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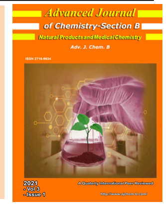

Original Research Article

Investigation of Parasites of Local Isolates of Theileria Anulata from Qazvin Province

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ABSTRACT

Theileria annulata is one of the most important bovine parasitic diseases transmitted to the host by hyaloma mites. Diagnosis of this disease is usually made by observing clinical signs as well as laboratory confirmation by preparing a spread of peripheral blood and lymph nodes. Observing the shapes inside red blood cells and schizonts inside leukocytes. Nowadays, with molecular methods such as PCR, it is possible to detect the specific DNA of this protozoan with high sensitivity and specificity. There are two highly pathogenic species of Theileria in cattle: Theileria parvae, which causes East Coast fever in East and Central Africa, and Theileria annulata, which causes tropical Theileria in the Mediterranean, Middle East, and Asia. Theileriae caused by Theileria parvae has a high mortality rate. The most common genes used in PCR are genes related to the Merozoite surface antigen of Theileria annulata (Tams1), the gene for the surface protein of Theleria (SP) or the ribosomal subunit gene (18s rRNA). Among these, the 18s rRNA gene is suitable for specific diagnosis and phylogenetic studies.

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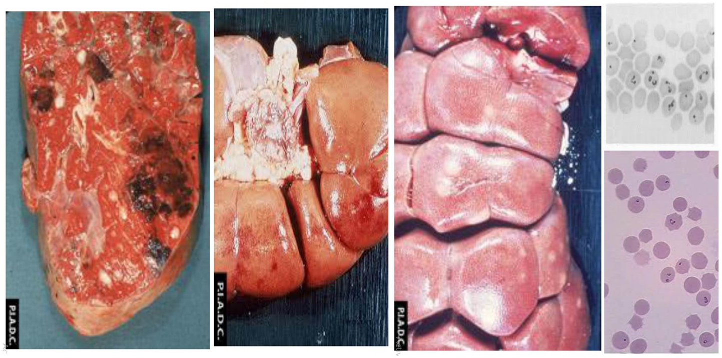
GRAPHICAL ABSTRACT



INTRODUCTION

Tillerosis is a protozoan parasitic disease caused by forced intracellular protozoa of the genus Tilleria that are transmitted by ticks to wild and non-wild toxins such as cattle, sheep and goats and cause disease in them. Infection with Theileria parasites due to health policies in some nations restricts the movement of livestock between countries. It can also reduce the production of infected livestock and increase mortality in susceptible livestock [1-3]. Theileria parasite is widely distributed in cattle, calves, and sheep in Africa, Asia, Europe, and Australia, and has a variety of vector-borne ticks and varies clinically from subclinical to subacute forms [4].

Despite its high prevalence, the Theileria annulata species has relatively low mortality, but because cows infected with the annulata species remain carriers for a long time, the infection is probably more important globally than the species [5]. Tylerosis is characterized by symptoms of fever, lymphoproliferative disorders, which may be associated with balcopenia as well as anemia. Infection in calves is often fatal and in older cows reduces their milk and meat production. European breeds in particular are susceptible to the disease, which currently prevents genetic improvement of herds in endemic areas [6].

One of the most important and effective methods of controlling tillerosis involves the use of livestock vaccination methods. Vaccination against typhoid has been carried out for more than 35 years using the bovine typhoid vaccine made by the Razi Institute in most parts of the country, and the prevalence of thallium, which estimates the economic effects of this disease, shows that this vaccine has had a good effect. But what is important is to get the amount and duration of safety after vaccination, because firstly, the production of vaccines is less than the country needs, and because the vaccine must be prepared locally, it cannot be imported from abroad, and if imported, the price of foreign vaccines Equal to the price of the domestic vaccine.

