



The prevalence of bovine Theileria amongst the cattles in the cattles farm of Borujency

Yaser Karimi Faradonbeh^{*1}, Morvarid Karimi Faradonbeh²,
Azarnushkarimi dastgerdi³, Azam malekmohammadi³

¹Graduated of Veterinary Medicine Faculty, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

²Student of Veterinary Medicine Faculty, Shahrekord Branch, Shahrekord University, Shahrekord, Iran

³Graduated of Veterinary Medicine Faculty, Shahrekord Branch, Shahrekord University, Shahrekord, Iran

Article published on April 30, 2017

Key words: Theileriosis, Piroplasm form, Cows, Borujen

Abstract

The current research intended to investigate the prevalence of bovine Theileria amongst the Cattles, in the cattle farm of Borujen city. To this end, 360 cows were studied, during December 2013 - December 2014, of which 22 cows were infected with Theileria Parasites. At the beginning, before entering any cattle farm, a questionnaire (Table 1) was designed. The questionnaire included items about the Cattles' sex, age, race, season and previous involvement with the disease. Next, in each season at the end of each period, samples of peripheral blood (blood samples from ear vessels) were collected from 90 Cattles and transferred to the laboratory. After preparing a blood smear and fixation by Methanol Alcohol, Giemsa Staining and Microscopic inspection of the smears of peripheral blood, the samples containing the Piroplasm forms of Theileria were recorded as positive samples. This study examined 4 different races, including Holstein, Brown Swiss, Hybrid and Native cows, which were classified into three groups of ageas 'under 1 year', '1-3 years' and 'above 3 years'. SPSS₁₉ statistical software as well as Excel₂₀₁₀ were used for research data analysis. Accordingly, the obtained results indicated that the most typical symptom of this disease was Swollen Lymph Nodes while Diarrhea was the least clinical symptom of the disease. Nevertheless, the highest degree of infection with the disease was related to the age group "under 1 year." Furthermore, the degree of infection was higher in the summer and in Holstein race in comparison to other cases, connoting a significant relationship.

*Corresponding Author: Yaser Karimi Faradonbeh ✉ Yaser.karimi688@gmail.com

Introduction

The phylum Apicomplexa comprises a large group of complex eukaryotic organisms known to be obligate parasites of vertebrates and invertebrates. These organisms share a common characteristic of having an apical complex which contains secretory organelles considered to be involved in invasion and/or establishment of the parasite in the mammalian or invertebrate host (Bishop *et al.*, 2004). The phylum is divided into four principal groups; the Coccidia, Gregarinasina (gregarines), Haemospororida (haemosporidians) and the Piroplasmorida (piroplasmids) (Adl *et al.*, 2012). The Piroplasmorida comprises two main genera (*Babesia* and *Theileria*) responsible for the economically important diseases of domestic and wild animals. New species of the piroplasmids are still being discovered and their full biology is not completely documented yet. Many of this order's parasites were formerly classified based on morphology, host cells in which schizogony occurs, the observation of piroplasms in the red blood cells associated with disease manifestation and host-vector specificity (Barnett, 1977). The genus *Theileria* is distinguished by infection of leukocytes by sporozoites, maturation of schizonts into merozoites and subsequent infection of red blood cells to form piroplasms (Barnett, 1977).

The *Theileria* species infect a wide range of both domestic and wild animals and are transmitted by ixodid ticks of the genera *Amblyomma*, *Haemaphysalis*, *Hyalomma* and *Rhipicephalus*. Most of these ticks are renowned for the large economic losses they cause to the agricultural industry due to disease outbreaks, mortalities, damage to hides and poor production in domestic animals (Bishop *et al.*, 2004). The expansion of wildlife husbandry and conservation has also made *Theileria* of wildlife important subjects of study. The *Theileria* can be grouped into schizont “transforming” and “non-transforming” species (Sivakumar *et al.*, 2014). Transforming parasites all group in the *T. taurotragi* clade (Sivakumar *et al.*, 2014), and uncontrolled proliferation of schizonts results in the pathologies associated with Corridor disease (*Theileria parva*), East Coast fever (*T. parva*),

