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Araştırma Makalesi ***Research Article***

Etiologic Evaluation of Late Term Abortions or Stillbirths in Some Small Ruminant Flocks of South Khorasan Province, Iran

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Abstract

In the present study, late term abortions in 477 (17.9%) of 2651 pregnant ewes and 129 of 680 pregnant goats (18.9%) from 17 small ruminant flocks of the South Khorasan province, east of Iran were investigated. Multistage cluster sampling technique was used to select the flocks and aborted animals. From which, 25 appropriate fetuses were chosen for bacterial and fungal contaminations. Blood samples were also taken from forty aborted and forty healthy age matched sheep. No microbial or fungal agents were detected in 45.4 % and 42.8% of sheep and goat,s fetuses respectively. The bacteria isolated from aborted fetuses of sheep and goat´s were as follows: *Escherichia coli* 27.2% and 7.1%, *Staphylococcus* spp. 9.0% and 7.1%, *Campylobacter fetus* 9.0% and 14.2%, *Staphylococcus* spp. and *Campylobacter fetus* together 9.0% and 0.0 %, *Klebsiellas* 0.0 % and 21.4%, *Aspergillus fumigatus* 0.0 % and 7.1%, respectively. The concentrations of total tri-iodothyronine (tT3), total thyroxin (tT4), copper, phosphorus and magnesium were significantly lower, but thyroid stimulating hormone (TSH) was higher in the aborted ewes compared to the healthy controls. The serum levels of calcium, zinc and vitamin E were not significantly different between the two groups. In conclusion, deficiencies of tT3, tT4, copper, phosphorus and magnesium, and increase in the TSH concentration could have taken part in the etiology of abortion in ewes. The most important cause of late term abortions was probably due to the maternal deficiencies (for example, iodine) in the concurrent drought in the province.

Özet

İran Güney Horasan Bölgesi'ndeki Küçük Ruminant Sürülerinde Geç Dönem Abortların veya Ölü Doğumların Etyolojik Değerlendirilmesi

Bu çalışmada, doğu İran'da Güney Horasan bölgesinde 17 küçük ruminant sürüsündeki 2651 gebe koyundan geç dönem abort yapan 477'si (%17,9) ve 680 gebe keçiden 17'si incelenmiştir. Sürülerin ve abort yapan hayvanların seçiminde çok aşamalı küme örnekleme tekniği kullanılmıştır. İçlerinden bakteriyel ve fungal kontaminasyona uygun 25 fötus seçilmiştir. Yaşları birbiriyle eş olan kırk adet abort yapmış ve kırk adet de sağlıklı koyundan kan örnekleri alınmıştır. %45,4 koyun ve %42,8 keçi fötusunda ne mikrobiyel ne de fungal bir ajan saptanamamıştır. Abort olan koyun ve keçi fötüslarından izole edilen bakteriler sırasıyla: *Escherichia coli* %27,2 ve %7,1, *Staphylococcus* spp. %9,0 ve %7,1, *Campylobacter fetus* %9,0 ve %14,2, *Staphylococcus* spp. ile *Campylobacter fetus* birlikte %9,0 ve %0,0, *Klebsiellas* %0,0 ve %21,4, *Aspergillus fumigates* %0,0 ve%7,1 olarak tespit edilmiştir. Abort yapan dişiler kontrol amaçlı sağlıklı dişiler ile karşılaştırıldığında total tri-iodotironin (tT3), total tiroksin (tT4), bakır, fosfor ve magnezyum konsantrasyonları önemli düzeyde düşük iken tiroid stimule edici hormon (TSH) daha yüksek bulunmuştur. İki grup arasındaki serum kalsiyum, çinko ve Vitamin E değerlerinin birbirinden farkı önemli düzeyde değildi. Sonuç olarak, tT3, tT4, bakır, fosfor ve magnezyum, ve TSH konsantrasyonundaki artış dişi koyun ve keçilerdeki abortun etyolojisinde yer alabilir. Geç dönem abortlarının en önemli sebebi bölgedeki eşzamanlı kuraklık nedeniyle annede meydana gelen eksikliklerdir (örneğin iyot).

