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**Table of Contents (İçindekiler)**

Volume (Cilt): 3 Number (Sayı): 2
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ZEUGMA BIOLOGICAL SCIENCE

ZEUGMA
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2022
v:3 n:2 p:1-11

# Ameliorative Effect of Poly-Herbal Mixture on *Caecal coccidiosis* in Broiler Chicks

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## Abstract

Caecal coccidiosis is disease of poultry which is caused by the *Eimeria tenella* (family of protozoan belongs to genus *Eimeria*). This is most prevalent ailment and results in decrease production and high death rates in broiler chicks**.** Extensive use of drugs as preventive therapy has created antimicrobial resistance. Poultry experts are thinking about the use of herbal plants and their combinations for the control of *Coccidiosis* in commercial poultry as alternatives. Holarrhena Antidysenterica and Azadirachta indica were reported to have anthelmintic, antifungal, antibacterial and antiviral activity. A total of 270 broiler chicks were equally divided into nine group from A to I. they were experimentally infected with a challenge of Eimeria tenella from group A to H. Group A to G were treated with poly herbal preparations and a routinely used drug. Group H was positive control but group I was negative control. Birds were observed for live body weight, hematological parameters and structural changes in cecum. Birds of group G and H showed highest ($P<0.05$) body weights, RBC, Hb and PCV as compared to positive control group. It was concluded that poly herbal mixture showed more anticoccidial effects to control this problem as compared to routine therapeutic drugs and hence they can be used as alternatives.

**Keywords:** Eimeria, broilers, hematology, histopathology

## Introduction

Coccidiosis is a parasitic affliction caused by apicomplexan protozoa of the genous *Eimeria.* It is a fowl ailment caused by microscopic protozoan which causes global economic losses of about $ 3 billion per year in commercial poultry (Dalloul and Lillehoj, 2006). The protozoan resides and multiplies within intestinal tract and cause tissue damages. It causes subclinical to clinical infection in birds with lower production and shedding infectious oocysts in the surrounding environment (Gerhold, 2015).

Poultry sector is the second biggest industry and is a feasible source of animal protein in Pakistan. The poultry industry was suffered by different destructive problems in term of mortality and morbidity. Coccidiosis is inversely affecting the economy and it has been reported round the year. A study was conducted to know the seasonal prevalence of the disease and it was found that the increased prevalence of infection during the rainy season enhanced the oocyst sporulation and subsequent spread (Dalloul and Lillehoj, 2006). High mortality and low weight gain in birds due to coccidiosis are major reasons for economical losses of farmers (Kothavade *et al*. 1996).

Coccidial infection can be diagnosed by observing the oocysts in the droppings of the live birds and by taking swabs from the dead birds. The location of lesions in the intestinal tract helps in estimating the specie of Eimeria. Age of birds and mortality can also help in estimating the Eimeria species. Eimeria species can also be identified on the basis of clinical signs and lesions (Habibi *et al*. 2016). Coccidiosis is a general infection in broiler industry and has been considered as a management problem around the world. Broilers are mostly affected by Coccidiosis and it is considered as the most detrimental disease because it caused an annual loss of US$ 127 million (Takara *et al*. 2002; El-Shall *et al*. 2022). Coccidia when enter into the body of birds, they start their replication and cause lesions in the intestinal tract (Ros *et al*. 2009). In the domestic fowl, *E. tenella* produced severe losses (Chapman, 2014) due to enteritis and hemorrhagic diarrhea. It has been reported that *Eimeria tenella* infected the cells of intestinal wall of caecum along with immunosuppression in broiler chicks (Vermeulen *et al.* 2001).

Coccidiosis might be controlled by excellent management practices, bio-security and bedding material (Nilsson *et al*. 2012). However, it is not possible to completely abolish and stop the disease without effective medication. A number of anticoccidial medicines are available which can either stop the coccidia by breaking their life cycle or directly kill them directly (Mallick *et al*. 2007). Several chemicals and ionophores were used in feed (Nogueira *et al*. 2009) which may cause hazardous effects in birds (Abbas *et al*. 2013) and drug residues for consumers. Eimeria tenella is an oval shaped protozoan and affect the epithelial lining cells of small intestine (Vermeulen *et al*. 2001) but it causes severe losses when attached on epithelial cells of cecum which may include decreased feed and water consumption, bloody diarrhea, weight loss, pigmentation loss, decreased feed conversion, high mortality and ruffled feathers in broiler chicks (Molina *et al.* 2000; Bachaya *et al.* 2012;Sharma *et al*. 2001).

People are using anticoccidial drugs in feeds since the start of poultry industry in Pakistan. But on the other side, a continuous use of synthetic drugs even in small quantity has negatively affected the defense mechanisms and produced resistance in birds against coccidiosis (Chapman 2009). The widespread emergence of antimicrobial resistance has developed in recent years about the safety of anticoccidial drugs and hazards on health of human, animals, birds and the environment (Zidar and Zizek, 2012). On 1st January 2008, The European Commission submitted a report (Arczewska-wlosek and Swaitkiewicz 2013) on the exodus of coccidiostats and histomonostats for its use as feed additives. According to the EC, there is presently no comparably effective substitution to coccidiostats. While the search for their natural replacement is direly needed.

In the backdrop of above, researchers have suggested the use of herbs for treatment of cecal coccidiosis (Arczewska-wlosek and Swaitkiewicz 2013). Some of the workers are involved in investigation of plants and plant derived products for the control of coccidiosis to save the poultry industry from heavy losses (Abdelnour *et al*. 2020). For this, Abbas *et al*. (2012) scientifically proved the anticoccidial effects of diverse herbs as a probable substitute to manage the avian coccidiosis. After this, many herbal products were experienced by the poultry farmers and found good results but they were unable to give scientific explanations about these products. But it is documented that these herbal products possess the anticoccidial, antimicrobial, antioxidant and anti-stress properties. They have been showing beneficial effects on gut micro flora with nutrigenomic effects and immune enhancement properties in birds (Hashemi and Davoodi, 2010).

In these circumstances, utilization of herbs is thought to be a safe, effective and economical way of controlling Coccidiosis (Abbas *et al.* 2012). *Azadirachta indica* has been used to cure

jaundice and liver diseases in many countries of the world. It has been traditionally used to treat ulcers, gastrointestinal disorders and acne vulgaris. Many researchers reported that it possessed the strong adaptogenic action and anti-oxidant properties (Craig, 1999). *Holarrhena antidysenterica* had many biological properties including immune stimulation, anti-inflammatory, vaccine adjuvant, anti-oxidant (Takara *et al.* 2002), anti-thrombosis (Molina *et al.* 2000), modulation of acetylcholine release (Barocci, 1999) and anti-stress effects (Bachaya *et al.* 2012) in animals and human. So, scientists are thinking that these plants may be a safe and effective substitution to control coccidiosis.

Keeping in view the above findings, the present study was planned to investigate the effects of Poly-herbal mixtures in controlling the Caecal coccidiosis in broiler chicks. This study has revealed the effects of herbal product on haematological parameters and cellular changes in intestinal mucosa at different doses over a period of 42 days in broiler chicks.

**Materials and Methods**

The current study was planned to investigate the effect of Poly-herbal mixture on *Caecal Coccidiosis* and its impact on haematology and histopathology of intestine in broiler chicks. Two hundred and seventy ($n$=270) healthy, day old broiler chicks in good health were purchased from local market and were kept at experimental poultry farm, College of Veterinary and Animal Sciences (CVAS), Jhang for 42 days. Feed and water were given ad-libitum. Birds were vaccinated against ND and IBD at day one and the booster dose of IBD at Day 8 and ND at day 10 were given to all birds.

The chicks were divided randomly into 9 equal groups from A to I with 30 chicks in each group at day 12. All groups were given same management, environment, feed and water at experimental farm. The groups of birds from A to F were given herbal mixtures but the group G was given an anticoccidial drug (Table 1). The group H had received only infection but no herbal treatment and had served as a positive control while the group I had given neither infection nor herbal treatment and had served as a negative control (Ros *et al*.2009).

The field isolate of *Eimeria tenella* was extracted from the broiler birds at sale points in the local market. The suspected cecum samples were examined in Parasitology Laboratory of CVAS, Jhang for *Eimeria tenella*. The positive samples were processed for extraction and sporulation of *Eimeria tenella* and the oocysts present in positive samples were separated through salt floatation technique as described by Bachaya *et al.* (2012) and preserved in 2.5% potassium dichromate solution. To make the challenge dose, counting of sporulated oocysts were executed by using Mac Master Chamber (Ros *et al.* 2009). For the induction of infection, 75000 sporulated oocysts per ml of distilled water were adjusted. The challenge dose of 1ml was administered to the broiler birds by using crop tube on day 14 as a single dose.

Leaves of *Azadirachta indica* (NL) and bark of *Holarrehna antidysenterica* (KB) were collected from the local market, dried and then grinded to form powder separately (Tipu *et al*. 2002). The powder was given to treatment groups in feed for 5 days from day 19 to day 23 daily in feed as given in Table 1.

Birds were examined for clinical signs continuously specially after administration of infection. All birds were weighed at the start and end of experiment. The clinical observations were recorded in the form of general body condition in terms of average body weight, morbidity and mortality throughout the period after infection.

Fifteen birds from each group were randomly selected and slaughtered at day 35 and remaining 15 birds were slaughtered at day 42. The blood samples were collected in vacutainer tubes coated with EDTA from each bird for hematological studies before slaughtering. Hematological parameters like red blood cell count (RBC), white blood cell count (TLC), hemoglobin (Hb) and Packed cell volume (PCV) were assessed by using hematology analyzer (Abbas *et al*. 2012).

Gross changes were noted in ceca in each slaughtered bird. The cecal length, hemorrhages on mucosal and serosal surfaces were observed carefully. The tissues (ceca) were processed for routine H and E staining (Bancroft and Gamble, 2008) for examination of microscopic changes like inflammation, congestion and necrosis of epithelium. After mounting, the tissue sections were examined under light microscope at different magnifying lenses i.e. 4x, 10x, 20x and 40x. Lesion scoring was done as following criteria.

No lesions found -

Mild intensity of lesions +

Moderate lesions ++

Severe lesions +++

The data were analyzed by ANOVA (Repeated measure ANOVA) and multiple comparisons were tested by Tukey's Test of statistical SPSS computer.

