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The Distribution and Prevalence Rate of Enterotoxemia in Sheep in East Azerbaijan Province, Northwestern Iran, in Spring 2008

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Abstract: Enterotoxaemic diseases, are the most important diseases caused by *C. perfringens* in ruminants. Ovine enterotoxaemia is of economic importance and has been reported to be produced by all five toxinotypes of *C. perfringens* (A through E) on the basis of its capability to produce major lethal toxins (alpha, beta, epsilon and iota toxins). Although, *C. perfringens* type D has been reported to be the dominant type causing enterotoxemia in sheep in Iran. This study was carried out, in the Northwestern of Iran to assess the distribution of Enterotoxemia and its prevalence rate in sheep at spring 2008. A total of 87802 sheep and lambs, belonging to 38 different large sheep farming zones, with size ranging from 200-8600 sheep from suburb of ten towns of East Azerbaijan province, were examined. The prophylaxis of enterotoxaemia in the animals was achieved by vaccination. Diagnoses of the disease were mainly based on the clinical examinations and classical bacteriological methods. Only in 128 (0.14%) animals with clinical signs of enterotoxemia including sudden death or abnormalities in digestive and/or nervous systems, *C. perfringens* were identified by classical bacteriological examinations. The average case fatality rate in this study was 40% (51/128) and the case fatality rates in each flock ranged between 0 and 80%. Prevalence rates in flocks of Ahar, Shabestar and Heris were highest in this study. No significant histological changes were found in the small or large intestine of any of the animals dying from enterotoxemia. Although, with the exception of only 2 animals that showed renal cortical hemorrhages, neither gross nor histological changes were observed in the kidneys of the animals that were necropsied immediately after death. These results suggested that the prevalence rate of enterotoxemia in sheep in East Azerbaijan province at last spring season was relatively low. However, it is strongly recommended that a regular vaccination schedule should be continued to prevent the occurrence of enterotoxemia in the province.

Key words: Enterotoxemia, clostridium perfringens, sheep, prevalence, east azerbaijan

INTRODUCTION

Most developing countries in the Asia, such as Iran, require improvement to the productivity of their agricultural systems. In Iran, as in neighboring countries, animal production is mostly at subsistence level. Increased productivity of small ruminants, the most important domesticated animals in this region, is restricted by various factors. It may be assumed that, one of the major limiting factors in sheep husbandry is infectious bacterial diseases, such as enterotoxaemia caused by Clostridium perfringens. This spore-forming, anaerobic bacterium is widespread in the environment and is frequently found in the intestinal tract of man and animals. It is responsible for gastrointestinal and enterotoxaemic diseases in animals and for food-poisoning, gangrene and enteritis necroticans in man (Petit *et al*., 1999). *C. perfringens* is a microorganism responsible for several forms of enterotoxaemia, which differs in clinical manifestations and in severity according to the toxigenic type involved and the specific toxins produced (Songer, 1996). *C. perfringens* is classified into five types (A, B, C, D and E) on the basis of the ability to produce four major lethal toxins, namely alpha, beta, epsilon and iota (a, b,e, i), which each cause a specific disease syndrome (Daube *et al*., 1994; Petit *et al*., 1999; Songer, 1996). The classical identification of the toxins is based on neutralization tests in mice or in the skin of guinea pigs (Sterne and Thomson, 1963). In recent years, various enzyme immunoassays have been developed (Naylor *et al*., 1987, 1997; Martin *et al*., 1988; El-Idrissi and Ward, 1992; Younan *et al*., 1994) and molecular methods have been adopted to identify the *C. perfringens* toxinotypes (Daube *et al*., 1994; Buogo *et al*., 1995;

