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Infectious and Non-infectious Causes of Abortion in Saanen and Alpine Goats

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ABSTRACT

Background: Infectious and non-infectious abortions are necessary to be evaluated in Saanen and Alpine breeds as they have been recently imported to Iran whith out enough information about their susceptibility to diseases in the country situation. The present study aimed to investigate the causes of abortion in Saanen and Alpine to compare their susceptibility to each identified abortion factor.

Methods: A total of 600 Saanen and 600 Alpine breeds were studied. Abomasal contents of foeti and serum samples from aborted does were analyzed by PCR and conventional culture methods and beta-hydroxy butyrate was measured in the serum of aborted animals.

Results: Among 1200 pregnant goats, 59 Saanens and 80 Alpines aborted their fetus. Escherichia coli was isolated from 4 Saanens and 3 Alpines. Trueperella pyogenes was isolated from 5 Saanens and 7 Alpines. Pregnancy toxemia was the cause of abortion in 12 Saanens and 30 Alpines which showed a significant difference between the two breeds. Six Saanens and 10 Alpines aborted their fetus following trauma.

Conclusion: As abortion is an economically important issue, especially for the breeds which we have less information about, the susceptibility of animals and the frequency of each abortifacient should be evaluated.

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Introduction

Saanen is one of the most milk-producing breeds worldwide. In the best condition, the average milk yield of Saanen, can be 10 times that of other goats (1). Alpine is another milk-producing breed but with less average milk yielding compared to Saanen (2). Because of this benefit, pure Saanen and Alpine have been recently imported to Iran and their raising has just been started in some special farms and industrial way in our country. Nevertheless, there isn't enough knowledge about the susceptibility of the new breeds to diseases especially endemic ones and their adaptability to Iran's geographical condition.

Reproductive performance of Saanen and Alpine is necessary to achieve a high productivity. Abortion is a reproductive failure which has also negative impacts on milk quantity and quality. However, causes of abortion in Saanen and Alpine and the possibility of difference in the breed susceptibility haven't been evaluated so far. Infectious and non-infectious abortions are financially important wastages which may have some differences in terms of the causes and the rate in the source of the imported animals. Various bacteria, viruses, parasites and fungi can lead the outcome of a pregnancy to abortion (3). The commonly diagnosed agents are *Chlamydia abortus*, *Campylobacter* sp., *Listeria* sp., *Coxiella burnetii*, *Brucella melitensis*, bluetongue virus, border disease virus, *Toxoplasma gondii* and Aspergillus sp. which their occurrence have been reported worldwide (3, 4, 5, 6). Other agents such as *Escherichia coli*, *Bacillus* sp. and *Trueperella pyogenes* have been less isolated as the main causes of abortion (3).

Toxic plants, genetic factors, metabolic diseases, nutritional deficiencies, and physiological factors are common non-infectious etiologies of abortion (3). Infectious abortions are more plausible than non-infectious ones and most of which pose serious zoonotic risks (4, 5, 6) so they need to be rapidly controlled.

Various factors such as defects in animal's health care, feeding and watering management in a pregnancy period can prepare the opportunity for the abortifacients to take place in a farm. Moreover, agro ecological factors have significant impacts on the occurrence of abortion which may leads non-native breeds to be more sensitive (7). There are studies in which some of the diseases were found affected by the type of breeds (8, 9, 10). For instance, Kaupke et al. in 2017 found a breed-related occurrence of *Cryptosporidium* infection in a population of goats in Poland (11). As the same results may be obtained in terms of abortion, and identifying the involved abortifacients is the first step for implying prevention programs, the present study assessed causes of abortion in Saanen and Alpine first and then compared the susceptibility of each of them to the factors.

Materials and Methods

Case selection

In a pregnancy period abortion in 600 Saanen and 600 Alpine breeds were processed. The cases were submitted to the laboratory from an industrial farm in Tehran province which had high sanitary condition and the animals had received the main vaccines against endemic diseases including Rev 1, enterotoxemia, PPR, goatpox and foot and mouth disease.

Sample collection

All foeti were autopsied and placentas were examined macroscopically for gross lesions. The aborted foeti with blood in abdominal cavity or skull and rib fractures that none of the infectious agents were isolated from, were considered as trauma (10).

