

ROLE OF CALCIUM, VITAMIN D AND VITAMIN D RECEPTORS IN PATHOGENESIS OF THEILERIOSIS IN CATTLE

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ABSTRACT

The present study was conducted to examine the levels of calcium, vitamin D and Vitamin D receptor (VDR) in naturally-infected cattle with bovine theileriosis for understanding their role in the pathogenesis of the disease. A total of 100 cattle were selected, based on clinical signs in three districts of Punjab, Pakistan. Fifteen healthy animals were kept as negative controls. Blood samples were collected in EDTA containing vacutainer for Giemsa staining to examine the intra-erythrocytic forms of *Theileria*. Serum was collected in yellow top vacutainer for Vitamin D and calcium determination while plasma was also extracted for the determination of VDR levels from EDTA containing tubes. Giemsa staining showed 23% positive animals by microscopy while 32% of animals were positive by PCR. Levels of calcium, vitamin D, and VDR were very significantly ($P < 0.001$) lower in *Theileria* affected animals in comparison to negative control. The results revealed that calcium, Vitamin D, and VDR might have a role in pathogenesis of bovine theileriosis.

Keywords: Bovine Tropical Theileriosis; Calcium; Vitamin D; VDRs; *T.annulata*

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INTRODUCTION

Theileria is an intracellular protozoan that can infect both wild and domestic cattle throughout the world. Ixodid and Hyalomma tick species are responsible for spreading this protozoan parasite in both vertebrate and invertebrate hosts. Two species of *Theileria* (*T.annulata* and *T.parva*) cause lymph proliferative disease (also called tropical theileriosis and the east coast fever) in cattle with high morbidity and mortality. One difference between the two is that *T.annulata* destroys red blood cells, leading to anemia, jaundice and infrequently hemoglobinuria (Mans *et al.*, 2015; Selim *et al.*, 2022). The occurrence and spread of these species across different regions influence the incidence of theileriosis in small and large ruminants (Ghulam *et al.*, 2022; Prajapati *et al.*, 2023). Normal incubation period for tropical theileriosis varies between 7 and 24 days (Jabbar *et al.*, 2015). This disease is seasonal, starting in the second half of April and adding to its large increase in June and July. Cases of theileriosis are commonly observed in summer or in a stormy season when ticks have greater action although sporadic episodes have been recorded during the year (Martinez, 2018). Various

molecular based methods have been employed for the identification of *Theileria* in various species using various biomarkers (Muhammad *et al.*, 2022; Faiza *et al.*, 2023).

As the disease progression in theileriosis is associated with low immunity there is need to study the factors affecting the immunity such as vitamin D and calcium. In order to clearly understand the pathophysiological significance of vitamin D it is vital to study the role of Vitamin D receptors (VDR). The association of these two factors in disease development and pathogenesis may contribute to the advancement of new diagnostic approaches targeting receptors. Therefore, the present study was designed to examine the levels of calcium, vitamin D, and VDR in cattle naturally infected with *Theileria* for understanding their role in the pathogenesis of the theileriosis in Punjab, Pakistan.

In addition, the progressive anemia is a diagnostically most crucial feature of theileriosis particularly hemolytic anemia due to the presence of intraerythrocytic presence of *Theileria* (Temiz *et al.*, 2014). However, there are certain aspects of pathogenesis that are still unclear. Although some previous studies explain the link of calcium with other blood disorders in humans such as thalassemia but there is no such study to

explain the association of calcium with hemolytic anemia in theileriosis (Lertsuwan *et al.*, 2018). Keeping in view these factors the present study was conducted to determine the role of calcium, Vitamin D and VDR in pathogenesis of bovine theileriosis.

MATERIALS AND METHODS

Experimental Design and Study Area: One hundred suspected cattle from different regions of Punjab (Lahore, Narowal, Sheikhpura) were selected to collect blood samples (about 10 mL) with the help of 10cc sterile needle from the jugular vein and approximately 3ml blood was collected in tubes having EDTA as an anticoagulant (Antwi-Baffour *et al.*, 2013). Remaining blood was transferred to serum vacutainers. Serum extracted from blood samples added to yellow top coagulant tube which was then centrifuged at 3000 rpm for five minutes. The serum samples were stored at -20°C till estimation. Plasma was also harvested after centrifugation of blood at 5000 rpm for 15 minutes and kept at -20°C till examination (Dua *et al.*, 2012). Fifteen healthy animals were kept as controls negative in the study.

