THE SCIENCE WORLD
A Monthly e Magazine
ISSN:2583-2212
May 2024 Vol.4(5), 1975-1982

Popular Article

Review on Small Ruminant Theileriosis

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https://doi.org/10.5281/zenodo.11428554

Introduction

In developing countries small ruminants play an important role in the economy of the marginal and poor farmers, their health and wellbeing will be directly reflected on the returns from the business. Tick infestation and tick-borne diseases are one of the major cause for the loss that occurs in farm, their prevalence is very high in tropical and sub-tropical countries like India. Sheep and goats (by expressing their behaviour of grazing and browsing respectively) they are very much prone to attack from ticks and eventually they may get infected with piroplasms. Though the piroplasmosis is common in small ruminants the study on it is very scarce compared to piroplasmosis in cattle.

Key words: Theileria, small ruminants, Koch blue bodies

Theileriosis is one of the common intracellular haem protozoan disease / piroplasmosis of small ruminants (which is transmitted through ticks). There are currently six Theileria spp. associated with ovine theileriosis; *Theileria lestoquardi*, *T. luwenshuni*, and *T. uilenbergi* are highly pathogenic (Schnittger *et al*., 2000), while *T. separata*, *T. ovis*, and *T. recondite* are less pathogenic and cause subclinical diseases in sheep and goats (Ahmed, 2006).

The most common Thelieria species are *Theileria hirci* (Malignant Theileriosis) – renamed as *T. lestoquardi*, *Theileria ovis* (Benign Theileriosis), *Theileria separata* (Mild Ovine Theileriosis). (Kaufmann,J, 1996)

Description and importance of the disease:

Theileriosis affects small ruminants in tropical and subtropical regions of Europe, Africa, the Middle East, Far East and Asia. The disease causes clinical signs in domestic ruminants, such as decreased weight and milk production and increased mortality, resulting in significant economic losses. The aetiologic agent is a protozoan transmitted by Ixodid ticks, belonging to the Haemaphysalis, Hyalomma and Rhipicephalus genera. Both internal factors (genetics, breed, lambing and lactation) and external factors (nutrition, other concurrent infections) influence susceptibility to infection. Although sheep and goat breeding is one of the most important economic resources in some regions of the world, parasitic protozoa are not well characterised in these species. (WOAH Terrestrial Manual, 2022)

Epidemiology

T. lestoquardi is most pathogenic Theileria sp. Of small ruminants with high morbidity and mortality rate. In India both malignant and benign theileriosis is common. There are also reports of *Theileria annulata* experimental infection in sheep and goats shows mild to moderate systemic illness. The morbidity rate in small ruminants infected with *T. lestoquardi* can approach 100%, with reported mortality rates of 46-100%. Clinical cases seem to be more severe in sheep than goats (Spickler, Anna Rovid. 2019)

Transmission

Theileria are transmitted by ticks acting as biological vectors, and can be transmitted transstadially. Transovarial transmission is not thought to occur. Genera of ticks reported to act as vectors include Rhipicephalus (*T. ovis, T lestoquardi*), Hyalomma (*T lestoquardi, T. separata*), Haemaphysalis (*T. luwenshuni*). *Theileria* spp. enter the body as sporozoites in the saliva of a feeding tick. (Spickler, Anna Rovid. 2019)

Lifecycle

When ticks attach themselves to the already infected host, they engulf blood (RBC has piroplasms), once they enter tick's midgut , they undergoes sexual phase of lifecycle produces gamonts (macro and micro). These gamonts unite to form zygote, this zygote migrates to salivary glands of ticks (motile kinetes) and becomes sporozoites. When the tick feed on goat/sheep blood they enter the blood stream of host, to the host's WBC (lymphocytes). In these cell's cytoplasm they under goes series of schizogony (producing schizonts) parallel to the host's infected lymphocytes replication and disseminated to daughter lymphocytes which is then followed by merogony (producing merozoites). These merozoites comes out of WBC by rupturing them and enter RBC in blood becomes piroplasms and then lifecycle continues.

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Published 25.05.2024

Theileria only passes from one tick stage to the next, and does not pass transovarially (Michael A. Sleigh, 1989)

Signalling pathways that are activated in Theileria transformed cells are,

- Mitogen activated protein kinase (MAPKs)
- Constitutive NF- κB activation
- The PI-3K pathway (Dobbelaere DA *et al.*,)