The abdominal cavity of each fetus was aseptically dissected and abomasal contents were aspirated into sterile syringes. Then the samples

Table 1. Primer sequences for molecular detection of the studied bacteria.

Bacteria	Sequence	Weight of band	Reference
Coxiella burnetii	TATGTATCCACCGTAGCCAGTC CCCAACAACACCTCCTTATTC	687 bp	(12)
Mycoplasma sp.	GGGAGCAAACACGATAGATACCCT TGCACCATCTGTCACTCTG-TTAACCTC	270 bp	(13)
Campylobacter sp.	GGATGACACTTTTCGGAGC CATTGTAGCACGTGTGTC	876 bp	(14)
Brucella sp	TGGCTCGGTTGCCAATATCAA CGCGCTTGCCTTTCAGGTCTG	223 bp	(15)
Listeria monocytogenes	CATTAGTGGAAAGATGGAATG GTATCCTCCAGAGTGATCGA	730 bp	(16)
Salmonella sp.	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284 bp	(17)

Table 2. The cycling parameters for each bacterium.

Bacteria		Initial denaturation	Denaturation	Annealing	Extension	Cycle number	Final extension
Coxiella Burnetii	Step 1	94 °C, 30s	94 °C, 30s	66-61°C, 1 min	72 °C, 1 min	5	-
	Step 2	-	94 °C, 30s	61 °C, 30s	72 °C, 1 min	30	72 °C, 10 min
Mycoplasma sp.		95°C, 5 min	94 °C, 1 min	50°C, 1 min	72 °C, 1 min	34	72 °C, 5 min
Campylobacter sp.		94°C, 1 min	94°C, 1 min	60°C, 1 min	72 °C, 1 min	25	72 °C, 5 min
Brucella sp.		93°C, 5 min	90°C, 1 min	60°C, 1 min	72 °C, 1 min	40	72 °C, 10 min
Listeria monocytogenes		80 °C, 10 min	94 °C, 3 min	64 °C, 30s	72 °C, 30s	30	72°C, 2 min
Salmonella sp.		95°C, 1 min	95°C, 30s	64°C, 30s	72 °C, 30s	35	72°C, 7 min

Table 3. Biochemical tests were conducted for confirmation of *E. coli*.

Catalase	oxidase	Nitrate reduction	gelatinase	IMViC	TSI	Urea	glucose	mannitol	Motility
+	-	+	-	++--	A/A H_2S- Gas +	-	+	+	+

Table 4. Biochemical tests were conducted for confirmation of *Trueperella pyogenes*.

Haemolysis	litmus milk	serum loeffler	Nitrate reduction	urease	gelatinase
β hemolysis	APCPRK	+	-	-	+

Table 5. Non-infectious causes of abortion.

The cause of abortion	Saanen	Alpine
Pregnancy toxemia	12 (2%)	30 (5%)*
Trauma	6 (1%)	10 (1.6%)

were packaged with ice packs and transmitted to the laboratory. Blood samples were taken from jugular vein of the aborted does and poured into the tubes which hadn't been treated with anticoagulant for the collection of sera (10).

Bacteriological assessment

Abomasal contents from 59 Saanens and 80 Alpines were cultured on blood and MacConkey agars using strile loop. The cultured plates were incubated for 48 hours at 37 °C. Then the isolated colonies were further biochemically examined according to the characteristics of the bacteria. The biochemical tests such as nitrate reduction, gelatinase, catalase, oxidase and urease were conducted for the confirmation (3).

DNA extraction and Polymerase Chain Reaction

DNA was extracted from the samples of abomasal contents using Roche High Pure PCR Template Preparation Kit (Roche, Germany) following the manufacturer's protocol. Then *PCr* amplicon amplifications were performed for the detection of *Chlamydia abortus*, *Coxiella burnetii*, *Brucella* sp., *Mycoplasma* sp., *Salmonella* sp., *Campylobacter* sp, and *Listeria monocytogenes*. The primer pairs used in this study are listed in table 1.