Introduction

Abortion is a multi factorial event in small ruminant flocks and may be idiopathic or due to infectious, nutritional, genetic factors, or even a combination of them (van den Brom et al., 2012). The great majority of abortion cases throughout the world is of microbial aetiology. Generally the major bacterial agents that have been implicated in abortion in small ruminants were as follow: *Brucella melitensis*, *Campylobacter fetus* subsp. *fetus*, *Chlamydophila abortus*, *Coxiella burnetti*, *Toxoplasma gondi* and *Border Disease* Virus; of lesser importance can be various other bacteria (e.g., *Salmonella* spp., *Mycoplasma* spp., *Leptospira* spp.) and viruses (Fthenakis, et al., 2012). Basically, any other bacteria that can find their way into the bloodstream are usually associated with abortion; some of these bacteria

are: *Arcanobacterium pyogenes* and *Bacillus* spp., *Escherichia coli*, *Histophilus somni*, *Pasteurella* spp., *Listeria* spp., *Staphylococcus* spp., and *Streptococcus* spp. The common mycotic agents including *Aspergillus fumigatus*, *Aspergillus* spp., and *Candida* spp., (Holler, 2012). Also there is a large number of literature suggesting the close correlation between the plasma levels of some elements and vitamins in the reproductive functions and abortion in ruminants (Grewal et al., 2011). Various minerals influence reproductive performance of small ruminants, deficiencies or imbalances of which may induce reproductive failure. Late pregnant and heavily lactating animals are especially susceptible to the calcium deficiency. Calcium and phosphorus deficiency are recognized more in livestock fed on concentrates and grazing on pastures respectively (Herdt and Hoff, 2011). Magnesium is a cofactor of some important enzymes such as choline esterase and ATPase. Magnesium supplementation is necessary for normal fertility (Soetan et al., 2010). Inadequate copper levels have been associated with delayed or suppressed estrus, decreased conception rate, infertility, and embryo death (Abdollahi et al., 2013). Zinc is used in the structure of many enzymes such as carbonic anhydrase, carboxy peptidase, and is a cofactor of many enzymes. Zinc deficiency leads to reproductive failure, abortion, and altered myometrial contractibility with prolonged labor. Zinc and vitamin E as part of the non-enzymatic antioxidant defense mechanisms are important in preventing of some infectious diseases (Aytekin and Aypak, 2011). The thyroid hormones are necessary for the growth, metabolism and function of all cells in the body (Radostits et al., 2007). The present study was focused to determine the role of some probable etiological agents in the late term heavy incidence of abortions.

Materials and Methods

Data collection was completed by using a questionnaire specifically prepared for the study. The questionnaires were completed by personal interviews with the owners who were requested to contact with the author or his co-workers as soon as they were faced with late term abortions was higher than five percent during a season (Fthenakis et al., 2012). The details of the questions are presented in Table 1. A cross sectional study was conducted on 17 small ruminant flocks (10 sheep and 7 mixed flocks containing both sheep and goats). Forty late term aborted ewes were picked from the flocks using a multistage cluster sampling technique. Forty pregnant or gestated ewes upon physical examination and serologically negative for brucellosis were chosen as the healthy control group. None of the animals in the control group revealed a history of abortion during the past three years. Jugular blood samples were taken during January to March 2008 within 24 hours after abortion. The whole aborted fetus and fetal membranes were submitted in an insulated container to the laboratory (Borel et al., 2014). The culture conditions for enrichment, isolation, and identification of pathogenic bacteria/fungi from aborted fetuses are given in Table 2. The blood samples were collected into 10 ml vacuum tube and chilled immediately and transported to the laboratory within 1°h of collection. Sera were collected following centrifugation at 750× *g* for 15 minutes, frozen and stored at −21 °C until further analysis. The concentrations of calcium, inorganic phosphorus and magnesium were determined using an automated biochemical analyzer (Biotecnica, Targa 3000, Rome, Italy) by commercial kits (Parsazmoon, Tehran, Iran) and routin methods (Abarghani et al., 2012). Thyroid stimulating hormone (TSH), total tri-iodothyronine (tT3) and total thyroxine (tT4) concentrations were determined by radioimmunoassay method using commercial kits (Immunotech Company, Radiove, Prague, Chech Republic) (Thienpont et al., 2013). Copper and zinc were determined by using an atomic absorption spectrophotometer (Shimadzu AA 670, Kyoto, Japan). Measurement of vitamin E (α-tocopherol) was performed by a rapid high-performance liquid chromatography (HPLC) method. The HPLC system was consisted of a solvent delivery pump (JASCO 980-PU, Tokyo, Japan), a reversed-phase column (Luna C18, 250 mm×4.6 mm; Phenomenex, CA, USA), and a UV–Vis detector (Jasco, UV-975, Tokyo, Japan) (Mohebbi-Fani et al., 2012). The diagnosis of brucellosis was completed by Rose Bengal plate test (RBPT) (Matope et al., 2011). The data were analyzed by descriptive statistics and independent *t*-test using SPSS 16/PC software. All values were expressed as mean and standard error (SEM), and $P<0.05$ was considered as statistically significant.

