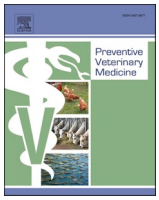
Preventive Veterinary Medicine 234 (2025) 106381

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed



Causes of abortion in Iranian goat herds and associated risk factors

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ARTICLE INFO

Keywords:
Abortion
Goat
Iran
Risk factors
Small ruminants

ABSTRACT

Abortion imposes a substantial economic burden on the global small ruminant industry, not only reducing herd productivity but also contributing to the spread of zoonotic diseases. This study examines the primary factors associated with abortion, both infectious and non-infectious, in 623 goat herds across Iran. A comprehensive evaluation was performed, incorporating herd history, laboratory results, and statistical analyses using univariate tests and multivariable binary logistic regression. Key findings revealed significant associations with abortion, including previous abortion history, gestational age of the aborted foetus, routine veterinary visits, mineral supplementation, and vaccination practices. Non-infectious factors, such as pregnancy toxemia, goiter, and deficiencies in vitamin E/selenium, were identified in herds with a low abortion prevalence (<10 %). Among the 623 herds studied, 277 (44.5 %) exhibited an abortion prevalence below 2 %, considered within normal limits, while the remaining 346 herds (55.5 %) experienced pathological abortion rates exceeding 2 %. The definitive cause of abortion was determined in 227 of the 346 abortion outbreaks analysed, accounting for 65.6 % of the cases. Infectious agents were identified in 40.7 % of the herds with abortion rates exceeding 2 %, with *Brucella melitensis* (9.5 %), *Chlamydia abortus* (7.8 %), and *Coxiella burnetii* (5.2 %) being the most prevalent pathogens. Multivariable binary logistic regression analysis revealed significant associations between abortion and several factors, including birth (OR=2.01, 95 % CI: 1.05–3.89, P=0.036), previous abortion history (OR=14.5, 95 % CI: 6.01–37.3, P<0.001), gestational age of the aborted foetus (OR=3.07, 95 % CI: 1.63–5.89, P<0.001), routine veterinary visits (OR=0.16, 95 % CI: 0.09–0.27, P<0.001), vaccination (OR=0.25, 95 % CI: 0.11–0.53, P<0.001), and mineral supplementation (OR=0.36, 95 % CI: 0.21–0.62, P<0.001). These findings underscore the diverse causes of abortion in Iranian goat herds, emphasizing the need to improve farmer awareness and access to commercial vaccines targeting infectious abortion agents to enhance herd productivity.

1. Introduction

Goat domestication began approximately 10,500 years ago in the Zagros Mountains of present-day Iran, leading to the development of over 20 million goats across 15 indigenous breeds in the country. Due to Iran's vast arid and desert terrain, these native breeds have been selectively bred to thrive in harsh desert conditions. In Iran, meat and milk are the primary products derived from goats, with annual production reaching 90,000 tons of meat and 287,000 tons of milk (Esmaeili et al., 2022). Today, goats are no longer associated with poverty and underdevelopment, and more than 60 % of the world's goat population is found in Asia. Recent studies highlight goats as a reference model for more advanced sectors of livestock farming (Boyazoglu et al., 2005). Goat breeding should now be viewed from a new perspective. The outdated stereotype of goats as "poor man's cattle" must be discarded, and goats should be recognized as a key driver of agricultural development in the 21st century (Escareño et al., 2012).

Goats play a crucial economic role in the livelihoods of Iranian livestock farmers and are essential to the development of rural and nomadic communities. While goat husbandry is often a secondary

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https://doi.org/10.1016/j.prevetmed.2024.106381
Received 9 June 2024; Received in revised form 17 October 2024; Accepted 5 November 2024
Available online 8 November 2024

agricultural activity in rural areas, it serves as the primary livelihood for nomads in the southern regions of Iran. As a result, goat abortions have significant impacts on animal and human health, food production, and the agricultural economy (Esmaeili et al., 2022).

Reproductive failures, including abortion, reduce the number of weaned offspring, increase the culling of aborted does, and limit the availability of replacement goats, thereby impeding herd productivity and causing significant economic losses for farmers. The causes of goat abortion can be broadly classified as either infectious or non-infectious. Although non-infectious agents account for a lower prevalence of abortion, they present challenges in investigation due to their complex nature (Pugh and Baird, 2012). Major non-infectious causes of goat abortion include pregnancy toxemia, vitamin E/selenium deficiency, trauma, stress-induced corpus luteum loss, and iodine deficiency, particularly in certain breeds like the Beetal (Raoofi et al., 2017; Pugh and Baird, 2012).