Giemsa staining: Staining was performed with freshly filtered and diluted Giemsa stain for 30-45 min and washed with distal water to remove extra stain followed by drying and adding one drop of cedar oil to analyze under oil immersion lens (Coles, 1986). For intra-erythrocytic forms of *Theileria* spp. *i.e.* piroplasm, blood smears were observed one by one under ×100 objective magnification. The parasite morphological characteristics were compared according to the characters defined by Soulsby (1982). For piroplasm's the smears were noted as negative if no parasites detected in 50 oil-immersion fields; whereas, even the presence of single piroplasm was recorded as positive for *Theileria annulata*.

Gene Target and *Theileria annulata* Primer: Specific DNA primers for *T.annulata* were used to amplify cytochrome b gene in the molecular lab of Pathology, UVAS. The mitochondrial genome's forensically informative nucleotide sequences (FINS) valuable for intra- and inter-species differentiation. mtDNA is more abundant and has high copy number per cell than nuclear genome (Branicki *et al.*, 2003). DNA primers designed manually for *Theileria annulata* reference sequences of the cattle from NCBI Genbank.

Forward primer Tann (F): 5'-AAG TAT AGC AAC TGC TTT TGT T - 3',

And reverse primer Tann (R): 5'- TCC TGC CAT TGC CAA AAG TC- 3'.

PCR for *Theileria annulata* (PCR mixture): For each PCR amplification, 2µl of DNA used as a template in a 20µL reaction mixture containing 10µL PCR master

buffer (Catalog NO. W1401-2 Wizbio solutions, Korea), 2µL of each primer and 4µl of Diethyl Pyrocarbonate (DEPC) treated water (Catalog NO. 7500923 INVITROGEN, USA).

Thermocycler Conditions for PCR: The reactions were performed in a GS482 thermocycler (G-STORM, UK) under the described conditions: 95 ° C for 30 seconds of denaturation, 58°C for 30 seconds of annealing and 72 ° C for 1 minute, followed by an extension of 10 minutes at 72°C. The reaction was cycled 40 times.

Determination of calcium, vitamin D status and VDR levels: Serum separated by centrifugation was used for the determination of Ca, Serum Vit. D and VDR levels. Calcium was determined using a flame photometer according to the manufacturer's instructions in the department of environmental sciences, UVAS, Lahore.

Serum vitamin D levels of the study groups were determined by using a quantitative sandwich ELISA kit (My BioSource). The kit's detection range was 15.6 ng/mL to 500 ng/mL, and its sensitivity was 2 ng/mL. In a similar manner, Vitamin D receptor (VDR) of the plasma sample from the research groups was ascertained by using a Quantitative Sandwich ELISA kit from (MyBioSource, Inc., USA) while adhering to the manufacturer's instructions. The Sandwich ELISA kit was designed to measure unoccupied sequence of human VDR qualitatively in a stepwise manner within the sample. To develop a 96- wells effectively a micro titration plate was used to generate a solid-phase antibody, a purified VDR antibody was used to coat the plate. Next, the serum samples with VDR were added into the right wells for the construction of the ELISA assay.

Subsequently, the formation of a third layer was involved in the formation of an antibody-antigen-enzyme antibody complex via the addition of horseradish peroxidase-labelled VDR antibody. Finally, TMB substrate solution was added into each well to allow the TMB substrate forming blue color after the wells had been washed plenty of times. Specifically at 450 nm, the color intensity change was observed after the addition of stop solution which halted the enzymatic activity. The OD of each sample was then compared to establish our data set for VDR concentration in each of the sample solutions (Al-Ghafari *et al.*, 2020).

The goal of the kit was to measure the VDR levels in undiluted bodily secretions, tissues, and fluids from cows. The sample used in this assay process was plasma. The kit had a sensitivity of 0.1 nmol/L. The kit's detection range was 1.56 mmol/L to 50 nmol/L.

Statistical analysis: Statistical analysis was performed using the statistical package for social sciences (SPSS) version 25.0. An independent sample *t* test was used to

analyze the significance of the differences. Data were expressed as Mean \pm SD.