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Uzal *et al*., 1997; Yoo *et al*., 1997). Enterotoxaemic diseases are the most important diseases caused by *C. perfringens* in ruminants. In sheep, enterotoxaemia has been reported to be produced by all 5 toxinotypes of *C. perfringens* (Songer, 1998), though toxinotype D (major toxins alpha and epsilon), the cause of pulpy kidney disease accounts for the largest proportion of losses due to *C. perfringens* (Radostitis *et al*., 2007). *C. perfringens* toxinotype A (major toxin alpha) causes enterotoxaemia, or yellow lamb disease, which occurs primarily in the western United States (McGowan *et al*., 1958). Young ewes and other adult sheep can also develop type C (major toxins alpha and beta) enterotoxaemia, known as `struck' (Sterne and Thomson, 1963). Enterotoxaemia in ruminants caused by type B (major toxins alpha, beta and epsilon) was described by Frank (1956) and *C. perfringens* toxinotype E (major toxins alpha and iota) is a rather uncommon cause of enterotoxaemia in small ruminants (Songer, 1998). The spores of *C. perfringens* types A, B, C and D are found in the soil and faces of healthy animals in areas where the disease is widespread as well as in the intestinal contents of animals with disease. Fatal infections may occur when predisposing factors allow rapid multiplication of *C. perfringens* in gut and the powerful toxins are released. Ovine enterotoxaemia is of economic importance in temperate climates. In the tropics, *C. perfringens* enterotoxaemia arises mainly at the beginning of the rainy season, associated with an oversupply of young fresh fodder plants (Songer, 1998).

Enterotoxemia caused by *C. perfringens* types D is worldwide in its distribution (Radostitis *et al*., 2007). Although, the disease has been recognized from 1950 in sheep in Iran by Razi Institute specialists (Tabatabayi and Firouzi, 2000), with attention to the high population of sheep and importance of the disease in the country especially Northwestern areas, such as Azerbaijan province, there have been a little investigations about distribution and epidemiology of the disease in such areas.

The present study was conducted to evaluate distribution and prevalence rate of *C. perfringens* infections in sheep at spring 2008 in East Azerbaijan province in Iran. In this study distribution and prevalence rate of enterotoxemia in sheep and lambs, occurring in 38 large sheep farming areas at surrounding of ten cities in East Azerbaijan province, the northwestern of Iran, were investigated.

MATERIALS AND METHODS

Animals and study design: A total of 87802 sheep and lambs 2 days to 5 years of age, were included in this study. The animals belonged to 38 different areas of 200-8600 animals, from suburb of the ten small or large towns of East Azerbaijan province, northwestern Iran (Tabriz, Ahar, Marand, Sarab, Malekan, Shabestar, Kaleibar, Heris, Ajabshir and charoymag). Geographical position of the towns in the province has been showed in Fig. 1. All the animals were subjected to regular vaccination against clostridial diseases with commercial vaccines, according to manufacturer's instructions. All the animals were surveyed, on the enterotoxemia disease during three months in the spring of 2008. Diagnoses of the disease were mainly based on the clinical findings and bacteriological examinations. A variety of clinical signs such as abdominal pain, bloat, mild to severe (blood-tinged to bloody) diarrhea, recumbency were the suspected clinical findings. As well as in some animals nervous symptoms (dullness, muscular tremors, opisthotonos and convulsions) were observed, while in other cases death was the only clinical sign observed. Samples for microbiological examination were taken from animals, which displayed the mentioned clinical signs.

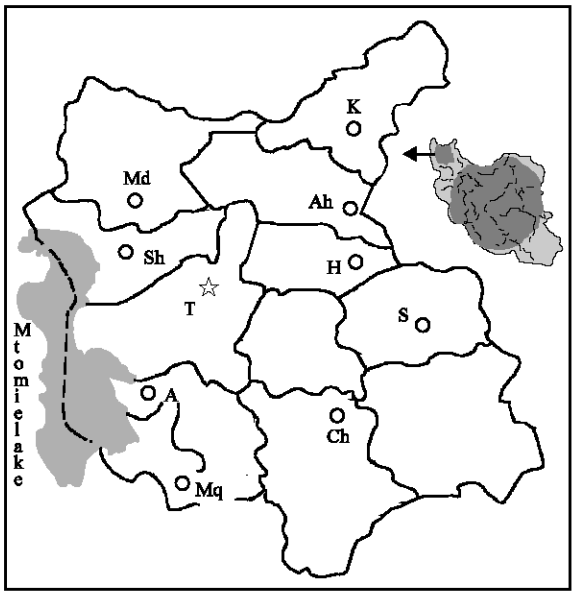

Fig. 1: Geographical map of the East Azerbaijan province, which is placed in the northwestern of Iran. Positions of Towns, which were surveyed on the presence of enterotoxemia in their sheep flocks are shown in the map. (T: Tabriz, Ah: Ahar, Md: Marand, S: Sarab, Mq: Malekan, Sh: Shabestar, K: Kaleibar, H: Heris, A: Ajabshir and Ch: Charoymag)