Definition of Tillerius

Thyroid agents affect a wide range of animals, mainly ruminants, and are particularly important as pathogens of domestic cattle, sheep, and goats in the tropics and subtropics. Exudates are transmitted. Diseases resulting from them are among the most serious barriers to raising domestic animals in most parts of Africa, Asia and the Middle East. Although native animals may not react strongly to the disease due to constant exposure to protozoa and relative resistance, their production may be reduced, especially in susceptible animals from disease-free areas to infected areas. They enter and sometimes the mortality rate reaches up to one hundred percent [7-9].

History of Tylerosis

In 1897, Koch observed small pyroplasmas in bovine blood in East Africa. In 1904, Tyler in South Africa defined a parasite as Piroplasma parvum. In the same year, two Russian scientists named Deshnkowski and Luz were able to find small pyroplasmas in the blood of Caucasian cows and named the parasite Pyroplasma annulatum. In 1907, during the division of Pyroplasma, Bettencourt and his colleagues identified the genus [10] Theileria for small parasites discovered in South Africa, and the name Pyroplasma parvum was changed to Theilria parvum [11]. In 1906, Tyler found another small parasite in a cow's blood and named it Pyroplasma mutans. In 1924, Serjan and his colleagues in Algeria named the Theileria Dispar the Theleria [12].

History of Tillerius in Iran

From 1934 to 1937 at the Razi Institute of Iran, Delpi et al. Studied cattle theileria in Iran closely and proved its similarity to the parasites they defined in the Caucasus, Algeria and Iraq. Later by Rafiei, Maghami, Hooshmand Rad and Hashemi in terms of vector mites, safety, tissue culture of parasites, etc [13] continued in the same institute and is still being studied. The parasites that have been named in the genus Theileria in cattle by various researchers are relatively numerous, but recent studies have shown that the genus Theileria in cattle includes certain species, which are described below [14].

Epidemiology

Occurrence of the disease and methods of its transmission

Theileria annulata is widely distributed in the world. Some of the countries that have reported the disease include: Iran, Turkey, India, Afghanistan, Pakistan, China, Russia, Egypt, Sudan, North Africa and Southern Europe [15]. The tilleria parasite is transmitted by tick vectors or biological transmission. That is, the protozoan must carry both its reproduction and part of its evolutionary cycle in the body of the vector tick (Cyclo-propagative) until it becomes actively and transferable to a healthy animal, and this transmission is carried out by the tick in a step-by-step manner [16].

Infection rate and mortality from the disease

In areas where the disease is endemic, almost all adult animals are infected and the disease is highly contagious, but the mortality rate is approximately 10-20% and is mainly limited to calves. But mortality in recently introduced non-native and susceptible cows may reach as high as 20-90%. Normally, the disease is stable in native livestock, but this balance is disturbed when non-native livestock enters the herd, resulting in heavier losses. In general, the mortality rate due to the disease is as severe as the parasite; it depends on the breed and individual sensitivity and the immune system of the infected animal [17].

Disease safety mechanisms

The process of lymphoproliferation is controlled by suppressive macrophages, which as an immune mechanism leads to recovery from the disease. Cows that have recovered from Theileria anulata infection generally show a long-lasting immunity but remain carriers. It should be noted that buffaloes may also play a role as carriers of the disease. The immunity developed against Theileria annulata, like Theileria parva, is mainly cell-mediated but weak in calves that die from the disease. Unlike Theileria parva, the immunity of Theileria parvola is apparently effective against all strains of the parasite. Theileria parva has cross-immunity with Theileria lorenzi, but this type of immunity does not exist in relation to other species of theileria, including Theileria annulata.

Theileria species cannot be kept in laboratory animals, so everything known about the safety of this infection is related to various experiments performed on cattle. It has been shown that Tileria-infected lymphocytes are attacked by the immune system, and the most important factors that play a role in the immune response are cytotoxic T lymphocytes, MHC class 1, and nonspecific cytotoxic cells.