Tropical theileriosis (*T. annulata*) in cattle and malignant theileriosis (*T. lestoquardi*) in goats and sheep (Bishop *et al.*, 2004; McKeever, 2009)

In eastern, central and southern Africa, a disease of major interest in this context is caused by infection with *Theileria parva*, an apicomplexan protozoan parasite which is transmitted by the brown ear tick (*Rhipicephalus appendiculatus*). *T. parva* infects both cattle and the African buffalo (*Syncerus caffer*), although it is believed that the parasite evolved with the buffalo in eastern Africa long before the introduction of cattle (Uilenberg, 1981). There are very few, if any, clinical signs in infected buffalo, whereas in cattle the parasite causes a severe, often fatal, lympho-proliferative disorder called East Coast fever (ECF). ECF results in considerable economic losses as a consequence of high mortality rates and the inability of livestock owners to introduce the more productive but highly susceptible European breeds of cattle into enzootic areas (Ben J. Mans *et al.*, 2015)

Theileria is transmitted to the Cattle by the bites of a tick named *Rhipicephalus* (Hayashida *et al.*, 2012). The most important prevention methods and immunization of Cattle against this disease are by hygiene, using a suitable byer for keeping Cattle, spraying Cattle sheds, flaming and fighting against ticks.

It is recommended that the site of Cattle be made of stone, cement and iron coated with cement plasters. Collecting the dung and manure of the Cattle, covering them and regularly shipping them out of the Cattle's site, as well as collecting and removing the straws and the residual food of the Cattle are also important. The more the light is allowed in the site of the Cattle, the less it is contaminated and infected with *Theileria*. Regular spraying of the Cattle's site against ticks by anti-tick poisonous serum in proper time and seasons is very important in the prevention of disease.

Another prevention method is Vaccination against Theileriosis; the vaccine is normally stored in liquid Nitrogen to -79°C. The vaccine is injected to non-pregnant Cattle in cold seasons (winter) when ticks are not active and immunizes the site for about one year.

Materials and methods

For the purpose of the current research, which was done from December 2013 to December, 360 cows

were studied in Borujen. At the beginning, before entering any cattle farm, a questionnaire (Table 1) was designed.

Table 1. The questionnaire

Row	gender	Clinical signs	Age	Season	Strain	Previous collision with disease
		Swollen Lymph Nodes				
		of Pale Mucous Membranes				
		Jaundice	≤1 years	summer	Brown Swiss	Unexposed
		Diarrhea	1-3 years	autumn	Holstein	Once exposure
			≥3years	Winter	Brown Swiss	Double Exposure
			Spring		Hybrids	
					Native	

The questionnaire included items about the Cattles' sex, age, race, season and previous involvement with the disease as well as a series of supplementary items including body temperature, respiration and heart rate. Next, in each season at the end of each period, samples of peripheral blood (blood samples from ear vessels) were collected from 90 Cattles and transferred to the laboratory. After preparing a blood smear and fixation by Methanol Alcohol, Giemsa Staining¹ and Microscopic inspection of the smears of peripheral blood, the samples containing the Piroplasm forms of Theileria were recorded as positive samples.

This study examined 4 different races, including Holstein, Brown Swiss, Hybrid and Native cows,

1 - Giemsa stain is used to differentiate nuclear and/or cytoplasmic morphology of platelets, RBCs, WBCs, and parasites. The most dependable stain for blood parasites, particularly in thick films, is Giemsa stain containing azure B. Liquid stock is available commercially. The stain must be diluted for use with water buffered to pH 6.8 or 7.0 to 7.2, depending on the specific technique used. Either should be tested for proper staining reaction before use. The stock is stable for years, but it must be protected from moisture because the staining reaction is oxidative. Therefore, the oxygen in water will initiate the reaction and ruin the stock stain. The aqueous working dilution of stain is good only for 1 day

which were classified into three groups of age as 'under 1 year', '1-3 years' and 'above 3 years'. SPSS₁₉ statistical software as well as Excel₂₀₁₀ were used for research data analysis.

