



Seroprevalence and Risk Factor Associated with Endemicity of *Theileria equi* Infection in Horses in Rajasthan State, India

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ABSTRACT

Background: Equine piroplasmiasis is a haemoprotozoan disease of equids and enzootic in Rajasthan prefecture of India. In endemic areas, the subclinical condition is more common, as infected equids recover from the disease and become latently infected. This study aimed to investigate the seroepidemiology and risk factors associated with the endemicity of *Theileria equi* infection in Rajasthan state.

Methods: A cross-sectional study on the seroprevalence of *Theileria equi*, was performed. Total of 151 serum samples collected from different areas of Rajasthan. The serum samples were screened by ELISA for assessment of seroprevalence of *T. equi* infection.

Result: Overall seroprevalence of *T. equi* was 49.66%. A total of 75 *T. equi* seropositive horses were detected and one horse was clinically positive for *T. equi* infection. The clinical signs observed were - fever, haemoglobinuria, mild colic, anaemia and icteric conjunctival mucous membrane. *Theileria equi* infection is endemic among horses in Rajasthan state and Ajmer district found the most endemic. Haematological observations in *T. equi* clinically infected and healthy horses were studied. A decreased haemoglobin concentration, packed cell volume, total erythrocytic counts were observed in *T. equi* infected horse in comparison to healthy horses. This observation showed anaemic condition in *T. equi* clinically infected equine. An increased concentration of liver enzymes - AST, ALP, GGT in *T. equi* infected equine was also recorded, which indicated the liver damage.

Key words: ELISA, Horses, Piroplasmiasis, Risk factors, Seroprevalence, *Theileria equi*.

INTRODUCTION

Equine piroplasmiasis is a tick-borne disease caused by intraerythrocytic protozoa, *Theileria equi* (Mehlhorn and Schein, 1998) and *Babesia caballi* (Nuttall and Strickland, 1910). The disease is endemic in the tropical and subtropical area of the world (Shkap *et al.*, 1998; Uilenberg, 2006; Vial and Gorenflot, 2006). Multiple tick's species of genera are transmitting both the protozoa. *Hyalomma*, *Dermacentor*, *Amblyomma* and *Rhipicephalus* are distributed worldwide and considered as a vector for these protozoa (de Waal, 1992; Ali *et al.*, 1996). These tick species are most prevalent in different Indian climatic zones (Geevarghese *et al.*, 1997). In Indian regions, *Hyalomma anatolicum anatolicum* transmits *T. equi* and *Theileria annulata* in equines and bovine, respectively (Chaudhuri *et al.*, 1969; Bhattacharyulu *et al.*, 1975). *Theileria equi* infection is endemic in India in many agro-climatic regions (Kumar *et al.*, 2013; Dahiya *et al.*, 2018; Sumbria *et al.*, 2018) and sporadic clinical cases have been reported (Gautam, 1976; Sharma *et al.*, 1982).

Equines infected with piroplasmiasis show different symptoms like high fever, haemoglobinuria, pale mucus membrane, icterus, petechial haemorrhage on the nictitating membrane, peripheral oedema and occasionally death (De Waal, 1992; Ambawat *et al.*, 1999; de Waal and van Heerden, 2004; Radostits *et al.*, 2006; Onyiche *et al.*, 2019). The disease condition observes as per-acute, acute, sub-acute or chronic (Uilenberg, 2006; Zobba *et al.*, 2008). In endemic areas, the subclinical condition is more common, as infected equids recover from the disease and

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become a latent carrier (de Waal and van Heerden, 2004). Transplacental transmission of *T. equi* infection from latently infected pregnant mare to naïve unborn neonate has been reported (Phipps and Otter, 2004; Allsopp *et al.*, 2007; Georges *et al.*, 2011; Chhabra *et al.*, 2012).

Direct and indirect methods are in vogue for diagnosis of equine piroplasmiasis. These methods include

microscopic blood smears examination, serological assays, cell culture (microaerophilous stationary phase, MASP technique) and molecular techniques *viz.* PCR/qPCR assays (Kumar *et al.*, 2009; Tirosch-Levy *et al.*, 2020). Direct diagnosis includes the demonstration of an intraerythrocytic form of protozoa in Giemsa stained blood or organ smear in the acute stage of infection (Nagore *et al.*, 2004). Nevertheless, it is challenging to demonstrate *T. equi* parasite in blood smears prepared from latently infected equines. Therefore, various serological assays developed to increase diagnostic specificity and sensitivity. These indirect serological methods prescribed for large scale epidemiological and seroprevalence studies on *T. equi* infection. Office International des Epizooties (OIE) prescribed competitive inhibition enzyme-linked immunosorbent assay (cELISA) for international trade and transportation of equines (OIE, 2004). EMA-2 recombinant antigen of *T. equi* has widely used in ELISA for seroepidemiological studies (Huang *et al.*, 2003; Kumar *et al.*, 2013).