Results

Based on data collected during the interviews, fifteen percent of flocks were grazed on the pastures. Twenty eight percent of flocks were fed manually. The remains were grazed on the pastures and received supplementary feeding which was usually barley, straw, turnips, beets, pistachio garden weeds, leaves, pine dried foliage, pomegranate skin and etc. One-third of the flocks were not received any vaccination within last year. In the other flocks, one or a combination of pox, enterotoxaemia, blackleg, and FMD vaccines were inoculated. Trace mineral salts were not provided in a regular program for flocks. Abortion was recorded in 477

out of 2651 pregnant ewes (17.9%) and 129 out of 680 pregnant goats (18.9%) during lambing and kidding season. Maximum abortion rates were occurred in the third and fourth parities. No new sheep or goat was added to the flocks within the last few months with the exception of three flocks, the remaining did not have any history of heavy abortions. The bacteria associated with abortions are listed in Table 3. All the aborted animals were serologically negative for brucellosis. Table 4 shows the means ± standard error (SEM) of the parameters measured in the aborted and healthy animals. The levels of T3, T4, phosphorus, magnesium, and copper in the sera of the aborted ewes were significantly lower than those in the healthy animals; and that of TSH was higher in the aborted animals than in the healthy controls ($P < 0.05$). The serum levels of calcium, zinc, and vitamin E were not significantly different between the two groups.

Table 1. Parameters covered by the questionnaires.
Tablo 1. Anket formlarının kapsadığı parametreler.

Date of Visit, Date of Abortion	**Name and Address**
Size and composition of the flock; The number of ewes, rams and lambs in the herd?	Current abortions in the flock? Proportion of abortion to the whole number of ewes in the flock
The history of previous abortion? Stage of pregnancy (First, middle, last)	History of the entrance of new animal to flock in the past few months
Previous deliveries in the flock	Vaccines used in the flock
Start and end time of lambing and kidding in the flock?	Drugs (antibiotics), deworming and … used in the flock
Overall health status of the flock?	Feeding type and amount (grazing on the pastures, manual, both) in the flock? Supplementary feed.
Overcrowding in the flock, shelter and buildings condition.	Any other comments

Table 2. Culture conditions of different pathogenic bacteria/fungi isolated from aborted fetuses.
Tablo 2. Abort fötuslardan elde edilen farklı patojenik bakteri ve mantarların kültür şartları.

Pathogenic Bacteria/Fungi	**Enrichment**	**Isolation Culture Condition**	**Identification**	**References**
E. coli	-	MacConkey agar, 35 ˚C, 24 h	Gram stain, biochemical tests	Feng et al., 2002
Staphylococcus spp.	Tryptic soy broth with 10% sodium chloride, 35˚C, 24 h	Blood agar, Mannitol salt agar, with egg yolk, 35˚C, 48 h	Gram stain, catalase, coagulase, API staph	Corry et al., 2012
Campylobacter fetus	-	Campylobacter selective agar supplemented by Vancomycin, PMB, Trimethoprim, 37˚C, 48 h, micro-aerophilic	Gram stain, biochemical tests	Sato et al., 2004
Klebsiella	-	MacConkey agar, 37˚C, 24 h	Gram stain	Baylis et al., 2000
Aspergillus fumigatus	-	Sabouraud dextrose agar supplemented by chloramphenicol, 25 ˚C, 5-7 days	Gram stain, wet smear	La Bombardi and Wang, 2013

Table 3. Numbers and percentages of microbial and fungal agents; approximately 25 suitable fetuses were selected from the flocks with abortion.

Tablo 3. Mikrobiyal ve fungal ajanların sayıları ve oranları; abort gözlenen sürülerden uygun olan yaklaşık 25 fötus seçilmiştir.

Microbial and Fungal Agents	Fetuses n=25			
	Goats		Sheep	
	N	%	N	%
E. coli	3	27.2	1	7.1
Staphylococcus spp.	1	9.0	1	7.1
Campylobacter fetus	1	9.0	2	14.2
Staphylococcus spp. + *Campylobacter fetus*	1	9.0	0	0
Klebsiellas	0	0	3	21.4
Aspergillus fumigatus	0	0	1	7.14
Nothing	5	45.4	6	42.8
Total	11	100	14	100

Table 4. Levels of tT3, tT4, TSH, Ca, P, Mg, Zn, Cu and Vitamin E in the aborted sheep and healthy controls, in South Khorasan province, Eastern Iran, (Mean ± SEM).

Tablo 4. Doğu İran Güney Horasan Bölgesi'nde abort yapmış ve sağlıklı kontrol koyunlarda tT3, tT4, TSH, Ca, P, Mg, Zn, Cu ve Vitamin E düzeyleri.

