



Theileria Annulate Mildly Virulent Strain Isolation For Creation of an Anti-Theileria Vaccine

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Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 14 Oct 2023	<i>It is established that it takes place the wide circulation of horned cattle theileriosis in the areas of Samarkand, Navoi, Djisak regions. Which differ with its environmental conditions? At the same time there is distinguished a low virulent strain from the Jf Samarkand region, which is suitable for manufacturing vaccines against theileriosis.</i>
CC License CC-BY-NC-SA 4.0	Keywords: Cattle, Theileriosis, Strain, Mites, Parasitic, Crobanr, Cryopreservation, Culture, Prevention, Parasitic Reaction

1. Introduction

The primary responsibility of livestock breeders is to supply the 35 million people of the Republic of Uzbekistan with a consistent and adequate supply of high-quality, secure animal products as well as the raw material sector. In Uzbekistan right now, there are around 14 million cattle. The main objective of veterinary science and practice is to protect this population of animals from parasitic and infectious illnesses. Various viral and parasitic illnesses cause substantial losses and a decline in animal output despite the extensive efforts made to combat them. Theileriosis in cattle is the invasive illness that has the biggest economic impact. By employing different enzymes to clean up tissues, advancements have been made in the technique for cultivating Theilerian cells. As a result, it was feasible to acquire a monolayer primary trypsinized culture of the invading *Theileria annulata*'s lymphoid cells.

The VIEV (Institute of Experimental Veterinary Medicine) anti-theileriosis vaccine demonstrated high reactogenicity, and during the vaccination of animals, one to 12% of sick animals with post-vaccination complications were isolated. In addition, this vaccine became unavailable to us as a result of the fall of the Soviet Union and currency dependence. This makes the creation of a liquid, domestic anti-theileria vaccine from a strain of the disease called *Theileria annulata*, which is only moderately virulent, a pressing issue. Theileriosis in cattle is the most pernicious illness, and the ixodid ticks *Hyalomma anatolicum* and *H. detritum*, which are common throughout the Republic, are the disease's vectors.

In light of this, it is crucial for the creation of the *Theileria* vaccine that a weakly virulent strain of *Theileria annulata* be isolated from several climatic regions (1). Therefore, it is crucial to design an anti-theileriosis vaccination based on a locally prevalent, minimally pathogenic strain.

Goals

Using this information as a foundation, the study's objective is to identify less pathogenic *Theileria annulata* strains from diverse climates that are ideal for the creation of an anti-*Theileria* vaccine.

Tasks

1. To investigate the epidemiology of theileriosis in cattle under various climatic and geographic situations in the Jizzakh area villages of Mugol, Bulungur, Samarkand, Istiqbol, Karmana, Navoi, and Oltinsoy.

2. Investigating the level of animal parasitism in the investigated farms.

3. Isolate a sparingly pathogenic strain of *Theileria annulata* from several habitats, including animal husbandry. to register and validate the strains, investigate their pathogenetic, hematological, and morphological characteristics, and develop a strain cryobank.

3. Results and Discussion

Studies on the epizootological situation of theileriosis were conducted in a variety of natural and climatic zones, farms, and settlements that varied in the quantity and sophistication of animal husbandry, including the Istiqbol farms in the Karmaninsky district of the Navoi region, the Mugol settlement in the Bulungur district of the Samarkand region, and the farm "Oltinsoy" in the Rashidov district of the Jizzakh region. Smears of peripheral blood from 50 heads of cattle were obtained in order to find parasitism on each farm. The Romanovsky Giemsa procedure was used to stain the smears, and then the samples were inspected under microscopes. conducted clinical trials on animals that were ill with theileriosis on their own and collected blood for study to find the theileria. Additionally, tick vectors were collected, and their family and *Theileria* infection were identified.

