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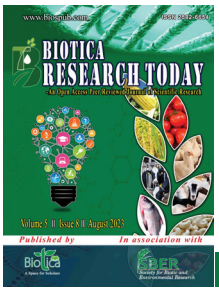

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Haemoparasites of Domestic Bovines and their Diagnostic Pattern: A Review

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Abstract

Haemoparasites, such as *Trypanosoma*, *Theileria*, *Babesia* and *Anaplasma* cause severe infections in both humans and animals, is having a significant impact on health and productivity of farm animals and humans. Conventional diagnostic methods rely on microscopy that are challenging, especially during chronic infection. Tick infestations, which are common in the region, pose a major problem for livestock, causing direct damage through blood loss and indirect damage through the transmission of protozoal parasites. Immunological and molecular techniques offer more accurate detection than microscopy. Serological tests are commonly used for antibody-based diagnosis, although specific reference tests are still lacking. Molecular techniques provide high sensitivity and are suitable for detecting infections in the latent phase and assessing the effectiveness of specific treatments. Nucleic acid-based techniques are convenient and accurate for disease diagnosis, particularly for detecting infections at low levels in carrier animals. In this review paper, elaborating different haemoparasites prevalent in Indian perspective as well as world scenario in the domestic bovines.

Keywords: Animal, Diagnosis, Haemoparasites, Microscopy

Introduction

Haemoparasitic infections caused by organisms like Trypanosoma, Theileria, Babesia and Anaplasma, can exert considerable effects on the health and productivity of both livestock and humans. These infections are frequently transmitted by arthropod vectors or via blood transfusion (Maharana *et al.*, 2016), contributing to their widespread impact on both animal and human populations. Veterinary medicine is particularly concerned with diseases such as trypanosomosis, theileriosis, babesiosis, anaplasmosis and microfilaria infection. Clinical signs of haemoparasitic infections can vary but often include symptoms such as anaemia, fever, anorexia, threatened abortion and even mortality in severe cases. Traditionally, diagnosis of these infections relied on microscopic examination of blood or tissue fluid samples to identify the infective stages of the parasites. However, this approach can be challenging, especially during the chronic stage of the infection.

Serological tests play a crucial role in diagnosing haemoparasitic infections, as they depend on identifying particular antibodies generated by the host's immune system in response to the infection. Employing methods like enzyme-linked immunosorbent assay (ELISA) or immunofluorescence assay (IFA), these tests can effectively detect the presence of parasite-specific antibodies in a patient's blood sample. They are particularly useful during the chronic phase of infection when the parasites may be difficult to detect directly. Serological tests provide a reliable and non-invasive method for diagnosing haemoparasitic infections, aiding in timely and accurate treatment decisions.

The advent of molecular diagnostic methods has brought about a revolutionary change in the detection of haemoparasitic infections. Approaches like Polymerase Chain Reaction (PCR) and nucleic acid hybridization have enabled the amplification and identification of precise DNA or RNA sequences from the parasites. PCR, being

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exceptionally sensitive, can detect even trace amounts of parasitic DNA, making it an invaluable tool for diagnosing chronic infections and distinguishing between various species or strains. Nucleic acid hybridization employs specific DNA or RNA probes to bind to complementary sequences of the parasite's genetic material, enabling their detection. These molecular methods provide rapid, accurate and specific results, aiding in early detection, treatment decisions and surveillance of haemoparasitic infections. In summary, nucleic acid-based techniques, such as PCR, have become the most convenient and accurate method for diagnosing haemoparasitic infections. These techniques offer improved sensitivity, specificity and convenience compared to traditional diagnostic methods and play a crucial role in disease surveillance and control.