Infectious causes of goat abortion, which include bacterial, protozoal, and viral agents, are widespread in small ruminants globally. Common infectious agents associated with goat abortion include *Brucella melitensis* (*B. melitensis*), *Chlamydia abortus* (*C. abortus*), *Coxiella burnetii* (C. burnetii), *Salmonella* spp., Border disease virus (BDV), and *Toxoplasma gondii* (*T. gondii*) (Pugh and Baird, 2012). The prevalence of infectious agents causing abortion varies across regions. For instance, research in South America by Dorsch et al. (2021) identified *Campylobacter fetus* subsp. *fetus*, *Listeria ivanovii*, C. abortus, *T. gondii*, *Neospora caninum*, and *Sarcocystis* spp. as leading causes of abortion in sheep and goats. In India, Sharma et al. (2008) reported that most abortion cases in sheep and goats were attributed to *B. melitensis*, *Listeria monocytogenes*, and *C. abortus*. Similarly, studies in Europe have highlighted the prevalence of various abortifacient pathogens, consistently noting *C. burnetii*, *C. abortus*, and *T. gondii* as major contributors to abortions in sheep and goats (Magouras et al., 2017; Jiménez-Martín et al., 2020). Notably, investigations conducted in Iran by Esmaeili et al. (2022) identified *C. burnetii*, *C. abortus*, and *B. melitensis* as the predominant infectious agents responsible for abortion in sheep flocks, with *C. abortus* reported as the cause of 23.5 % of abortions in sheep and goats.

Given that published information on goat health and production is more limited compared to that of sheep and cattle, this study, conducted in one of the world's most goat-populated countries, provides valuable insights into goat diseases and serves as a useful guide for improving productivity in this species. Enhancing reproductive performance in goat herds is essential for increasing the production of kids, meat, and milk. Therefore, identifying the causes of abortion and the associated risk factors is critical. This study aims to identify the prevalent causes of goat abortion and their related risk factors within Iranian goat herds.

2. Materials and methods

2.1. Design and case selection

Between April 2015 and March 2019, a total of 623 clinical cases of caprine abortion in Iranian goat herds were investigated in a cross-sectional study. The research included various local and imported goat breeds, such as Mahabadi, Adani, Bakhtiyari, Torki-Ghashghaei, Saanen, Alpine, Murcia-Granada, Markhoz, Najdi, Raeini, Tali, and Nadoshan.

During the kidding season, active surveillance was conducted to monitor abortion occurrences, with each herd included in the study visited and examined by a veterinarian. Abortion rates were calculated by dividing the number of aborted does by the total number of pregnant does in each herd, with sampling performed once per herd. After collecting herd history from farmers and performing clinical examinations, aborted fetuses were transported to the laboratory on ice for necropsy and sampling.

General information regarding each herd and animal was documented in Table 2, with each parameter classified as either zero (absent) or one (present). Herds with an abortion prevalence of less than 2 % were designated as control herds, as this rate is considered normal in goat populations. A herd in this study was defined as a population of goats with at least 50 individuals.

A case was defined as the submission of one or more fetuses or tissues from a single herd to the diagnostic laboratory. Cases included intact fetuses or tissues from field necropsies, provided that fresh tissues such as brain, heart, lungs, liver, kidneys, and abomasal contents were available for evaluation. Abortion investigations involved the assessment of clinical history and necropsy findings. Since embryonic death is often not visible to farmers due to the small size of the embryo and its inaccessibility, this study focused on cases of abortion and stillbirth, with no sampling conducted for embryonic death. The gestational age of necropsied fetuses was estimated using crown-rump length measurements, the presence or absence of a developed hair coat, and incisor tooth eruption (Noden and Lahunta, 1985).

2.2. Study area

The geographic area of the study encompassed Iran's territory, covering 1648,000 square kilometers, located between longitudes 44° to 63° E and latitudes 25° to 39° N. Iran experiences highly diverse weather patterns, ranging from desert areas in the central regions to mountainous areas and coastal strips in the north and south. These varied geographical conditions have contributed to the development of numerous goat breeds and breeding systems, resulting in many distinct epidemiological units. Factors such as goat breed, geographical conditions, annual rainfall, shared land borders with eastern and western neighboring countries, and the potential for common grazing with herds from neighboring countries, along with farmers' practices and breeding systems, have led to the emergence of different epidemiological units in this study.

2.3. Sampling

The target population consisted of goat herds within different epidemiological units. The sample size was determined based on data from the Iran Veterinary Organization's database, which clustered the goat population into epidemiological units with designated locations in the geographic information system (GIS). Goats within each epidemiological unit were kept under the same conditions. Thus, in this study, an epidemiological unit was defined as herds maintained under identical conditions. Herds with abortion rates both above and below 2 % within the same epidemiological unit were selected for the study.

The final sample size was calculated using an expected abortion prevalence of 10 % at the herd level, a 95 % confidence level, a desired absolute precision (d) of 0.07, and the following formula: $n = (z1\text{-}\alpha/2)^2 (p.q)/d^2$. A total of 62 clusters were identified, and 10 goat herds were sampled from each cluster according to GIS data, with each sample representing a herd. During herd visits, any freshly aborted fetuses were necropsied, with samples pooled and mixed, and ultimately, one representative sample from each herd was tested.

Before blood collection, the hair around the jugular region of the goats was trimmed. Blood sampling was performed aseptically by obtaining 2 ml of blood via jugular venipuncture. The collected blood was evenly divided between a sterile collection tube for serum, intended for Beta-hydroxybutyrate (BHB) analysis, and a second tube containing ethylenediaminetetraacetic acid (EDTA) anticoagulant for PCR analysis of the Bluetongue virus (BTV). The samples were stored at −20°C until further processing.

Blood samples were collected exclusively from does displaying clinical signs consistent with bluetongue infection (e.g., bloody nasal and oral discharge, swelling and edema of the lips and gums, and a swollen, cyanotic tongue) as well as pregnancy toxemia (characterized by hind limb edema or emaciated does that had aborted twins or more). Additionally, aseptic samples from the brain, heart, liver, stomach

Table 1
Infectious and non-infectious causes of abortion in goat herds in Iran are distributed according to the percentage of abortion rate.