According to the research, the invasion rate of cattle is 7% in the Samarkand region, 6% in the Navoi region, and 12% in the Jizzakh region. Field isolates were obtained to explore the biological, pathogenic, and virulent characteristics of pathogens from spontaneously ill animals from each farm under investigation and cryopreserved. According to research done to ascertain the ratio of tick-carrying species, in the Samarkand region's natural and pasture settings, *Hyalomma anatolicum* ticks make up 98% of the population, *H. detritum* ticks 2%, in the Navoi region they are respectively 95 and 5%, and in the Jizzakh region they are 94 and 6%.

The Samarkand region's land experiences a temperate climate. The Nurata, Turkestan, and Zeravshan mountains surround the area on three sides, with the average yearly temperature being +16.5 °C, the average January temperature being 0.2 °C, and the average July temperature being 27 °C. Temperate, rainy, and chilly weather are greatly influenced by mountain ranges. It is clearly a continental climate with strong seasonal variations, in contrast to the climate of the Navoi and Jizzakh areas. Temperatures can get as high as 40 °C in the summer and as low as -15 °C in the winter. cold humidity makes it easy to handle both hot and cold temperatures.

At the age of 9 to 11 months, three groups of animals, each with three heads, were used in experiments to examine the pathogenic, virulent, and morphological qualities of pathogens as well as the hematological characteristics in theileriosis. Using a dosage of 20 ml administered subcutaneously, the first group of animals were exposed to the isolated strain from the Samarkand region, which has a gentler and more humid environment, and the second through third groups to the hotter and drier climate of the Navoi and Jizzakh regions.

Clinical, parasitological, and hematological tests were carried out daily and every 3–4 days on experimental animals. According to the studies, the incubation period for theileriosis in animals exposed to the isolated *Theileria annulata* strain from the Samarkand region was 20 days, compared to 16 days for those exposed to the theileriosis caused by the isolated strain from the Navoi and Jizzakh regions (Table 1).

Table 1: Pathogenic properties of *Theileria annulata* isolated from various natural and climatic conditions

Gr.	Kolgol	Method of infection	16 days after infection		18 days after infection		20 days after infection	
			T°C	A parasite. Reaction.%	T°C	A parasite. Reaction.%	T°C	A parasite. Reaction.%
1	3	The isolated strain from Samarkand region	39,6	-	39,7	-	40,4	2+-0,02
2	3	The isolated strain from the Navoi Region	40,5	2+-0,02	41,1	4+-0,04	41,6	12+-0,03

3	3	Selected strain from Jizzakh region	40,6	3+-0,02	40,9	5+-0,02	41,3	9+-0,02
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We may thus draw the conclusion that the theileria strains obtained from the Samarkand region have a comparatively low pathogenic potential. According to morphological research, the percentage of *Theileria annulata* isolated from the Samarkand region that is in the oval form is 40.3%, compared to round, 26.7%, comma-shaped, 20.3%, punctate, and missing cruciform. Pathogens from the Jizzakh region have the following forms: oval 35.3%, round 26%, comma-shaped 26.6%, punctate 14%, and cruciform 1%. Pathogens from the Navoi region have oval 34.6%, round shapes 21.6%, comma-shaped 27%, punctate 14%, and cruciform 2% (Table 2).

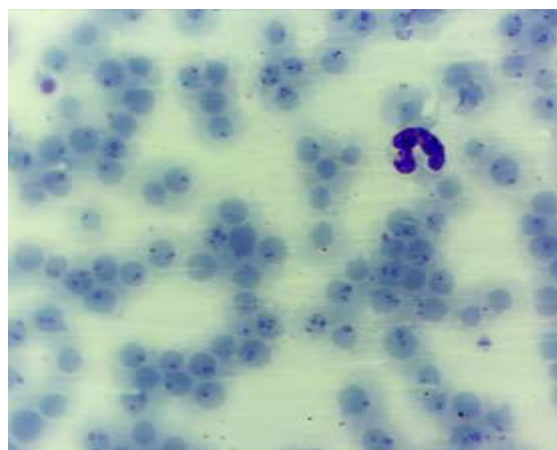


Figure 1: Parasitic reaction and morphological indicators of theileria taken from smears from animals infected with the Navoi strain