**Results**

The present study was planned to find out the anticoccidial effects of phytochemicals in broiler birds. The birds were experimentally infected with cecal coccidiosis to compare the curative effect of phytochemicals with a routine anticoccidial drug. The broiler chicks have the ability to gain weight in a short period of about 42 days. Birds suffering from coccidiosis showed impaired growth and low weight gain. Amprolium is used to treat this disease in broiler chicks on commercial level. Our results showed that the birds of group F and G receiving treatment of polyherbal mixture and amprolium respectively gained highest ($P<0.05$) live body weight (Table 2). The birds of group E showed less live body weight at day 35 but the weight was improved gradually over a period of one week and they gained higher weight as compared to groups A to D.

All the infected groups showed morbidity and mortality as given in Table 2. The morbidity in treatment groups was significantly ($P<0.05$) higher than negative control group but comparable with positive control group in our experiment. Our results showed that there was lowest mortality of birds in group F which was treated with higher doses of polyherbal mixture.

The results of blood parameters at day 35 and 42 are given in Table 3 and 4 respectively. The blood parameters are affected with coccidial infection as there is blood loss in this disease. An early ameliorative effect of anticoccidial agents can prevent the blood loss with normal blood parameters. Our results showed that red blood cell count, hemoglobin concentration and packed cell volume in birds of groups F and G were comparable with negative control group. The white blood cells perform a defensive role against infections and their number is increased in infections. In our study, the values of white blood cells in group F and G were significantly ($P<0.05$) lower than positive control but comparable with negative control group.

There are many Eimeria species which are affecting the intestine on specific locations. Eimeria tenella is specifically damaging the cecum and producing lesions in this part of intestine. The lesions are produced in wall of intestine which can be observed with naked eye and confirmed by microscopic examination. In our study, the stained tissue sections of group G showed the lowest percentage at day 35 with 46.6% of positive cases. Group G was treated with highest dose of polyherbal mixture and this group showed highest recovery from the disease and only 6.6% positive cases were observed at day 42 of this trial.

**Discussion**

Coccidiosis is caused by Protozoan parasites of the genus *Eimeria* (Coccidia: *Eimeriidae*) which live and multiplies in the intestinal tract. These parasites caused an enteric disease of chickens which caused economical losses in poultry worldwide (El-Shall et al. 2022). Economic importance of this disease is because of production losses and high morbidity resulting from an acute, bloody enteritis and high mortality rates. Eimeria species show specific predilection sites in intestinal tract and hence intestinal lesions of the infection are different depending on the species (Blake *et al*. 2020). About 1800 *Eimeria* species affect the intestinal mucosa of different mammals and birds but seven species of *Eimeria* including *E. tenella, E. necatrix*, *E. acervulina,*

*E. maxima, E. brunetti, E. mitis*, and *E. praecox* are the causative agents of coccidiosis in chickens.

This topic is specifically important due to the manifestation of a widespread anticoccidial resistance of *Eimeria* species and the problems linked with drug residues (Hashemi and Davoodi, 2010). There is a long list of chemotherapeutic agents which are being used in poultry industry all over the world. Many hazardous effects of drugs have also been reported on health and performance of broilers. This is the reason that people are thinking about alternatives to avoid antimicrobial resistance and health hazards of chemotherapeutic agents.

Performance of broiler chicks is denoted by live body weight (LBW) gain in a specific period of 42 days (Remmal *et al*. 2011). In our experiment, coccidiosis affected the live body weight of broilers chicks and different herbal products were used to control the disease separately and in combinations. Herbal products were compared with a drug (Amprolium) in our research work. The herbal mixture was equally effective against coccidiosis and the live body weight was similar to amprolium treated group. Our these findings were strongly supported by Zaman *et al*. (2012) who found *A. indica* as a cheap alternative agent against Eimeria species. They also observed similar effects of amprolium of live body weight of broiler chicks. But their work was differernt to our research as they workded on Eimeria tenella only. In another stuyd, Biu *et al*. (2006) observed the same effects of A indica on live body weight of broiler chicks but the number of birds per group was only four and histopathological changes were not correlated with coccidiosis in their study.

In our study, there was significant reduction in red blood cell count, hemoglobin concentration and packed cell volume in experimentally infected group of birds. This is similar to the findings of Ellakany *et al*. (2011) who observed the lower values of RBC, Hb and PCV in broiler chicks infected with E. tenella. The birds treated with herbal mixture showed higher values of red blood cell count, hemoglobin concentration and packed cell volume in our stuyd which was in line with the findings of Oyagbemi and Adejinmi (2012) who observed an increase in RBC, Hemoglobin concenration and packed cell volume of birds treated with A. indica. The reports of National Research Council showed that *A.indica* caused an increase in RBC count, WBC

count and antibodies production when given orally to birds (Council, 2002) which also support our findings.

The structural changes in cecum of positive control were more pronounced as compared to treatment group with polyherbal mixture and amprolium in our study. The similar histological changes were observed by Tsiouris *et al*. (2021) where they experimentally infected birds with Eimeria species and used a poly herbal mixture containing H. antidysentrica as a major constituent of the herbal preparation. In another study, Dkhil *et al*. (2013) reported the ameliorative effects of *A. indica* in Eimeria infection in rats which also support our findings of less histologic changes in intestine of birds in *Eimeria tenella* infection in broilers.

In conclusion, the constant use of chemotherapeutic substacne as feed additives against coccidiosis are creating drug resistance in broilers. They are producing a threat to consumers in terms of drug residues in meat. In this scario, the the herbal products are the best alternatives which are cost-effective and readily available in many areas of developing countries like pakistan. The herbal products are equally effective against coccidiosis as that of drugs but they are quite safe for birds as well as consumers. We summarized that herbal plants used in our research were effective and showed preventive, therapeutic and growth promoting effects against coccidiosis.

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**Table 1** Groups of broiler chicks receiving infection and treatments of herbal mixture.

| Groups | Infection | Treatment | Dose | No. of birds |
|---|---|---|---|---|
| **A** | *E.tenella* oocysts | NL powder | 2g/kg of feed | 30 |
| **B** | *E.tenella* oocysts | NL powder | 3g/kg of feed | 30 |
| **C** | *E.tenella* oocysts | KB powder | 3g/kg of feed | 30 |
| **D** | *E.tenella* oocysts | KB powder | 4g/kg of feed | 30 |
| **E** | *E.tenella* oocysts | NL + Mixture KB | 2g/kg of feed + 3 g/kg of feed | 30 |
| **F** | *E.tenella* oocysts | NL + Mixture KB | 3g/kg of feed + 4g/kg of feed | 30 |
| **G** | *E.tenella* oocysts | Amprolium | 1ml/liter of water | 30 |
| **H** | *E.tenella* oocysts | ------ | ------- | 30 |
| **I** | ---------- | ---------- | ------- | 30 |

**Table 2. Clinical signs of coccidiosis observed in broiler chicks from day 14 to 42 of the trial.**

| Groups | Average body weight at Day 35 (g) | Average body Weight at day 42 (g) | Morbidity (%) | Mortality (%) |
|---|---|---|---|---|
| **A** | **1462** | **2014** | **76.6** | **36.6** |
| **B** | **1665** | **2215** | **80** | **33.3** |
| **C** | **1575** | **2115** | **86.6** | **40** |
| **D** | **1645** | **2232** | **83.3** | **30** |
| **E** | **1427** | **2565** | **83.3** | **30** |
| **F** | **1968** | **2605** | **90** | **13.3** |
| **G** | **1967** | **2570** | **86.6** | **20** |
| **H** | **1320** | **1690** | **90** | **46.6** |
| **I** | **1987** | **2680** | **6.67** | **6.67** |

**Table 3 Mean values of blood parameters at day 35 in experimental birds.**

| Groups | Hb (g/dl) | RBC | WBC | PCV (%) |
|---|---|---|---|---|
| **A** | 8.9 ± 0.4[a] | 1.81±0.8[a] | 7.94±3.55[a] | 26.5±1.0[a] |
| **B** | 8.8 ± 0.6[a] | 2.59 ±0.4[b] | 7.68±6.61[a] | 27.0±0.9[a] |
| **C** | 8.9 ± 0.4[a] | 1.80 ± 0.5[a] | 7.94±3.55[a] | 26.8±0.9[a] |
| **D** | 9.0 ± 0.4[a] | 2.61 ± 0.4[b] | 7.94±3.55[a] | 27.9±1.0[a] |
| **E** | 9.1 ± 0.4[a] | 1.78±0.4[a] | 7.94±3.55[a] | 28.3±0.7[b] |
| **F** | 9.4 ± 0.4[b] | 3.50 ±.33[c] | 5.83±3.69[a] | 31.0±2.0[c] |
| **G** | 9.6 ± 0.3[c] | 3.47 ± .12[c] | 5.68±5.13[b] | 34.7±0.7[d] |
| **H** | 7.4 ± 0.1[d] | 0.67 ± .05[d] | 8.71±5.15[c] | 21.7±1.0[c] |
| **I** | 10.3 ± 0.4[e] | 4.22 ± .12[c] | 5.83±3.69[b] | 34.6±0.7[d] |

Mean ± SD, the values with different superscripts has significant difference at P<0.05

**Table 4 Mean values of blood parameters at day 42 in experimental birds**

| Groups | Hb (g/dl) | RBC | WBC | PCV (%) |
|---|---|---|---|---|
| **A** | 9.3±0.5[a] | 1.83±.09[a] | 7.89±4.31[a] | 28.5±.6[a] |
| **B** | 9.5±0.7[ab] | 2.59±.04[b] | 7.63±7.19[a] | 28.3±1.0[a] |
| **C** | 9.5±0.1[a] | 1.81±.07[c] | 7.63±7.19[a] | 28.1±0.7[a] |
| **D** | 9.4±0.7[a] | 2.59±.05[b] | 7.56±9.04[a] | 28.5±0.7[a] |
| **E** | 10.0±0.4[bd] | 1.77±.04[a] | 7.63±7.19[a] | 30.2±0.9[b] |
| **F** | 9.9±0.4[a] | 3.48±.40[c] | 5.62±5.62[b] | 34.2±0.9[c] |
| **G** | 9.9±0.4[ab] | 3.42±.14[c] | 5.66±5.49[b] | 34.3±0.8[c] |
| **H** | 7.1±0.5[c] | 0.66±.06[d] | 8.86±5.02[c] | 21.8±0.7[d] |
| **I** | 10.5±0.2[d] | 4.27±.14[c] | 5.84±3.21[b] | 34.4±0.7[c] |