RESULTS

A total of 100 cattle from Lahore, Sheikhpura and Narowal were collected on the basis of clinical signs such as fever (41-42 °C), nasal discharge, difficulty in walking, recumbency, melena, decreased milk production, straw yellow urine, anemia, difficulty in getting up, corneal opacity, pale mucous membrane, diarrhea (yellowish softness), enlargement of the superficial lymph nodes, brisket edema and presence of ticks in the body that were supposed to be infected with the bovine theileriosis.

Giemsa Staining results of intraerythrocytic bodies showed 23% positive animals by microscopy while 32% of animals were positive by PCR out of 100

samples. On microscopy samples showing intraerythrocytic bodies were considered positive, while in PCR, amplification of a 460 bp fragment of the cytochrome-b gene detected on a 1.5% agarose gel was confirmed positive. PCR products for this gene were sequenced. *Theileria annulata* specific PCR products run on 1.5% agarose gel are shown in figure 1 and 2.

From PCR positive samples serum calcium levels were determined and a statistically significant decrease ($P<0.001$) in the mean serum calcium level ($6.18 \pm 0.73\text{mg/dL}$) compared with the controls ($9.19 \pm 1.15\text{mg/dL}$) was observed. In addition, it was determined that there was a statistically significant difference ($P<0.001$) between the groups in terms of vitamin D levels ($23.05 \pm 3.93\text{ng/mL}$) with the control ($M = 41.3$, $SD = 3.1$) and vitamin D receptors ($1.270 \pm 0.27\text{ng/mL}$) compared to the control (1.93 ± 0.66) as shown in table 1.

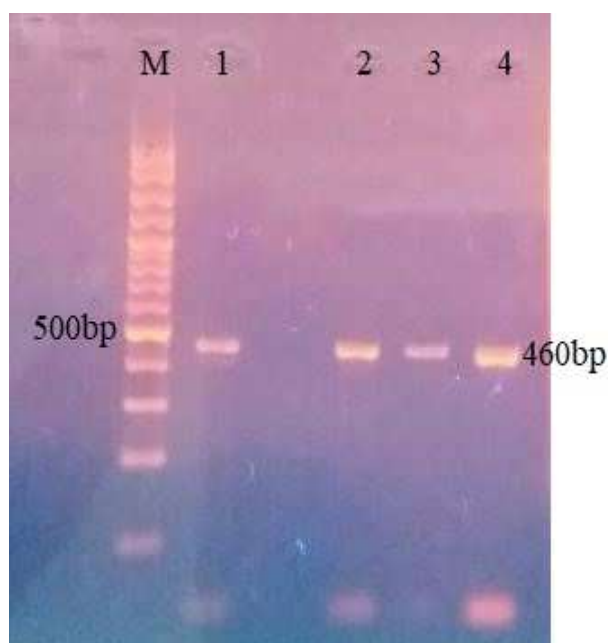


Figure 1. *Theileria annulata* specific PCR products. M: Molecular marker. Lane 3,4,5 was +ve samples of cattle with theileriosis showing 460bp. Lane:1 was kept as a positive control and Lane:2 Negative control

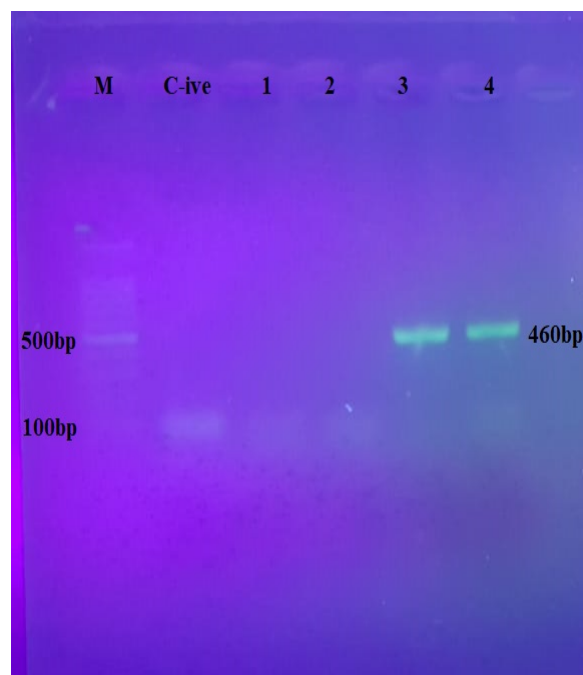


Figure 2. *Theileria annulata* specific PCR products. There was no PCR amplification from cattle isolates for samples 1 and 2 and there was amplification (1.5% agarose gel) for samples 3 and 4 with sized 460 bp. M = Molecular marker, C-ve = Negative control