PCR was conducted using 2 µM of each primer, 2.5 µl of 10X buffer, 1.5 mM of $MgCl_2$, 200 µM of dNTPs, 0.3 U of Taq DNA polymerase, and 5 µl of DNA template. The final volume of reaction mixture was amounted to 25 µl using nuclease free water. Amplification was carried out in the

automated DNA thermal cycle. The cycling parameters for each test are demonstrated in table 2. The desired amplified products were analyzed using 1% agarose gel. A mixture of 10 µl safe stain with Tris Borate EDTA buffer was resolved in the gel. Then electrophoresis was performed and the products were visualized under a UV transilluminator.

Serologic testing

For the diagnosis of pregnancy toxaemia, beta-hydroxy butyrate was measured in the serum samples using the Williamson-Mellanby enzymatic method (Commercial kit, Biorex Fars Company, Shiraz, Iran) and the animals that showed a BHB concentration greater than 3 mmol/l were considered suffering from pregnancy toxaemia (18).

Statistical analysis

The results were statistically analyzed using t-test and the analysis was done by Statistical Analysis Software (SAS, 2013), version of 9.4. $P<0.05$ was considered significant.

Result

During the study period, 59 (9.8%) of Saanens and 80 (13.3%) of Alpines aborted their foeti. There was no difference between the two breeds in abortion rate. None of the bacteria were detected by PCR and observation of the bands belonged to the positive controls showed accuracy of the amplifications. In the microbial culture *Escherichia coli* was isolated from 4 Saanens (0.6%) and 3 Alpines (0.5%). Moreover, *Trueperella pyogenes* was isolated from 5 Saanens (0.8%) and 7 Alpines (1.1%). The results of the biochemical tests for confirmation of the suspected colonies are given in tables 3 and 4. The incidence of non-infectious abortion in Saanens and Alpines is given in table 5.

Discussion

The current study evaluated the susceptibility of Saanen and Alpine to some of the most common infectious and non-infectious factors. The formers were the causes of pregnancy failure in 1.5% of Saanens and 1.6% of Alpines. Using the molecular method, none of the major abortifacients were detected and the results were due to the high hygiene condition and strict quarantine protocols in such industrial farms. However, among the studied pathogens, only *E. coli* and *T. pyogenes* were isolated in microbial examination of the specimens.

Contrary to our results, different studies have identified *Brucella melitensis*, *Chlamydia abortus*, *Campylobacter fetus*, *Coxiella burnetii*, *Mycoplasma* sp. and *Salmonella* as the most frequent infectious causes of abortion in different areas of Iran. This shows differences in health management and organized vaccination plan between extended small ruminant raising and industrial ones. Bacteria such as *E. coli* and *T. pyogenes* are less considered in abortion studies since there are still major causes which small ruminants are struggling with. Moreover, even though vaccination with Rev1 is the strategy of Iran veterinary organization against *B. melitensis*, it is still the first infectious agent that should be considered in abortion cases especially in small ruminants (19).

In some of the caprine herds in Chahaimahal va Bakhtiari province, Iran. 2017, abomasal contents were collected for the detection of *B. meliensis* DNA by PCR method which identified 16.4% of aborted fetuses infected with the bacterium (20). Another seroprevalence study in Urmia showed rather same percentage in sheep (16.55%) (21).

Although brucellosis has been eradicated in most of the European countries, it is one of the common causes of abortion in some less developed ones. The recorded results of Farag et al. in 2021 in Egypt showed that the most prevalent causes of abortion were due to brucellosis (17%), Salmonellosis (12%) and listeriosis (11%) respectively (22). The identified agents were prevalent in Egypt and the samples had been collected from local farms which needed higher hygiene condition.

Recently *Chlamydia abortus* has been considered in abortion cases and despite lack of attention of studies to this bacterium, they have showed high frequency of chlamydial abortion in sheep and goats in Iran. Hamedi et al. in 2020 detected *Chlamydia abortus* in 23.5% of fetuses by PCR method (23). In the cross-sectional study of Esmaeili et a. in 2021, 834 vaginal swab samples from aborted small ruminants were taken from 83 flocks belonged to different areas of Iran and were then tested for *C. abortus* DNA by real-time PCR. The authors detected the bacterium in 24.1% of the samples (24).

