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Study of the Bacterial and Nutritional Causes of Diarrhea in Alpine and Saanen Kids

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ABSTRACT

Background: Infectious diarrhea in young goats is one of the most common and serious threats to the animals' health, influencing the profitability of the goat industry. Considering the need to investigate the factors involved in the occurrence of neonatal diseases, especially diarrhea, to improve livestock health and increase productivity, especially in Iran, the present study was conducted with the aim of investigating the bacterial agents involved in the diarrhea of Alpine and Saanen kids.

Methods: Between 1421 goats (714 Saanen and 704 Alpine) under the supervision, 254 goats (17.8%) of different ages died due to diarrhea sign. Samples from the liver, heart, and lungs were taken during the necropsy process, and bacteriological examinations were performed.

Results: Forty-four kids died of non-infectious cause. Diarrhea caused by bacterial agents (10.9%) was much more than non-infectious agents (3.1%). Significantly more so than at other ages, the bacterial agent was isolated from the age of less than 2 months. Isolated bacterial agents included *Escherichia coli*, *Staphylococcus aureus* (12.3%), *Pseudomonas* (7.1%), *Bacillus* (11%) and *Klebsiella* (2.6%). *E. coli* was isolated frequently (63.2%) , and mostly at the age of less than 2 months. The highest rate of isolation of bacterial agents was from the heart, especially *E. coli*.

Conclusion: The findings of the current research showed that in Saanen and Alpine goats reared in intensive rearing systems, *E. coli* bacteria has a high contribution to the occurrence of digestive problems and diarrhea, which can be avoided by controlling environmental health, improvement and preventing the density of parturitions in a short time.

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Introduction

Human populations have traditionally depended heavily on goats for their livelihoods, especially in rural and less favored areas. Goats are kept in many different ways and for many different purposes (1). In many regions of Iran, it is customary as a traditional work for their livelihood and income earning (2, 3). Diarrhea, a common and frequent disease with gastrointestinal dysfunction in animal husbandry, will affect young ruminants growth and often causes death in these animals (4). Despite the abundance of scientific knowledge on neonatal small ruminant livestock disease and mortality, survival rates have not significantly increased as a result of this knowledge. The reason might be that researchers haven't paid much attention to neonatal morbidity and have instead concentrated on examining and addressing problems brought on by economic factors (5). Infectious diarrhea in neonates and young goats is one of the most serious threats to the animals' health, influencing the profitability of the goat industry (6, 7). A number of factors contribute to the intestinal mucosa's strong secretion or decreased absorption, according to its physiological mechanism (5). In goats, diarrhea is a very common sign that can be caused by a range of factors, including a sudden change in feed, ingestion of poisonous plants, infection, or in more severe cases, a viral, fungal, or bacterial infection (1, 8).

The general consensus is that infectious diseases like diarrhea, are the primary cause of mortality in goat kids. which enteric pathogens are primarily responsible for. More than 50% of kid mortality is attributed to diarrhea brought on by bacterial agents like *Escherichia coli*, according to reports (6). The bacterial agents that contribute to the mortality of the lambs' and goats' kids were studied in Jordan. Since the start of the kidding season, the kids have been under observation. Diarrhea was responsible for 59.75% of infant deaths in kids. *E. coli*, *Pasteurella multocida*, *Clostridium perfringens*, and *Staphylococcus aureus* are some of the bacteria that cause kids mortality. The most prevalent bacterial species that killed infants in lambs and goats was *E. coli* (63.4%) (9). According to a study, diarrheal kids intestinal microbiota was differ significantly from that of healthy kids (4). Therefore, a precise diagnosis of the bacterial causes of diarrhea in goat kids is required. For the confirmatory diagnosis of bacterial infection, detection of specific bacterial pathogens is done by conventional method of culturing microorganisms on agar plates, followed by standard biochemical identifications. Postmortem examination of dead neonatal kids can be useful (1, 10).