Characteristic	<2 %, N = 277 (44 %)[a]	2–4.9 %, N = 116 (19 %)[a]	5–9.9 %, N = 80 (13 %)[a]	10–19.9 %, N = 70 (11 %)[a]	20–29.9 %, N = 37 (5.9 %)[a]	30–50 %, N = 33 (5.3 %)[a]	>50 %, N = 10 (1.6 %)[a]	> 2 %, N = 346 (55.8 %)[a]	Overall N = 623	p-value[b]
Infectious agents										<0.001
Negative	265 (95.7 %)	100 (86.2 %)	39 (48.8 %)	37 (53 %)	23 (62 %)	5 (15.1 %)	1 (10 %)	205 (59.3 %)	470 (75.5 %)	
Positive	12 (4.3 %)	16 (13.8 %)	41 (51.2 %)	33 (47 %)	14 (38 %)	28 (84.9 %)	9 (90 %)	141 (40.7 %)	153 (24.5 %)	
Diagnosis reached										<0.001
No	257 (92.8 %)	25 (21.6 %)	28 (35 %)	37 (52.9 %)	23 (62 %)	5 (15.1 %)	1 (10 %)	119 (34.4 %)	376 (60.3 %)	
Yes	20 (7.2 %)	91 (78.4 %)	52 (65 %)	33 (47.1 %)	14 (38 %)	28 (84.9 %)	9 (90 %)	227 (65.6 %)	247 (39.7 %)	
Brucella melitensis										<0.001
Negative	277 (100 %)	116 (100 %)	78 (97.5 %)	64 (91.4 %)	31 (83.8 %)	19 (57.6 %)	5 (50 %)	313 (90.5 %)	590 (94.7 %)	
Positive	0 (0 %)	0 (0 %)	2 (2.5 %)	6 (8.6 %)	6 (16.2 %)	14 (42.4 %)	5 (50 %)	33 (9.5 %)	33 (5.3 %)	
Chlamydia abortus										<0.001
Negative	277 (100 %)	116 (100 %)	79 (98.8 %)	65 (92.9 %)	33 (89.2 %)	20 (60.6 %)	6 (60 %)	319 (92.2 %)	596 (95.7 %)	
Positive	0 (0 %)	0 (0 %)	1 (1.2 %)	5 (7.1 %)	4 (10.8 %)	13 (39.4 %)	4 (40 %)	27 (7.8 %)	27 (4.3 %)	
Coxiella burnetti										<0.001
Negative	277 (100 %)	116 (100 %)	74 (92.5 %)	61 (87.1 %)	35 (94.6 %)	32 (97 %)	10 (100 %)	328 (94.8 %)	605 (97.1 %)	
Positive	0 (0 %)	0 (0 %)	6 (7.5 %)	9 (12.9 %)	2 (5.4 %)	1 (3 %)	0 (0 %)	18 (5.2 %)	18 (2.9 %)	
***Campylobacter* spp.**										<0.001
Negative	277 (100 %)	116 (100 %)	77 (96.2 %)	66 (94.3 %)	35 (94.6 %)	33 (100 %)	10 (100 %)	337 (97.4 %)	614 (98.6 %)	
Positive	0 (0 %)	0 (0 %)	3 (3.8 %)	4 (5.7 %)	2 (5.4 %)	0 (0 %)	0 (0 %)	9 (2.6 %)	9 (1.4 %)	
***Salmonella* spp.**										0.002
Negative	277 (100 %)	116 (100 %)	76 (95 %)	67 (95.7 %)	37 (100 %)	33 (100 %)	10 (100 %)	339 (98 %)	616 (98.9 %)	
Positive	0 (0 %)	0 (0 %)	4 (5 %)	3 (4.3 %)	0 (0 %)	0 (0 %)	0 (0 %)	7 (2 %)	7 (1.1 %)	
***Listeria* spp.**										0.015
Negative	277 (100 %)	114 (98.3 %)	76 (95 %)	70 (100 %)	37 (100 %)	33 (100 %)	10 (100 %)	340 (98.3 %)	617 (99 %)	
Positive	0 (0 %)	2 (1.7 %)	4 (5 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	6 (1.7 %)	6 (1 %)	
***Mycoplasma* spp.**										0.005
Negative	277 (100 %)	116 (100 %)	76 (95 %)	68 (97.2 %)	37 (100 %)	33 (100 %)	10 (100 %)	340 (98.3 %)	617 (99 %)	
Positive	0 (0 %)	0 (0 %)	4 (5 %)	2 (2.8 %)	0 (0 %)	0 (0 %)	0 (0 %)	6 (1.7 %)	6 (1 %)	
Other bacteria										0.051
Negative	277 (100 %)	114 (98.3 %)	77 (96.3 %)	69 (98.6 %)	37 (100 %)	33 (100 %)	10 (100 %)	340 (98.3 %)	617 (99 %)	
Positive	0 (0 %)	2 (1.7 %)	3 (3.7 %)	1 (1.4 %)	0 (0 %)	0 (0 %)	0 (0 %)	6 (1.7 %)	6 (1 %)	
Escherichia coli										0.2
Negative	275 (99.3 %)	114 (98.3 %)	76 (95 %)	70 (100 %)	37 (100 %)	33 (100 %)	10 (100 %)	340 (98.3 %)	615 (98.7 %)	
Positive	2 (0.7 %)	2 (1.7 %)	4 (5 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	6 (1.7 %)	8 (1.3 %)	
***Trueperella* spp.**										0.11
Negative	277 (100 %)	113 (97.4 %)	80 (100 %)	70 (100 %)	37(100 %)	33(100 %)	10 (100 %)	343 (99.1 %)	620 (99.5 %)	
Positive	0 (0 %)	3 (2.6 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	3 (0.9 %)	3 (0.5 %)	
Coxiella & Chlamydia										0.054
Negative	277 (100 %)	116 (100 %)	80 (100 %)	68 (97.2 %)	37 (100 %)	33 (100 %)	10 (100 %)	344 (99.4 %)	621 (99.7 %)	
Positive	0 (0 %)	0 (0 %)	0 (0 %)	2 (2.8 %)	0 (0 %)	0 (0 %)	0 (0 %)	2 (0.6 %)	2 (0.3 %)	
Chlamydia & Brucella										0.2
Negative	277 (100 %)	116 (100 %)	80 (100 %)	69 (98.6 %)	37 (100 %)	33 (100 %)	10 (100 %)	345 (99.7 %)	622 (99.9 %)	
Positive	0 (0 %)	0 (0 %)	0 (0 %)	1 (1.4 %)	0 (0 %)	0 (0 %)	0 (0 %)	1 (0.3 %)	1 (0.1 %)	
Fungi spp.										0.6
Negative	275 (99.3 %)	114 (98.3 %)	78 (97.5 %)	70 (100 %)	37 (100 %)	33 (100 %)	10 (100 %)	342 (98.9 %)	617 (99 %)	
Positive	2 (0.7 %)	2 (1.7 %)	2 (2.5 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	4 (1.1 %)	6 (1 %)	