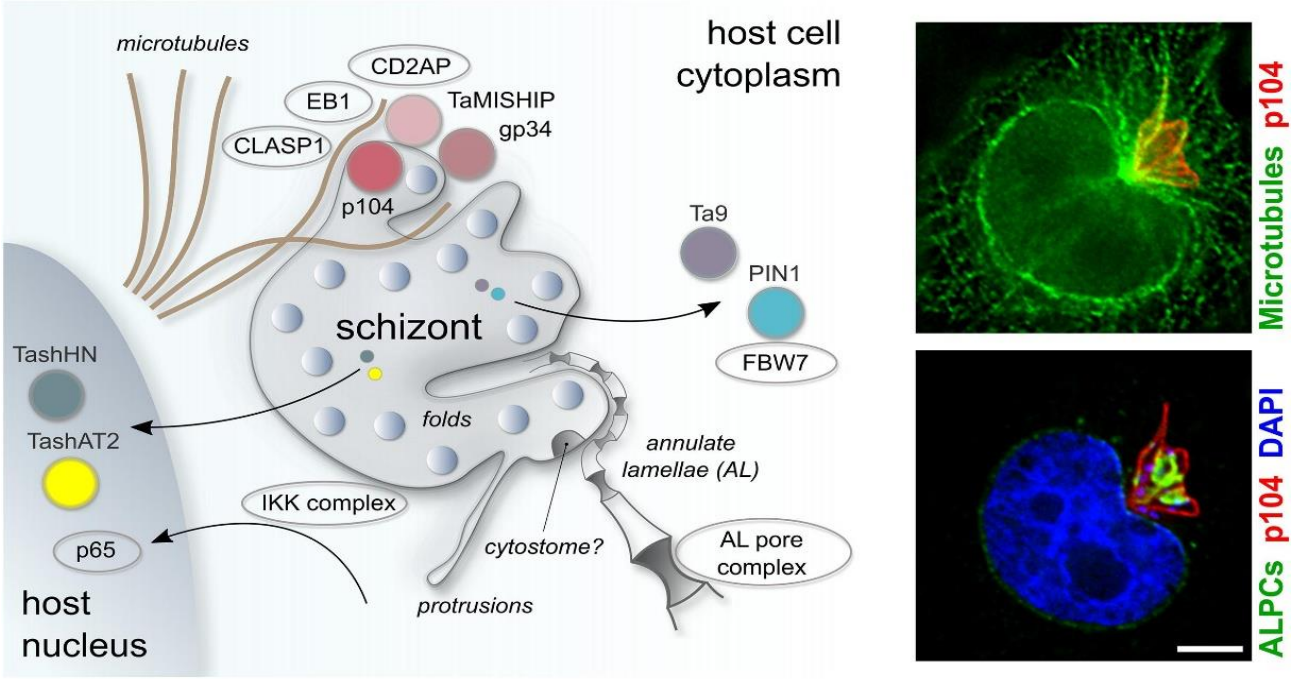

Figure 1 - Schematic representation of key host-parasite interactions in Theileria annulata infected leukocytes. Theileria schizonts export proteins into the host nucleus (TashAT, TashHN) or cytoplasm (PIN1, Ta9), where they have the potential to interact with host proteins (FBW7) to modify the host phenotype. Theileria interacts closely with host MTs, mediated in part by a protein complex on the schizont surface comprising bovine CLASP1, CD2AP, EB1 and the schizont proteins p104 and MISHIP. The IKK complex is recruited to the parasite surface, enabling the nuclear localization of NF-kB subunits (p65) to the host nucleus. Porous annulate lamellae that align closely to the schizont membrane are depicted, as are structural elements of the parasite plasma membrane such as dynamic protrusions and a potential cytostome. Fluorescent images show host MTs, labeled with anti-CLASP2 antibodies (green, top panel) and host ALPCs, labeled with anti-RanGAP1 (green, bottom panel) in relation to the schizont membrane, which is labeled with anti-p104 (red). The scale bar is 5 μm

Source - Woods K *et al.*, 2021. Theileria's Strategies and Effector Mechanisms for Host Cell Transformation: From Invasion to Immortalization.

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Post mortem lesions

At necropsy, some affected sheep showed enlargement of lymph nodes, enlarged and icteric liver, mucosal ulceration of abomasum and lung oedema. Histopathological findings of focal necrotic foci in the liver, interstitial oedema of the lung, enteritis with mononuclear infiltration and lymphadenopathy were in agreement with gross pathology. (Ramesh kumar *et al*.,2022)

Clinical signs

Unlike other haem protozoan diseases, there are no specific clinical signs that indicated theileriosis in infected animals but the overall signs noticed mostly leads to suspicion on theileriosis like infected animal showing anorexia, anaemia with swollen lymph nodes (generalized) in tick infested animals.

The most common clinical signs include high fever, dullness, prolonged anorexia leading to loss in body condition and appearance, ruffled hair coat, generalized swollen lymph nodes, digestive disturbances like intermittent diarrhoea/constipation, sometimes abortion, lateral recumbence, followed by death of animal. Due to severe lung oedema animal shows respiratory signs like cough, breathing difficulty etc.

On clinical examination, animal will be dehydrated, pale mucus membrane, tachycardia, alteration in the blood count – low RBC count, low Hb levels, Decreased PCV, Leucocytosis.

Diagnosis

Haematology (not so specific but helps for tentative diagnosis)

The most common method of examination is peripheral blood smear (giemsa stain) reveals piroplasms.

Salient morphology

In RBC

The organisms occur in RBC in different forms as following

- Bacilliform or comma shaped elongated trophozoites with either round/oval chromatin mass
- Round form of trophozoites with reduced cytoplasm appearing in tetrad or singy
- Oval or pyriform trophozoites
- A specific type of trophozoite with velum

In lymphocytes (As schizont form called Koch blue bodies).