Table 1. Comparison of biochemical parameters of *Theileria* infected cattle with healthy control

Variable	<i>Theileria</i> Infected Group (n=32)	Control Group (n=15)	p value
Calcium (mg/dL)	6.18 ± 0.73	9.19 ± 1.15	0.001***
Vitamin D (ng/mL)	23.05 ± 3.93	41.37 ± 3.18	0.001***
Vitamin D Receptor (ng/mL)	1.270 ± 0.27	1.93 ± 0.66	0.001***

Values are presented as mean \pm standard deviation. *P* value by independent sample *t*-test. *** $P<0.001$ = strongly significant. N=number of animals.

DISCUSSION

Bovine tropical theileriosis is a major issue of livestock in Pakistan with high mortality and morbidity. The socio-economic losses due this disease cannot be neglected in low-income countries (Goyal *et al.*, 2018).

Bovine theileriosis has a profound effect on serum biochemical and hematological parameters. Hemolytic anemia is a diagnostically most crucial feature of theileriosis (Omer *et al.*, 2003). It is stated that anemia in animals mainly occurs due to presence of *Theileria* inside the RBCs. (Radostitis *et al.*, 2010; Katsogiannou *et al.*, 2018). According to previous studies improvement of anemia might be attributed to the stimulation of endogenous erythropoietin production by nandrolone decanoate and antioxidant potential of vitamins A, D3, E and H (Singh *et al.*, 2012). However, the role of Vitamin D with anemia is not very clear. But few studies explained this mechanism that the administration of 1.25 (OH) D increased the erythroid expansion (Aucella *et al.*, 2003) and thus improves anemia.

The objective of the present study was to explore the role of Vitamin D, Calcium and vitamin D receptors (VDR) in pathogenesis of bovine tropical theileriosis. Firstly, the *Theileria* suspected sampled were confirmed by PCR. The PCR results showed that 32 (32.0%) of total 100 blood samples from suspected animals were positive for *Theileria annulata*. In current study, Cytochrome b gene was used for identification of *Theileria* which is proven and preferred method for the identification of an organism or species. This technique has an advantage over proteins in terms of increased information richness, uses DNA to determine the biological origin of a casework sample. Multiple research studies have employed the cytb gene's nucleotide sequence, which provides species-specific information, in forensic and phylogenetic analyses (Parson *et al.*, 2000).

Calcium (Ca) levels in the present study were significantly ($P < 0.001$) lower in infected cattle than the healthy control (Denizhan *et al.*, 2017). A decrease in Ca level ($P < 0.01$) has also been reported in theileriosis by previous studies (Criado., 2006; Hasanpour *et al.*, 2008; Dua *et al.*, 2012). This decrease in Calcium level might be due to hypoproteinemia, decrease in appetite, intestinal malfunction and kidney damage associated with theileriosis in cattle (Omer *et al.*, 2002) or a possible decrease in vitamin D level which is the regulating factor of calcium metabolism.

It was also observed that vitamin D deficiency (< 30 ng/ml) was more significantly ($P < 0.001$) common in animals positive for *Theileria* as compared to control. Our results were in line with the hypothesis that the low level of vitamin D lead to decrease in calcium levels and thus predispose to theileriosis. Vitamin D levels may be affected due to chronic illness in case of theileriosis (*T. annulata*) and less exposure to the sun. The best measure

of vitamin D status is 25OHD serum, the values are not considered immunologically acceptable until the 25OHD serum groups are > 30 ng/ml (Nelson *et al.*, 2012). Investigations found an association between inflammation and the low level of 25-hydroxyvitamin D3 [25 (OH) D3] in serum (Meckel *et al.*, 2016) which could be associated to tropical theileriosis in in this study.

T. annulata metabolizes hemoglobin producing free radicals that increase oxidative stress in infected animals. This increase oxidative stress could be associated with decreased vitamin D levels in infected animals (Dimri *et al.*, 2013). However, further research work is required to explore these aspects of pathogenesis. Moreover, the role of Vitamin D in pathogenesis of theileriosis can be helpful in drug discoveries against *Theileria*. It is reported that vitamin D ligands increase the production of cathelicidin, which is an effective antimicrobial and antimalarial peptide (Nelson *et al.*, 2012). As *Theileria* is closely related to *Plasmodium* (Morrison *et al.*, 2006) and it has recently been discovered that cathelicidin has antimalarial properties *in vitro* (Parra *et al.*, 2013). It might be possible that cathelicidin may has anti-theilerial properties so further studies should be conducted to determine the efficacy of cathelicidin against tropical theileriosis.