Knowledge Gap

The abundant tick population in the region poses a significant health concern for free-ranging animals, including Mithun. Ectoparasitic infestations, especially ticks, are a prevalent and economically significant issue that affects Mithun and other livestock species. Ticks inflict both direct and indirect harm on their hosts. Direct harm occurs through blood loss, leading to reduced growth rates, decreased milk production and devalued hides due to tick bites. Indirect harm arises from the transmission of protozoal parasites, causing severe diseases. Currently, the diagnosis of tick-borne diseases primarily relies on the conventional method of microscopic examination of infected samples. However, immunological methods offer enhanced accuracy and specificity compared to microscopy. These methods employ specific antibodies to detect tick-borne pathogens, providing more reliable results. Molecular detection techniques like PCR are also valuable for handling a large number of samples with higher sensitivity. These techniques offer automation and upgradability advantages, making them efficient for prevalence studies of veterinary and zoonotic diseases. By identifying specific DNA or RNA sequences of the pathogens, molecular techniques accurately detect the presence of tick-borne diseases. In conclusion, although microscopy remains a common diagnostic method for tick-borne diseases, immunological methods and molecular detection techniques offer increased accuracy, specificity and sensitivity. These advanced techniques are particularly useful for prevalence studies of diseases with veterinary and zoonotic importance, enabling more effective disease surveillance and control measures.

Review of *Microfilaremia* in the Context of National and International

Microfilaria, primarily transmitted by Culicoides mosquitoes, is commonly found in Mithun without causing clinical symptoms. The presence of microfilaria has been observed in Mithun from Arunachal Pradesh and Nagaland through post-mortem examinations. *Setaria digitata* has been identified as the species through gross morphology analysis and PCR amplification of marker genes (Chamuah *et al.*, 2015).

In Arunachal Pradesh and Nagaland, Mithun (*Bos frontalis*) commonly harbor *S. digitata*, a long and milky-white worm, within their peritoneal cavity. The host's blood contains microfilariae, the larval stage of this filarial nematode. *S. digitata* follows an indirect life cycle, using Culicine mosquitoes as intermediate hosts. Although adult worms usually cause mild fibrinous peritonitis and are non-pathogenic, the larval forms are considered pathogenic and responsible for cerebral nematodiasis (Tung *et al*., 2003). The prevalence of *S. digitata* infection is influenced by climate and the presence of suitable mosquito vectors (Chamuah *et al.*, 2021), with mosquitoes from the genera Aedes, Culex, Anopheles and Armigeres acting as transmitters of this nematode. Within 8-10 days of being ingested by mosquitoes, the microfilariae undergo two molts and develop into infective third-stage larvae (L3 larvae) in the thoracic muscles of the mosquitoes (Chamuah *et al.*, 2021; Perumal *et al.*, 2016). Infected mosquitoes can then transmit L3 larvae to other susceptible hosts during blood meals. In the host, the L3 larvae reach sexual maturity within 8-10 months, thus completing the life cycle of *S. digitata* (Chamuah *et al.*, 2021).

Overall, *S. digitata* infection in Mithun is characterized by the presence of microfilariae in the blood and adult worms in the peritoneal cavity. While adult worms are generally non-pathogenic, the larval forms can cause health issues. The transmission of *S. digitata* relies on mosquito vectors and the completion of its life cycle is influenced by environmental factors and the availability of suitable vectors.

International Context

Besides the peritoneal cavity, adult *S. digitata* worms have been documented in various locations including the urinary bladder, epicardium of the heart, lungs and mesenteric lymph nodes. Infection with *S. digitata* can result in a severe condition known as cerebrospinal nematodiasis (epizootic cerebrospinal setariosis or kumri or lumbar paralysis) in unnatural hosts like horses, sheep and goats. This condition arises due to the migration of microfilariae into the central nervous system (Chamuah *et al.*, 2021). Additionally, there have been reports of congenital cases of setariosis. Although *S. digitata* infections are prevalent in cattle across India, ranging from 77 to 95%, reports of *S. digitata* infections in Mithun are exceedingly rare (Chamuah *et al.*, 2015).

National Context

Chamuah *et al*. (2015) conducted a pilot study to determine the prevalence of *S. digitata* microfilaria in Mithun, a livestock species, in the North-eastern region of India. They collected blood samples from 41 adult Mithun, regardless of age and sex and used the modified knott technique for screening. Among the tested animals, 10 (12.19%) were positive for microfilaria infection. For further analysis, they examined a total of 110 Mithun during slaughter and at various local tribal rituals and ceremonies in Arunachal Pradesh and Nagaland to obtain adult parasites. Nine individuals (8.2%) were found to harbor *Setaria* adult parasites in their peritoneal cavity and the morphological characteristics confirmed their identification as *S. digitata*.