Bacteriological investigation: Gram-stained smears were prepared for direct microscopic examination from faecal samples or by scraping the intestinal mucosa from the

animals, which showed the mentioned clinical signs or suddenly dead ones. Additionally, samples were aseptically obtained from liver, lungs, kidneys, heart, small (ileum) and large intestine (colon) contents from some of the suddenly died animals, kept on ice and cultured within 1 h on 5% sheep blood agar and incubated under anaerobic conditions for 24 h at 40°C. Following macroscopic and microscopic evaluation isolates confirmed as *C. perfringens* (Younan *et al*., 1994) were cultured in modified Gordon medium (Gordon *et al*., 1957) and the toxin pattern (beta and epsilon) was subsequently determined by EIA in some of them (Naylor *et al*., 1987; Martin *et al*., 1988).

Histopathology: Necropsy was performed on the animals, when possible, within 6 h of death. Samples of the duodenum, ileum, colon, liver, spleen, kidney and lung were collected and fixed in 10% neutral buffered formalin for 24-72 h. Fixed samples were dealcoholised after dehydration and luminosity. Blocks were embedded in paraffin wax, sectioned at 5 mm, stained with hematoxylin and eosin and finally evaluated under a light microscope.

RESULTS AND DISCUSSION

In this study, 123 sheep and lambs were totally recognized as the affected animals with enterotoxemia on the basis of clinical findings and bacteriological investigation. Thus, prevalence rate of the disease in sheep in the East Azerbaijan county was 0.14% (128/87802) (Table 1).

Different studied epidemiological areas, number of evaluated sheep in each of them, number of affected animals and prevalence rates in each area are described in Table 2.

Enterotoxaemia caused by *C. perfringens* depends not only on the presence of the bacteria, or more precisely their toxins, but also on other factors that alter the balance in the digestive tract. After a sudden shift to food rich in proteins and/or carbohydrates, undigested items of high nutritional value reach the small intestine. This creates optimal conditions for growth of *C. perfringens*, which may eventually produce large quantities of toxins that may lead to an outbreak of fatal per acute toxicosis (Barker *et al*., 1993).

The pathogenicity of *C. perfringens* is closely related to the production of major lethal toxins (alpha, beta, epsilon and iota toxins) and other toxins, including enterotoxin (Ozturk, 1996). The patterns of production of the toxins are different, depending on the *C. perfringens* type. Therefore, the patterns have been used to type the bacterium into types A, B, C, D and E. There were geographical differences in the prevalent types of the bacterium. Also, the type could be different depending on the animals' species in the area (Cho *et al*., 1990). Possible prevention and control strategies for enterotoxaemia should be based on combined immunoprophylaxis and management strategies that avoid abrupt changes in sheep husbandry (Blobel and Schliesser, 1995).

Existence of enterotoxemia in Iran was discovered the years after 1945. Firstly, it occurred in foreign breed sheep in Heidarabad husbandry institute. To date the disease is almost prevalent in most cities of the country (Tabatabayi and Firouzi, 2000). The presence of *C. perfringens* in the fasces of sheep in East Azerbaijan was concerned. Because sheep die per acutely of enterotoxaemia and since dead animals are used immediately as food by the local population, it was not possible to get more samples from diseased animals.