The economic importance of the disease

In many parts of the Middle East and Asia, the disease is a major constraint on herd expansion programs as nearly 200 million cows are at risk. In addition to other tick-borne diseases such as babesiosis and anaplasmosis, tropical theileriae imposes severe limitations on herd production due to a lack of age-related resistance to Theileria anulata. Tillerosis has directly and indirectly caused significant damage in East, Central and South Africa, costing about US $ 165 million a year. Economic losses from Tillerosis, livestock mortality. It also includes a reduction in the production of the animals involved, such as a reduction in milk production and a reduction in their growth rate, and the indirect economic losses of the disease include the imposition of various costs to control the disease, such as the use of acaricides and drug treatment.

Significantly, these costs have increased dramatically over the past 20 years. It was found that by immunizing cattle against the disease, the mentioned figure will be reduced by 40 to 68%. Also, in this study, the risk of infection with the disease was estimated to be 4.8% in immunized cows and 23.5% in unimmunized cows, which was a significant difference. Similarly, the risk of mortality due to the disease in immunized cows was 0.9% and in unimmunized cows was 20.3% and this difference was significant. It is estimated that about one million cows die each year from Tillers disease, and the economic loss from these deaths is estimated at US $ 168 million per year.

Identification of Theileria anulata in Iran

In 1314, with the arrival of 16 purebred cows from France and the loss of 12 of them, Tillers was considered for the first time in Iran. In 1315, research on identifying the disease began and with the efforts of parasitology staff at Razi Institute Under the leadership of Dr. Delpi, it was determined that the cause of acute thyroiditis in Iran is theileria anulata. Indigenous Iranian cows, especially in non-mountainous areas, are resistant to thallium due to the contamination of the environment and their frequent exposure to parasitic strains, and their mortality is not more than 20%. In a study by Dr. Hashemi Fesharaki et al. In relation to the rate of deaths due to tropical tillers in Iran, it was found that the mortality rate in the acute form of the disease in purebred and mixed Iranian cows is 80 and 40%, respectively, and economically tillers caused by Theileria annulata is the most important bovine disease in Iran and is a serious threat to the development and improvement of herd production. The reason for the prevalence of Theileria anulata in Iran is the favorable climate of our country for the growth and reproduction of mites carrying this species, especially Hyaloma anatolicum anatolicum. In addition, this mite has adapted to the climate of different regions of Iran. According to a study conducted between 1998 and 1999 in the Iranian province of Kurdistan (where Tilleriosis is endemic), 680 ticks were isolated from 107 cows infected with Tillerius anilata as follows:

92.35% of Hyaloma anatolicum excavatum, 5.14% of Hyaloma marginatum, 1.7% of Hyaloma asiaticum asiaticum and the rest were mites. According to another study, the prevalence of tropical tilriosis was widespread with the distribution of hard mites such as hyaloma excavatum and hyaloma detritum, which are the main carriers of the disease, is consistent.

Biology of vector mites

All mites are vertebrate parasites and feed only on these hosts. Mites spend a long time apart from their host among plants or in cracks in the ground and cracks in the walls of buildings. Therefore, they are well accustomed to living without nutrition, and many of them are resistant to drought. Among the two groups of hard and soft mites, all carriers of different species of Theileria are in the group of hard mites, such as: genus Hyaloma, Rip cephalos, Hemaphysalis and Ambiloma.