(*continued on next page*)

Table 1 (*continued*)

Characteristic	<2 %, N = 277 (44 %)[a]	2–4.9 %, N = 116 (19 %)[a]	5–9.9 %, N = 80 (13 %)[a]	10–19.9 %, N = 70 (11 %)[a]	20–29.9 %, N = 37 (5.9 %)[a]	30–50 %, N = 33 (5.3 %)[a]	>50 %, N = 10 (1.6 %)[a]	> 2 %, N = 346 (55.8 %)[a]	Overall N = 623	p-value[b]
Border Disease										0.001
Negative	277 (100 %)	116 (100 %)	75 (94 %)	70 (100 %)	37 (100 %)	33 (100 %)	10 (100 %)	341 (98.5 %)	618 (99 %)	
Positive	0 (0 %)	0 (0 %)	5 (6.2 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	5 (1.5 %)	5 (0.8 %)	
Toxoplasma gondii										0.6
Negative	269 (97.1 %)	111 (95.7 %)	77 (96.4 %)	70 (100 %)	37 (100 %)	33 (100 %)	10 (100 %)	338 (97.7 %)	607 (97.4 %)	
Positive	8 (2.9 %)	5 (4.3 %)	3 (3.8 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	8 (2.3 %)	16 (2.6 %)	
Vit E/selenium deficiency										<0.001
Negative	276 (99.6 %)	70 (60 %)	80 (100 %)	70 (100 %)	37 (100 %)	33 (100 %)	10 (100 %)	300 (86.7 %)	576 (92.5 %)	
Positive	1 (0.4 %)	46 (40 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	46 (13.3 %)	47 (7.5 %)	
Pregnancy toxaemia										<0.001
Negative	273 (98.6 %)	101 (87 %)	69 (86.3 %)	70 (100 %)	37 (100 %)	33 (100 %)	10 (100 %)	320 (92.5 %)	593 (95.2 %)	
Positive	4 (1.4 %)	15 (13 %)	11 (13.7 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	26 (7.5 %)	30 (4.8 %)	
Goitre										<0.001
Negative	276 (99.6 %)	107 (92.2 %)	80 (100 %)	70 (100 %)	37 (100 %)	33 (100 %)	10 (100 %)	337 (97.4 %)	613 (98.4 %)	
Positive	1 (0.4 %)	9 (7.8 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	9 (2.6 %)	10 (1.6 %)	
Congenital defects										0.3
Negative	276 (99.6 %)	113 (97.4 %)	80 (100 %)	70 (100 %)	37 (100 %)	33 (100 %)	10 (100 %)	343 (99.1 %)	619 (99.4 %)	
Positive	1 (0.4 %)	3 (2.6 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	3 (0.9 %)	4 (0.6 %)	
Trauma										0.5
Negative	276 (99.6 %)	114 (98.3 %)	80 (100 %)	70 (100 %)	37 (100 %)	33 (100 %)	10 (100 %)	344 (99.4 %)	620 (99.5 %)	
Positive	1 (0.4 %)	2 (1.7 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	0 (0 %)	2 (0.6 %)	3 (0.5 %)	

[a] Statistics presented: n (%)
[b] Statistical tests performed: chi-square test of independence; Fisher's exact test

Table 2
Analysis of the risk factors associated with abortion in goat herds of Iran.