In the present study, 128 (0.14%) samples tested by clinical examinations and microbiological culture were

Table 1: Prevalence rate of enterotoxemia in sheep in East Azerbaijan province in the spring of 2008

	No. of sheep	No. of positive (f)	Prevalence rate (%)
Examine for enterotoxemia[a]	87802	123	0.14

[a]On the basis of clinical signs and bacteriological culture

Table 2: Distribution of evaluated sheep zones in different towns, number of surveyed areas pertaining to the towns, number of animals affected with enterotoxemia based on clinical signs and culture results and local and total prevalence rates of enterotoxemia in the study

Town	No. of studied areas	Sum no. of sheep populations	No of positive animals[a]	No of rapidly died animals	No of animals with clinical signs[b]	Prevalence rate (%)
Tabriz	2	8724	5	3	2	0.057
Ahar	6	8700	48	17	31	0.550
Marand	6	24700	21	10	11	0.080
Sarab	4	7250	7	5	2	0.096
Malekan	1	4500	4	2	2	0.090
Shabestar	8	9793	16	4	12	0.160
Kaleibar	3	6700	3	0	3	0.040
Heris	5	9435	16	6	10	0.170
Ajabshir	1	4000	3	0	3	0.080
Charoymag	2	4000	5	4	1	0.013
Total	38	87802	128	51	77	0.140

[a]Results of culture, [b]Abdominal pain, bloat, mild to severe diarrhea, recumbency and nervous symptoms such as dullness, muscular tremors, opisthotonos and convulsions

positive for enterotoxemia caused by *C. perfringens* type D, during spring season in 2008 (Table 1 and 2). Enterotoxemia caused by *C. perfringens* types D is a disease of ruminant animals primarily of lambs and is worldwide in its distribution (Radostitis *et al*., 2007). The common practice of vaccination against this disease will reduce its prevalence. The prevalence of the disease in flocks varies a great deal but seldom exceeds10% (Radostitis *et al*., 2007). Reports from countries around the world have reported prevalence rates of enterotoxemia ranging between 1 and 100% (Greco *et al*., 2005; Radostitis *et al*., 2007). In a survey in North America in 2 feedlots, the disease had an annual prevalence of 3.14 and 1.46% (Radostitis *et al*., 2007). In Turkey, the prevalence of enterotoxemia has been reported to be range between 38.63 and 50%. In these studies, various results were obtained and type D was reported to be the most dominant *C. perfringens* type causing enterotoxemia in sheep (Ozturk, 1996; Ozcan and Gurcay, 2000). These differences may be the consequence of environmental factors, host potencies and control efforts. At current spring the amount of the raining was too low to adequately grow of all type of fodder and grass in pastures in approximately all areas of East Azerbaijan thus, it may be one of the reasons of relatively low prevalence rate of the enterotoxemia in this survey. On the other hand, the occurrence of disease is associated with highly nutritious diets (Radostitis *et al*., 2007) thus, presence of lowly nutritious diets in pastures and farms because of the low amounts of raining in the spring was another reason of relatively low prevalence rate of the enterotoxemia in this survey.

Enterotoxemia ranked third in importance as a cause of death despite a policy of vaccination and the costs of prevention programs were the largest expenditure of all disease prevention programs in the feedlots. The highest incidence of the disease is in suckling lamb between 3 and 10 weeks of age. The risk for disease in this age group is highest when ewes are grazed on lush pastures that result in profuse lactation. The disease can occur following rain in set stocked flocks and in flocks newly introduced to lush pastures is often manifest 5-14 days after introduction (Radostitis *et al*., 2007).

Enterotoxemia causes more economic loss among feedlot- and pasture-reared lambs than all other diseases combined, if vaccination is not applied (Songer, 1996). Fortunately, in East Azerbaijan province, most sheep farmers strictly vaccinate their ewes against enterotoxemia and more than half of the owners that do vaccinate give a double dose of vaccine. Nonetheless, it is well known that initial vaccination should be followed by a booster 4-6 weeks later in order to promote protective immunity (De la Rosa *et al*., 1997). Additionally, vaccination of pregnant ewes is also important for transferring the passive immunity through colostrums to the newborn lambs. Therefore, the vaccination programs against enterotoxemia in sheep are most probably another reason for the low incidence of the disease in East Azerbaijan. Furthermore, *C. perfringens* type D has been reported to be the dominant type causing enterotoxemia in sheep in Iran; On the other hand, to date, most vaccines prepared and administered to immunize sheep against type D, which may also be another reason for the low incidence of the disease in East Azerbaijan.

It is well known that enterotoxemia causes considerable economic loss to the sheep industry due to a high fatality rate, decreased productivity and increased treatment costs (Greco, 2005). The average case fatality rate in this study was 40% (51/128) and the case fatality rates in each flock ranged between 0% (in Kaleibar and Ajabshir) and 80% (In Charoymag) (Table 2). In literatures, the case fatality rate of the enterotoxemia may reach up to 100% (Radostitis *et al*., 2007).

