.46	*0.02*	1.190901 - 16.74557
Calving	3rd vs. 2nd	6.71	*0.00*	1.699738 - 26.54932
Mastitis	Yes vs No	14.39	*0.00*	4.168355 - 49.71589
Diarrhea	Yes vs No	10.07	*0.00*	2.597476 - 39.09351
Abortion	Yes vs No	10.07	*0.01*	2.597476 - 39.09351
Arthritis	Yes vs No	6.29	*0.02*	1.311419 - 30.24644
Overcrowding	Yes vs No	29.72	*0.00*	10.04008 - 88.01832
Introduction of animals	Yes vs No	20.75	*0.00*	3.540337 - 121.6162
Seasons	Wintering vs Dry season	10.07	*0.01*	2.597476 - 39.09351
Eco-climatic zones	SNS vs. SSS vs. Sahel	3	*0.03*	1.090342 - 8.254292
Transhumance	Yes vs No	19.37	*0.00*	6.02416 - 62.33798
Cattle markets	Presence vs. Absence	33.2	*0.00*	6.82469 - 161.5077

4. Discussion

The objective of this study is to determine the serological and microbiological prevalence of contagious agalactia in small ruminants to establish banks of strains and positive controls as well as the risk factors and the spatialization of this disease. The serological prevalence obtained shows that Mali is not free from this disease because the overall prevalence at the herd level was 32.94%, of which 25 to 40.48% were obtained from Kayes to Mopti and the District of Bamako. The infection of these regions by Mycoplasma agalactiae seems to be linked on the one hand to the farming system and their eco-climatic situation. The individual seroprevalence of 4.51% obtained is slightly lower than that of a 2003 study where a prevalence rate of 8.3% was obtained in Mali Niang M. [12]. The species most infected by this disease was sheep because at the level of each herd cases were recorded, unlike goats where the cases seem to be concentrated in a few herds. Sheep seem more exposed to infection than goats. Serological prevalence studies have been carried out in Spain Verbisck-Bucker and al.[13], in Jordan MADANAT and al.[14] with 11.2% and 5% respectively, in Brazil Em D and al.[15] with an individual seroprevalence of 7.8% and herd of 25.9%). Compared to the risk factors significantly associated with seroprevalence, many authors have reported that the quasi-systematic attack of the udder is very often the first and sometimes the only one observed in lactation, then the ocular and articular symptoms then the polyarthritis (carpus, tarsus) as well as conjunctivitis or keratitis and less frequently, diarrhea and especially abortions. Pneumonia is more common in young people (and goats) Bergonier and al.[16]. For some authors, the risk factors for contagious agalactia were the season (summer), calving rank, sex (females), age (young), production cycle (calving), density, polyarthritis G VB and al.[17]. Females were the main risk factors for contagious agalactia were sex (female) at 10.1% prevalence and animal category (female in production) for 11.1% Em and al.[15]. Numerous studies of isolation and identification of Mycoplasma agalactiae by culture and PCR have been carried out on samples of milk, swabs and aborts, particularly in Iran where, out of 102 samples of milk and swabs, 19 (18.62%) tested positive by isolation and PCR Bayatzadeh and al.[18]; 25 milk samples (47.2%) positive by PCR at Ma Hajizadeh and al.[19]; out of 78 runts 24 (30.8%) were infected with Mycoplasma agalactiae Hosein Abadi and al.[20]. In Kurdistan, a bacteriological prevalence of 32.6% due to Mycoplasma agalactiae was obtained Khezri and al.[11]; in India with a bacteriological prevalence rate of 16% Khezri and al.[21] and in Spain where a bacteriological prevalence rate of 32.9%was obtained in 2008 and 2009 and 42.7% in 2010 Ariza-Miguel and al.[22]. In Iran, the molecular and microbiological prevalence rates of contagious agalactia were 19% and 8.51% Pooladgar and al.[23],14.8% and 36.0% in culture and PCR on milk, swab samples Shamsaddini Bafti and al.[24]. Some authors have mentioned the persistence of Mycoplasma agalactiae in bulk milk after two years Tardy and al.[25] and others were able to identify by PCR Ma in goat 29 (74.3%) were positive in culture and 38 (97.4%) positive and PCR Pourbakhsh and al.[8]. Of 40 identified samples of Mycoplasma 11 samples (27.5%) were of the genus Mycoplasma agalactiae ER and al.[10]**.** Most African countries are probably affected by contagious agalactia because outbreaks have been described in North Africa, Mauritania, Senegal, Guinea, Guinea-Bissau, Togo, Ivory Coast, Ghana, Nigeria, Cameroon, Niger, Chad, Sudan, Ethiopia, Kenya, Mozambique and Zimbabwe Bergonier and al. [16]. Of 35 serum samples 11.35% were due to Mycoplasma agalactiae Loureiro and al.[26]**.** The control strategies currently implemented by several European countries are the control of animal movements, current diagnostic methods, antibiotic therapy, vaccination and disinfection Loria and al.[27].

5. Conclusion

The results of the study confirmed the presence of contagious agalactia in small domestic ruminants in Mali because in addition to serological indices, Mycoplasma agalactiae (pathogen responsible for this disease) was isolated and then identified. There are serological indices in Mali which, in the absence of vaccination against contagious agalactia caused by Mycoplasma agalactiae, can be considered as post-infectious antibodies. The control of this disease is envisaged by sensitivity to the usual antibiotics and vaccination with the development of candidate vaccine based on local strains.

6. Conflict of Interest
Not available

7. Financial Support
Not available

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