To gain insights into the parasite's genetic profile, the researchers performed sequence analysis of ribosomal (12S rDNA, 28S rDNA and ITS-2) and mitochondrial (Cytochrome

C Oxidase subunit 1-COX1) regions. The ribosomal DNA regions showed a 98% similarity with *S. digitata*, while the mitochondrial COX1 gene displayed 99% similarity with an S. digitata isolate from Sri Lanka (Acc. No. EF179382) and 87% similarity with *S. labiatopapillosa.* The observed epidemiological pattern of Setaria infection in Mithun aligned with previous findings. In this study, the intensity of *S. digitata* microfilaria infection was higher in older animals compared to younger ones and prevalence increased during warmer seasons due to heightened mosquito activity (Chamuah *et al.*, 2021). Gender did not appear to influence the occurrence of *S. digitata* infection in Mithun.

Considering the favorable environment for mosquito vectors in the North-eastern region of India, the researchers recommended implementing vector control measures, such as appropriate insecticide use, to mitigate the incidence of *S. digitata* infection in Mithun. They emphasized the importance of providing adequate care and guidance to Mithun owners to reduce the parasitic burden and improve production performance in the hilly regions of North-eastern India.

Review of Haemoprotozoan Parasites in the International Scenario

1. Babesia

Piroplasmosis, a disease caused by various species of Babesia, a parasitic protozoan, has a significant impact on the health of both wild and domestic animals, leading to outbreaks with economic implications in livestock (Amorim *et al.*, 2013). Among the Babesia species, *B. bovis*, *B. bigemina* and *B. divergens* are particularly associated with bovine babesiosis and are considered the most virulent. While *B. bovis* and *B. bigemina* are prevalent in tropical and subtropical regions worldwide, *B. divergens*, which is also zoonotic, is common in Europe (Elsify *et al.*, 2015).

Transmission of piroplasmosis occurs when infected ticks release Babesia sporozoites into the host's bloodstream during blood feeding. These sporozoites invade the host's red blood cells (RBCs) and transform into merozoites (Elsify *et al*., 2015). The asexual multiplication of merozoites within RBCs leads to their destruction, causing symptoms such as hemolysis, anemia and jaundice in infected animals. In the case of *B. bovis* infection, the sequestration of infected RBCs in vital internal organs' capillary beds can result in nervous and respiratory symptoms (Elsify *et al.*, 2015). The severity of Babesia infections varies depending on factors such as the specific species, host characteristics and the immune response. Severe cases can lead to significant morbidity and mortality in livestock, causing economic losses through reduced productivity, including decreased milk production and weight loss.

Controlling and preventing piroplasmosis involves employing various strategies. Essential measures include tick control through the use of acaricides, effective pasture management and the utilization of tick-resistant livestock breeds. These measures aim to reduce the tick population and disrupt the transmission cycle of Babesia parasites. Additionally, researchers are actively investigating the development and deployment of effective vaccines to protect livestock from Babesia infection and mitigate the disease's impact.

It is crucial to note that the information provided is based on general knowledge of piroplasmosis. Implementing control and prevention strategies require staying updated with the latest research findings and considering regional variations in the disease.

2. Theileriosis

The main causes of severe theileriosis in cattle are *Theileria parva* and *T. annulata*. Nevertheless, outbreaks of theileriosis resulting from the harmless parasite *Theileria orientalis* have also been observed in various countries. Unlike most Babesia species, Theileria sporozoites infect and reproduce within host leukocytes, undergoing schizogony and merogony; while *T. parva* and *T. annulata* prompt rapid leukocyte proliferation, transforming the infected cells, *T. orientalis* does not induce leukocyte proliferation and is considered a non-transforming parasite (Elsify *et al.*, 2015).

Merozoites released from schizont lysis infect red blood cells (RBCs), with *T. annulata* and *T. orientalis* displaying efficient multiplication in RBCs, while *T. parva* shows less pronounced merogony in these cells. Geographically, *T. parva* is endemic to eastern, central and southern Africa, *T. annulata* is prevalent in North Africa, Southern Europe and Asia and *T. orientalis* has a global distribution (Elsify *et al.*, 2015).