Prevalence rates in flocks of Ahar, Shabestar and Heris were highest in this study. On the other hand, the evaluated flocks in these cities have high intensity. This suggests a high hazard from all of toxinotype in places where animals are concentrated (Aschfalk *et al*., 2002). Moreover, hygienic procedures aren't applied in flocks of mentioned areas especially in Ahar and Heris. This emphasizes the hygienic danger from transporting animals and the introduction of animals from other places and in places where livestock is gathered (Aschfalk, 2002). In addition, the winter season in last year was very long in Ahar state and flocks are kept indoors for more than 6 months. During the spring, plants grow very fast in pastures and the flocks are put out to pasture and graze large amounts of green plants in a short period. In contrast, summer is very short and cereal crops are harvested in a short period. Grazing animals in the pastures are moved to these fields and allowed to consume large amounts of grain, mostly wheat and barley, in a short period of time, which are well-known predisposing factors for enterotoxemia in sheep (Songer, 1996). Therefore, consuming nontolerable amounts of lush graze and grains in a short period of time may play a role in the relatively high occurrence of enterotoxemia in these areas. In the present study, the mortality rate in the flocks ranged between 0 and 0.2%. Therefore, the result of the present study suggests that uncontrolled disease may cause major economic loss to the sheep industry in East Azerbaijan, where 1000,000

sheep are present. On the other hand, economic loss caused by clostridial diseases can be prevented with proper management and vaccination timing (Songer, 1998). Thus, it can be suggested that this proper vaccination programs maintain to provide strong immunity against both type A and D in the sheep of East Azerbaijan province.

No significant histological changes were found in the small or large intestine and other organs of any of the animals dying from enterotoxemia. This finding agrees with previous observations indicating that no major intestinal changes occur in sheep enterotoxemia (Bullen and Batty, 1957). It has been demonstrated experimentally that when epsilon toxin is inoculated into legated intestinal loops of sheep, no morphological alterations are produced in the small intestine, whereas severe inflammatory changes are produced in the colon (Fernandez and Uzal, 2002). It has been suggested that in cases of enterotoxemia in sheep, epsilon toxin is readily absorbed in the small intestine thus not reaching the colon in concentrations high enough to produce lesions (Uzal and Kelly, 1998). Clostridium perfringens type D enterotoxemia has been called historically pulpy kidney disease (Radostitis *et al*., 2007) and the changes in the kidneys of sheep dying from this disease have been called nephrosis (Barker *et al*., 1993). However, in the present study, with the exception of only 2 animal from group Ahar that showed renal cortical hemorrhages, neither gross nor histological changes were observed in the kidneys of the animals that were necropsied immediately after death, which supports the hypothesis that the so-called pulpy kidney lesion is a postmortem phenomenon. The assertion that pulpy kidney disease is a postmortem phenomenon suggests that epsilon toxin accelerates the postmortem decomposition of kidneys. Although, this is possible, it has not been proven and no information is available in the literature comparing the speed of autolysis in animals with enterotoxemia and animals dying from other causes. But even if this was proven to be true (i.e., that autolysis is accelerated in animals dying from enterotoxemia) the evaluation of the speed of autolysis is a very subjective parameter of little, if any at all, diagnostic value. Histology of the kidney should, therefore, not be considered as a diagnostic indicator in sheep enterotoxemia. In this study, excess pericardial fluid with or without fibrin strands was found in 30 of 51 animals dying of enterotoxemia. This change, when present, would support a presumptive diagnosis of enterotoxemia in sheep. In this study, glucose was not measured in pericardial or other bodily fluids.

CONCLUSION

It seems that the prevalence rate of enterotoxemia in sheep in East Azerbaijan province at last spring season was relatively low, which may be due to a common practice of vaccination against the disease and lack of adequately raining. However, it is strongly recommended that a same regular vaccination schedule be implemented to prevent the incidence of enterotoxemia in the province. This vaccine should provide adequate protective immunity, especially against *C. perfringens* type A and D.

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