Results and discussion

From 360 Cattles under study, 22 sample cows were infected with Theileria Protozoan. The symptoms including 11 cases of Swollen Lymph Nodes, 10 cases of Pale Mucous Membranes, 4 cases of Jaundice and 1 case of Diarrhea were reported to be most prevalent in Cattles. Nonetheless, the highest degree of infection was related to the age group "under 1 year", in Holstein race and in the summer in comparison to other cases, connoting a significant relationship. On the contrary, native races as well as the age group 'above 3 years' were recorded to be the least infected cases.

Table 2. Clinical symptoms observed in the cattles Suffering from Theileria in Borujen.

	Swollen Lymph Nodes	of Pale Mucous Membranes	Jaundice	Diarrhea
Frequency	11	10	4	1
Frequency Percent	42/3	38/46	15/38	3/86

According to Table 2, the most typical symptom of Theileria is Swollen Lymph Nodes; whereas, Diarrhea is the least common clinical symptom.

Table 3. The degree of infection with Theileria protozoan in the cattles

	Total studied animals	Infected animals
Frequency	360	22
Frequency Percent	100	6/11

Table 3 shows that from all the 360 Cattles studied in this research, 22 cases (% 6.11) were infected with Theileria Parasite.

Table 4. The degree of infection with Theileria Annulata in both male and female sexes of the Cattles

	Total infected animals	Male animals infected	Female animals infected
Frequency	22	7	15
Frequency Percent	100	31/82	68/18

As presented in Table 4, amongst the infected Cattles, 7 cases (%31.78) were male while 15 cases (%68.18) were female cows. It was concluded that there was not any statistically significant relationship between the sex of the cows and the infection with parasite ($P \leq 0.5$).

Table 5. The degree of infection with Theileria protozoan in terms of age

	≤ 1 years	1-3 years	≥ 3 years
Frequency	13	5	4
Frequency Percent	59/1	22/72	18/18

The results of Table 5 illustrates that the highest degree of involvement with Theileria was in the age group "under 1 year" while the age group "above 3 years" had the lowest degree of infection. There was not any statistically significant relationship between the age of the cows and the degree of infection ($P \leq 0.5$).

Table 6. The Degree of infection with Theileria protozoan in terms of season.

	Spring	summer	Autumn	Winter
Frequency	6	14	2	0
Frequency Percent	27/27	63/63	9/1	-

According to Table 6, summer was reported to have the most cases of infection with Theileria. On the contrary, no reports on the cases of infection with Theileria in winter were recorded. Thus, there is a statistically significant relationship between the incidence of Theileria and different seasons of the year ($P \leq 0.5$).

Table 7. The degree of infection with Theileria protozoan in terms of race

	Holstein	Brown Swiss	Hybrids	Native
Frequency	11	6	4	1
Frequency Percent	50	27/27	18/18	4/55

Table 8. The degree of infection with Theileria protozoan in terms of the type of previous involvement with the disease

	Unexposed	Once exposure	Double Exposure
Frequency	21	1	0
Frequency Percent	95/45	4/55	-

In the study of Shahqolian *et al*, it was indicated that the highest degree of infection was observed in May and June in Shahrekord, and that sex does not have any significant effect on this disease (Brown., 1990).

Doroudchi *et al* found that Theileriosis caused by T. Annulata is one of the prevalent disease in tropical and subtropical regions; they concluded that the highest degree of involvement with this protozoan was mostly in June and July (Doroodchi., 1363).

Tutushin found that the highest degree of infection with Theileria was associated with June and July in Kazakhstan (Tutushin., 1985).

In their study in Zahedan, Mozafarri *et al* found that the highest degree of infection occurred in summer while winter had the lowest degree of infection. They reported that the most susceptible race was Holstein whereas Indigenous (Native) races were the most resistant ones (Young *et al.*, 1988). Furthermore, since summer had the highest degree of incidence of the Theileria, it was concluded that there was a

statistically significant relationship between the occurrence of the disease and different seasons of the year.