Recovery from clinical diseases caused by Babesia and Theileria parasites often leads to animals becoming carriers and subclinical infections may be common in animals resistant to clinical piroplasmosis (Elsify *et al.*, 2015). Detecting carriers and subclinical infections is vital to assess the risk posed by these parasites. Epidemiological surveys provide valuable data to evaluate past parasite control programs and make necessary adjustments to control strategies (Kumar *et al*., 2018).

Although microscopic examination of Giemsa-stained blood smears is a common and simple method for identifying blood parasites, it may lack sensitivity and specificity as a diagnostic tool during the carrier stage with low parasitemia. Currently, DNA detection techniques like PCR assays are preferred in epidemiological investigations due to their specificity, sensitivity and ability to detect active infections (Elsify *et al.*, 2015).

In Egypt, where cattle, buffalo and sheep are major sources of meat, milk and related products, clinical diseases caused by Theileria and Babesia species are prevalent among cattle and buffaloes (Elsify *et al.*, 2015). This leads to economic losses due to reduced productivity, expensive veterinary treatments and occasional animal deaths. Previous epidemiological studies conducted in Egypt have reported the presence of *B. bovis* and *B. bigemina* in cattle, buffaloes and ticks (Elsify *et al.*, 2015). However, these investigations were often limited to specific provinces, lacking simultaneous detection of both Babesia and Theileria parasites. Future studies should also consider the presence of *T. orientalis*, which has been reported in multiple countries, to gain a more comprehensive understanding of the parasite landscape in Egypt.

3. Trypanosomiasis

Surra, a disease caused by the parasite *Trypanosoma evansi*, was initially prevalent in camels in sub-Saharan Africa and primarily transmitted mechanically by certain types of flies. However, the disease has now spread globally, affecting a wide range of hosts including camelids, equines, cattle, buffaloes, sheep, goats, pigs, dogs, carnivores, deer, gazelles, elephants and even vampire bats. Untreated Surra poses a significant threat to camels, equines and dogs, often leading to fatal outcomes (Gangwar *et al*., 2019). Clinical signs, such as anemia, weight loss, abortion and death, may vary in severity and manifestations across hosts and locations. *T. evansi* is known for its immunosuppressive nature, which can impact the host's immune response and have implications for concurrent diseases or vaccination efforts.

To detect and characterize *T. evansi* infections in different animal hosts, researchers have turned to molecular diagnostic techniques, particularly polymerase chain reaction (PCR) (Migri *et al*., 2016). In a study involving mice, PCR was able to detect *T. evansi* infection 24 hours earlier than the commonly used micro-hematocrit centrifugation technique (MHCT). Furthermore, PCR allowed for the detection of *T. evansi* in organs even when the parasites were undetectable in the blood (Behour *et al.*, 2019). Similarly, PCR assays were employed in blood samples from camels, with certain primer sets showing higher sensitivity in detecting *T. evansi* infection.

In another study conducted by Elata *et al.* (2020), a PCR-based assay was used to detect and characterize *T. evansi* in goats from Cebu, Philippines. The study found a detection rate of 33.9% for *T. evansi* in the goat population, with a slightly higher prevalence in male goats and younger goats. Interestingly, two of the positive samples showed co-infection with a trypanosome species similar to *T. theileri*. Sequence analysis of a specific region called ITS1 confirmed the presence of both *T. evansi* and the *T. theileri*-like trypanosome in the goats.

These molecular detection studies highlight the importance of PCR-based assays in diagnosing and identifying *T. evansi* infections in various animal hosts, including mice, goats and cattle. By utilizing specific primers and sequence analysis, these techniques provide sensitive and accurate detection of the parasite, allowing for better understanding of the disease's distribution, prevalence and potential co-infections in different geographic regions and animal populations.

4. Rickettsia

Anaplasmosis, also known as gall sickness, is an infectious disease caused by the bacterium *Anaplasma marginale* (Maharana *et al.*, 2016). This bacterium resides and multiplies within the red blood cells of its host and is primarily transmitted through tick bites. However, mechanical transmission through biting flies and blood-contaminated fomites can also occur and infected cows can pass the infection to their unborn calves. *Bovine anaplasmosis* is prevalent in tropical and subtropical regions, mainly caused by *A. marginale* and *Anaplasma centrale* (Maharana *et al.*, 2016). These bacteria invade red blood cells, leading to symptoms such as anemia, fever and other clinical signs. Tick control programs and infection screening are crucial for controlling and preventing anaplasmosis. Adult cattle are more susceptible and can become carriers for life. Wild ruminants, especially cervids, serve as reservoirs for various Anaplasma species.