		Resulted from univariate chi-square analysis		Resulted from multivariable logistic regression analysis	
Variables	**Categories**	**OR (95 % CI)**	**p-value**	**OR (95 % CI)**	**p-value**
Birth	Multiple	1 (Ref.)	<0.001	1 (Ref.)	0.036
	One	0.44 (0.32–0.61)		2.01 (1.05–3.89)	
Herd Type	Rural	1 (Ref.)	0.008	1 (Ref.)	0.12
	Nomadic	1.55 (1.12–2.15)		1.53 (0.89–2.63)	
Herd size	<100	1 (Ref.)	<0.001	-	0.4
	>100	5.07 (3.00–8.92)		1.36 (0.65–2.92)	
Abortion history	No	1 (Ref.)	<0.001	1 (Ref.)	<0.001
	Yes	2.58 (1.62–4.23)		14.5 (6.01–37.3)	
Gestational age of aborted fetus	<3month	1 (Ref.)	<0.001	1 (Ref.)	<0.001
	>3month	2.08 (1.46–2.97)		3.07 (1.63–5.89)	
History of Rev−1 vaccination	No	1 (Ref.)	<0.001	1 (Ref.)	0.7
	Yes	0.33 (0.23–0.46)		0.87 (0.47–1.61)	
Contact with other herds	No	1 (Ref.)	0.2	-	-
	Yes	0.49 (0.13–1.49)		-	
Routine visits of veterinarian	No	1 (Ref.)	<0.001	1 (Ref.)	<0.001
	Yes	0.14 (0.09–0.21)		0.16 (0.09–0.27)	
Vaccination	No	1 (Ref.)	<0.001	1 (Ref.)	<0.001
	Yes	0.12 (0.07–0.21)		0.25 (0.11–0.53)	
Mineral supplementation	No	1 (Ref.)	<0.001	1 (Ref.)	<0.001
	Yes	0.24 (0.16–0.34)		0.36 (0.21–0.62)	
History of Stillbirth	No	1 (Ref.)	0.5	-	-
	Yes	0.68 (0.22–2.07)		-	

contents, and lungs were collected from aborted fetuses for culture and molecular detection of infectious agents.

2.4. Bacteriological culture

Fresh samples of brain, heart, lung, liver, and abomasal fluid were inoculated onto MacConkey and blood agar (Merck, Germany) for

bacterial isolation, following standard methodologies. Two sets of plates, one for aerobic and the other for anaerobic cultures, were incubated at 37°C for 48 hours. Bacterial growth was inspected after 16–20 hours of incubation, and in cases of no or slow growth, the plates were incubated for an additional 24 hours. Colony selection for subculturing was based on colony morphology and the absence of post-mortem contamination. Colonies were then subcultured and identified using morphology, Gram staining, and routine biochemical tests.

Bacteria other than *Trueperella pyogenes* (*T. pyogenes*), *Brucella melitensis*, *Campylobacter* spp., *Mycoplasma* spp., *Salmonella* spp., *Chlamydia abortus*, *Escherichia coli* (*E. coli*), *Listeria monocytogenes* (*L. monocytogenes*), or *Coxiella burnetii* were classified as 'other bacteria'. Mixed cultures containing fewer than three distinct bacterial colony types without confirmed pathogenic bacteria were not considered positive for an etiological agent.

2.5. Mycologic examination

To diagnose mycotic infections, 1–3 milliliters of abomasal contents were collected using a sterile syringe. Additionally, lung tissue, eyelid, and skin samples (if dermatitis was evident) were collected, depending on the availability of fetal tissues. In cases where gross lesions were observed on aborted fetuses, a small amount of skin material was scraped using a sterile scalpel. This material, along with macerated fetal lung tissue and 0.2 milliliters of abomasal content, was spread separately onto Sabouraud dextrose agar (SDA) plates containing 1000 units per milliliter of penicillin G. The plates were incubated at both room temperature and 37°C. Daily examinations were performed during the first week, followed by twice-weekly inspections for the next two weeks.

Samples showing detectable mycotic growth were transferred to potato dextrose agar (PDA) plates and slants, in addition to SDA plates. PDA was used as the growth medium for slide cultures, which were incubated for 3–7 days before staining with lactophenol cotton blue. Filamentous isolates were identified based on gross and microscopic characteristics. To minimize bias from environmental contamination, fungal diagnoses were based on the observation of disseminated hyphal elements or yeast, with or without positive cultures from non-placental sites.

2.6. Molecular analysis

Nucleic acid (DNA and/or RNA) extraction was performed on 25 mg of tissue samples using the High Pure PCR Template Preparation Kit (Roche, Germany), following the manufacturer's instructions. The diagnosis of each abortion agent was conducted using PCR assays according to the methodology described by Esmaeili et al. (2022) for the following pathogens: *Mycoplasma* spp., *T. pyogenes*, Border disease virus, Bluetongue virus, *Campylobacter* spp., *T. gondii*, *B. melitensis*, *L. monocytogenes*, *C. burnetii*, *Salmonella* spp., and *C. abortus*.