Mean ± SD, the values with different superscripts has significant difference at P<0.05

**Table 5 Histopathological findings at Day 35 in broiler birds of different groups.**

| Groups | Total no of Birds | Nil (-) | Mild (+) | Moderate (++) | Severe (+++) | Positive % |
|---|---|---|---|---|---|---|
| **A** | 15 | 5 | 5 | 2 | 3 | 66.6 |
| **B** | 15 | 6 | 5 | 2 | 2 | 60.0 |
| **C** | 15 | 4 | 6 | 3 | 2 | 73.3 |
| **D** | 15 | 4 | 7 | 3 | 1 | 73.3 |
| **E** | 15 | 5 | 7 | 1 | 2 | 66.6 |
| **F** | 15 | 8 | 5 | 1 | 1 | 46.6 |
| **G** | 15 | 7 | 4 | 3 | 1 | 53.3 |
| **H** | 15 | 1 | 3 | 4 | 7 | 93.3 |
| **I** | 15 | 14 | 1 | 0 | 0 | 6.6 |

**Table 6: Histopathological findings at Day 42 in broiler birds of different groups**

| Groups | Total no of Birds | Nil (-) | Mild (+) | Moderate (++) | Severe (+++) | Positive % |
|---|---|---|---|---|---|---|
| **A** | 15 | 6 | 4 | 3 | 2 | 60.0 |
| **B** | 15 | 7 | 5 | 2 | 1 | 53.3 |
| **C** | 15 | 6 | 5 | 2 | 2 | 60.0 |
| **D** | 15 | 9 | 3 | 2 | 1 | 40.0 |
| **E** | 15 | 8 | 4 | 2 | 1 | 46.6 |
| **F** | 15 | 8 | 4 | 2 | 1 | 6.6 |
| **G** | 15 | 9 | 4 | 1 | 1 | 40.0 |
| **H** | 15 | 2 | 4 | 3 | 6 | 86.6 |
| **I** | 15 | 13 | 1 | 1 | 0 | 13.3 |

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# Review on Trypanosomiasis and their prevalence in ruminants

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## Abstract

Trypanosoma is a protozoan infection that has the potential to harm both humans and animals in practically every corner of the world. Cattle, buffaloes, sheep, and goats, including members of the family Camelidae, are among the ruminants that may be infected with the pathogen. The economic impact of this condition in various countries throughout the world was evaluated in this review research, which ran from 2018 to 2022. Iran, Syria, Iraq, Eastern Thailand, Nicaragua, Central Africa, Nigeria, Uganda, Indonesia, Philippines, Ecuador, Brazil, and Saudi Arabia were among the countries where the disease was studied in depth. In Pakistan, it is dire need to investigate it in wide range. In addition to the differences in diagnostic procedures, including blood smears, buffy coat smears, giemsa stained, serological testing, hematocrit centrifugation, and molecular analysis, PCR are used in these countries, the prevalence of trypanosoma is also different in each of these countries. In the United States, the prevalence of trypanosoma is the same as it is in other countries. In the current research, an investigation on the distribution and prevalence of trypanosoma infection in various countries was carried out using information from previously published research. The published literature from 2000 to 2022 was gathered using Google Scholar and PubMed. A total of 16 papers were published between 2018 and 2022 that looked at the frequency and distribution of trypnosoma infection around the world. According to published data, camel trypanosomiasis is more common in Saudi Arabia than in other countries, and PCR is used to diagnose the disease in approximately 85 percent of clinical and non-clinical cases. As a result, accurate diagnostic tests should be used to initiate or maintain quick medication or management of the condition, since failure to do so in the early stages may result in high mortality.

## Introduction

Trypanosomes are haemoprotozoans members of the trypanosoma genus, tryanosomatidae family, kinetoplastida order, and sarcomastigophora phylum. trypanosoma species and subspecies infect many hosts, including animals and people, and cause widespread disease. A

trypanosoma is an organism derived from trypano, which means "borer," and Soma, which means the body (Shah & Khan, 2019). Tsetse fly species are known to spread Trypanosomes. The relative relevance of their transmission, on the other hand, is determined by the intensity of their connections with vulnerable hosts.

For this reason, the survival of livestock and small ruminants, as well as horses, in the Gambia is jeopardised as long as these insects are allowed to persist in the country. Tsetse fly (Kuye,2020). Trypanosomiasis is associated with anaemia, which is the most common clinical sign. The signs of an infection in animals include high fever, reduced hunger, anorexia, sharp fall in milk production, lymph nodes enlarged, and an overall decline in health. The presence of jaundice with hemoglobinuria is unusual. It is also possible that trypanosomiasis is causing milk production to have reduced output fecundity has been decreased in animals. In contrast, mortality has increased (De Gier, Cecchi, Paone, Dede, & Zhao, 2020). Pathogenic trypanosomes that infect and kill domestic animals constitute a significant source of illness and mortality in these species. These viruses are a significant impediment to economic development in Africa. Their detrimental influence on the economies of South America and Asia is becoming more pronounced (Wilkowsky, 2018).

The disease has a significant economic impact in India; however, it is impossible to estimate the financial losses due to a lack of precise epidemiological statistics and an inability to collect sufficient data. More than half of the world's cattle, hundred million buffaloes, and twelve million camels are at risk of extinction because of this epidemic. It is responsible for 30% of morbidity and 3% of death in the population of the United States. However, the economic losses produced by surra in Asia may be more significant than those caused by African trypanosomes; in terms of the cost of meat and dairy products, it is estimated that they will cost 1.3 billion US$ (Kristjanson, Swallow, Rowlands, Kruska, & De Leeuw, 1999).

Trypanosoma affects significant number of domestic and wild animals around the world, including mules, horses, donkeys, camels, buffalo, sheep and a herd of goats, cows, pigs, dogs, elephants, deer, foxes, tigers, and jackals, and causes a persistently high but intermittent temperature, anemia, weight loss, edema-affected regions, anxious symptoms, and abortion among other symptoms. Animal and poultry producers suffer huge losses as a result of it. World Health Organization (WHO) states that animal trypanosomiasis is currently a permanent constraint on cattle productivity across Asia, Africa, and Latin America. According to the World Health Organization, the disease's geographic spread is still in flux (Desquesnes *et al*., 2013).

**Trypanosomiasis**

The members of the genus trypanosoma are the causal agents' African human trypanosomiasis (also known as African sleeping sickness) and American trypanosomiasis (also known as Chagas' illness). Insects transmit Blood-borne diseases (Barrett et al., 2003). It is a vector-borne disease spread mainly through blood-sucking insects for example tabanus, haematopota, atylotus, chrysops, stomoxys, and heamatobia (Otto *et al.,* 2010).

*Trypanosoma vivax, Trypanosoma congolense*, *T. brucei*, *T. simiae*, and *Trypanosoma evansi* are the most prevalent pathogenic trypanosome species. Cattle have the highest prevalence of the first three. *T. congolense* is the most commonly seen parasitic trypanosome in cattle (Algehani, Jaber, Khan, & Alsulami, 2021). The transmission of African animal trypanosomiasis is carried out by various trypanosome species (AAT), including trypanosoma congolense, *T. vivax*, *T. Godfrey*, *T. simiae*, and *T. brucei*. subspecies are responsible for

transmitting African human trypanosomiasis (Isaac *et al.,* 2016) also include trypanosome species spread mainly through biting vectors such as tsetse flies (Rahman, Goreish, Yagi, Rajab, & Gasmir, 2008).

Animal trypanosomiasis is an endemic disease in tropical regions of Asia, Africa, and South America, and it may cause both human and animal disease (Aregawi, Agga, Abdi, & Büscher, 2019). One of the biggest obstacles establishing animal husbandry and breeding and livestock-related businesses in Africa is a lack of available land for grazing. In addition, in tropical Africa, it harms mixed farming, human health, and livelihood. Tsetse flies are Africa's most prevalent vector of the illness. The chronic illness is characterized by a depressed mood, fitful fever, anorexia, anemia, diarrhea with a bloody tinge, and adenopathy, associated with petechiae on the mucosa. If left untreated, it may result in abortion and death (Maichomo, Orenge, & Gamba, 2021). Animal trypanosomes may be found in ruminants, camels, equines, pigs, and carnivores, among other animals. In addition to decreasing livestock output, the illness harms livestock producers' wealth and the overall community's nutritional status (Holt, Selby, Mumba, Napier, & Guitian, 2016).

The parasite Trypanosoma evansi causes trypanosomiasis, which is endemic in the majority of tropical areas, including Africa, Latin America, and Asia. The principal targets of *T. evansi* illness are camels and horses, which are conveyed mechanically by biting flies and other vectors. This illness has also impacted other animal species, including goats, sheep, cattle, donkeys, and buffaloes (Al-Abedi, Spray, Hussein, & Gharban, 2018). Surra also known as "Mal de caderas" is a trypanosomal infection caused by the *Trypanosoma evansi* parasite that affects both domestic and wild animals, causing extensive disease and death. It is the most prevalent trypanosomal disease of domestic and wild animals. The parasite is mechanically disseminated by biting flies and is present in a broad variety of geographical places across the globe (Monzón, Mancebo, & Roux, 1990; Truc *et al.,* 2013).

The parasite *T. congolense* lives inside the body's blood vessels. The *T. brucei* travel outside and invade other parts of the body. The virulence mechanisms, host-pathogen connections, and bodily consequences of the two species are all highly different. It can be grown in vitro for *T. congolense*, but it can't be done for *T. brucei*, which can't be done that way (Gray, Ross, Taylor, & Luckins, 1984; Gray, Ross, Taylor, Tetley, & Luckins, 1985).

**Worldwide distribution**

Survivors of Surra are found in tropical and subtropical parts of Northern Africa, Southeast Asia, Central and South America, and the Caribbean. Surra, and the causative agent *T. evansi*, are also found in tropical and subtropical regions of Central and South America (Raina, Kumar, Sridhar, & Singh, 1985). *Trypanosoma evansi* is a salivation trypanosome that is extensively spread in Southern America, Africa, and Asia, among other places (Aregawi *et al.,* 2019). In camels from northern Kenya, epizootic diseases have been found. A further result of this study is that the same infections were present in camel keds obtained from the same studied animals. These flies may be helpful in the xenodiagnosis of haemopathogens that are circulating among camels (Kidambasi, Masiga, Villinger, Carrington, & Bargul, 2020).