Anaplasma infection affects red blood cells, resulting in symptoms such as fever, anemia, jaundice, brownish urine, loss of appetite, depression, physical deterioration, tremors, constipation, pale mucous membranes and labored breathing. During the acute phase, microscopic examination of well-prepared thin smears is suitable for diagnosing anaplasmosis. However, it may not reliably detect pre-symptomatic or carrier animals and can be influenced by debris. Diagnosis is commonly achieved through serological tests, such as ELISA using recombinant antigens like MSP5. These tests exhibit high sensitivity and specificity, although cross-reactions among different Anaplasma species can occur.

For Anaplasma detection, nucleic acid-based diagnostic methods are widely employed, including PCR-ELISA, semi-nested PCR and real-time PCR. These molecular techniques offer reliable and accurate detection, particularly for low-level infections and carrier animals. By targeting specific DNA or RNA sequences, these assays provide greater sensitivity and specificity compared to other methods.

Haemoprotozoan Disease Diagnosis in the National Scenario

1. Babesiosis and Theileriosis

The diagnosis of haemoprotozoan diseases in India follows a pattern that involves a combination of clinical evaluation, laboratory testing and microscopic examination of blood samples. The specific diagnostic pattern may vary depending on the suspected haemoprotozoan disease and the available resources in different healthcare settings. Here is a general overview of the diagnosis pattern for haemoprotozoan diseases in India.

• *Clinical Evaluation*: Healthcare professionals assess the patient's medical history, including symptoms, travel history, exposure to ticks or animals and any relevant epidemiological factors. Clinical symptoms can vary depending on the specific haemoprotozoan infection but may include fever, fatigue, anemia and organ-specific signs.

• *Blood Sample Collection*: Blood samples are collected for laboratory testing. The collection method can include venipuncture or finger prick, depending on the specific requirements and available facilities.

• *Laboratory Testing/ Microscopic Examination*: Thin and thick blood smears are prepared and stained using specific dyes such as Giemsa or Wright stain. Skilled laboratory technicians or pathologists examine the stained smears under a microscope to detect the presence of haemoprotozoan parasites within red blood cells. Morphological features, such as the shape, size and arrangement of the parasites, are assessed to identify the species.

• *Polymerase Chain Reaction* (*PCR*): PCR-based tests are

employed to amplify and detect the genetic material (DNA or RNA) of haemoprotozoan parasites. PCR is highly sensitive and specific, allowing for species identification and differentiation, even in cases where the parasitemia is low. PCR-based tests are particularly useful for confirming the presence of parasites and differentiating between various species.

• *Serological Tests*: Serological tests detect specific antibodies produced by the immune system in response to haemoprotozoan infections. Enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody (IFA) tests are commonly used serological tests for haemoprotozoan diseases. These tests can indicate exposure to the parasites and may be used for screening or confirmation, but they may not always distinguish active from past infections.

• *Additional Tests*: Additional tests may be performed depending on the specific haemoprotozoan disease and the patient's clinical presentation. These may include complete blood count (CBC), liver function tests, renal function tests and imaging studies to evaluate organ involvement and overall patient health.

It is important to note that the diagnosis pattern can vary across different regions and healthcare facilities in India. Disease occurrences in India have been reported by different authors from time to time as follows.

In Punjab, India, the prevalence of *Babesia bigemina* in ticks infesting cattle was investigated using PCR. It was found positive results in 1.48% of tick egg mass samples and 4.44% of samples using nested PCR (nPCR). For unfed larval stages, PCR showed 1.48% positivity and nPCR showed 7.41% positivity. Prevalence rates did not significantly differ across agro-climatic zones or cattle age groups (Bal *et al.*, 2016).

Kakati *et al.* (2015) used PCR assays to amplify DNA fragments of *B. bigemina*, *T. orientalis* and *A. marginale*. Positive rates were 64.91% for *B. bigemina*, 21.05% for *T. orientalis* and 14.03% for *A. marginale*. No amplification was observed for *B. bovis* and *T. annulata*.