Theileria equi infection reported to be endemic in Rajasthan (Kumar *et al.*, 2013), but its epidemiological information is limited. This study aimed to investigate the seroepidemiology and risk factors associated with the endemicity of *T. equi* infection in Rajasthan state in India.

MATERIALS AND METHODS

Animals and sampled area

One hundred fifty one horses were screened for equine piroplasmiasis. Samples from these animals were collected from different areas of Rajasthan including Ajmer, Barmer, Bikaner, Nagaur and Pali districts (Fig 1A). The animals were sampled as per guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) after due approval from the Institute Animals Ethics Committee (IAEC).

Sample collection and serological examination

Blood was withdrawn aseptically from the jugular vein and collected in sterile clot activator vacutainers. Serum was separated after centrifugation at 4000 g for 25 min and kept immediately at -20°C till further processing. Blood smears from each horse were prepared on a glass slide at the time of blood collection and fixed with methanol. Blood smears were stained using Giemsa staining (Himedia Laboratories, India) and processed further for microscopic examination for any evidence of haemoparasites including *T. equi* (Henry, 1992).

Serum samples were screened by ELISA, developed at the equine piroplasmiasis laboratory of National Research Centre on Equines (NRCE) for detection of antibodies against *T. equi* (Kumar *et al.*, 2013; Bhagwan *et al.*, 2015). Briefly, ELISA plate coated with recombinant protein (rEMA) and incubated overnight at 4°C. Next day the plate was blocked with 3% BSA-PBS for 1 h followed by washing with

PBS containing 0.05% Tween-20 (PBS-T) four times. The test serum samples including control positive or negative were diluted in 1% BSA-PBS (1:200 dilution) and 50 µl added was to ELISA plate in duplicate wells. The plate was incubated at room temperature (RT) for 1 h. Thereafter it was washed with PBS-T. Rabbit anti-horse IgG HRP conjugated antibody was used as secondary antibody. It was diluted in 1% BSA-PBS and added to the wells of the ELISA plate. The plate was incubated at RT for 1 h. After that, the plate was washed again with PBS-T. The substrate solution of O-phenylenediamine dihydrochloride, OPD (Sigma Aldrich) was prepared as per manufacturer instructions and added to each well of the ELISA plate. The ELISA plate was incubated in the dark for 5 min at RT. The development of orange-brown colour was stopped by adding 50 µl of 3 M H₂SO₄. The ELISA plate was read at an absorbance of 492 nm (OD₄₉₂) in ELISA plate reader (BioTek, USA). The ELISA OD₄₉₂ cutoff point was determined by calculating the relative per cent positivity (RPP) as per the following formula. Test serum sample showing RPP >20 was considered as positive.

$$RPP = \frac{OD_{492} \text{ of tested sample} - OD_{492} \text{ of negative control sample}}{OD_{492} \text{ of positive control sample} - OD_{492} \text{ of negative control sample}} \times 100$$

Haemato-biochemical analysis

Haematological parameters on the collected whole blood were analysed manually. Haemoglobin (Hb); packed cell volume (PCV); total erythrocytes count (TEC) and total leukocytes count parameters (TLC) were estimated as per prescribed methodology. Biochemical parameters such as aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin and gamma-glutamyl transferase (GGT) were analysed using commercial kits (Transasia Biomedicals Ltd., India).

Risk factors analysis

A customised questionnaire was prepared and information related to sampled area, age, sex and managerial practices were collected from the equine owners at the time of sample collection. The questionnaire also included a description of 'unorganised farms' and 'organised farms'. At 'unorganised farms' equine owners practise inappropriate managerial practices *viz.* stables with kaccha (imperfect and crude) floor, neglected sanitation and unstable feeding programmes; while 'organised farms' owners pursue appropriate technical managerial schedule.

Statistical analysis

The data were statistically analysed and compared by GraphPad Prism version 5.0 software (San Diego California, USA). The association of seroprevalence of *T. equi* with respects to different sampled geographic areas of Rajasthan and epidemiological risk factors were statistically analysed by Pearson's chi-square test.