In contrast to other geographical places, it seems that the medical and economic consequences of *T. evansi* are pretty varied. It mainly affects camels and horses in Africa. It is typically regarded as a moderate or minor infection in cattle in the rest of the world. Horses, dogs, and buffalo (Bubalus bubalis) are the most frequently affected; animals in Latin America; nevertheless, it is also regarded as a weak or inconsequential infection in cattle. In Asia, on the

other hand, it influences water buffalo, horses, and pigs and cattle (Holland *et al*., 2005; D Tuntasuvan & Luckins, 1998; Darunee Tuntasuvan, Sarataphan, & Nishikawa, 1997).

According to researcher Hasan *T. evansi*, the virus is quite uncommon in Pakistan's Punjab region (Hasan *et al*., 2006). According to research conducted in the country by Mauritania, camel trypanosomiasis was projected to be widespread in the country, particularly in forested regions near streams. The greatest rise in frequency was seen in the animals between the ages of five and 10 years (Dia, Diop, Aminetou, Jacquiet, and Thiam, 1997).

According to Van Vinh Chau, the first reported *T. evansi* in Vietnam occurred in 1997. The patient had previously been healthy. According to him, a raw flesh wound that happened during the meat-processing technique was the cause of the disease (Zambrano Salazar, 2021).

**Life cycle and vector**

A large number of hematophagous flies are carriers of the trypanosomes parasite, present in several hosts' internal and extracellular fluids. Hematophagous insects are responsible for its mechanical transmission (Luckins & Dwinger, 2004).

**Table-1 Prevalence of Trypanosome in ruminants.**

| Researcher | The year | Country | Sample | Total of Sample | Type of Species | Method for analysis | Percentage |
|---|---|---|---|---|---|---|---|
| Asghari | 2022 | Iran | *Camelus dromedarius* | 134 | *T. vivax* *T. evansi* | Blood microscopy and PCR analysis | Blood microscopy showed 2.98% While PCR analysis showed 30.6% |
| Gomes | 2021 | Brazil | Bovine | 623 | *T. vivax* | Giemsa stained blood smears, PCR molecular analysis | Blood smear 0.3% PCR analysis 18.9% |
| Elobaid | 2021 | Saudi Arabia | *Camelus dromedarius* | 454 | *T. evansi* | wet and thick smear film PCR- ITS1 and RoTat | wet and thick smear film 3.1% and 3.5% PCR- ITS1 and RoTat 19% |
| Chávez-Larrea | 2021 | Ecuador | Cattle | 20 | *T. vivax* | PCR- viCatL and DTO155 | PCR- viCatL and DTO155 15% |
| Bahrami | 2021 | Iran | *Camelus dromedarius* | 167 | *T. evansi* | Blood smear examination PCR molecular analysis | Blood smear examination 6% PCR molecular analysis 8.4% |
| Setiawan | 2021 | Indonesia | Cattle | 100 | *T. evansi* | microscopic observation PCR- ITS2 | microscopic observation 1% PCR- ITS2 3% |
| Kizza | 2021 | Uganda | Cattle | 460 | *T. vivax* *T. congolense* *T.evansi* | ITS-PCR assay | *T. vivax*+ *T.congolense* =20.4% *T.evansi* 10.2% |
| Oyewusi | 2019 | Nigeria | Sheep | 243 | *T. vivax* *T. congolense (Savannah)* *T. congolense* (forest) *T. congolense* (Kilifi) *T.brucei brucei* | HCT method PCR analysis by primers | HCT method found no positive PCR analysis by specific primers shows the following results. *T. vivax* 23.05% *T. congolense (Savannah)* 13.38% *T. congolense (Forest)* 7.82% *T. congolense (Kilifi)* 0% *T. brucei brucei* 4.53% |

| Researcher | The year | Country | Sample | Total of Sample | Type of Species | Method for analysis | Percentage |
|---|---|---|---|---|---|---|---|
| Maganga | 2020 | Central Africa | Sheep goats | 146 sheep 140 goats=286 total | *Trypanosoma spp.* | PCR-IST1, IST2, ITS3, and ITS4 analysis | By PCR-IST1, IST2, ITS3, and ITS4 in goats *T.vivax* 2.14%,*T. simiae* 5.71%,*T. simiae tsavo* 5%,*T. theileri* 0%,*T. brucei* 0.71%,*T. congolense* 0.71% In sheep *T.vivax* 0%, *T. simiae* 18.49%, *T. simiae* 0%, *T. theileri* 1.37%, *T. brucei* 0%, *T. congolense* 0% |
| Megasari | 2020 | Syria | Cattle | 207 | *T. evansi* | RoTat1.2-PCR amplified ITS1 region | RoTat1.2-PCR amplified ITS1 region shows 13% positive |
| Shaeel | 2020 | Iraq | Cattle | 150 | *Trypanosoma spp.* | Microscopy examination PCR assay | Microscopy examination 5% PCR assay 14% |
| Metwally | 2021 | Saudi Arabia | Camelus dromedarius | 400 | *T. evansi* | Thin blood smears microscopy ITS-1 and ITS-2 PCR assay | Thin blood smears microscopy shows 0% ITS-1 and ITS-2 PCR assay showed 85.5% |
| Panja | 2019 | Eastern Thailand | Buffaloes | 153 | *T. evansi* | PCR assay analysis | PCR assay analysis shows 21.92% |
| Bonilla | 2021 | Nicaragua | Sheep | 30 | *T.vivax* | Blood smears microscopy PCR analysis | Blood smears microscopy shows 73.3% PCR analysis 36.7% |
| Mirshekar | 2019 | Iran | Iranian dromedary camel | 370 | *T.evansi* | Micro-hematocrit centrifugation technique and PCR analysis | Micro-hematocrit centrifugation technique 11.89% PCR analysis 31.5% |
| Elata | 2020 | Philippines | Goat | 251 | *T. evansi* | PCR- TS1 | PCR- TS1 33.9% |

Trypanosomes have complicated life cycles that comprise both proliferative and differentiation cell divisions in nature. The parasite's capacity to organise its cell cycle in order to accomplish these several divisions is important for infecting both the host and the vector (Wheeler, Gull, & Sunter, 2019).After reaching high concentrations slender stage cells circulate in the blood of the mammalian host transform into stumpy stage cells, which have entered the cell cycle and cannot reproduce. It is considered that the stumpy stage is the only capable of successful vector transmission. It performs two crucial functions: first, it regulates the parasite load in the host, and second, it serves as a transitional step among the parasite burden and the host (Schuster *et al.,* 2021).

If exposed to the environment, it may move through the circulatory system's spectrum of body fluids, including the lymphatic system and cerebrospinal fluid, as well as the placenta. Parasites migrate from liquids to organs, with the most common parasite disorders affecting the brain and central nervous system (CNS). However, as soon as the tsetse fly consumes blood, parasites in trypomastigote form begin to travel to the insect vector's midgut, where the processes occurring inside the vector may be seen for the first time. Following the entry into the midgut, trypomastigote forms continue to increase and migrate down the esophagus and hypopharynx, finally reaching the salivary gland. Certain strains of these bacteria are contagious, whereas others are not. They can grow and convert into highly pathogenic metacyclic forms (Sharma *et*

*al.,* 2009).

Contaminated blood is briefly kept in the crop, which acts as a storage site and allows the tsetse to absorb more blood each meal, or it is transferred directly to the midgut. Trypanosomes move to the proliferative procyclic stage after passing through the midgut. Once implanted in the midgut, parasites must migrate through the peritrophic matrix. This protective sleeve divides the bloodmeal from the midgut tissue that surrounds it. Parasites are thought to do so by swimming up the endotrophic space to the proventriculus, the site of peritrophic matrix development, from whence they may enter the ectotrophic area (Rose *et al.,* 2020).

**Symptoms**

The animals infected with Nagana and Surra display clinical symptoms such as sporadic fever, anemia, lymphadenopathy, wasting, loss of condition, pallor (pallor of the skin), abortion, lacrimation (milking), weakness, and lethargy. They lag behind the rest of the herd. Diseases are prevalent on a global scale. It is possible to have an acute, subacute, or chronic (Namangala & Odongo, 2014). Numerous studies have shown that trypanosoma damages cells and affects various physiological organs. Additionally, the parasite alters the chemical composition of the buffaloes' blood, increasing their bodies' oxidative stress (Pandey *et al.,* 2015; Hussain *et al.,* 2018).

Some ruminants, such as cows, buffaloes, sheep, and goats, can die from *T. vivax* infection because of the high fever and anemia caused by the sickness, leading to death (Gonzatti *et al*., 2014). Animals exposed to high fevers and induced anemia due to *T. vivax* are at risk of contracting a severe and usually fatal sickness. Ruminants such as cattle, buffaloes, sheep, and goats are particularly vulnerable ( Gonzatti, Gonzál ez-Baradat, Aso, & Reyna-Bello, 2014).

Acute anorexia with increased body temperature and severe generalized edema is the most prevalent clinical signs of ill camels. Additionally, the chronic form of trypanosoma infection manifests in noticeable muscle atrophy, an undulant fever, pale mucous membranes, gradually decreasing body weight, and ascites, among other symptoms, are more common. In certain situations, diseased dromedaries emit a characteristic odor of urine ketones, which may be identified by their smell (Constable *et al.,* 2017).

The symptoms of *T. evansi* infection are severe and sometimes fatal, mainly when the sickness is in its late stages. The severity of the illness may vary from chronic to potentially lethal. Eventually, it results in progressive weakness, depletion, lymph node enlargement, and death (Saleh *et al.,* 2009; Herrera *et al.,* 2002).

According to Eyob, E., and Matias, microscopic examination of trypanosomiasis is a dangerous disease that can spread to a wide range of domestic and wild animals, as well as humans, and it can be tough to treat. The spleen, liver, heart, lungs, brain, and kidneys are all affected by *T. evansi* infection, as are many other vital parts of the body. *Trypanosoma evansi* is a disease that affects many camel species, causing much money to be lost because of the high cost of medicine, high death rates, weight loss, and decreased animal production caused by the disease (Eyob & Matios, 2013). Antioxidant enzymes were more elevated in camels naturally infected with *T. evansi* than in those not infected with the virus. When the mean values of the positive group were compared to the mean values of the opposing group, there was a significant difference (G6PDH and G.R.). It did not change BUN levels, serum creatinine, or total protein. However, the A/G ratio did change because of this. There was a lot of hypoalbuminemia and hyperglobulinemia in the blood. Damage to the liver may be why there is not enough albumin

and much globulin in the blood (Ahmad, Butt, Muhammad, Athar, & Khan, 2004; Chaudhary & Iqbal, 2000).