According to the findings of Brown *et al.*, Holstein was the most vulnerable race to Theileria Protozoan; On the contrary, indigenous (native) races were less sensitive to the disease, or if any, they show the least clinical symptoms (Tutushin *et al.*, 1985). Nevertheless, they found that the most susceptible and the most resistant races were respectively Holstein and Indigenous races. Therefore, there was a statistically significant relationship between the occurrence of Theileria and different races.

Hayashida *et al.* found that indigenous races infected in endemic areas may either be able to endure the disease or suffer from a mild disease under clinical supervision. Whereas, Non-indigenous (Non-native) cows were more sensitive and vulnerable and showed severe symptoms or even die in case of catching the disease (Hayashida *et al.*, 2012).

In this study done in Borujen, it was indicated that the most typical symptom of this Theileria was Swollen Lymph Nodes while Diarrhea was the least observed clinical symptom of the disease. Furthermore, Cattles aged under 1 year had the most degree of involvement with the disease in comparison to the other age groups.

One of the main points of the present research was the degree of infection with Protozoan Theileria in terms of the previous involvement of the Cattles with the disease. Accordingly, the results showed that Cattles which experienced infection with Theileria, for the first time, were less resistant to the disease. On the contrary, Cattles with previous involvement with Theileria had shown a relative resistance that reduce the degree of infection; this implies a significant relationship between the degree of infection with Theileria protozoan and Cattles' previous involvement with the disease.

References

AdISM, Adl AG. 2012. The revised classification of eukaryotes J. Eukaryot. Microbiol 429–493.

Barnett SF, Kreier JP. 1977. Parasitic Protozoa, vol. IV, Academic Press, New York, USA 77–113.

Bishop R, Bishop A, Musoke S, Morzaria M, Gardner V. 2004. Nene Theileria: intracellular protozoan parasites of wild and domestic ruminants transmitted by ixodid ticks Parasitology 271–283.

Brown GD. 1990. Control of tropical Theileriosis of cattle. Veterinary Parasitology, 31–36.

Doroodchi MM. 1363. Evaluation of serum proteins in infected cattle. Finally, a Doctor of Veterinary Medicine, Tehran University, pages 25–20.

Florin M, Schnittger L. 2009. Piroplasmids and ticks a long-lasting intimate relationship. Frontiers in bioscience. A journal and virtual library 14, 3064–3073.

Gharavi MJ. 2003. Clinical Parasitology. 3th Ed. Tehran. Teimorzahed Publisher: 91–8.

Hayashida K, Hara Y, Abe T, Yamasaki C, Toyoda A, Kosuge T, Suzuki Y, Sato Y, Kawashima S, Katayama T, Wakaguri H, Inoue N, Homma K, Tada Umezaki M, Yagi Y, Fujii Y, Habara T, Kanehisa M, Watanabe H, Ito K, Gojobori T, Sugawara H, Imanishi T, Weir W, Gardner M, Pain A, Shiels B, Hattori M, Nene V, Sugimoto C. 2012. Comparative genome analysis of three eukaryotic parasites with differing abilities to transform leukocytes reveals key mediators of theileria-induced leukocyte transformation. MBio 3(5).pii: e 12-00204.

McKeever DJ. 2009. Bovine immunity – a driver for diversity in Theileria parasites? Trends Parasitol 269–276.

Sivakumar T, Hayashida K, Sugimoto C, Yokoyama N. 2014. Evolution and genetic diversity of Theileria Infect. Genet. Evol 250–263.

Tutushin MI. 1985. Distribution and seasonal and age variation of bovine theileriosis in the south of Kazakhstan Veterinary Bulletin 55, 1482.

Uilenberg G, Irving AD, Cunningham MP, Young AS. 1981. Advances in the Control of Theileriosis, Martinus Nijhoff Publishers, The Hague, Boston, London 4–37.

Young AS, Groockok CN, Kariuki DP. 1988. Integrated control of tick and tick borne disease of cattle. *Africa parasitology* **96**, 403-411.