RESULTS AND DISCUSSION

Microscopic examination of blood smears

Stained blood smears were microscopically examined for the presence of *T. equi* parasites, if any. A total 151 blood smears were examined, only one blood smears was positive for *T. equi* protozoa, which was clinically infected (Fig 1B).

Clinical findings and seroprevalence

The symptoms such as fever, haemoglobinuria, mild colic, anaemia and icteric conjunctival mucous membrane were observed in horses with clinical infection by *T. equi* parasite. Out of 151 serum samples collected from the study area, 75 (49.66%) were seropositive to *T. equi* infection. In Ajmer area maximum percentage of equids were seropositive to *T. equi* infection, followed by Nagaur, Bikaner, Pali and Barmer area (Fig 1C).

Haemato-biochemical analysis

Most of the parameters of Haemato-biochemical parameters (Hb, PCV, TEC, GGT, ALP and total bilirubin, Table 1) did not differ significantly among apparently healthy and *T. equi* sero-positive horse. Whereas, values of TLC and AST differ significantly among these two groups (Table 1). However, these haemato-biochemical parameters in *T. equi* clinically infected horse differ significantly from apparently healthy and *T. equi* seropositive equines (Table 1). These observations in *T. equi* clinically infected horse were indicative of anaemic and liver damage condition.

Risk factors analysis

Relative risk factors involved in *T. equi* infected/seropositive equines were analysed (Table 2). In Ajmer area, a more significant number of *T. equi* seropositivity was observed

Table 1: Haemato-biochemicals alteration in *Theileria equi* seropositive and infected equids.

| Parameters | Apparently healthy animals | Seropositive animals | Clinically infected animals |
|--|----------------------------|---------------------------|-----------------------------|
| Hb (g/dl) | 11.69±0.27 | 11.59±0.30 | 8.2 ^c |
| Packed cell volume, PCV (%) | 39.54±0.74 | 39.03±0.89 | 26.2 ^c |
| Total erythrocytic count, TEC (10 ⁶ /cmm) | 8.67±0.29 | 8.33±0.34 | 4.4 ^c |
| Total leucocytes count, TLC (10 ³ /cmm) | 9.83±0.24 ^a | 9.02±0.29 ^b | 6.2 ^c |
| Serum aspartate aminotransferase, AST (U/L) | 333.29±9.10 ^a | 368.69±11.58 ^b | 445 ^c |
| Gamma-glutamyl transferase, GGT (U/L) | 24.52±1.58 | 26.37±1.62 | 57 ^c |
| Alkaline phosphatase, ALP (U/L) | 491.03±26.43 | 492.33±28.85 | 784 ^c |
| Total bilirubin (mg/dl) | 1.50±0.049 | 1.56±0.044 | 2.55 ^c |

Note: Numerals with different superscripts, differ significantly (p<0.05).