The diagnosed bovine babesiosis has outbreaks in Punjab (Bal *et al.*, 2016; Bhat *et al.*, 2017). Among 465 at-risk cattle, 6.02% showed morbidity, 3.01% mortality and a 50.00% case fatality rate. *Babesia bigemina* was confirmed using molecular diagnosis (PCR) on blood smears from infected animals. Successful treatment was administered.

2. Trypanosomiasis

Trypanosomiasis, a significant disease affecting wild animals worldwide, is relatively limited in India. An effort was undertaken to collect available literature on trypanosomiasis prevalence, clinical features, diagnosis, treatment and control specifically in India. In the country, clinical disease and outbreaks are more frequently observed in captive Indian wildlife, showing similar clinical symptoms as compared to other regions. Notably, carnivores consuming affected meat contribute to feline and canine outbreaks. Flared-up trypanosomiasis occurs in free-living wild animals due to drought, starvation and concurrent diseases, which compromise their trypano-tolerance. Stress-induced immunosuppression further weakens trypanotolerance in wild animals. Studies conducted in the Mumbai region revealed a high prevalence of *Trypanosoma evansi* infection in buffaloes, indicating their role as reservoir hosts and an increased risk of infection. An outbreak of trypanosomiasis in camels resulted in fatalities, accompanied by various observed symptoms.

Treatment involved the use of anti-trypanosomal drugs along with supportive therapy. The overall prevalence of trypanosomosis in buffaloes was found to be 15.25%. In Karnataka, both *Trypanosoma evansi* and *T. theileri* were identified. Effective detection of carriers and strain identification was achieved through serological methods and PCR-based diagnostics. *T. evansi* was associated with abortion cases and tabanid flies were identified as vectors. Specific injections and plant extracts were utilized for curing infections.

Studies in Punjab (Sharma *et al*., 2013) and Telangana state (Kumar *et al.*, 2018) assessed prevalence using duplex PCR and Giemsa staining. *Trypanosoma evansi*, *Babesia bigemina* and dual infections were detected. Among bovines, *Trypanosoma* spp. showed the highest prevalence, followed by *Theileria* spp. and *Babesia* spp. A study compared PCR and blood smear examination for *Trypanosoma evansi* detection, revealing positive samples by PCR but limited findings in blood smears.

These studies highlight the prevalence of *Trypanosoma evansi* and other haemoprotozoan infections in different regions of India and the use of various diagnostic methods, including PCR and microscopy, to assess pathogenicity in different animal species (Ganguly *et al.*, 2020).

3. Rickettsia

The study analyzed prevalence in different zones in India and continents worldwide (Paramanandham *et al.*, 2019). The Central zone in India had the highest prevalence at 61%, while the West and South zones had lower prevalences of 6%. South America had the highest prevalence at 82% among continents.

Different diagnostic methods were used, with serological methods showing higher prevalence rates in India (34%) and worldwide (46%), while blood smear examination had lower rates in India (7%) and worldwide (21%). Nucleic acid-based techniques had higher sensitivity.

In India, the prevalence of anaplasmosis was higher in cattle (12%) compared to buffaloes (2%). However, the overall prevalence in India was lower than the worldwide average, with South America experiencing a higher prevalence. Anaplasmosis has a significant impact on dairy animals, leading to reduced productivity and economic losses. It is essential to implement effective measures to control Anaplasma infections, especially in high-risk areas, to enhance the economic benefits of dairy farming.

Conclusion and Future Prospect

Haemoparasite can greatly impact livestock, leading to mortality and reduced productivity. Traditional diagnostic methods based on microscopy have limitation in sensitivity and specificity. However, advancement in technology

has improved the diagnosis of haemoparasitic infection. Serological test are fast but lacks specificity. Molecular techniques like PCR offer highly sensitive and accurate detection, even in the latent phase (Charaya *et al.*, 2021). These methods can differentiate between similar parasites and are not affected by the host's immune response. Combining traditional and molecular approaches enhances accuracy and facilitates targeted treatment. These advanced diagnostic techniques contribute to managing and controlling haemoparasitic diseases, improving animal health and productivity.

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