There is no denying that all goats, including Alpine and Saanen, which are important to economic and ecological field systems, are susceptible to infections and non-infectious diarrheal causes (1, 11). To enhance animal health and boost productivity, it is necessary to look for factors that contribute to the occurrence of neonatal diseases, particularly diarrhea, as gastrointestinal infections are likely the first disease in goat kids. Additionally, there aren't many reports of diarrhea caused by bacterial agents in Iranian goats that are being bred. While Iran frequently uses extensive systems to breed goats, the current study was carried out in an intensive breeding system. Iranian breeders have recently gained interest in Saanen and Alpine goat breeding, which is primarily done through intensive systems. Therefore, the current study was conducted with the aim of investigating the bacterial agents involved in the diarrhea of Alpine and Saanen goat.

Materials and Methods

Animals

Current study was conducted from July 2017 to April 2018. A number of 1421 kids (714 Saanen and 704 Alpine) were monitored for diarrhea after birth. Two hundred and fifty-four kids (132 Saanen and 122 Alpine) died due to diarrhea sign. These kids were reared under intensive system; Additionally, they had received vaccinations for brucellosis, smallpox, PPR, FAD, and enterotoxemia according to the guidelines of the Iran veterinary organization. Immediately after birth, the goat kids were separated from their mothers and were fed three times for 24 h with colostrum heated at 56°C. After receiving

mother's milk for up to a week, the kids were fed milk powder until they were weaned.

Postmortem examination and Sampling

All 254 kids that died due to diarrhea were necropsied. The thorough postmortem examination carried out immediately after death. kids with obvious necropsy signs of Abomasum bloat, diarrhea, metabolic acidosis, gastrointestinal hyperemia, and Abomasum rupture were considered as casualties due to non-infect agents. Samples were taken from the heart, lungs, and liver of other kids without signs of nutritional diarrhea for bacteriological examination. Special care was always taken to avoid contamination as possible. In total, bacterial samples were taken aseptically from 210 kids (108 Saanen and 102 Alpine).

Bacteriological Examination

Aseptic samples taken from heart, lung, and liver were inoculated aerobically at 37°C on blood agar and McConkey agar (Oxoid, United Kingdom) for 48 hours. If bacterial growth occurred (mixed culture), different colonies were selected with respect to morphology of them for complementary tests (isolation and identification) mentioned by Markey et al (12). The colonies on primarily incubation were repeatedly sub-cultured until pure culture with homogeneous colonies were obtained. In order to differentiate *Staphylococcus* species, various tests were used such as pigment production, hemolysis, coagulase test, DNase test, alkaline phosphatase, urease test, fermentation of mannitol and maltose and antibiotic susceptibility against polymyxin B and novobiocin. To differentiate *Pseudomonas* species, colonial morphology, pigment production, gelatinase production, ability to growth at the 5°C and 42°C, urease test, oxidation of glucose, lactose, and maltose and arginine dehydrolase were used. To identify different genus and species of *Enterobacteriaceae*, indole production, methyl red (MR) and Voges-Proskauer (VP) tests, Growth on Simon's citrate, lactose fermentation, H2S production, lysine decarboxylase test, and urease tests were used. To differentiate *Bacillus* species, colonial morphology, susceptibility test to penicillin, citrate hydrolysis, motility, VP, Indole production, catalase, nitrate reduction, and production of H2S were used.

Statitistical Analysis

Data were analyzed using SPSS 25.0 software (SPSS Inc., Chicago, IL). The results were analyzed statistically using chi-square tests and ANOVA. $P \leq 0.05$ indicates a significant difference.

Results

Among the number of 254 dead kids, 44 (3.1% of the total number of births) deaths (24 Saanen (1.7%) and 20 Alpines (1.4%)) were caused by Non-bacterial causes of diarrhea, which often died in less than 24 hours due to overfeeding from milk bottles or buckets, etc. In terms of age, there were twenty-nine kids under two months (65.9%) (16 Saanen and 13 Alpine), twelve kids between two and three months (27.3%) (6 Saanen and 6 Alpine), and three kids between three and six months (6.8%) (1 Saanen and 2 Alpine). The rate of deaths caused by nutritional factors between different age groups was statistically significant ($p \leq 0.05$). At the age of seven days, milk replacer was used to feed the kids, which led to an increase in diarrhea cases in the 7 to 8-day-old animals. An increase in the incidence of diarrhea was observed twelve hours after the first feeding of the kids with milk buckets. Abomasum bloat was the most common sign in the necropsy of kids that died due to diarrhea caused by nutritional factors (Table 1). Under two months of age, 65.9% of diarrhea cases were brought by non-bacterial agents, 27.3% between two and three months, and 6.8% between three and six months.