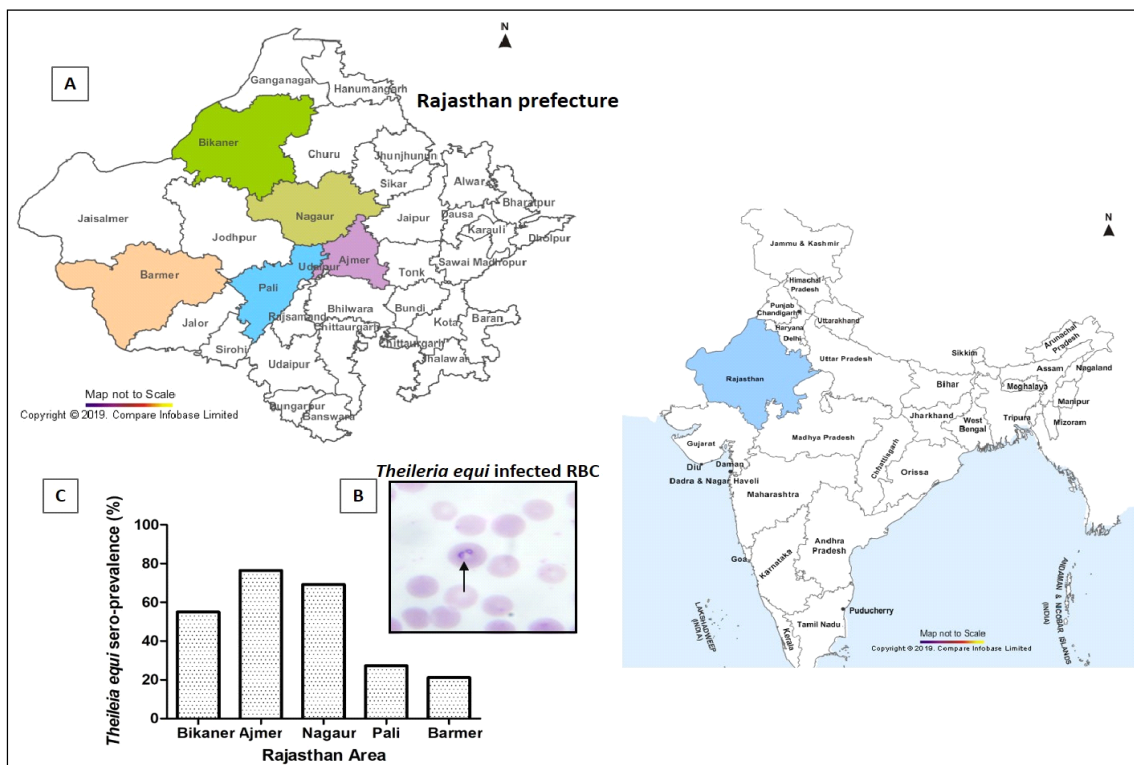


Fig 1: Geographical distribution of sampling area from Rajasthan state (A). Microphotograph depicts *T. equi* infected RBC (B). Bar diagram represents sero-positivity of *Theileria equi* infection in ELISA in different districts (C).

Table 2: Different variables identified to determine their relative risk associated with seroprevalence of *T. equi* in equids of Rajasthan, India.

| Factors | Variables | Sample positive/total samples tested (% positive) | Chi square value | Odds ratio | |
|----------------------|--------------------------------------|---|-----------------------|---------------|----------------------------------|
| | | | | Relative risk | Confidence interval ^b |
| Areas | Zones | | 26.413* | a | |
| | | Ajmer | 13/17 (76.47) | | |
| | | Nagaur | 27/39 (69.23) | 0.692 | 0.187-2.568 |
| | | Bikaner | 22/40 (55) | 0.367 | 0.104-1.335 |
| | | Pali | 06/22 (27.27) | 0.115 | 0.027-0.498 |
| Animal - Age | Barmer | 07/33 (21.21) | 7.155 | 0.083 | 0.020-0.335 |
| | Age (yrs) | | | a | |
| | ≤ 1 | 10/30 (33.33) | | | |
| | 1-5 | 33/54 (61.11) | | 3.143 | 1.233-8.011 |
| | 6-10 | 27/53 (50.94) | | 2.077 | 0.819-5.268 |
| Age (months) | ≥ 10 | 5/14 (35.71) | 6.490 | 1.111 | 0.294-4.205 |
| | 0-2 | 3/13 (23.07) | | a | |
| | 3-4 | 3/12 (25) | | 1.111 | 0.177-6.970 |
| | 5-6 | 2/3 (66.66) | | 6.667 | 0.437-101.732 |
| | Up to 12 | 2/2 (100) | | xxx | xxx |
| Sex | Male | 12/35 (34.28) | 4.313* | 0.439 | 0.200-0.965 |
| | Female | 63/116 (54.31) | | xxx | xxx |
| Management practices | Organised farm | 24/69 (34.78) | 11.263* (Significant) | a | 0.166-0.632 |
| | Unorganised farm | 51/82 (62.19) | | a | |
| | Kept with other livestock species | 38/89 (42.69) | 4.215* (Significant) | 0.503 | 0.261-0.973 |
| | Kept without other livestock species | 37/62 (59.67) | | a | |
| | Presence of tick infestations | 29/46 (63.04) | 4.734* (Significant) | 2.188 | 1.073-4.460 |
| | Absence of tick infestations | 46/105 (43.80) | | a | |
| | Pacca houses | 30/80 (37.5) | 10.078* (Significant) | 0.374 | 0.179-0.672 |
| | Kachha houses | 45/71 (63.38) | | a | |
| | Control adopted by the owner | 34/97 (35.05) | 23.183* (Significant) | 0.171 | 0.081-0.362 |
| | Without control adopted by owner | 41/54 (75.92) | | a | |

*: Chi square value is Significant (p>0.05) indicating the association of factor w.r.t. positive per cent of infection;b: 95% confidence Interval.

as compared to the other sampling areas. The equines at Barmer area were at least relative risk (0.083 times) than at Ajmer area for *T. equi* infection.

Analysis of age-related data indicated higher seroprevalence of *T. equi* in horses of age 1-5 year. Whereas, ≥ 10 years old horses were at least risk. Seroprevalence of *T. equi* was higher in foals of 4 to 12 months age ($n=10$). Whereas, neonate foals (0-2 and 3-4 months) were at least risk.