2.7. Pregnancy toxaemia

Pregnancy toxemia in pregnant goats was diagnosed based on Beta-Hydroxybutyrate (BHB) levels and the presence of subcutaneous edema in the lower limbs. BHB concentration was measured in serum samples using the Williamson-Mellanby enzymatic method, employing a commercial kit provided by Biorex Fars Company, Shiraz, Iran. Goats with a BHB concentration exceeding 3 mmol/L, along with subcutaneous edema in the lower limbs, were identified as suffering from pregnancy toxemia, in accordance with the criteria established by Pugh and Baird (2012).

2.8. Final diagnosis

The results of gross pathological examinations during necropsy, along with findings from microbiological, molecular, and serologic testing, were comprehensively integrated and interpreted on a case-by-case basis. Criteria for trauma included observations such as head hemorrhage, rib fractures, intrathoracic hemorrhage, and/or ruptured liver in full-term kids, with no infectious agents detected in any examined tissues.

Vitamin E/selenium deficiency was diagnosed using the method described by Liu et al. (1996), while fatal congenital defects were identified through fetal examination. In cases where the thyroid gland showed macroscopic enlargement, it was isolated and weighed. A mean ratio of thyroid weight (g) to body weight (kg) equal to or greater than 0.40 g/kg was considered diagnostic for goiter, following the criteria outlined by Knowles and Grace (2007).

2.9. Statistical analysis

Categorical data were presented as frequencies and percentages. To assess the association between various factors and abortion in goat herds, univariate analyses were performed using the chi-square test or Fisher's exact test, as appropriate. Variables with a p-value below 0.2 in the univariate analysis were considered for inclusion in a multivariable binary logistic regression model. This regression analysis was used to identify independent risk factors for abortion in goat herds, with results reported as odds ratios (ORs) and 95 % confidence intervals (CIs). A p-value of less than 0.05 was considered statistically significant. Goat herds with an abortion prevalence of less than 2 % were considered the control group and compared to herds with an abortion prevalence greater than 2 %. All statistical analyses were conducted using R software (version 4.3.1).

3. Results

Among the 623 herds studied, 277 (44.5 %) exhibited an abortion prevalence below 2 %, which was considered within normal limits, while the remaining 346 herds (55.5 %) had a pathological abortion prevalence exceeding 2 %. The definitive cause of abortion was determined in 227 of the 346 abortion outbreaks analyzed, accounting for 65.6 % of the cases.

As shown in Table 1, infectious agents were identified as the cause of abortion in 153 herds (24.5 %, 153/623). Among herds with an abortion prevalence below 2 %, infectious agents were isolated in 12 herds (4.3 %, 12/277), whereas in herds with abortion rates exceeding 2 %, this figure rose to 141 (40.7 %, 141/346). The predominant infectious agent identified was *B. melitensis*, detected in 9.5 % (33/346) of the surveyed outbreaks, followed by *C. abortus* (7.8 %, 27/346), *C. burnetii* (5.2 %, 18/346), *Campylobacter* spp. (2.6 %, 9/346), *T. gondii* (2.3 %, 8/346), *Salmonella* spp. (2 %, 7/346), *L. monocytogenes* (1.7 %, 6/346), *Mycoplasma* spp. (1.7 %, 6/346), Border Disease virus (1.5 %, 5/346), and fungi (1.1 %, 4/346), including *Aspergillus fumigatus* and *Candida albicans*.

Bacterial agents were implicated in abortions in 124 herds (35.8 %), viral agents in 5 herds (1.5 %), and fungal agents in 4 herds (1.1 %). Non-infectious causes, such as vitamin E/selenium deficiency (13.3 %), pregnancy toxemia (7.5 %), and goiter (2.6 %), were primarily identified in herds with an abortion prevalence ranging from 2 % to 5 %.

Univariate analyses revealed significant associations between abortion and factors such as parity, herd type, herd size, abortion history, gestational age of the aborted fetus, history of Rev-1 vaccination, routine veterinary visits, adherence to vaccination protocols according to the Iran Veterinary Organization (IVO), and mineral supplementation, all with p-values <0.05 (Table 2). Multivariable binary logistic regression analysis indicated that does with a history of abortion were at significantly higher risk of experiencing abortion compared to those without such a history (OR: 14.5; 95 % CI: 6.01–37.3; $p<0.001$) (Table 2).

4. Discussion

Abortion significantly impacts the profitability and health of goat farms. This study represents the first comprehensive epidemiological assessment of abortion-causing agents in Iranian goat herds, highlighting bacterial pathogens such as *B. melitensis*, *C. abortus,* and *C. burnetii* as the primary abortifacients.

The final diagnosis of the cause of abortion was more successful in outbreaks compared to sporadic cases and low-prevalence situations. In this study, a definitive diagnosis was reached in 85 % of herds with an abortion prevalence exceeding 30 %. Consistent with our findings, a previous study in Turkey (Sakmanoğlu et al., 2021) reported a final diagnosis rate of 62 % for abortion causes. In contrast, other studies reported lower success rates, approximately 30 %, in diagnosing abortion causes in sheep and goats (Dorsch et al., 2022). Factors such as hormonal, metabolic, genetic, and developmental disorders, which are often challenging to confirm using conventional diagnostic methods, may contribute to the failure to reach a final diagnosis of abortion. However, infectious agents may account for a significant proportion of undiagnosed abortions (Anderson, 2007).