Hard mites of hyaloma detritum, anatolicum anatolicum

The geographical distribution of Tileria annulata is consistent with its vector ticks, so that in the Mediterranean margin, the hyaloma detritum mite and in Central-West Asia, Egypt and Sudan, the species Hyaloma anatolicum anatolicum is the causative agent of the disease. The genus Hyaloma is transmitted to the host through the saliva of the mite during feeding. Theileria anulata spermatozoa mature and enter the saliva when the tick attaches to the host, and usually the tick must have been attached to the host for 48-72 hours before it became infected. However, if the ambient temperature is high, sporozoites are able to infect the body of the mite on the ground and may attach to the host within the first few hours. They enter the body of Theileria anulata. Transmission between ticks, such as Theileria parva, does not occur through the ovary. Its transmission in the tick is step by step. In vivo, the host Theleria sperozoites undergo a complex ring cycle that is accompanied by the proliferation of schizonts within white blood cells and the proliferation of pyroplasmas within red blood cells, which will then be fully described. Hyaloma mites are accustomed to humid tropics, arid climates, and sparsely populated areas. These mites live around mammals or livestock in cracks in the walls. Hyaloma mites evolve in one, two, or three hosts, and some species vary between two and three hosts, depending on the host and climatic conditions.

Evolution of Theileria annulata

Mahlhorn and Shen believe that prior to the discovery of the electron microscope, the classification of theilerias was questionable, and their full nature was unclear and perhaps unclear. The original was partially known, and in the case of gametogony forms, although zygote-like forms have been described by Serjan et al. Different gametogonies were examined and compared in more detail, the different stages of gametogony, sprogony are described below.

24 to 96 hours after eating blood, the forms inside the red blood cell of Theileria anulata enter the body of the hungry novice and reach its intestine, after changes in them, they turn into almost spherical forms, ie microgamont and macro-gamont. In the lumen of the infected intestine, spindle-shaped microgammots form erythrocyte rings, which then turn into several thread-like micrograms after the nucleus divides and the cytoplasmic growths develop. The core of the microgammon is divided into four small parts and each of them is formed towards one of the thread protrusions and four micrograms are produced. The microgams are 9 to 12 microns long and 0.25 microns wide. Ring shapes are also converted into round shapes, 34 microns in diameter, called microgammons, which are then converted to microgams. The number of microgammons and microgams is far greater than the number of microgammons and microgams.

Zygotes usually appear in the intestinal epithelial cells six days after the tick eats. Zygotes have a spherical shape and empty space at their center, the nucleoplasm of the nucleus is uniform and chromosomal elements are not understood. Twelve days after the hyaloma excavatum eats blood, the zygotes become deformed and elongated, in other words, they move from a fixed non-moving state to a moving form called a kinet. After peeling the pupae and turning them into adults, the kinet leaves the lining cells of the intestine and migrates through the hemolymph to the cells of the salivary glands.

Effect of physical and chemical factors

Alderwalen Bogen found that defibrillated blood was infectious for at least 19 days if stored at 12 °C. Serjan et al. found that when citrated or fibrin-free blood is stored at a variable temperature of 0-25 °C. Theileria annulata survives for up to 9 days. They recommended that infected citrate blood be used for immunization within 3 days of blood sampling. Infectious agents survive this period even when exposed to North African temperatures.

Isolation of pure isolates of Theileria annulata

Serjan et al. proved that the two-host mite Hyaloma mauritanicum (detritum) is only able to transmit Theileria anulata and is not able to transmit Theileria mutans and other bovine blood parasites. This specific relationship provided a suitable method for the pure isolation of Theileria annulata isolates.

Pathogenicity of Theileria annulata

One of the main factors in the pathogenesis and pathogenesis of the protozoan Theileria annulata is the induction of severe proliferation of lymphocytes and their transformation into lymphoplasts. Cells infected with Theileria annulata, interleukin alpha 1, interleukin beta 1, interleukin 6, interleukin 10, and tumor necrosis factor produce alpha, but do not produce interleukin 2 and interleukin 4. T lymphocyte proliferation is directly related to the production of interleukin alpha 1 and interleukin 6. Cells infected with Tilriaparva protozoa produce interleukin alpha 1, interleukin 2, interleukin 10, and interferon-gamma. Only cells infected with Theileria parva are able to produce interferon-gamma, but cells infected with Theileria parvata are not able to produce interferon-gamma, and interferon-gamma plays an important role in the protozoan pathogenicity of Tilria parva.