A high *T. equi* seroprevalence was observed in female horses ($n=63$; 54.13%) as compared to male horses ($n=12$; 34.28%) indicating that females are significantly more at risk than male equine population.

Equine farm management practices influence the incidence of *T. equi* infection remarkably. The equines reared at the organised farm were significantly at lower risk of contracting infection with *T. equi* as compared to equines reared at the unorganised farm. Likewise, equines were significantly at less risk (0.503 times) when kept with other animal species than reared without any other animal species. The equines infested with tick-vectors were significantly two times (2.188) more inclination of getting *T. equi* infection as compared to those who were not infested with ticks. Flooring in the stable is a significant risk factor towards maintaining proper drainage and sanitation. Equines reared at kaccha house were more at risk than equines kept on pacca floor. The risk of getting infected with *T. equi* decreased significantly in equines, where owners adopted control measures such as anti-tick spray and bathing of equines etc.

Equine piroplasmiasis is the economically significant disease of horses, donkeys, mules and zebras. Tick vectors are ubiquitous and responsible for the spread of parasite in the equine population. A *T. equi* infected horse showing clinical signs of the disease condition was observed in the present study. Similar clinical observations were recorded by other researchers also (Hailat *et al.*, 1997; Radostits *et al.*, 2006; Balkaya *et al.*, 2010; Garba *et al.*, 2011; Behera *et al.*, 2012; Hussain *et al.*, 2014).

Very high seroprevalence of *T. equi* infection has been reported from Rajasthan state (Kumar *et al.*, 1997; Kumar *et al.*, 2013). In the present study also very high *T. equi* seropositivity was recorded in the samples collected from different areas of Rajasthan state (Fig 1). *Theileria equi* seroprevalence was higher in the horses of Ajmer district followed by Nagaur, Bikaner, Pali and Barmer areas. Ajmer area has the highest livestock density among the sampled region (Livestock Census All India Report, 2019). High livestock density help in the propagation of the infected tick vectors, which may be responsible for the highest seroprevalence of *T. equi* infection. Livestock density also influences tick biology and tick's control measures. Similar observation reported by different researchers (Salim *et al.*, 2008; Kumar *et al.*, 2013; Hussain *et al.*, 2014). Decreased haemoglobin concentration, packed cell volume and total erythrocytic counts indicated anaemic condition in *T. equi* clinically infected horse (Table 1). *Theileria equi* infection

inflicts lipid peroxidation of infected erythrocytes membrane, making it more fragile. This process eventually leads to its lysis (Ambawat *et al.*, 1999). That may be the reason for the anaemic condition of the *T. equi* infected horse. An increased concentration of liver enzymes (AST, GGT and ALP) in *T. equi* infected horse (Table 1) is associated with hepatocytes necrosis and centrilobular degeneration. These findings are in agreement with earlier reports (Camacho *et al.*, 2005; Zobba *et al.*, 2008; Kumar *et al.*, 2008).

Seropositivity of *T. equi* infection among different regions was analysed based on age, gender and managemental practices (Table 2). The female equine population was more seropositive to *T. equi* infection as compared to the male's population. Males (stallion) are more scattered as compared to female (mare) population, as stallion usually used for the breeding purpose only. Hence, the comparatively male equine population is at lesser risk than females.

We also collected serum samples from a dam and their foals. *Theileria equi* antibodies observed in neonates (0 to 4 months of age) in the present study, indicating transcolostrum transfer of specific antibodies. Equine neonates are naïve at birth and they acquire *T. equi* immunity from colostrum of their preimmune dam (de Waal and van Heerden, 1994; Kumar *et al.*, 2008). This passive immunity is transitory and disappears after some time. Kumar *et al.* (2008) observed antibodies titre in naïve foals up to 63 to 77 days post-foaling.

A significantly higher incidence of *T. equi* infection at unorganised farms may be attributed to unhygienic management practices e. g. open grazing system, absence of grooming practices etc. This managemental practice increases the probability of getting infested with *T. equi* infected ticks (Kouam *et al.*, 2010; Moretti *et al.*, 2010; Abutarbush *et al.*, 2012; Steinman *et al.*, 2012; Peckle *et al.*, 2013). Nevertheless, infected ticks' infestation is responsible for making the equids *T. equi* seropositive (Bhagwan *et al.*, 2015). Kaccha housing conditions are responsible for the propagation of ticks breeding.