However, the liver function tests revealed a significant rise in the lactate dehydrogenase enzyme (LDH) activity and globulin levels, total and indirect bilirubin. In contrast, a substantial decrease in the movement of the alkaline phosphatase enzyme was seen (Baldissera et al., 2017).

Trypanosome infection increased the WBC count in the orally and intraperitoneally treated groups to 4.3 109 WBC/mm3 and 6.7 109 WBC/mm3, respectively (P 0.050). Similarly, the percentage of lymphocytes in the infected group was lowered relative to the non-infected group. Still, it rose in the treated groups (P 0.050). RBC count and hemoglobin content were significantly lower (P 0.050) in the untreated infected group than in the non-infected group. However, both were greatly improved after LSSE treatment. The hematocrit (HCT) was markedly lower in the untreated infected group than in the control group (P 0.050). After therapy, it returned to near-normal levels. AST and ALT levels were significantly higher in the untreated infected group than in the uninfected control group (P 0.050). However, LSSE treatment restored AST and ALT to near-normal levels (Al-Otaibi, Al-Quraishy, Al-Malki, & Abdel-Baki, 2019).

This investigation focused on several clinical indications, including rectal temperature, feed intake, overall body condition, and weakness and dullness. Rough hair coat, expansion of peripheral lymph nodes, runny nose, and quick pulse are examined (Scholar, 2021).

The findings of Amin's study revealed that *T. evansi* infection in bulls, which included the negative influence of *T. evansi* on the reproductive system of the animals, was connected mainly with histological changes in the animals' testicular tissue. The purpose of this study was to assess the changes in reproductive testicular parameters and the incidence of reproductive failure in dromedary bulls during the breeding season. Infected Camelus dromedarius were studied using a variety of diagnostic methods after infection with *T. evansi*, including examination of oxidative stress, testicular lesions, semen features, hormone levels, and hematological and biochemical parameters, among other things (Amin, Noseer, Fouad, Ali, & Mahmoud, 2020).

The lungs and other organs, including the liver, intestinal tract, kidneys (including testicles), bone marrow (including cerebellum), brain (including cerebellum), and testicles, had interstitial tissues filled with immune cells from other bodies (including bone marrow). There were also trypanosomes in the lungs and other parts of the body. Also, B- and T-cell responses were found in the lymphatic system. However, the findings showed that the lymph nodes, spleen, and bone marrow were immune-suppressed (Dargantes, Campbell, Copeman, & Reid, 2005).

Hypoglycemia may occur as a result of trypanosoma infections. Trypanosomes have been shown to cause a decline in blood glucose levels at periods of high parasitemia, presumably as a result of utilizing glucose for metabolic activity (Gutiérrez Cabrera, Corbera Sánchez, Juste de Santa Ana, Doreste, & Morales Fariña, 2006)(Silva, Sequeira, Santos, & Tiago, 2013).

Another possibility for the leukopenia seen at the beginning of the infection is that leukocytes are undergoing apoptosis, as shown in rats infected with *T. brucei* (Happi, Milner Jr, & Antia, 2012). Previous studies have indicated that erythrocytes can shield neutrophils from apoptosis via glutathione and catalase metabolism, contributing to the neutrophilic decline. However, for this to be confirmed, an additional study must be done in this area(Aoshiba, Nakajima, Yasui,

Tamaoki, & Nagai, 1999). According to this research, the number of eosinophils in the blood of cattle exposed to *T. vivax* had decreased, which is consistent with earlier findings (Dagnachew et al., 2015).

As reported here, brain tissue from infected subgroups of mice was shown to have vacuolar degeneration and a moderate mononuclear cellular infiltration in the cerebellum. When *T. evansi* infected Swiss albino mice, the alterations in their brain tissue were identical to those seen in the brain of infected Swiss albino mice (Bal et al., 2012). In addition to being more sensitive, DNA-based approaches benefit from detecting trypanosomes down to the subspecies level. They can also tell if there are mixed infections (Isaac et al., 2016).

*T. evansi* has been associated with neurologic symptoms in horses, cattle, deer, and buffaloes during the final phase of natural infection, although there has been little research into this in horses (Seiler, Omar, & Jackson, 1981; D Tuntasuvan et al., 2000; Darunee Tuntasuvan, Sarataphan, & Nishikawa, 1997).

**Diagnosis**

Due to the absence of clinical signs in camels, numerous approaches with varying sensitivity and specificity may be used to diagnose human trypanosoma infection. For the identification of *Trypanosoma spp*., standard detection approaches such as microscopic examination of fresh or stained blood smears are used. However, they may lack the sensitivity and specificity necessary to identify certain parasites (Salim et al., 2011).

Trypanosoma may be identified in the blood using a light microscope at a magnification of 40X. A microscopic examination of blood samples stained with Giemsa staining may be undertaken later in the procedure (Mafie et al., 2018).

Microscopy is essential in diagnosing HAT since it detects motile trypanosomes and fixes and stained organisms in the bloodstream. Microscopy of spinal fluid must be done after a HAT diagnosis is made to see if the patient has reached the neurological stage of the illness, which is very important in deciding what kind of treatment the patient needs (Brun, Blum, Chappuis, & Burri, 2010).

In the last few years, Carl Zeiss and FIND have made an LED microscope that is so bright that it can be used for both fluorescence and bright field microscopy, called the Primo Star iLED. Acridine orange-based fluorescence microscopy and traditional Giemsa stain microscopy were compared in this investigation to see whether a parasite concentration and RBC lysis step may improve the method's sensitivity in detecting trypanosomes in the blood (Biéler et al., 2012).

Historically, *Trypanosoma spp*. was recognized using standard trypanosome detection methods, such as microscopic examination of fresh or stained blood smears (microscopy). Regrettably, this technique lacks the necessary sensitivity and specificity. The World Organization for Animal Health (OIE) has approved the card agglutination test for *T. evansi* as a rapid diagnostic test. For the identification of trypanosomosis in camels, serological tests have been designed and evaluated. Antibodies are detected using the CATT T.evansi and other assays (Songa & Hamers, 1988).

To conduct the formol gel test (FGT), one milliliter of serum was mixed well with two drops of robust formalin solution (which contained 37 percent formaldehyde). When the serum started to coagulate immediately and turned opaque, positive findings were proclaimed (Jacobson,

2004).

Additionally, the antigen preparation utilized in the enzyme-linked immune sorbent test has been used to identify (ELISA). ELISA each trypanosome isolate was tested in triplicate using an ELISA plate under similar conditions. Two weeks before the infection, negative serum samples from each sample were collected and used as controls. Specimens were collected on days 1, 2, 10, 18, 24, 30, 39, 47, and 61 of the experimental infection. Sensitized plates containing 20 g of antigen per well were washed five times with washing buffer (W.B.) and blocked for an hour at 37°C with 200 l/well of 5% skim milk in PBS. Before adding 100 l/well of each serum diluted 1:200 in W.B., the plates were washed five times with W.B. Each ELISA plate was pre-inoculated with positive and negative control sera. After an hour of incubation at 37°C, the dishes were washed five times with W.B., and 100 conjugate rabbit peroxidase-conjugated anti-(any sample) IgG Pierce were added. The Immunocore antibody was diluted at 1:5000 in W.B. and incubated for 60 minutes at 37°C. Following incubation, the plates were washed three times, and 100 l of the substrate solution, ABTS 2% H2O, was added and incubated for 45 minutes at 37 °C. Photometrically, absorbance was determined at 405nm using an ELISA reader (Parra-Gimenez & Reyna-Bello, 2019).

Many molecular diagnostic methods have been used to diagnose camel trypanosomiasis, including conventional and real-time polymerase chain reaction (PCR) tests. They are more sensitive than other methods and have the benefit of being able to categorize parasites at the subspecies level, which is not achievable with other systems(Barghash, Abou El-Naga, El-Sherbeny, & Darwish, 2014; Elhaig, Youssef, & El-Gayar, 2013)

Molecular analysis targeting the internal transcribed spacer 1 (ITS1) region has been used in epidemiological research because it enables the identification of many different trypanosome species using a single polymerase chain reaction (PCR (Salim, Bakheit, Kamau, Nakamura, & Sugimoto, 2011). The use of molecular technologies, such as the typical polymerase chain reaction (PCR), is beneficial for sampling big animals during field research initiatives (Behour, Aboelhadid, & Mousa, 2015). The unique molecular identification and characterization of each sample are dependent on special primers for each species used. In the past, the primers for amplifying the internal transcribed spacer (ITS1) in DNA is a sequence that is transcribed during the transcription process (ITS1CF and ITS1BR) were designed to do so (Constantine *et al.,* 2007).

**Prevention**

In the fight against AAT and the tsetse fly vector cycle, a number of different strategies have been used. It has been decided to adopt vector control measures as well as the diagnosis and treatment of animals afflicted with AAT. Trypanocides such as isometamidium chloride (ISM), diminazene acetonate (DA), and ethidium bromide are the only three presently available trypanocides that may be used to treat or prevent the spread of trypanosomes (Barrett, Coombs, & Mottram, 2004). Mechanical transmissions do not just cause the spread of trypanosomosis in the environment through vectors. Animal mobility also helps spread the disease (Holmes, 2013).

Current and future attempts to successfully eradicate AAT and its vectors in Sudan will need reliable information on the disease's geographic spread. Although epidemiological research has been conducted throughout the years, the prevalence of tsetse and AAT has not been geo-referenced or harmonized. Planning, implementing, and monitoring field engagement needs this kind of data. An project to map the prevalence of Tsetse and trypanosoma infections in

Sudan has been launched by Sudan's VRI (Vector Research Institute). The FAO's technical assistance includes the Atlas of Tsetse and AAT, according to this study (Cecchi et al., 2014; Cecchi, Paone, Herrero, Vreysen, & Mattioli, 2015). The African trypanosomosis programme's tsetse and tsetse Atlas is part of that programme (PAAT).