Transmission of *T. annulata* occurs rapidly by the means of the lymphatic system and other organs and encourages the production of TNF- α and the IFN- γ . These cytokines interrupt the "physiological integrity" of the host. Furthermore, the appearance of organisms in the adrenal and pituitary glands causes destruction of the endocrine-immune system. Increase levels of TNF- α , IL-1, & IL-6 (cytokines) cause anorexia, fever, depression, and disseminated hemorrhages formed by infected mononuclear cells (Graham *et al.*, 2001). In short, low levels of Vitamin D (25OHD3) in animals with the (clinical) disease might suggest that these animals have abridged "innate immune responses" thus attenuating immunity to fight infection. The target organs of vitamin D are bones, intestines, parathyroid glands and kidneys (Elder and Bishop, 2014). The immune system is affected by vitamin D deficiency. The effect of vitamin D insufficiency is observed in macrophages at the cellular level as they cannot function in the absence of vitamin D. Therefore, chemotaxis, phagocytosis and the production of proinflammatory cytokines cannot be performed (Cantorna, 2000). Vitamin D plays its immunomodulatory role through the vitamin D receptor (Christakos *et al.*, 2013).

It was also observed that plasma VDR levels were lower in animals with theileriosis (*T. annulata*) than in controls ($P < 0.001$). The lowest concentration of VDR was probably due to a severe vitamin D deficiency in animals with chronic theileriosis; therefore, less 25 (OH) D3 were available for conversion to the active form, which also leads to a lower expression of VDR. The

decrease in VDR levels would in turn be responsible for the reduced effect of the adequate immune and inflammatory response mediated by vitamin D. Calcium and phosphate serum levels changes cause differences in the expression of VDR in the target tissue (Issa *et al.*, 1998). In light of these findings, we conclude that low VDR levels can influence theileriosis, regardless of vitamin D levels. However, this is the initial research that evaluates the plasma VDR levels in cattle with theileriosis; therefore, further research is required to evaluate the relationship between VDR and theileriosis in bovines. For the highest point of our data, there is no previous study for investigation of plasma levels of VDR in the bovine theileriosis.

As far as the association of weather is concerned the samples were mostly collected in mid-March and September therefore the predisposition of low nutrients related to sun exposure is not possible. According to available data, reasonable 25(OH)D3 serum concentrations that range 40-80 ng/mL are ideal for cattle. As a rule of thumb, if exposure of sun is inadequate, supplemental feeding 30 to 50 IU daily of vitaminD3/kg of BW should achieve that range for cattle (Nelson and Merriman. 2014).

Conclusion: These results explain that calcium, Vitamin D and Vitamin D receptors might have a role in pathogenesis of theileriosis. Vitamin D deficiency should be considered a risk factor in cattle infected with tropical region theileriosis.

Authors Contributions: Dr. Raheela Akhtar and Dr. Uzma Farid Durrani conceived and designed the project. Khurram Khan and Mehwish Naseer collected the samples, performed staining and designed specific primers for the experiment and ran PCR to execute the experiment. Beenish Zahid analyzed the data collected from the experiments. Muhammad Usman Jamil helped in critical revisions of the article. All authors interpreted the data, critically revised it for important updates and approved the final version.