In various studies, including ours, bacterial pathogens were identified as the leading infectious cause of abortion in goat herds. Research from Turkey in 2021 also found that bacteria were the primary cause of abortion outbreaks in sheep and goats (Sakmanoğlu et al., 2021). Our study revealed that *B. melitensis*, *C. abortus*, and *C. burnetii* were the most prevalent causes of abortion, particularly in outbreaks where abortion prevalence exceeded 10 %. Although caprine brucellosis has been eradicated in some developed countries, it remains endemic in many developing regions. Several studies in Iran have emphasized the role of *B. melitensis* in small ruminant abortion. For instance, Esmaeili et al. (2022) reported *B. melitensis* as the third leading infectious cause of abortion in sheep flocks, while Behroozikhah et al. (2012) identified *B. melitensis* biovar 1 as the predominant biovar among Iranian sheep and goat populations. Similarly, Tekle et al. (2019) isolated B. melitensis from 12.5 % of aborted goats in Ethiopia.

C. abortus is widely recognized as a major cause of small ruminant abortion globally. Reports from Argentina (Di Paolo et al., 2019) and Iran (Esmaeili et al., 2021; Esmaeili et al, 2015) have also documented *Chlamydia* isolation in most cases of caprine abortion. Goats are also reservoirs for *C. burnetii*, which can cause abortion and reproductive disorders. Outbreaks of caprine abortion due to *C. burnetii* have been reported in Egypt (Selim et al., 2018) and Switzerland (Magouras et al., 2017).

In the present study, *Mycoplasma* spp. and *Listeria* spp. were detected in 1.7 % of aborted foetuses, consistent with findings from Esmaeili et al. (2022) in Iranian sheep flocks. Pourbakhsh et al. (2017) successfully identified *Mycoplasma* spp. in 15 % of buck semen samples via PCR in 2017, which can contribute to infertility and abortion.

Numerous studies worldwide have identified *Campylobacter* spp. as a cause of abortion in small ruminants. New Zealand researchers found that *Campylobacter* spp., *T. gondii*, and *Salmonella brandenburg* were major causes of abortion in their herds (West, 2002). A 2022 study of Iranian sheep flocks reported that *Campylobacter* spp. was responsible for 3.7 % of abortions (Esmaeili et al., 2022).

Previous studies have documented various bacterial agents involved in goat abortion globally. Esmaeili et al. (2023) reported that *Escherichia coli* was isolated from 5 % of goat abortions. Another survey in southern Iran found that *Brucella* spp., *Salmonella* spp., *Campylobacter* spp., and *E. coli* were isolated in 20.5 %, 19.6 %, 7.5 %, and 26.1 % of aborted foetuses, respectively, using conventional culture methods (Firouzi, 2006). Research shows that endotoxins released from gram-negative bacteria like *E. coli* and *Salmonella* can induce abortion by triggering prostaglandin release (Schlafer et al., 1994).

Improper hay and silage storage in extensive and rural farming systems can pose risks of abortion due to mycotoxins and fungi when pregnant goats are fed moldy hay or poor-quality silage (Pugh and Baird, 2012). The viral factors contributing to abortion in sheep and goats have not been thoroughly studied worldwide, leading to varying results across regions. A study in Iran reported a 0.8 % detection rate for Border disease virus (BDV) in aborted foetuses. Kaleibar et al. (2014) reported that 11.3 % of suspected ewes with reproductive failure tested positive for the BDV genome.

Toxoplasma infection was identified in 2.3 % of aborted fetuses in this study. The overall seroprevalence of this infection among Iranian goats is estimated to be 27 %, with no significant difference between bucks and does (Sharif et al., 2015). Castaño et al. (2016) demonstrated that parasite proliferation in placental and fetal tissues can lead to abortion at any stage of gestation, depending on the concentration and virulence of the protozoan strain. Prasad Sah et al. (2019) detected *T. gondii* DNA in 5.2 % of aborted goat foetuses using PCR.

The varying outcomes of studies worldwide may stem from factors such as different breeding systems, breed sensitivities, geographic and climatic conditions, vaccination strategies, vaccine quality, eradication campaigns, antibiotic use before abortion, sampling techniques, laboratory methods, and farmer training (Anderson, 2007; Esmaeili et al., 2023).

Regarding non-infectious causes of abortion, 7.8 % of fetuses in herds with abortion prevalence of 2–5 % were diagnosed with goiter. Goats, due to their selective feeding habits, are more prone to iodine deficiency compared to other ruminants. Congenital goiter, a non-inflammatory enlargement of the fetal thyroid gland, is more common in goats. Certain breeds, like Boer, Beetal, and Angora, are particularly susceptible (Pugh and Baird, 2012). Several studies have documented iodine deficiency in Iranian goats (Raoofi et al., 2017). In India, 18 % of stillbirths in kids were linked to congenital goiter (Singh et al., 2003). In South Africa, Angora goats grazing on thiocyanate-rich alfalfa pastures experienced an outbreak of goiter-related abortions (Bath et al., 1979). In parts of Iran, Brassicaceae plants, which can induce goiter, are often grazed during winter, coinciding with pregnancy, leading to goiter-related abortions and stillbirths.