**Vaccination and treatment**

Currently, there are four main medicines that are used to treat HAT. These are suramin, pentamidine, melarsoprol, eflornithine (difluoro-methyl ornithine, or DFMO), and nifurtimox (Fairlamb, 2003).Most of these medications were developed in the first part of the twentieth century. Since 1981, no new medicine for human use has been licensed. A drug named DB 289, a dimidine derivative, has just completed its second clinical study (Legros *et al.,* 2002). Treatment for the early stages of rhodesiense and gambiense disorders is accomplished by the use of suramin and pentamidine. There is just one medicine that can be used to treat both forms of HAT in its late stages: arsenical melarsoprol. Individuals who take treatment may absorb it into their brains (Legros *et al.,* 2002). Nifurtimox may be taken orally for up to two months, whereas DFMO is administered intravenously for five weeks. However, the DFMO is not widely used, and it is considered too costly for most people to use daily. Also, DMFO is exclusively effective against *T. b. gambiense*, thus use it just against this infection (Burri & Brun, 2003).

Trypanosomiasis medicines are already available, but they have considerable side effects, and the parasite is becoming more resistant to the pharmaceuticals being used to treat it (Kizza *et al.,* 2021). However, diminazene aceturate is potentially dangerous to the host when treating T. evansi infection in domestic animals. (Do Carmo *et al.,* 2015).

To fight trypanosomiasis, it is crucial to keep researching new, safe treatments (Adeyemi et al., 2018). Indigofera oblongifolia was given to sick mice, and the parasite count in their blood was substantially lower than in control blood samples. Active compounds such as phenol, quinines, saponins, and coumarin have been shown to protect the body (Nassef, El-Melegy, Beshay, Al-Sharaky, & Al-Attar, 2018). Despite the use of well-known anti-trypanosome medications including Nagano, Cymelarsan, and Antrycide, most blood samples confirmed positive for Trypanosoma evansi failed to react to either of the two regimens (Shahjahan, Vani, & Devi, 2005).

The incidence of sickness increases at an alarming rate, reaching epidemic proportions. With the lack of a viable mammalian vaccination and a scarcity of affordable and effective drugs, illness management has become more problematic (Aksoy, 2003).

The availability of accurate and sensitive diagnostic instruments for controlling *T. evansi* infection is an absolute need. DNA-based PCR detection methods, on the other hand, satisfy all of these requirements (Li *et al*., 2020).

It is thought about if regular doses of medicine don't work. This is called drug resistance. When treatment doesn't work, it may be because of things other than parasite resistance to medications. Having parasites in animals that have been treated might mean they have a new infection, not a recurrence, in places where they are at risk (Rowlands *et al*., 2001). They don't know why the animal isn't healthy enough, how to use the medicine (like complicated treatment or long breaks between treatments), or the animal doesn't get enough of the drug. In this case, low-quality medicines could be to blame. Either because they were stored incorrectly or because they used counterfeit products, this could be the reason (Sutcliffe *et al.,* 2014).

As little as 0.5 and 0.25 mg/kg of body weight were used to treat mice that had been infected with the *T. evansi*. Six compounds were used. Besides that, they were better than the most common medications, such as suramin, diminazene, and quinapyramine. The three diamidine compounds, D.B. 75, DB 867, and DB 1192, met all of the criteria for being chosen. They could be used as preclinical candidates to fight off *T. evansi* infection (Gillingwater *et al*., 2009).

*T. congolense* and *T. brucei* isolates were tested in vivo, and the results revealed that the single-dose mouse test is still the gold standard for quickly identifying single or multiple resistance.Although the test does not quantify the number of parasites eliminated, it does give a means of determining if parasites react to dosages of the treatment used in veterinary medicine. There are methods for optimizing drug sensitivity studies that do not need microscopy. For instance, PCR methods may be used instead of microscopy to detect trypanosomes in the blood (Tran *et al.,* 2014).

The maximal dose evaluated in this experiment was hundred percent effective against *T. evansi*. On the other hand, Cordycepin was not curative in vivo when administered alone; instead, it lengthened the animals' lives when given therapy. Due to the rapid deamination of Cordycepin (3′-deoxyadenosine) to 3′-deoxyinosine, the trypanosomal enzymes inosine and deoxyinosine hydrolases may inactivate and destroy Cordycepin (Rottenberg *et al.,* 2005).

Drug resistance may emerge as a consequence of the abuse of trypanocidal drugs. Many reports of drug resistance to pharmaceuticals such as isometamidium chloride and diminazene aceturate have been produced in the context of these therapies (Nuryady, Widayanti, Nurcahyo, & Fadjrinatha, 2019).

**Discussion**

There have been fifteen articles examining the frequency and distribution of trypanosomiasis in animals worldwide. This review summarized sixteen reports to ascertain the present state of trypanosomiasis and its spread rates in a variety of countries worldwide, including Iran, Syria, Iraq, Eastern Thailand, Nicaragua, Central Africa, Nigeria, Uganda, Indonesia, the Philippines, Ecuador, Brazil, and Saudi Arabia. The current research attributes variations in the prevalence and spread of *Trypanosoma evansi* among countries to geographic location, animal management techniques, seasons, and the animals' breed, sex, and age. Certain nations seem to be more concerned with infection-control strategies with a low infection rate. A PCR experiment demonstrated that the parasite was responsible for 85.5% of trypanosomiasis infections in Saudi Arabian camels. Saudi Arabia's camel population has expanded dramatically in recent years (Faye, 2015), in 2017, there were over 500,000 camels in the Riyadh area, representing tremendous growth (FAOSTAT, 2019). The prevalence numbers for each species vary significantly among nations, depending on the diagnostic technique employed and the geographic region covered by reports. Camels had the highest estimated prevalence values of any species. Cattle, buffaloes, goats, sheep, and sheep were the following animals on the list.

A substantial source of revenue and food for many people who live in many regions of the globe, camels are an essential source of money and food. *Trypanosoma evansi* infections are less prevalent in camel markets in locations where people are better knowledgeable of how to feed and care for their camels, administer medications, and how vital it is for camel owners to sell their camels for a reasonable price. Sheep and goat infections are often regarded as moderate or non-threatening. A superficial corneal ulcer and retinochoroiditis were seen in goats that had been artificially infected, although there were no visual impairments (Morales *et al.,* 2006). Surra was detected in both acute and chronic manifestations in the camel. It has

called a critical case when severe fever, anemia, infirmity, and death. If they are sick for a long time, they are more likely to have chronic issues. The condition is devastating and has three-year duration. It includes the physical manifestations of disease like intermittent fever, dullness, progressive weakness, loss of appetite, and edema in the central parts. Young animals are more likely to get sick, but the disease affects animals of all ages. Camels may have lymph glands that grow and become infected in the inguinal region. *Trypanosoma evansi* also caused dromedary camels in the Canary Islands to have abortions and die at a high rate as babies (Gutierrez *et al.,* 2005).

According to different researches, Surra was discovered in Pakistan. Tehseen *et al*. (2015) assessed *T. evansi* infection in camels in Pakistan's Cholistan Desert using parasitological, serological, and molecular techniques. Their results were published in Parasitology, Serology, and Molecular Research. Abbasi *et al.* (2014) conducted comparative research. Immunodiagnosis is the only successful approach in field circumstances, particularly in RDT (Rapid Diagnostic Test) environments. However, there is no antigen capture test available that can be used to detect active infections, which is a severe drawback. The use of whole trypanosome lysates as antigens in the currently used ELISA-based antibody detection techniques has been limited, resulting in the problem of antigen standardization. The few articles that employed a unique recombinant protein as an antigen to address standardization problems were often found to be inadequately sensitive (Desquesnes *et al.,* 2009).

This review discovered that *Trypanosoma evansi* infections are frequent in numerous nations on a broad scale. Despite this, there is very little understanding of how animals and people acquire it. *Trypanosoma evansi* spreads to many animals through the bites of flies. Different species can cause a wide range of diseases that can significantly impact animals' health and the economy.

Take note of the study's flaws, including reporting bias and other issues. It could not conduct a statistical review of publication bias due to the lack of variance in diagnostic test categories, geographic location, breed of animals collected, and study era.

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# Review on anaplasmosis in different ruminants

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## Abstract

*Anaplasma* bacteria, particularly *A. marginale*, *A. ovis*, and *A. phagocytophilum*, have attracted researchers' attention in recent years. To a lesser extent, it has to do with the pathogenicity of these bacteria for farm animals and people alike. Anaplasmosis, which is a disease caused by numerous anaplasmosis species, is a major concern for animal producers. Ixodes, Dermacentor, Amblyomma, and Rhipicephalus ticks are the most frequent vectors of Anaplasma bacteria, which may be found in almost every region of the globe. Eukaryotic cells' vacuoles are host to obligatory intracellular bacteria of the *Anaplasma* genus. The obligatory intracellular bacteria Anaplasma centrale, *A. bovis, A. ovis,* and *A. marginale*, infect animal (mostly ruminants) red blood cells and monocytes. Dogs are its primary host, although it may also infect humans and domestic animals. In this study, we discussed six Anaplasma species and their vectors from across the globe.

## Introduction

Anaplasmosis is a minor clinical illness caused by intraerythrocytic rickettsia of the genus Anaplasma (Rar, Tkachev et al. 2021). Depressive symptoms, debility, decreased milk production, weight loss, miscarriage, severe anaemia, and jaundice have been reported in endemic areas due to infections with parasites *Anaplasma ovis* and *Anaplasma marginale* (*A. marginale*) (Hosseini-Chegeni and Tavakoli 2020). Bacteria belonging to the genus Anaplasma are members of the kingdom Prokaryote and, more particularly, of the family Anaplasmataceae. There are rickettsial tick infections called Anaplasma species that cause anaplasmosis, which is the most common form of anaplasmosis in ruminants like camels and cattle. Anaplasmosis is a serious disease that causes large financial losses, especially in subtropical and tropical regions (Omer, Elfehid et al. 2021). Anaplasma species were thought to be parasites until genetic studies showed that they are rickettsial bacterial pathogens that belong to the Anaplasmataceae family (Dumler, Barbet et al. 2001). Sir Arnold Theiler developed the term "yellow bag" or "gall sickness" in 1910 (Theiler and Therapeutics 1910, Kocan, de la Fuente et

al. 2010). A disease of ruminants in tropical and subtropical regions, as well as many temperate ones, began to be widely recognized after Theiler's studies. At least 20 species of ticks transmit Anaplasma (Marchette and Stiller 2018); although Rhipicephalus (Boophilus) microplus is the primary transmission agent (Aubry, Geale et al. 2011, Marchette and Stiller 2018). Other modes of transmission include biting insects or fomites contaminated with blood. Because it lacks a normal cell wall and is unable to synthesize lipopolysaccharide and peptidoglycan, this rickettsial bacterium is more closely resembles to protozoa (Brayton, Kappmeyer et al. 2005). Infections with Anaplasma are more prevalent in cattle than in buffaloes (Rajput, Hu et al. 2005). There are approximately 20 different types of ticks involved in the disease's dissemination, which is the primary mode of transmission (Marchette and Stiller 2018). The, Rhipicephalus, Hyalomma Ixodes, Dermacentor and Boophilus species of ticks are of importance, as well as numerous more ticks (Estrada-Pena, D'Amico et al. 2017). Anaplasmosis is more frequent during the hot, humid, and rainy seasons because of the high quantity of ticks (El-Metenawy 2000). Hemorrickettsial disease anaemia is prevalent in subtropical and tropical regions of the globe (Iqbal, Mukhtar et al. 2019). One of the most frequent bovine hemoparasitic diseases in Pakistan, with a prevalence of 4–75.6%, is this parasitic disease (Khan, Zahoor et al. 2004). The livestock and cattle population in Khyber Pakhtunkhwa are suffering from a serious health issue (Ali et al. 2019).