REFERENCES

- Al-Ghafari, A. B., K.S. Balamash and H.A. AL-Doghaither (2020). Serum vitamin D receptor (VDR) levels as a potential diagnostic marker for colorectal cancer. *Saudi. J. Biol. Sci.* 27: 827-832. doi: 10.1016/j.sjbs.2020.01.006
- Antwi-Baffour, S., E. Quao, R. Kyeremeh and S.A. Mahmood (2013). Prolong storage of blood in EDTA has an effect on the morphology and osmotic fragility of erythrocytes. *Int. J. Biomed. Sci. Eng.* 1(2): 20-23. doi: 10.11648/j.ijbse.20130102.11
- Aucella, F., R.P. Scalzulli, G. Gatta, M. Vigilante, A.M. Carella and C. Stallone (2003). Calcitriol increases burst-forming unit-erythroid proliferation in chronic renal failure. *Nephron Clin. Pract.* 95(4):121-127. <https://doi.org/10.1159/000074835>
- Branicki, W., T. Kupiec and R. Pawlowski (2003). Validation of cytochrome b sequence analysis as a method of species identification. *J. Foren. Sci.* 48: 83-87. doi: 10.1520/JFS2002128
- Cantorna, M.T. (2000). Vitamin D and Autoimmunity: Is Vitamin D Status an environmental factor affecting autoimmune disease prevalence? *Proc. Soc. Exp. Biol. Med.* 223(3): 230-233. <https://doi.org/10.1177/153537020022300303>
- Christakos, S., M. Hewison, D.G. Gardner, C.L. Wagner, I.N. Sergeev, E. Rutten, A.G. Pittas, R. Boland, L. Ferrucci and D.D. Bikle (2013). Vitamin D: beyond bone. *Ann. N. Y. Acad. Sci.* 1287(1): 45-58. <https://doi.org/10.1111/nyas.12129>
- Criado A, Martinez AJ, Buling A, Barba JC, Merino S, Jefferies R, Irwin PJ (2006) New data on epizootiology and genetics of piroplasms based on sequences of small ribosomal subunit and cytochrome b genes. *Vet Parasitol* 142:238–247 10.1016/j.vetpar.2006.07.004
- Coles, E. (1986). In: *Veterinary Clinical Pathology*. 4th Ed. WB Saunders Company. Philadelphia and London.
- Denizhan, V., S. Kozat, C. Özkan and S.S.Tuncer (2017). Evaluation of serum cobalt, copper, iron, calcium, phosphorus and magnesium concentrations in cattle naturally infected with *Theileria annulata*. *Atatürk University J. Vet. Sci.* 12(2): 111-117. <https://doi.org/10.17094/ataunivbd.347680>
- Dimri, U., M.C. Sharma, S.K. Singh, P. Kumar, R. Jhambh, B. Singh, S. Bandhyopadhyay and M.R. Verma (2013). Amelioration of altered oxidant/antioxidant balance of Indian water buffaloes with subclinical mastitis by vitamins A, D 3, E, and H supplementation. *Trop. Anim. Health Prod.* 45(4): 971-978. DOI 10.1007/s11250-012-0319-6
- Dua, K., M. Kumar, R. Ranjan, S.K. Uppal and L.D. Singla (2012). Levels of certain minerals in blood of cattle naturally infected with *Theileriaannulata*. *J. Vet. Parasitol.* 26(1):89-90.
- Elder, C.J. and N.J. Bishop (2014). Ricketts. *Lancet* 383(9929):1665s-1676. [http://dx.doi.org/10.1016/S0140-6736\(13\)61650-5](http://dx.doi.org/10.1016/S0140-6736(13)61650-5)
- Faiza, A., M. Rehman, S. Gulbeena, A. Kamran, A.H. Mian and S. Muhammad (2023). Identification and molecular characterization of *Theileria annulata* with associated risk factors in naturally infected camels from selected districts in