In total, bacterial samples were taken aseptically from 210 kids (14.8% of the total number of births). One hundred and eight kids were of Saanen breed (7.6%); 75 kids were less than two months old, 21 kids at weaning age between two and three months, and 12 kids between three and six months. One hundred and two Alpine kids (7.2%); 73 kids were

less than two months old, 12 kids were weaned, and 10 kids were between three and six months old. Among 210 deaths caused by infectious agents, bacterial agents were isolated from 155 kids (80 Saanen and 75 Alpine) (Table 3). Bacterial agents were caused 78% of diarrhea cases under two months old, 14.3% between two and three months, and 7.7% between three and six months. Intestinal inflammation and signs of septicemia were the necropsy sign of kids with bacterial diarrhea.

Following bacteriological investigations, *E. coli* was the most isolated bacterial agent with 63.2%. Then *S. aureus* (12.3%), *Pseudomonas* (7.1%), *Bacillus* (11%), *Klebsiella* (2.6%), and *Streptococcus* (3.8%) were respectively the most bacterial agents isolated from the samples taken from organs which should naturally be sterile.

E. coli was significantly more than other isolated bacterial agents ($p \leq 0.05$) and the most isolated bacterial agents were found in the heart tissue (Table 2). In a significant way, the isolation of the bacterial agent from the age ≤2 months was more compared to other ages ($p \leq 0.05$) (Table 2). In a significant way, the casualties at the age of ≤2 months were more than other ages ($p \leq 0.05$) (78%), and casualties at the age of 2-3 months were more than at the age of 3-6 months ($p \leq 0.05$).

Discussion

According to the current study findings, the overall casualty rate was 17.9%, that 73.8% of these losses were caused by bacterial agents and 3.1% were caused by nutritional factors. Less than two months of age saw a higher percentage of nutritionally related losses (66%). at the age of seven days, milk replacer was used to feed the kids, which caused an increase in the incidence of diarrhea in 7-8 day old kids. milk replacer is a recommended practice and this reduces feed costs (13, 14). Additionally, problems that might arise when mother's milk is fed naturally will be avoided (15). However, the nutritional stress of switching from mother's milk to milk powder can cause microbial and nutritional diarrhea (16). It is necessary to monitor more carefully when separating weaker kids and forming groups of equal strength when feeding from the shared bucket of milk because goats of the same age may not always be the exact same size or weight. This is done to prevent overeating, diarrhea, and death in the stronger kids and to prevent malnutrition of weaker kids.

An increase in cases of diarrhea among the goat kids was observed 12 hours after the first feeding of the kids with Shared milk buckets. The kids could choose the frequency and amount of milk they consumed. Considering the difference in sucking ability in different goat kids, when 5 kids use shared milk buckets (with a common milk bucket), it causes overeating in stronger kids. After overeating, diarrhea appeared in these kids. Using shared milk buckets forces the kids to drink head down, which keeps the esophageal groove open and results in milk being swallowed in the rumen instead of the abomasum. Bélanger-Naud et al. in 2021 pointed out the higher diarrhea prevalence in the use of such feeding methods as one of the performance indicators (14). Due to the increased resistance of the kids at the age of 2 months and the habituation of feeding from shared milking buckets, less deaths due to milk overeating were observed at this age. In order to learn more about diseases and mortality patterns in the goats that were subjected to intensive breeding, Rshaduzzaman et al. conducted a study on them (400 goats). Fatalities in goats under 3 months of age were 29%, Diarrhea was the leading cause of death for goats under three months of age, Additionally, deaths were decreased(22%) in goats older than 3 months; diarrhea was the least common cause of death at these ages (17). In a study by Erman Ali et al., the prevalence of diarrhea in infants (under 3 months) (59.52%) was significantly ($P<0.001$) higher than that of young goats (12.47%) and adults (13.42%). 1007 goats were examined in total for this study. Diarrhea was clinically diagnosed in 149 goats., and the overall prevalence of diarrhea in goats was 14.80% (18).