Parameter (unit)	Controls (n=40)	Abortion (n=40)
T3 (ng/ml)	1.40±0.04	0.57±0.076*
T4 (µg/dl)	5.92±0.11	4.93±0.14*
TSH (µIu/l)	0.30±0.11	0.96± 0.02*
Calcium (mg/dl)	9.73±0.24	9.66±0.13
Phosphorus (mg/dl)	7.52±0.21	6.91±0.36*
Magnesium (mg/dl)	2.64±0.05	1.69±0.07*
Zinc (µg/dl)	101.08±2.93	101.53±6.37
Copper (µg/dl)	148.24±9.64	91.40±9.23*
Vitamin E (µg/ml)	1.40±0.18	1.44±0.18

*In each row, indicates significant differences at P<0.05

Discussion

An incidence risk of abortion cases less than 5% during a season is considered acceptable (Fthenakis et al., 2012). In the present study, abortion rate was about 17.9% and 18.9% in sheep and goats, respectively. The most prevalent bacterial isolates from the fetuses of aborted ewes were *Escherichia coli* followed by *Staphylococcus* spp., and *Campylobacter fetus*, while the isolates from the fetuses of aborted goats were *Klebsiellas*, followed by *Campylobacter fetus*, *Staphylococcus* spp., *Escherichia coli*., and *Aspergillus fumigatus*.

One unanticipated finding was that, 45% of sheep fetuses and 42% of goat fetuses were not infected to any of microbial and fungal agents. The finding of negative brucella test was in contrast to the result of Menzies (2011), which stated that abortion in the third trimester of pregnancy in sheep was mostly caused by *Brucella melitensis* or *Brucella ovis*. Results showed that concentrations of tT3 and tT4 were significantly lower in the aborted group (P<0.05) and TSH value was also significantly greater compared to the control group (P<0.05). A possible explanation for late term abortion might be that iodine deficiency and hypothyroidism was resulted in the inadequate tyrosine synthesis and decreased blood concentrations of T4 and T3. This is detected by the hypothalamus and pituitary gland, stimulating an increase in TSH production (Mansourian, 2011). Iran is an endemic region for iodine deficiency (Nasehi et al., 2012). In an experimental study, severe iodine deficiency was produced in sheep. After a period of five months iodine deficiency was evident with the appearance of goiter, low T3 and T4 values and elevated

TSH levels (Potter et al., 1982). Iodine deficiency may be due to a deficient iodine intake or secondarily conditioned by intake of goitrogen feeds (Herdt and Hoff, 2011). In addition to iodine deficiency in South Khorasan, many foodstuffs have goitrogenic effects and inhibit the activity of the thyroid. Vegetables such as cabbage, soybeans, lentils, linseed, peas, peanuts and all of the *cruciferous* (mustard-like) plants possess goitrogens such as thiocyanate and goitrin, which is especially prevalent in *Brassica* family. They interfere with the process of trapping iodine by the thyroid, and their effects can be counteracted by increasing levels of iodine in the ration (Ozmen et al., 2005). Status of phosphorus, magnesium, and copper in the sera of the aborted ewes were significantly lower than the healthy control group ($P<0.05$) which coincide with findings of Unanian and Feliciano-Silva (1984) and Lloyd et al. (1993) in sheep abortions and perinatal lambs deaths. Copper is essential element for ruminants and its deficiencies occur in grazing animals in many parts of the world. Copper deficiency has closely associated with abortion (Sakhaee and Kazeminia, 2011). In this study, the low levels of copper in the aborted animals come in agreement with the reports of Hidiroglou (1979), Unanian and Feliciano-Silva (1984) and Sakhaee and Kazeminia (2011). Zinc, calcium, and vitamin E levels were almost the same in both groups with no significant differences. Zinc deficiencies have been associated with abortion, but naturally occurring zinc deficiency is rare in livestock (Kumar et al., 2011). This observation in the zinc level was confirmed by the results of Hidiroglou (1979) and Pond and Wallace (1986). Results suggested that maternal deficiencies such as hypothyroidism, hypocuprosis, hypophosphatemia and hypomagnesaemia may have been presented as main causative reasons of the late term abortions. When the nutritional deficiency is suspicious as an ingredient of abortion, correcting the nutritional needs in the diet is important. Vitamin and mineral supplementation may be of help to prevent or reduce the incidence of abortion. Probably the most important underlie factor for late term ovine and caprine abortions was due to a recent and concurrent drought in the province. Future studies on the current topic especially for the prevention and management of abortion with the use of feed supplements contains micro and macro minerals are therefore recommended. Another important practical implication is prevention of overcrowding, and stress especially for young heavily pregnant animals. Reduce environmental stress, preventing the spread of diseases, regular vaccination and de-worming program also recommended.

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