**Anaplasmosis**

The obligatory intra-erythrocytic bacteria of the genus Anaplasma cause anaplasmosis, more often known as gall sickness, in ruminants. Other ruminants, such as water bison, buffaloes, antelopes from Africa, and various deer species, may also get infected and suffer from chronic sickness. One of the most common causes of anaplasmosis is the transmission of newly formed erythrocytes by ticks and biting flies, as well as surgical equipment like needles and dehorning and castration equipment. Subtropical and Tropical regions (40 N–32 S) across the world, including central and South America, United States, southern Europe, Africa, Asia, and Australia, are known to harbor anaplasmosis cases (Khamesipour, Dida et al., 2018). The absence of a typical cell wall and the inability to synthesize lipopolysaccharide and peptidoglycan make this rickettsial organism more closely connected to protozoa (Brayton, Kappmeyer et al. 2005). Infections with Anaplasma are more common in cattle than in buffaloes (Rajput, Hu et al. 2005). Increased hemolytic anaemia, in addition to jaundice, decreased milk production, fever, abortion, hyperexcitability, and rare occurrences of quick mortality are also symptoms of the condition (Gay, Hinchcliff et al. 2000).

**Taxonomic classification of Anaplasma bacteria**

Within the order of Rickettsiales, considerable reorganizational modifications were achieved in 2001. As a consequence, certain bacterial genera were renamed as the new names for the Ehrlichiaceae, and certain bacterial genera had their classifications changed as a consequence (Dumler, Barbet et al. 2001). They belong to the Prokaryote kingdom, and Anaplasmataceae is their genus. When Sir Arnold Theiler discovered that "marginal spots" seen in stained erythrocytes of ill cattle were causal agents for a particular disease, he created the genus Anaplasma in 1910 (Theiler and Therapeutics 1910; Theiler 1911).

**History**

Anaplasma species were thought to be parasites until genetic studies showed that they are rickettsial bacterial pathogens that belong to the Anaplasmataceae family (Dumler, Barbet et al. 2001). Sir Arnold Theiler coined the term "yellow bag" to describe gall illness in 1910

(Theiler and Therapeutics 1910, Kocan, de la Fuente et al. 2010). Salmon and Smith found inclusion in calf erythrocytes in 1894, which was the first time anyone had heard of *A. marginale*. Sir Arnold Theiler discovered bacteria in the erythrocytes of South African cattle in 1910 and published the first comprehensive description of the organism (Kocan, De la Fuente et al. 2003). *A. marginale* and *A. centrale* are two separate subspecies of erythrocytes, with the latter more prone to developing "marginal points" in calves' erythrocytes. This sub-specie was shown to be slight dangerous to domesticated animals than others (Kocan, De la Fuente et al. 2003). Animal-pathogenic anaplasmosis species like *A. ovis*, A. platys (currently called E. platys), and *A. bovis* were discovered in the following years (Harvey, Simpson et al. 1978, Kuttler 1984). Human anaplasmosis (HGA) was detected for the first time in the United States in 1994 following years of investigation (Tylewska-Wierzbanowska, Chmielewski et al. 2001).

Europe has seen tick fever instances in calves, lambs, and goats since 1780. However, despite the fact that the cause of this ailment has yet to be discovered, the symptom description is quite similar. By discovering tick fever in small ruminants and thereafter in dogs, the disease of anaplasmosis has been revolutionized. Animals perished from the sickness on occasion, although its cause was unknown. Major reorganizational changes occurred within the Rickettsiales order in 2001. Some bacterial genera, as well as their families, were reclassified as a result of this reorganization (Dumler, Barbet et al. 2001). 150 years after the initial report, the sickness was ultimately identified to a bacterium, E. phagocytophila (now known as *A. phagocytophilum*) (Jenkins, Fallowfield et al. 2001).

After considerable investigation, it was discovered that anaplasmosis may infect people; the first instance of HGA was discovered in the US in 1994. There is an *A. phagocytophilum* agent that caused it (a human granulocytic anaplasmosis agent that was initially defined as a granulocytic ehrlichiosis agent) (Dumler et al., 2001). This disease was first identified in Europe in 1996 in Slovenia (Petrovec et al., 1997), and was first reported in Poland in 2001 Tylewska-Wierzbanowska and colleagues.

## Types / Taxonomy

### *Anaplasma marginale*

*Anaplasma marginale* is a tick-borne intraerythrocytic pathogen that infects just its particular host. Most often, infected hosts are ruminants, such as cattle (Kocan, de la Fuente et al. 2010, Zhang, Lv et al. 2016). Biting flies and the majority of tick species transmit the parasite either physiologically or mechanically. Fever, generalised sadness, loss of weight, developing anaemia, and icterus are all symptoms of A. marginale illness (Fereig, Mohamed et al. 2017). It is the most virulent species worldwide, causing epidemics more often than other anaplasmosis species, which is the most common tick-borne haemorickettsial illness (Atif, Khan et al. 2013). It is the most hazardous species, is an intraerythrocytic parasite that produces mild to severe epidemics in small ruminants (George, Bhandari et al. 2017).

### *Anaplasma centrale*

*Anaplasma centrale* is found in the red blood cells of ruminants, mainly in cattle. It creates concentrations in the cell's center, as opposed to *A. marginale*. This bacterium may be found all over the world. Despite differences in appearance and virulence, *A. centrale* and *A. marginale* are closely related. *A. centrale* is the most common cause of minor anemia in cow infections (Rajput, Hu et al. 2005). A. centrale, according to Theiler, is less harmful to cattle than A. marginale, however it may sometimes produce resistance to the latter. As a

consequence, it is used to develop live vaccine strains that give immune protection against bovine anaplasmosis. This vaccine is manufactured in Israel, Australia, Latin America, and Africa (Kocan, De la Fuente et al. 2003).

### *Anaplasma bovis*

Anaplasma bovis is a bacterium that belongs to the Anaplasma genus, the Anaplasmataceae family, and the Rickettsiales order. It was identified in 1936 while researching Theileria sp. transmission (Brouqui, Parola et al. 2007). It is recognized as a tick-borne pathogen in buffalo and cattle and, more importantly, has a deleterious effect on cow output (Dumler, Barbet et al. 2001). *A. bovis*, like other members of its family, is an obligate intracellular bacterium that lives inside cells and may spread to other cells throughout the body. Anaplasma bovis is passed from ticks to vertebrates through the bit of ticks that are infected with it (Brouqui, Parola et al. 2007). So, vertebrates are important to their lifetime. Recently, *A. bovis* was also found in mosquitoes, but the particular roles they play in maintaining and transmitting the disease aren't clear (Guo, Tian et al. 2016).

Several clinical symptoms, such as elevated fever, weight loss, and reduced milk production, were associated with anaplasmosis in cattle infected with Anaplasma bovis. It sometimes causes abortions and mortality during the severe phase of the illness (Rar, Golovljova et al. 2011). The majority of diseased buffalo and cattle, however, are asymptomatic. Aside from the two ruminants described above, DNA of *A. bovis* has been found in Mongolian gazelles, deer, raccoons, cottontail rabbits, rats, goats, sheep, dogs, wild cats, pigs, Eastern rock sengi, and monkeys all across the globe (Gofton, Waudby et al. 2017). Furthermore, *A. bovis* disease in these animals does not result in scientific signs.

### *Anaplasma ovis*

Anaplasma ovis are obligate intracellular bacterium carried by ticks. This Anaplasma species is thought to be reservoirs in wild ungulates (Kauffmann, Rehbein et al. 2017). Contrary to *A. phagocytophilum*, *A. ovis* causes ovine anaplasmosis and is much more host-specific. This disease mostly affects ovine and caprine red blood cells, but it has also been discovered in wild ungulates such as red and reo deer (Jiménez, Benito et al. 2019).

So far, just a few cases of *A. ovis* infection in humans have been recorded (Chochlakis, Ioannou et al. 2010). Despite its widespread distribution in the Mediterranean basin, Anaplasma ovis has only been identified on a few of occasions in Central Europe and has yet to be discovered in Northern Europe (Stuen 2016). Ticks belonging to the genera Rhipicephalus, Dermacentor, and Hyalomma are assumed to be the vectors of the illness, although there is no fresh evidence to support this theory (Friedhoff 1997).

*A. ovis* has just been discovered in sheep keds (Melophagus ovinus) (Zhang, Wang et al. 2021). Severe anemia, serious weakness, anorexia, and weight damage are the most common clinical symptoms in sheep, although they only appear when the animals are in poor health (Lacasta, Ferrer et al. 2020). Hemoglobinuria has been documented in a sheep herd in Hungary (Hornok, Elek et al. 2007), and icteric corpses of *A. ovis* positive lambs have recently been recorded in Spain (Lacasta, Ferrer et al. 2020). *A. ovis* produces hemolytic anaemia, which results in a reduction in hemological markers such as RBC, Hb, and PCV in sheep experimentally infected with the disease (Yasini, Khaki et al. 2012).

*A. ovis* is a worldwide problem, not only in developing nations, according to current studies by

a significant number of scientific institutions. The United States, Italy, and Hungary have all reported the presence of these microorganisms (Hornok, Elek et al. 2007). However, it should be emphasized that the restoration of old agricultural methods (pasturage on meadows) may aid in the spread of *A. ovis* in that region. Some experts say that modern climate change has the potential to contribute to the spread of this bacterium.