There are other studies which all shows the agents we examined in the current paper are amongst the most prevalent infectious abortifacient agents in Iran (19, 23, 24, 25, 26). Amouei et al in 2019, found infectious pathogens in 31.4% of aborted fetuses of sheep, goat and cattle in Mazandaran province, Iran in which *Toxoplasma gondii* was detected as the most prevalent agent followed by *Neospora caninum* and *Brucella melitensis* [25]. Mahdavi Roshan et al. in 2018 detected *Brucella melitensis*, *Coxiella burnetii* and *Salmonella abortusovis* in 35% of fetuses in Sistan region [26].

Infectious agents are more frequently detected in ovine and caprine abortion cases and many of which are zoonotic (27). We only identified *E. coli* and *T. pyogenes* as infectious agents and their frequency didn't have differences in Saanens and Alpines. The detected bacteria are neglected in abortion cases. These agents can find their way through bloodstream and may cause sporadic abortion (28). However, in most cases they aren't involved as the main pathogens (3, 25). The current studied farm had high sanitary condition and the animals had received the main vaccines against endemic diseases in Iran such as brucellosis. As a result, we didn't expect to detect other pathogens which their occurrence is related to hygienic level of a farm (29). In addition, several diseases may negatively impact reproductive situation (30) and the fact that most common diseases were controlled in the studied flock, leads the pregnant animals to be encountered with less abortifacient pathogens. Moreover, there are other bacteria, virus and parasites that may be involved in an abortion while we didn't examine their presence so it needs further studies in both Saanens and Alpines.

Non-infectious etiologies of abortion are usually rarely investigated and their incidence is less in small ruminants in comparison with bovines and mare (31), though there are other factors which lead non-infectious abortions remain undiagnosed such as difficulty in diagnosis, lack of laboratories equipment for the approving of non-infection cases and the fact that generally abortion factors accompanied by less pathognomonic signs in fetus. As a result, most laboratories tests are based on the detection of infectious agents. However, there is a demanding need for consideration of non-infectious factors which jeopardize pregnancy outcome and there should be more emphasis on their diagnosis, prevention and treatment.

In the present studied flock, we identified pregnancy toxemia and trauma as non-infectious causes of abortion and abortion due to pregnancy toxemia was significantly more in Alpine ($p<0.05$) which represents its higher susceptibility.

Energy requirements for a pregnant goat when carries a single fetus is 1.5 times that of a non-pregnant one and it increases by a factor 2 when she has two foeti (8). During last weeks of pregnancy, glucose level has a crucial role in fetal growth (32) and energy deficiency leads to hypoglycemia especially in animals with more than one fetus. Subsequently, the growth of fetus might be disturbed and abortion occurs (33).

In the experimental study of Affan et al. in 2022, energy intake was reduced by 50% in twelve goats to induce pregnancy toxemia which caused abortion between days 21 and 40 post induction (34). Pregnancy toxemia occurrence is related to improper food management such as poor nutrition, improper methods of food preparation and deprivation. Therefore, control of pregnancy toxemia through recognition of more susceptible animals in a flock and preparing of high energy rich feed with mineral supplements for poor body score condition animals are important issues to prevent its economic adverse effects specifically abortion (35, 36).

The other important non-infectious cause of abortion was trauma which showed no statistically difference between Saanen and Alpine (table 5). Trauma is not a common cause of abortion [36] and in our investigation, the incidence of physical abortions was 1.6% and 1% in Saanen and Alpine respectively. Nevertheless, in severe trauma in which amniotic vesicle, fetal heart or fetal vessels rupture, abortion may take place (36).

Conclusion

Small ruminants are needed for human community daily life but their productivity is constrained by abortion. Although the newly introduced animals are profitable for our food security policies, they have high susceptibility to diseases and information gap in terms of infectious and non-infectious conditions, their dynamics and distribution may lead to huge economic losses. There are neither comprehensive studies on the occurrence of diseases, nor experiment of raising Alpine and Saanen in an industrial level. So we need monitoring programs for diagnosis of abortigenic pathogens especially infectious ones. Since infectious causes of abortion require implementation of effective disease control strategies and rapid diagnosis at laboratories. Otherwise the agents may spread among other animals in a flock. In addition, a defined protocol of raising the breeds based on disease and climate situation of Iran is necessary and this study was a step for preparing such protocol.

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Ethics approval and consent to participate

This study did not require an ethics license.

Conflict of interest

The authors declare that they have no conflict of interest.

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