The protrusion of Theileria anulata causes a rapid proliferation of lymphocytes, and the proliferation of lymphocytes first takes place in the lymph node near the site of the vector tick bite. Because cells infected with Theileria annulata protozoa cause T lymphocytes to proliferate, improper proliferation and activation of T lymphocytes disrupts the host's immune response to the above protozoa and therefore the animal body cannot prevent the progression of pathogenesis.

Theileria annulata protozoa are able to infect bovine monocytes. These spermatozoa infected both adult monocytes and immature monocytes, but infected most adult monocytes. The host metalloproteinase matrix reduces the deformation and metastasis of infected host cells to the macrolyson of Theileria anulata. These protozoa were infected with tropical thalliosis and then slaughtered in different stages of the disease of diseased calves and using histological techniques and chemical cell safety, pathogenicity and the spread of schizonts of this protozoan were studied.

Schizont-infected cells spread rapidly through the lymphatic tissues from the pre-scapular lymph node to the farthest lymph node, and schizont-infected cells rapidly spread to non-lymphatic organs. Seven days after inoculation of sporozoites into calves, protozoan schizonts were observed in the liver, kidney, lung, abomasum, and adrenal glands, and 12 days after inoculation of sporozoites into calves, protozoan schizonts were observed in the heart. The results of this study showed that mononuclear cells infected with Theileria anulata protozoan play an important role in the development of clinical signs of tropical tylerosis and its tissue lesions, and secretion of cytokines and proliferation of phagocytic mononuclear cells play an important role in the pathogenesis of Tillerius.

The protozoan spirocytes of Theileria anulata become macrochisontitis after invading lymphocyte cells. Also, the protozoan Tillaria anulata is capable of invading sheep and goat leukocytes and transforming into macrochisontitis. Infection of sheep and goats with Theileria annulata protozoa has been little studied. Induction of nonspecific proliferation of lymphocytes interrupts the detection of protozoan antigen and the appropriate immune response. Proinflammatory cytokines produced by cells infected with Theileria anulata play a central role in damaging the protozoan Theileria anulata and the protozoan escaping from the host immune system. These macrophages are activated by the high gamma-interferon host produced by proliferating lymphocytes. When activated, these macrophages, which are activated by prostaglandin production, suppress lymphocyte proliferation.

Factors affecting pathogenicity and disease severity

The pathogenicity and severity of the disease caused by the protozoan Theileria annulata depend on the strain of the protozoan, the susceptibility of the infected animal, and the number of sporozoites of this protozoan, which are received by the vector tick.

Clinical Signs of Tropical Tylerosis

Clinical signs of this disease were observed in four heads that were exposed to adult hyaloanatomic anatomical mites on the nipples of this mite, after a 7-day incubation period and an 11-day pre-exposure period. Increased body temperature (fever), enlarged parotid lymph nodes, hyperemia of the mucous membrane of the eye and their edematousness, petechiae bleeding and bleeding spots were observed in the mucous membrane of the eye. There were also tears and discharge from the nose on the tenth day, and finally on the thirteenth day the calves became immobile, their eyelids swollen and did not eat. Their forearm lymph nodes were swollen and three calves died from the disease.

Symptoms of autopsy

In addition to jaundice of mucous membranes and yellowish discoloration of body tissues and muscles, which are obvious lesions of infection with Theileria anulata, other lesions that are more noteworthy include: carcass hyperemia, extensive pulmonary edema, emphysema, along with hydrothorax. Hydropericardium or increased pericardial fluid volume and dilation of the heart. Hemorrhagic spots and abundant foam are seen in the airways. The carcass is extremely thin and we see ecchymotic and petechiae bleeding in the tissues on most serous surfaces and mucous membranes of various internal organs and body fats.

Fig. 1. Cows' lungs with thyroiditis: Pay attention to the foci of bleeding on the lungs and foamy fluid inside the interlobular wall.