The results of the present study showed that the bacterial agent was isolated from 73.8% of deaths. According to Table 3, no statistical difference was observed between Saanen and Alpine breeds in terms of sensitivity to bacterial diarrhea agents. The most

isolated bacterial agent in present study was *E. coli* (63.2%) (table 3). In the study of Zaki et al., *E. coli* with a prevalence of 58% was introduced as the main cause of diarrhea in 100 goat kids aged 2 days to 3 months (19). also, Shabana et al investigated 310 goats (117) and sheep (193) with diarrhea, *E. coli* was the most-prevalent agent in both sheep (34.7%) and goats (30.7%) (20). Most cases of diarrhea were prevalent in half-births. This means that at the beginning of the kidding season, when the hall was just disinfected and the density of kidding was low, and as a result, the number and density of kids was low, the incidence of diarrhea was also observed less. With the increase in births and less maintenance in terms of changing and burning the litter, fewer kids in each pen, washing dishes, and feeding equipment, etc., the incidence of diarrhea also increased. Considering that *E. coli* is an environmental bacterium, its occurrence is more likely in such conditions.

In 2022, Shrivastava et al examined 200 stool samples collected from diarrhea cases of goats for the presence of *E. coli*. The prevalence of *E. coli* in goats with diarrhea was 87%. In this study, the highest prevalence of diarrhea with *E. coli* was observed in the age group of 0-14 days (35%). Then in the age group 30-15 days with a prevalence of 25.5%, 30-45 days 10%, 45-60 days 4.5%, 90-60 days 5%, 120-90 days 4.5%, and 3-6 months 5.2% (21). In the present study, the isolation of *E. coli* at the age of less than 2 months was more frequent in Alpine and Saanen kids (Table 2). In the study of Shabana et al., the prevalence of bacterial enteropathogens in the age group of 0-12 months was significantly higher than in older age groups (20). In Shrivastava's study, the incidence of bacterial diarrhea caused by *E. coli* in decentralized farms (extensive system) was 71.9% higher than in centralized farms (intensive system) by 43.6%. The prevalence of infection in extensive system farms may be due to lack of timely and sufficient colostrum feeding, malnutrition in free grazing and litter contamination. On the other hand, rearing under intensive system with daily cleaning and periodic disinfection will make the conditions unsuitable for infection transmission. The lower prevalence of *E. coli* in intensive farms may be due to better hygiene compared to open farms (21).

Sharif et al. in 2005 examined 55 cases of lambs and goats bacteriologically. Bacterial samples were taken from heart, liver, lung, and intestine. The causes of neonatal mortality in lambs and kids were: diarrhea (59.75%), respiratory diseases (13.3%), unknown causes (12.34%), and accident (8.39%). Bacteria responsible for neonatal mortality were: *E. coli*, *Pasteurella multocida*, *Clostridium perfringens* and *S. aureus*. diarrhea was the main sign accompanying the death in about 60% mortality of lambs and kids. In this study, *E. coli* was the most common bacterial species identified as the cause of infant mortality in lambs and goats and constituted 63.4% of the total bacteria isolated (9).

In our study, other isolated bacteria are *S. aureus*, *Bacillus*, *Klebsiella*, *Streptococcus* and *Pseudomonas*. diarrheic animals can harbor these pathogens and serve as sources of infection to healthy animals and humans (22). In a study by Mohamed, in order to identify the bacterial factors associated with diarrhea in lambs and goat kids. 150 rectal swab samples of diarrheal lambs and goats aged 1 to 60 days were examined bacteriologically. Also, in this study, *E. coli* (29.7%), *Klebsiella* (14.3%), *Pseudomonas* (12.1%), *Proteus* (5.5%), *Salmonella* (3.3%), *Shigella* (1.1%), *Serratia* (2.2%), and *Citrobacter* (3.3%) was isolated. *E. coli* was introduced as the most common cause of diarrhea in lambs and goats (23). In 2024, Nasr et al. examined 1200 lambs for bacterial enteritis. 650 showed enteritis (16.54%).