The stages are

- Roughly round trophozoite in lymphoblast
- Trophoblast with dividing nuclei

1978

- Macroscizont containing large chromatin granules
- Macromerozoite
- Microschzont containing smaller chromatin granules
- Micromerzoite (Mandal, S.C, 2006)

Theileria hirci – Erythrocytic piroplasms are round to oval in the majority of cases about 18% are rod shaped and a small percentile are Anaplasma like. Round forms measure 0.6 – 2 µm in diameter (E J L Soulsby, 1982.)

In the cytoplasm of lymphocyte, the organism divide. Few or more number of organisms are found in it. This is the confirmatory diagnostic feature during the examination of blood smear. Lymph node biopsy (aspiration) reveals Koch blue bodies. Precautions should be taken before interpreting the results as false positive and false negative diagnosis may can happen (should be differentiated from artefacts like stain particles)

Sometimes in early stages, piroplasms may not appear in the peripheral blood smear but Koch blue bodies may appear in aspiration cytology of lymph node. so if blood smear is negative for piroplasms it always not indicative of animal is not suffering from theileriosis.

Advanced diagnostic tests

- DNA extraction and estimation of DNA concentration from clinical samples
- PCR, sequencing and phytogenetic analysis
- Molecular investigations on outbreaks of ovine theileriosis among sheep and goats in Haryana, India. (Kumar R *et al*., 2022)
- Indirect fluorescent antibody tests, ELISA, Reverse line blot, loop-mediated isothermal amplification, restriction fragment length polymorphism (Ahmed H et al.,2015)
- PCR amplification is preferable molecular technique, mostly used for detection of piroplasms during recent years. (Muhammad Riaz *et al*., 2017)

Treatment

To eliminate the protozoan parasite, the common antiprotozoal drugs used are

1) Napthoquinones

Buparvaquone (@2.5mg/kg B.W I.M - in 48 hrs interval 2nd dose should be administered)

Parvaquone is another napthoquinone but less frequently used

Mechanism of action – they are chemically similar to ubiquonone, which serves as electron carrier between flavoproteins in cellular respiration. Due to their structural similarity to ubiquonone, 1979

napthoquinones have shown to selectively block the mitochondrial electron transport and related processes such as ATP and pyrimidine biosynthesis in susceptible protozoa. (Harpal Singh Sandhu, 2013)

2) **Tetracyclines**

As napthoquinones are costly for marginal farmers to purchase, tetracycline drugs are commonly used theilericides in the field conditions

Common tetracyclines are Oxytetracycline @ 20mg/kg B.W intravenously after diluting with normal saline for 5 days (M Geetha *et al*., 2021)

Tetracycline antibiotic can also be used. Oral route is more common than parenteral route

Sheep: 10mg/kg P.O 2 times daily upto 5 days (Harpal Singh Sandhu, 2013)

3) **Use of quinozolines like Halofuginone lactate orally is also followed in many areas but not much common in the field**

If any concurrent infections or diseases like Anaplasmosis, secondary ketosis etc are present specific treatment should be given.

Supportive therapy

- Antihistamines like chlorphenaramine/phenaramine should be administered before giving a dose of napthoquinones to prevent unnecessary allergic reactions
- Tick should be eliminated (can be done by using ectoparasiticide / sub cutaneous administered of Ivermectin @ 0.2mg/kg BW)
- Use of NSAIDS (anti-inflammatory, anti-pyretic) like flunixin meglumin, meloxicam etc.
- Fluid therapy (animal is starving, loss of condition, recumbence), care must be taken not to over hydrate the animals as affected animals already having anaemia and hypoproteinemia that a cause haem dilution and death.
- Blood transfusion in severe anaemic animals
- Haematinics can be used, they aid in rejuvenating erythrocytes along with vitamin supplementation like vitamin B12
- OTC therapy can show negative impact on ruminal microbes, once the therapy is done oral supplementation of digestive tonics and prebiotics is necessary.

Prevention and control

Only way to control theileriosis is by controlling tick infestation and disrupting pathogen's lifecycle, it can be done by implementing integrated pest management programmes, anti-tick vaccines, 1980

pasture/grazing land management etc., especially in endemic areas.

If any animal in the herd is positive for Theileiria, isolation is important and tick control treatment should be followed in the shed and sick/weak animals should get checked

No proper vaccines available but for for *T. lestoquardi*, an attenuated live vaccine based on inoculation of schizont-infected leukocytes has been used for the control of malignant theileriosis and successfully applied in some countries. (WOAH Terrestrial Manual, 2022)

Conclusion

Controlling theileriosis in small ruminants is very important as it directly or indirectly shows negative impact on economy and business returns. The efficient way to control the theileriosis by tick control. Educating the owners about tick infestation and tick-borne diseases is crucial for that field veterinarians and extension agents should participate actively. Understanding the pathogenesis and life cycle of Thelieria protozoan can aid in better diagnosis and faster treatment. Always prevention is better than cure.

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