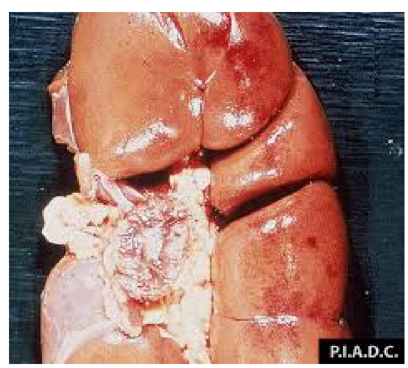


Fig. 2. Bovine kidney with telariosis: Pay attention to the petechiae on the surface of the renal cortex and the marked enlargement of the lymph node near the navel of the kidney.

Fig. 3. Petechiae bleeding on the surface of the heart epicardium.

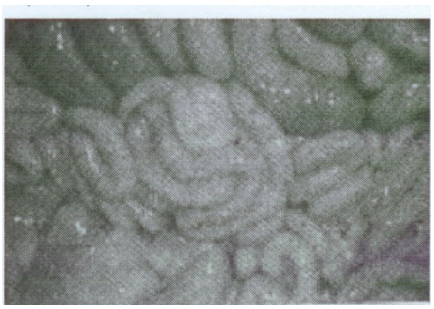


Fig. 4. Petechiae bleeding on the intestinal serosa.

The liver is large, purple in color and sometimes brick in color. The gallbladder is sometimes larger than normal and contains code bile and is dark in color. The spleen is full of blood and enlarged and its tissue is soft and does not have its natural consistency. The bladder is often full and the urine inside is dark blood in color and the mucosa is thickened due to swelling. The kidneys are covered with gelatinous and bloody fluid and are relatively larger than normal, and the kidneys are generally less damaged than other animal organs.

Wateryness, swelling, and bleeding in the lymph nodes (especially the pre-scapular, proximal, mediastinal, mesenteric, and abdominal lymph nodes) and unique volcanic lesions in the abomasal mucosa and rarely in the intestines, esophagus, tongue, and gums. Are other visible symptoms after death.

During infection with Theileria annulata, bovine leukocytes produce eight new metalloproteinases. Some researchers have identified these enzymes as toxic agents, the presence of which may explain some of the pathological features of the disease.

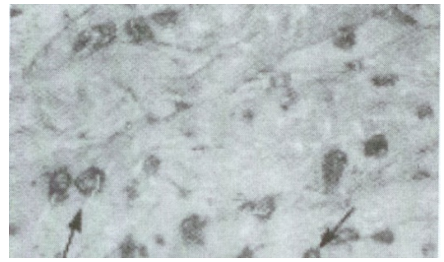


Fig. 5. Abdominal wounds caused by Theileria annulata and Theileria parva.

Specifically, the possibility of metastatic features of parasite-infected cells, volcanic ulcers and cachexia in tropical Tillerosis has been identified with the manifestation of these metalloproteinases.

White spots may appear on the liver, kidneys, and gastrointestinal tract from lymphocytic infiltrates or small lymphoid nodules (called pseudo-infarcts). In cases where the disease has persisted for a long time, the lymphatic organs of the lost animal may be small and degenerated.

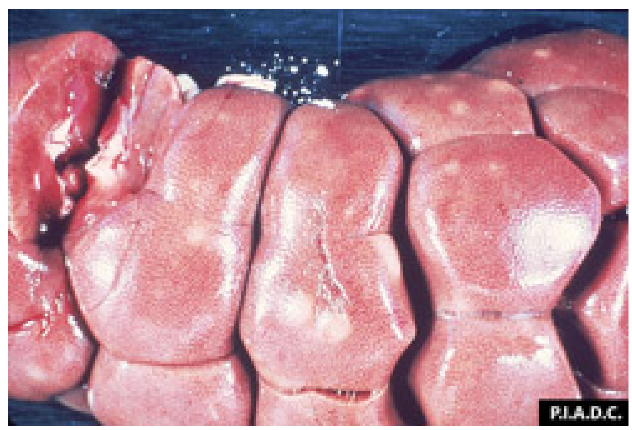


Fig. 6. White spot due to lymphocytic infiltration on the surface of the renal cortex