### *Anaplasma platys*

*Anaplasma platys* is a gram-negative, intracellular bacterium believed to be spread by Rhipicephalus sanguineus sensu lato brown dog ticks (Ramos, Latrofa et al. 2014). It is usually detected in dogs, although natural infections have been reported in cats, foxes, wild boars, red deer, and goats (Pereira, Parreira et al. 2016). Majority of infected dogs, blood loss may occur, and co-infection with other vector-borne infections deteriorates A. platys infection (Iatta, Sazmand et al. 2021).

Modern vector competence research, on the other hand, seems to provide strong evidence that R. sanguineus sensu stricto is a biological vector of A. platys (Snellgrove, Krapiunaya et al. 2020). This tick is also a vector for Ehrlichia canis, an Anaplasmataceae disease associated with A. platys. Co-infections with A. platys and E. canis are common in dogs (LanzaPerea, Zieger et al., 2014). Clinical signs of A. platys and E. canis infections in dogs can vary from asymptomatic to severe, including high fever, depression, weakness/lethargy, anorexia, lymphadenopathy, splenomegaly, thrombocytopenia, and weight loss (Mylonakis and Theodorou 2017).

Infections with Anaplasma platys may cause petechiae, whereas infections with E. canis can cause nasal bleeding in some dog breeds. Ecchymosis and cyclical changes in platelet counts occur in dogs with A. platys infections, most likely as a result of an infection-related decrease in thrombocytes and a host-induced immune response. While the infection is likely to be spontaneously managed by the canine immune system, the illness-associated immunological suppression and changing platelet levels may have a negative influence on the animal's health.

### *Anaplasma phagocytophilum*

*Anaplasma phagocytophilum* causes human, horse, and canine granulocytic anaplasmosis (Chvostá, Pitalská et al. 2018). Several *A. phagocytophilum* genetic variations have been identified in free-living and domestic animals in Europe (Földvári, Jahfari et al. 2014). Ixodes ricinus is the most common genotype in Europe, but other species of Ixodes are thought to be involved in the transmission of this disease. Ixodes persulcatus transmits the pathogen in Eastern Europe and temperate Asia, whereas Ixodes scapularis and Ixodes pacificus transmit it in North America (Jahfari, Coipan et al. 2014, Jaarsma, Sprong et al. 2019). When it comes to transmitting gram-negative TBPs (such as *A. phagocytophilum* ), the ecology of Ixodes species vectors and reservoir vertebrate hosts determines their ecological niche (Jaarsma, Sprong et al. 2019). Several molecular marker analyses demonstrated the presence of several genetic variations in *A. phagocytophilum*. Furthermore, some degree of host specificity has been documented for the various genetic variations at both the tick and vertebrate host levels (Kauffmann, Rehbein et al. 2017). *A. phagocytophilum's* genetic structure and complicated epidemiology are similar to the variety of genospecies in the Borrelia burgdorferi sensu lato complex (Jaarsma, Sprong et al. 2019). There are no significant differences in the robustness and consistency of genetic variant categorization in A. phagocytophilum using groEL as compared to methods that use the 16S-rDNA gene sequence, the ankA and the msp4 gene sequences (Battilani, De Arcangeli et al. 2017). There have been eight distinct genetic clusters,

separated into four ecotypes based on their geographic distribution and their relationship with vertebrate or vector species, found by Jaarsma et al. via the DNA sequence of a groEL fragment as the basis for their research. (Jaarsma, Sprong et al. 2019).

**Table 1. Pathogens characteristic of the genus *Anaplasma***

| Etiological agent | | Disease | Vector | Infected host | Infected cell |
|---|---|---|---|---|---|
| before 2001 | After 2001 | | | | |
| *Ehrlichia bovis* | *Anaplasma bovis* | Bovine anaplasmosis | *Haemaphysalis* sp.*Rhipicephalus* sp.*Amblyomma* sp. | ruminants farming, small mammals | Monocytes |
| *Anaplasma ovis* | *Anaplasma ovis* | Bovine anaplasmosis | *Dermacentor* sp. | small ruminants (goats, sheep) | Red blood cells |
| *Anaplasma marginale* | *Anaplasma marginale* | Bovine anaplasmosis | *Ixodes* sp.*Dermacentor* sp. | ruminants farming | Red blood cells |
| *Anaplasma centrale* | *Anaplasma centrale* | Bovine anaplasmosis | *Ixodes* sp.*Haemaphysalis* sp. | ruminants farming | Red blood cells |
| *E. equiE. phagocytophila* Czynnik HE | *Anaplasma phagocytophilum* (HGA agent) | HGA anaplasmosis | *Ixodes* sp.*Dermacentor* sp. | small ruminants forming and humans, wild, horses, dogs, | Granulocyte |
| *E. platys* | *Anaplasma platys* | Canine cyclic thrombocytopenia | *Rhipicephalus sanguineus* | Dogs | Platelets |

## Tick morphology and behavior

Ticks are the world's most lethal arthropod disease vectors, second only to mosquitoes in vector competence (Benelli, Maggi et al. 2017). Ehrlichiosis, Lyme disease, anaplasmosis, Powassan virus, piroplasmosis and Rocky Mountain spotted fever are all illnesses transmitted by ticks. (Ebani, Rocchigiani et al. 2017). About 900 different species of ticks may be found in the two major families of Argasidae and Ixodidae, as well as one in the Nutalliellidae, which only has one specie (*Nuttalliella namaqua*, Bredford) (Guglielmone, Robbins et al. 2014). Hard ticks, also known as Ixodidae, are the most prevalent vectors of human parasitism. However, Argasidae, sometimes known as soft ticks, do bite people sometimes (Dantas-Torres and Otranto 2016). There are a wide range of tick species that vary from location to region and often infect animals as reservoir hosts for a variety of disease pathogens (Piesman and Eisen 2008).

Host density and the weather may have a significant impact on the activity of ticks, which in turn influences the probability of tick-borne illnesses being transmitted to the host (Hubálek, Halouzka et al. 2003). However, ticks' cognitive endogenous elements in host seeking/contacting behaviour are also worth exploring. During host searching, hard ticks

(Ixodidae) conduct a behaviour known as "questing" in order to increase their chances of encountering a suitable mammal host. The tick will crawl up a blade of grass or other similar plant component and then wait with its forelegs spread. When a host passes by, it brushes up against the tick's forelegs, which contain Haller's organ, and the tick grips hold of the host (Randolph and Storey 1999, Perret, Guigoz et al. 2000).

It's difficult to predict exactly how a tick will react to an unfamiliar host. According to research, an increased concentration of CO2 in the air, i.e., exhalations from a host, is a crucial factor affecting the behavior of questing people. Ticks assume an expectant attitude (in passive species) or migrate towards the source of the gas when the concentration of carbon dioxide in the air changes (in active species). The aroma of a host, as well as its body temperature, may have a considerable impact on the tick's decision. Other stimuli that ticks respond to include pheromones, urine, animal noises, and an environmental element called light intensity (Buczek and Magdon, 1999). Stimulating factors differ and define various tick species.

Tick behavior may also be impacted by diurnal and seasonal patterns unique to particular populations. Ambient temperature influences seasonal activity, whereas prospective ambient temperature, host activity, humidity influence and diurnal activity.

**Anaplasma bacteria vectors worldwide**

The anaplasma-carrying common tick, Ixodes ricinus, is the most common tick in Europe Cafiso, Bazzocchi et al. (2016) use the term "European sheep tick" to refer to Ixodes ricinus. One of the most frequent types of ticks is the arachnid that feeds on blood, the Ixodida order, of the genus Ixodes (Muders 2015). Its parasitic actions on vertebrates, including humans, generate a broad spectrum of pathological alterations, some of which are life-threatening for their victims (swelling, inflammatory disorders of the skin, allergies, and paralysis). There are three distinct hosts for extranidamental ectoparasites such as I. ricinus, which feed on migratory animals in the open and have a three-stage life cycle (Medlock, Hansford et al. 2013). The life cycle of Ixodes ricinus includes all three stages: lymph, larva, and adult (imago). It is a temperate climate-adapted species that exhibits seasonality, or the reliance on the seasons for activity and progression through the life cycle at each stage. In temperate regions, the tick's life cycle may continue for up to three years, but it can live much longer under ideal climatic circumstances (Lindquist 2014). Additionally, studies have indicated that high temperatures and low humidity have a detrimental influence on the lifetime of host-seeking nymphs throughout the warm months of the year, especially when the relative humidity falls below 82% (Ginsberg, Rulison et al. 2014).

In Pakistan, according to Hussain et al. ticks have been identified on cattle, buffalo, and forty diverse species of ticks on goats and sheep. Ticks infesting buffaloes and cattle have been observed in Pakistan as well (2021). A total of two new tick species have been found as a result of this study, which was carried out on tiny ruminants (goats and sheep). The introduction of these two new species has resulted in an increase in the tick fauna in the United States. Ninety-nine tick species present in Pakistan's rural and urban areas have been recognised for the first time, including D. marginatus, Ha.punctata, Rhiphicephalus sanguineus, Rhiphicidephalus microplus, Rhiphicidephalus annulatus, and Hyalomma excavatum, among others (Ramzan *et.,* al.2020). It is possible that this study may result in the discovery of new species in Pakistan. In addition to the ability to transmit harmful infections, these two new species have the potential to wreak enormous economic damage. A number of other species have been discovered in Pakistani cattle. It is necessary to do research on tick species from different locations in Pakistan.

In spite of the fact that DNA testing has shown the existence of Anaplasma and Ehrlichia species in Iranian cattle, the infections' vectors have garnered much less attention than the pathogens themselves. In Iran, until recently, only a few studies examined the presence of Anaplasma and Ehrlichia spp. in ticks, showing that the north of the nation and other regions had infected insects (Tajedin et al. 2016). Because of their ability to transmit diseases like Ehrlichia and Anaplasma, ixodid ticks are vital to their continued existence in the natural world (Han, R. et al., 2019). Since A. marginale has been associated with Hyalomma species, very little study has been done on the idea that these parasites might be involved in the spread of Anaplasma and Ehrlichia species (Shkap et al. 2009). Domestic animals in Baluchistan and Sistan are often infected with Hyalomma, Anaplasma, and Ehrlichia spp.

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