They observed that the amount of enteritis in the intensive system (83.70%) was higher than the extensive system (46.80%) and the mortality rate was 4.16%. Bacteriological examination of fecal samples revealed that 190 (29%) of the lambs were positive for pathogenic bacteria culture. The most isolated bacteria were *E. coli* (65 case) other bacteria included; *Salmonella* (10 case), *Clostridium* spp. (15 case), *Proteus* Spp. (25 case), *Shigella* (20 case), and *Klebsiella* (15 case) (24).

Table 1. The frequency of post-mortem examination sign caused by nutritional diarrhea by age of the kids.

	≤ 2 months		2-3 months		3-6 months		
	Saanen	Alpine	Saanen	Alpine	Saanen	Alpine	**Total (n)**
Escherichia coli	41	43	5	3	3	3	98 (63.2%)
Staphylococcus aureus	6	5	3	2	2	1	19 (12.3%)
Pseudomonas	4	3	2	1	0	1	11 (7.1%)
Bacillus	5	5	2	3	2	0	17 (11%)
Klebsiella	2	2	0	0	0	0	4 (2.6%)
Streptococcus	2	3	1	0	0	0	6 (3.8%)
Total (n)	60	61	13	9	7	5	155 (100%)

Table 2. The abundance of bacteria isolated from the heart, kidney, and liver of dead kids.

	≤ 2 months	2-3 months	3-6 months	Total
Abomasum bloating	26 (23%)	11 (9.75%)	-	32.7%
diarrhea	19 (16.8%)	5 (4.4%)	3 (2.65%)	23.9%
Metabolic acidosis	19 (16.8%)	5 (4.4%)	-	21.2%
Gastrointestinal hyperemia	14 (12.4%)	5 (4.4%)	3 (2.65%)	19.45
Abomasum rupture	3 (2.65%)	-	-	2.65%

Table 3. Frequency of isolated microbial agents by breed and age.

	Isolated bacterial agents (n)					
	Escherichia coli	*Staphylococcus*	*Pseudomonas*	*Bacillus*	*Klebsiella*	*Streptococcus*
Heart	25	7	5	9	-	6
Liver	13	6	-	-	-	-
Lung	11	-	-	-	-	-
Heart+Liver +Lung	14	6	6	-	-	-
Heart+ Liver	12	-	-	8	4	-
Liver +Lung	12	-	-	-	-	-
Heart + Lung	11	-	-	-	-	-
Total	98	19	11	17	4	6

In the current study, 78% of deaths from infectious agents were related to the age of less than two months, at this age, *Escherichia* was isolated from 84 diarrheal kids (41 Saanen and 43 Alpine). While at more than 2 months of age, this figure showed a significant reduction. In the study of Abdou et al. in Kuwait, by examining the diarrheal samples of 556 sheep (334) and goats (222), in addition to mentioning *E. coli* as the most dominant enteropathogen causing diarrhea, they identified less than three months old goats as the most vulnerable age group, which showing diarrhea sign 2.38 times more than others (22). In the present study, with increasing age of the kids, it was evident that there was a decrease in mortality and the isolation of *E. coli*. At the age of less than ten days, there is the

highest sensitivity to *E. coli*, and with increasing age, the kid's resistance increases. In the study of Ndegwa et al., the most cases of *E. coli* were isolated from weaned goats. In the mentioned study, the stx1, stx2 and hly genes, Virulence genes associated with *E. coli*, peaked around weaning (60, 63 and 52%) respectively (25).

In the present study, the isolation of bacterial agents from the heart tissue was more than that of the liver and lungs, and it was statistically significant. This shows the importance of heart tissue in helping diagnosis. According to the results of the current study, it can be claimed that if it is possible to culture only one tissue, microbial culture should be done from the heart tissue. The heart tissue is anatomically protected against bacterial contamination and the possibility of cross-contamination is less, therefore one can have more confidence in the result of isolating the agent.

Conclusion

The findings of the present study showed that *E. coli* bacteria have a high contribution to the occurrence of digestive system problems and diarrhea in Saanen and Alpine kids in the intensive rearing system, which can be reduced by controlling the environmental health, including litter hygiene, lactation hygiene, and avoiding overcrowding. Following the increase in age, the amount of digestive problems with nutritional origin decreases, also, deaths due to overeating are more at the age of less than two months. A quick and correct diagnosis following the observation of diarrhea and deaths caused by diarrhea in the herd can prevent further losses.

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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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