In the neurological form of tillers, post-mortem lesions include sporadic hemorrhages on the surface of the brain, meningeal thrombosis, ventricular hemorrhage, and cerebral hemorrhage in infected animals. With regard to the symptoms seen in various forms of tropical thyroiditis, it should be noted that in the super-acute form of the disease, usually only lymph node hyperplasia and extensive bleeding are found. But in the more common cases, in the acute form of the disease, the subcutaneous tissues contain numerous hemorrhages and petechiae, which are especially common in tropical Theileriae caused by Theileria anulata. The lymph nodes are usually enlarged, but in chronic cases they may be wrinkled. It should be noted that the common pulmonary symptoms of East Coast fever are less common in tropical theilrium. A foaming exudate around the nostrils is often seen in livestock that die from East Coast fever. Visual emphysema and severe pulmonary edema are common, the lungs become red and full of fluid, and the trachea and bronchi often contain foamy fluid.

Parasitological tests

This procedure is based on showing a parasite in red blood cells or stained blood, biopsy of lymph nodes, liver and spleen. Diagnosis of schizonts should be sent to a laboratory, while merozoites are detected in blood smears.

Clinical infection of Theileria anulata in live animals can be detected by observing pyroplasmas in the peripheral blood and by identifying schizonts in blood smears, lymph node biopsies, liver and spleen. In necropsy, schizonts may be found in smears prepared from most internal organs. In red blood cells, the pyroplasm caused by Theileria anulata is usually seen in round, oval or annular shapes (0.5-1.5 microns). Bar and comma shapes (1.6 microns) and anaplasmic bodies may also be observed.61 There is usually more than one parasite per red blood cell, so that in cases of severe infection, two to nine protozoa are observed. And the severity of the infection is usually very high and 80-70% of the red blood cells are infected. By Giemsa staining, the cytoplasm of each protozoan is blue and with a red cermatin grain Seen at one end.

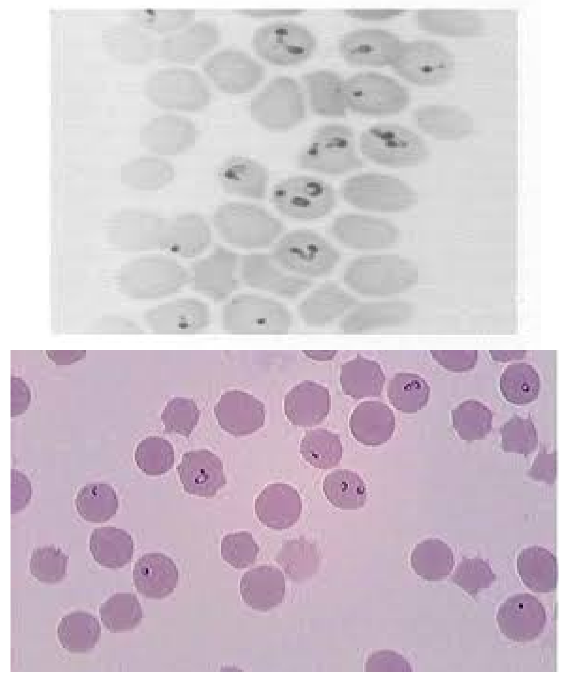


Fig. 7. The erythrocyte form and pyroplasm of Theileria anulata

In the cytoplasm of lymphocytes, smears prepared from biopsy of the lymph nodes and spleen and blood smears are found in the thesis of Theileria anulata, sometimes referred to as Koch blue bodies, because after staining with Giemsa, the cytoplasm It turns bright blue, and because schizonts were first identified and described by Koch, they are called Koch blue bodies. There is usually no more than one schizont in each white blood cell.