CONCLUSION

Theileria equi is endemic among horses of the Rajasthan region and clinical infection is common. *Theileria equi* seroprevalence was higher in the horses of Ajmer district followed by Nagaur, Bikaner, Pali and Barmer areas. Significantly, higher incidence of *T. equi* infection was observed at unorganised stud farms. This managemental practice increases the probability of getting infested with *T. equi* infected ticks. Risk factors play a vital role in maintaining the endemicity of *T. equi* infection.

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Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Authors' contributions

Rakesh J. Bhojani and Vikram S. Dewal performed the experiments. Anju Chahar collected samples and Kruti D. Mandal and Sneha L. Chauhan perform risk analysis, Rajender Kumar performed ELISA. Yash Pal performed statistical calculations and prepared the graphs Sanjay Kumar conceptualised, designed and supervised the whole study. Also drafted the final version of manuscript. All authors read and approved the final manuscript.

REFERENCES

- Abutarbush, S.M., Alqawasmeh, D.M., Mukbel, R.M., Al Majali, A.M. (2012). Equine babesiosis: Seroprevalence, risk factors and comparison of different diagnostic methods in Jordan. *Transboundary and Emerging Diseases*. 59(1): 72-78.
- Ali, S., Sugimoto, C., Onuma, M. (1996). Equine piroplasmosis. *Japanese Journal of Equine Science*. 7(4): 67-77.
- Allsopp, M.T., Lewis, B.D., Penzhorn, B.L. (2007). Molecular evidence for transplacental transmission of *Theileria equi* from carrier mares to their apparently healthy foals. *Veterinary Parasitology*. 148(2): 130-136.
- Ambawat, H.K., Malhotra, D.V., Kumar, S., Dhar, S. (1999). Erythrocyte associated haemato-biochemical changes in *Babesia equi* infection experimentally produced in donkeys. *Veterinary Parasitology*. 85(4): 319-324.
- Balkaya, I., Utuk, A.E., Piskin, F.C. (2010). Prevalence of *Theileria equi* and *Babesia caballi* in donkeys from Eastern Turkey in winter season. *Pakistan Veterinary Journal*. 30(4): 245-6.
- Behera, S.K., Banerjee, P.S., Garg, R., Maharana, B.R. (2012). A case of *Babesia equi*. *Indian Veterinary Journal*. 89(12): 87-88.
- Bhagwan, J., Kumar, A., Kumar, R., Goyal, L., Goel, P., Kumar, S. (2015). Molecular evidence of *Theileria equi* infection in *Hyalomma anatolicum* ticks infested on sero-positive Indian horses. *Acta Parasitologica*. 60(2): 322-329.
- Bhattacharyulu, Y., Chaudhuri, R.P., Gill, B.S. (1975). Transstadial transmission of *Theileria annulata* through common ixodid ticks infesting Indian cattle. *Parasitology*. 71(1): 1-7.
- Brüning, A. (1996). Equine piroplasmosis an update on diagnosis, treatment and prevention. *British Veterinary Journal*. 152(2): 139-151.
- Camacho, A.T., Guitian, F.J., Pallas, E., Gestal, J.J., Olmeda, A.S., Habela, M.A., Telford, S.R., Spielman, A. (2005). *Theileria (Babesia) equi* and *Babesia caballi* infections in horses in Galicia, Spain. *Tropical Animal Health and Production*. 37(4): 293-302.