Pregnancy toxemia, while not typically causing high abortion prevalence, is more common in Iranian goats than sheep due to higher prolificacy, fewer fat reserves, and post-parturition feeding practices in extensive systems. About 60 % of fetal growth occurs in the last six weeks of pregnancy, making inadequate energy intake during this period a risk factor for pregnancy toxemia, especially in goats carrying multiple fetuses (Pugh and Baird, 2012). Affected goats often exhibit subcutaneous edema in the lower limbs, with Alpine goats being particularly susceptible. Esmaeili et al. (2023) reported higher sensitivity to pregnancy toxemia in Alpine goats than in Saanen goats. In contrast, Iranian sheep exhibit lower incidence due to fat tails and smaller litters. Simpson et al. (2019) found that 12 % of Boer goats with multiple fetuses aborted despite treatment for pregnancy toxemia, while Lima et al. (2012) reported that pregnancy toxemia accounted for 18.5 % of adult goat deaths.

Vitamin E/selenium supplementation has been investigated for its impact on flock production and fertility, with selenium deficiency being a known cause of abortion in goats. In Iran, vitamin E/selenium deficiency was linked to sporadic abortions (Esmaeili et al., 2022). A Swiss study found that 2 % of goat abortions were due to vitamin E/selenium deficiency (Chanton-Greutmann et al., 2002). Most regions of Iran are selenium-deficient, leading many farms to administer two injections of vitamin E/selenium to pregnant goats as a preventive measure.

In this survey, a significant number of herds with an abortion prevalence of less than 20 % were unable to determine the cause of abortion. Non-infectious factors, such as unbalanced nutrition, genetic abnormalities, environmental influences, hormonal imbalances, and exposure to toxic substances, are highly diverse and often lead to sporadic abortions, making them challenging to detect in many cases. Furthermore, the lack of reference values for mineral concentrations in fetal tissues complicates the confirmation of mineral imbalances. Additionally, toxic agents responsible for abortion may not be present in the analyzed

tissues, further hindering accurate diagnosis (Anderson, 2007). Enhanced diagnostic methods for non-infectious agents would increase the likelihood of obtaining definitive results in these cases. Various conditions in goats can also impact the foetus and placenta, potentially leading to abortion. These conditions include haemoconcentration, circulatory failure, anaemia, fever, endotoxemia, and respiratory disease. Abortions resulting from these factors typically do not yield isolated infectious agents from the foetus, nor do necropsies reveal significant diagnostic lesions.

Multivariate binary logistic regression analysis revealed a correlation between the gestational age of aborted foetuses and the occurrence of abortion. The majority of aborted foetuses were found to be over three months old, a period during which fetal demands on the does are particularly significant. This finding can be attributed to two primary reasons: First, primary abortion agents typically induce abortion during late pregnancy. Second, early embryonic death (EED) and late embryonic death (LED) often lack specific signs, and the aborted materials may be insignificant, making it difficult for farmers to detect them. Consequently, goats may remain in the breeding season, leading to their return to estrus and subsequent pregnancy.

Furthermore, statistical analysis in this survey revealed a significant relationship between abortion and the absence of mineral supplementation in goat feed. This finding underscores the importance of mineral supplementation in improving health conditions and stimulating the immune system (Suttle, 2010). A recent survey of Iranian sheep flocks also reported a similar relationship between mineral supplementation and abortion in ewes (Esmaeili et al., 2022).

Finally, this survey revealed that farmers who adhered to the vaccination schedule recommended by the International Veterinary Organization (IVO) and maintained regular visits from veterinarians experienced lower prevalence of abortion. It is well established that herds employing these practices generally exhibit better health and breeding conditions. Vaccines targeting diseases such as brucellosis and contagious agalactia have been shown to effectively reduce abortions. Additionally, routine veterinary visits can contribute to the prevention of various diseases, including abortion. Al-Majali (2005) also identified a lack of vaccination in goat herds as a risk factor for abortion. Analysis using multivariable logistic regression indicated that a history of previous abortions is a significant risk factor for abortion in Iranian goat herds. Similar findings have been reported in studies of sheep and goat herds conducted by other researchers (Esmaeili et al., 2022).

In conclusion, abortion control requires a comprehensive approach addressing both infectious and non-infectious factors. Laboratory analyses, combined with clinical and epidemiological data, are essential for timely abortion diagnosis and effective preventive measures.

Funding

This research was supported by University of Tehran Grant Number: 390612.

CRediT authorship contribution statement

Mohammadreza Ghorani: Writing – original draft, Investigation, Conceptualization. **Hossein Esmaeili:** Writing – review & editing, Writing – original draft, Software, Project administration, Methodology, Funding acquisition, Conceptualization. **Seyed Mehdi Joghataei:** Methodology, Investigation, Formal analysis. **Zeinab Hamidiya:** Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sergio Villanueva-Saz:** Writing – review & editing, Writing – original draft. **Delia Lacasta:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to thank (Janine E. Davies) for constructive criticism of the English revision.

Author's contributions

Conceived and designed the experiments (H.E., M.G., and Z.H.); performed the sample collection (Z.H., and M.J.); did the laboratory examination (M.J., and Z.H.); wrote the manuscript (H.E., D.L., M.G., and S.V.S.); did the statistical analysis (H.E., and Z.H.); did the project management (H.E.); reviewed the manuscript (H.E., S.V.S., and D.L.). All authors have read and agreed on the manuscript.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki.

Data availability

The data that support the findings of this study are available from the corresponding author upon request.

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