But in advanced cases, two or three separate schizonts may be seen inside it. Schizonts come in two forms. Macro chisons that contain up to 8 cores while microchisons have up to 36 small cores.

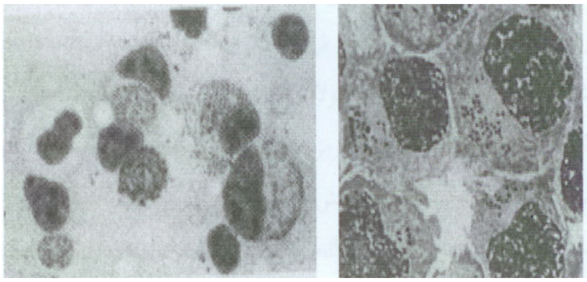


Fig. 8. Macrochisonate (Koch blue bodies) and Theleria anulata microchisontime in lymph node proliferation

Following infection with Theileria anulata, cows that survive and recover from the disease carry small numbers of pyroplasms in the erythrocytes for long periods of time, which is very difficult to isolate in stained blood smears. It is very difficult to detect the parasite. Therefore, blood contamination with pyroplasmas is low and without schizonts usually introduces a healed animal and a carrier. Surveys of livestock that survive under farm conditions have been widely used to assess the prevalence of disease carriers and cattle exposed to Theileria anulata infection.

Clinical pathology (clinical pathology)

In blood smear tests and lymph node biopsies, pyroplasmas within erythrocytes and schizonts in macrophages and lymphocytes will be visible. Theileria annulata schizonts are more likely to be present in liver tissue than those seen in lymph node smears, but are almost indistinguishable from Tilleria parva schizonts. It should be noted that Theleria parva schizonts can sometimes be detected in circulating lymphocytes and mainly in enlarged lymph node biopsy smears stained with Giemsa. In addition, the pyroplasmas of Theileria annulata are mostly oval (0.5-1.5 microns), which are different from the comma-shaped pyroplasmas and rod-shaped Theleria parva.

Conclusion

In one study, specific blood parameters were measured in Holstein cattle that were naturally infected with Theileria anulata. Hematological analysis showed a significant reduction in these factors. Red blood cell count, hematocrit level, hemoglobin level, mean red blood cell hemoglobin concentration (MCHC) and white blood cell count include:

Lymphocytes, neutrophils, monocytes, eosinophils and basophils. Hemoglobin levels, hematocrit, and red blood cell counts markedly decreased significantly from day 16 onwards. In the early stages of the disease, there is mild leukocytosis due to lymphocytosis, but later leukopenia occurs due to the destruction and removal of infected lymphocytes, although some sources have reported leukopenia without leukocytosis. On the other hand, in comparison with infected animals with control (non-infected) animals, a significant increase in mean corpuscular volume and number of reticulocytes in peripheral blood was observed.

Anemia due to the destruction of red blood cells contains pyroplasms, but other factors that may be involved in the development of anemia include hemolysis due to autoimmune reactions and poor bone marrow response to anemia. The release of toxins by schizont tilleria affects the hematopoietic system and reduces their activity, causing anemia in the patient. Autoimmunity is associated with schizonetic changes. In other words, the lack of schizophrenic leukocytes in anemia caused by a decrease in red blood cells is not significant, but with an increase in their number, this type of anemia is very noticeable. On the other hand, the destruction of red blood cells is associated with a decrease in hemoglobin and an increase in their phagocytosis, but the recent phenomenon alone is not an effective factor in the development of anemia. Researchers have differing views on the type of anemia, with microcytic, normocytic, and macrocytic anemias reported. According to MCHC, some anemia is mentioned as normochromic and some as hypochromic.

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