- Chaudhuri, R.P., Srivastava, S.C., Naithani, R.C. (1969). On the biology of the Ixodid tick, *Hyalomma (Hyalomma) anatolicum* Koch, 1844 (Acarina: Ixodidae). *Indian Journal of Animal Sciences*. 39(3): 257-268.
- Chhabra, S., Ranjan, R., Uppal, S.K., Singla, L.D. (2012). Transplacental transmission of *Babesia equi (Theileria equi)* from carrier mares to foals. *Journal of Parasitic Diseases*. 36(1):31-3.
- Dahiya, R., Salar, R.K., Mandal, K.D., Kumar, R., Tripathi, B.N., Pal, Y. and Kumar, S. (2018). Risk factor analysis associated with *Theileria equi* infected equines in semi-arid and sub-humid ecological enzootic zones of India. *Veterinary Parasitology, Regional Studies and Reports*. 12: 17-21.
- de Waal DT, van Heerden J. (2004). Equine Piroplasmosis. In: *Infectious Diseases of Livestock*, [(ed.) Coetzer JAW]. Oxford University Press, Capetown, South Africa, 425-434.
- De Waal, D.T. (1992). Equine piroplasmosis: A review. *British Veterinary Journal*. 148(1): 6-14.
- de Waal, D.T., Van Heerden, J. (1994). Equine Babesiosis. In: *Infectious Diseases of Livestock with Special Reference to Southern Africa*. [Coetzer, J.A., Thomson, G.R., Tustin, R.C., (eds.)], Oxford University Press, Oxford. 295-304.
- Garba, U.M., Sackey, A.K., Tekdek, L.B., Agbede, R.I., Bisalla, M. (2011). Clinical manifestations and prevalence of piroplasmosis in Nigerian royal horses. *Journal of Veterinary Advances*. 1(1): 11-5.
- Gautam, O.P. (1976). Equine babesiosis; A severe outbreak in a stud farm at Hissar. *Indian Veterinary Journal*. 53: 546-551.
- Geevarghese, G., Fernandes, S., Kulkarni, S.M. (1997). A checklist of Indian ticks (Acari: Ixodoidea). *Indian Journal of Animal Sciences*. 67(7): 566-574.
- Georges, K.C., Ezeokoli, C.D., Sparagano, O., Pargass, I., Campbell, M., D'Abadie, R., Yabsley, M.J. (2011). A case of transplacental transmission of *Theileria equi* in a foal in Trinidad. *Veterinary Parasitology*. 175(3-4): 363-366.
- Hailat, N.Q., Lafi, S.Q., Al-Darraj, A.M., Al-Ani, F.K. (1997). Equine babesiosis associated with strenuous exercise: Clinical and pathological studies in Jordan. *Veterinary Parasitology*. 69(1-2): 1-8.
- Henry, M.M. (1992). Hemolytic Anemia. In: *Current Therapy in Equine Medicine*, [Robinson N.E., (ed)], 3rd Ed. W. B. Saunders, New York, USA, 495-501.
- Huang, X., Xuan, X., Yokoyama, N., Xu, L., Suzuki, H., Sugimoto, C., Nagasawa, H., Fujisaki, K., Igarashi, I. (2003). High-level expression and purification of a truncated merozoite antigen-2 of *Babesia equi* in *Escherichia coli* and its potential for immunodiagnosis. *Journal of Clinical Microbiology*. 41(3): 1147-1151.
- Hussain, M.H., Saqib, M., Raza, F., Muhammad, G., Asi, M.N., Mansoor, M.K., Saleem, M., Jabbar, A. (2014). Seroprevalence of *Babesia caballi* and *Theileria equi* in five draught equine populated metropolises of Punjab, Pakistan. *Veterinary Parasitology*. 202(3-4): 248-256.
- Kouam, M.K., Kantzoura, V., Gajadhar, A.A., Theis, J.H., Papadopoulos, E., Theodoropoulos, G. (2010). Seroprevalence of equine piroplasm's and host-related factors associated with infection in Greece. *Veterinary Parasitology*. 169(3-4): 273-278.