Table 2: Morphological indicators of *Theileria annulata* isolated from different climatic and geographical conditions of the Republic

Region	Animal numbers	Morphological indicators of the causative agent of theileriosis				
		Round	Oval	Commas	Punctate	Cruciform
Samarkand region	1	29	41	21	9	-
	2	28	42	19	11	-
	3	26	39	21	14	-
	Average	27,6	40,3	20,3	11,3	-
Navoi region	1	22	38	24	12	2
	2	18	34	29	16	3
	3	25	32	28	14	1
	average	21,6	34,6	27	14	2
Jizzakh region	1	24	30	29	15	2
	2	29	36	21	14	
	3	25	37	30	9	
	average	26	35,3	26,6	12,6	

Thus, the conducted morphological studies of theileriosis pathogens - *Theileria annulata* found that in the Samarkand region, round and oval forms of theileria are superior to comma-shaped and punctate, and cruciform theileria are absent, which indicates the low virulence of theileriosis pathogens, while in Navoi and Jizzakh regions, round and oval forms are relatively less and comma-shaped and dot-shaped forms are superior, in addition, cruciform forms of theileria are found, which indicates the virulence of pathogens. It is known that in the pathogenesis of theileriosis, metabolic disorders, intoxication, impaired hematopoiesis, the functions of the circulatory, lymphatic and nervous systems

are manifested, as a result of which animals die. Anemia, vascular infiltration, and bleeding are specifically seen as a result of intoxication and poor hematopoiesis. (figure 2).



Figure 2: Anemia of mucous membranes, infiltration and hemorrhage in an animal with theileriosis, Jizzakh region, farm Oltinsoy

Studying the impact of isolated strains from different parts of the Samarkand, Navoi, and Jizzakh regions on animal hematopoiesis is crucial in this respect for the development of an antitheyleriosis vaccine. On three groups of animals in each of three heads, experiments were conducted to examine the impact that isolated strains from various climatic and geographic situations had on animal hematopoiesis. *Theileria annulata*, an isolated strain, was transmitted to the first group of animals from the Samarkand, second group from the Navoi, and third group from the Jizzakh districts. Every 3–4 days, the test animals collected blood for a hematological investigation. As a result of the conducted studies, a weak effect on hematopoiesis was established than in infected animals with a strain of Navoi and Jizzakh regions (Table 3).

Table 3: Influence of theileriosis causative agent - *Theileria annulata* on hematopoiesis of animals isolated from different regions

Gr.	Kol.	Source	Before infection			18 days after infection			21 days after infection		
			Er.	Lak.	Hb	Er.	Lak.	Hb	Er.	Lake	Hb
1	3	Mild and humid climate	6,7	8,8	9,2	6,2	8,2	9,0	6,2	7,6	6,2
2	3	Hot and dry climate	6,6	9,2	9,6	5,0	7,6	6,8	4,2	6,0	4,0
3	3	Hot and dry climate	6,8	8,6	8,8	5,2	7,8	6,2	4,2	6,2	4,0

Hematological research revealed that, in comparison to theileria isolated from the areas of Navoi and Jizzakh regions, the theileriosis strain of *Theileria annulata* obtained from the Samarkand region had minimal impact on the hematopoiesis of the animal organism.

Note: *Erythrocytes in million/μl, Leukocytes in thousand/μl, G/l*

As a result of the research work carried out, we came to the following conclusions:

4. Conclusion

It has been discovered that theileriosis is pervasive in the Samarkand, Navoi, and Jizzakh regions, where it is also often carried by ticks, *Hyalomma anatolicum* and *H. detritum*. *Theileria annulata*, an isolated strain from the Samarkand region, exhibits less virulence than similar isolates from the Navoi

and Syrdarya regions and has no impact on hematopoiesis. A mildly contagious strain that can be used to create an antitheyleriosis vaccine has been identified, registered, approved, and stored in a cryobank.

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