- Kumar, S., Kumar, R., and Sugimoto, C. (2009). A perspective on *Theileria equi* infections in donkeys. *The Japanese Journal of Veterinary Research*. 56(4): 171-180.
- Kumar, S., Kumar, R., Gupta, A.K., Dwivedi, S.K. (2008). Passive transfer of *Theileria equi* antibodies to neonate foals of immune tolerant mares. *Veterinary Parasitology*. 151(1): 80-85.
- Kumar, S., Kumar, R., Gupta, A.K., Yadav, S.C., Goyal, S.K., Khurana, S.K., Singh, R.K. (2013). Development of EMA-2 recombinant antigen based enzyme-linked immunosorbent assay for seroprevalence studies of *Theileria equi* infection in Indian equine population. *Veterinary Parasitology*. 198(1-2): 10-17.
- Kumar, S., Malhotra, D.V., Dhar, S. (1997). Serodiagnosis of *Babesia equi* infection-a comparison of Dot-ELISA, complement fixation test and capillary tube agglutination test. *Veterinary Parasitology*. 69(3-4): 171-176.
- Livestock Census All India Report-20th. (2019). Ministry of Agriculture Department of Animal Husbandry, Dairying and Fisheries, Krishi Bhawan, New Delhi. <http://www.dahd.nic.in/about-us/divisions/statistics> accessed on 20th August 2020.
- Mehlhorn, H., Schein, E. (1998). Redescription of *Babesia equi* Laveran, 1901 as *Theileria equi* Mehlhorn, Schein 1998. *Parasitology Research*. 84(6): 467-475.
- Moretti, A., Mangili, V., Salvatori, R., Maresca, C., Scoccia, E., Torina, A., Moretta, I., Gabrielli, S., Tampieri, M.P., Pietrobelli, M. (2010). Prevalence and diagnosis of *Babesia* and *Theileria* infections in horses in Italy: A preliminary study. *The Veterinary Journal*. 184(3): 346-350.
- Nagore, D., Garcia-Sanmartin, J., Garcia-Pérez, A.L., Juste, R.A., Hurtado, A. (2004). Detection and identification of equine *Theileria* and *Babesia* species by reverse line blotting: Epidemiological survey and phylogenetic analysis. *Veterinary Parasitology*. 123(1-2): 41-54.
- Nuttal, G.H., Strickland, C. (1910). Die parasiten der pferdepiroplasmose response der biliary fever. *Zentralbl Bakteriol*. 1(2).
- Office International des Epizooties (OIE) (2004). Equine Piroplasmosis. In: *Manual of Standard for Diagnostic Tests and Vaccines for Terrestrial Animals*. 5th Ed.
- Onyiche, T.E., Sukanuma, K., Igarashi, I., Yokoyama, N., Xuan, X. and Thekiso, O. (2019). A review on equine piroplasmosis: Epidemiology, vector ecology, risk factors, host immunity, diagnosis and control. *International Journal of Environmental Research and Public Health*. 16(10): 1736. doi: 10.3390/ijerph16101736.
- Peckle, M., Pires, M.S., dos Santos, T.M., Roier, E.C., da Silva, C.B., Vilela, J.A., Santos, H.A., Massard, C.L. (2013). Molecular epidemiology of *Theileria equi* in horses and their association with possible tick vectors in the state of Rio de Janeiro, Brazil. *Parasitology Research*. 112(5): 2017-2025.
- Phipps, L.P., Otter, A. (2004). Transplacental transmission of *Theileria equi* in two foals born and reared in the United Kingdom. *Veterinary Record*. 154(13): 406-408.
- Radostits, O.M., Gay, C.C., Hinchcliff, K.W., Constable, P.D. (2006). *A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats*. Veterinary Medicine. 10th Ed. W. B. Saunders Co., Philadelphia, USA: 2045-50.
- Salim, B.O., Hassan, S.M., Bakheit, M.A., Alhassan, A., Igarashi, I., Karanis, P., Abdelrahman, M.B. (2008). Diagnosis of *Babesia caballi* and *Theileria equi* infections in horses in Sudan using ELISA and PCR. *Parasitology Research*. 103(5): 1145-1150.
- Sharma, S.N., Tanwar, R.K., Gahlot, A.K., Yadav, J.S., Rajvanshi, D.S. (1982). Note on clinical studies in *Babesia equi* infection in ponies. *Indian Journal of Animal Sciences*. 52: 1001-1003.
- Shkap, V., Cohen, I., Leibovitz, B., Pipano, E., Avni, G., Shofer, S., Giger, U., Kappmeyer, L., Knowles, D. (1998). Seroprevalence of *Babesia equi* among horses in Israel using competitive inhibition ELISA and IFA assays. *Veterinary Parasitology*. 76(4): 251-259.
- Steinman, A., Zimmerman, T., Klement, E., Lensky, I.M., Berlin, D., Gottlieb, Y., Baneth, G. (2012). Demographic and environmental risk factors for infection by *Theileria equi* in 590 horses in Israel. *Veterinary Parasitology*. 187(3-4): 558-562.
- Sumbria, D., Singla, L.D. and Kaur, P. (2018). Sero-prevalence and risk factor analysis of *Theileria equi* infection in equids from different agro-climatic zones of Punjab (India) by Indirect Immunofluorescence Antibody test. *Veterinary Parasitology, Regional Studies and Reports*. 13: 18-20.
- Tirosh-Levy, S., Gottlieb, Y., Fry, L.M., Knowles, D.P. and Steinman, A. (2020). Twenty years of equine piroplasmosis research: Global distribution, molecular diagnosis and phylogeny. *Pathogens (Basel, Switzerland)*. 9(11): 926. doi: 10.3390/pathogens9110926.
- Uilenberg, G. (2006). *Babesia*-A historical overview. *Veterinary Parasitology*. 138(1-2): 3-10.
- Vial, H.J., Gorenflot, A. (2006). Chemotherapy against babesiosis. *Veterinary Parasitology*. 138(1-2): 147-160.
- Zobba, R., Ardu, M., Niccolini, S., Chessa, B., Manna, L., Cocco, R., Parpaglia, M.L. (2008). Clinical and laboratory findings in equine piroplasmosis. *Journal of Equine Veterinary Science*. 28(5): 301-308.