- Punjab, Pakistan. Pakistan Vet. J. 2023, 43(1): 79-84. DOI: 10.29261/pakvetj/2022.084
- Ghulam, Y., A. Kamran, N. Umer, I.R. Muhammad and Z.A. Muhammad (2022). First genetic evidence of *Trypanosoma theileri* in indigenous cattle in Southern Punjab province of Pakistan. Pakistan Vet. J. 2022, 42(3): 322-327. DOI: 10.29261/pakvetj/2022.034
- Goyal, P., A. Chahar, R.K. Tanwar, Fakhruddin and S.S. Soni (2018). Serum biochemical study to cattle-calves naturally infected with *Theileria annulata* confirmed by nested polymerase chain reaction in Bikaner district of Rajasthan. Int. J. Curr. Micro. App. Sci. 7(2): 2927-2932. DOI: 10.20546/ijcmas.2018.702.355
- Graham, S.P., D.J. Brown, Z. Vatansver, D. Waddington, L.H. Taylor, A.K. Nichani, J.D. Campbell, R.E. Adamson, E.J. Glass and R.L. Spooner (2001). Proinflammatory cytokine expression by *Theileria annulata* infected cell lines correlates with the pathology they cause *in vivo*. Vacci. 19 (20-22): 2932-2944. DOI: 10.1016/s0264-410x(00)00529-6
- Hasanpour A., G.A. ghaddam and A. Nematollahi (2008). Biochemical, hematological, and electrocardiographic changes in buffaloes naturally infected with *Theileria annulata*. Kor. J. Para. 46(4):223-7. DOI: 10.3347/kjp.2008.46.4.223
- Issa, L., G. Leong and J. Eisman (1998). Molecular mechanism of vitamin D receptor action. J. Inflamm. Res. 47(12): 451-475. DOI: 10.1007/s000110050360
- Jabbar, A., T. Abbas, H. A. Saddiqi, M.F. Qamar and R.B. Gasser (2015). Tick-borne diseases of bovines in Pakistan: major scope for future research and improved control. Parasit. Vect. 8(1):283. DOI: 10.1186/s13071-015-0894-2
- Katsogiannou E.G., L.V. Athanasiou, G. Christodouloupoulos and Z.S. Polizopoulou (2018). Diagnostic approach of anemia in ruminants. J. Hellenic. Vet. Med. Soc. 69(3): 1033-1046. DOI: 10.12681/jhvms.18866
- Lertsuwan K., K. Wongdee, J. Teerapornpantakit, N. Charoenphandhu (2018). Intestinal calcium transport and its regulation in thalassemia: interaction between calcium and iron metabolism. J. Physiol. Sci. 68(3):221-232. DOI: 10.1007/s12576-018-0600-1
- Mans, B.J., R. Pienaar and A.A. Latif (2015). A review of *Theileria* diagnostics and epidemiology. Int. J. Parasitol Parasites Wildl. 4(1): 104-118. <https://doi.org/10.1016/j.ijppaw.2014.12.006>
- Meckel, K., Y.C. Li, J. Lim, M. Kocherginsky, C. Weber, A. Almoghrabi, X. Chen, A. Kaboff, F. Sadiq, S.B. Hanauer, R.D. Cohen, J. Kwon, D.T. Rubin, I. Hanan, A. Sakuraba, E. Yen, M. Bissonnette and J. Pekow (2016). Serum 25-hydroxyvitamin D concentration is inversely associated with mucosal inflammation in patients with ulcerative colitis. Am. J. Clin. Nutr. 104(1):113-120. <https://doi.org/10.1016/j.jep.2022.115655>
- Martinez M. E. (2018). The calendar of epidemics: Seasonal cycles of infectious diseases. PLoS pathogens, 14(11), doi: 10.1371/journal.ppat.1007327
- Morrison, W. and D. McKeever (2006). Current status of vaccine development against *Theileria* parasites. Parasit. 133(S2): 169-187. DOI: 10.1017/S0031182006001867
- Muhammad, A. Z., R. Azhar, M. Uzma, M. Saba, A.A. Farhan, A. Asgar, H. Kashif, A.R. Muhammad, A. Sidra, S. Faisal, M. Rehman and O. Mosaab (2022). Epidemiological investigation and development of loop mediated isothermal amplification for the diagnosis of ovine theileriosis. Pakistan Vet. J. 42(3): 370-375. DOI: 10.29261/pakvetj/2022.039
- Nelson, D., T.A. Reinhardt, J.D. Lippolis, R.E. Sacco and B.J. Nonnecke (2012). Vitamin D signaling in the bovine immune system: a model for understanding human vitamin D requirements. Nutri.4(3):181-196. DOI: 10.3390/nu4030181
- Nelson, C.D. and K.E. Merriman (2014). Vitamin D metabolism in dairy cattle and implications for dietary requirements. 25th Annual Florida Ruminant Nutrition Symposium, Florida, USA: 4-5.
- Omer, O.H., K. El-Malik, O. Mahmoud, E.M. Haroun, A. Hawas, D. Sweeney and M. Magzoub (2002). Haematological profiles in pure bred cattle naturally infected with *Theileria annulata* in Saudi Arabia. Vet. Parasitol. 107(1-2):161-168. DOI: 10.1016/s0304-4017(02)00094-8
- Omer, O.H., K. El-Malik, M. Magzoub, O.M. Mahmoud, E.M. Haroun, A. Hawas and H.M. Omar (2003). Biochemical profiles in Friesian cattle naturally infected with *Theileria annulata* in Saudi Arabia. Vet. Res. Commun. 27(1):15-25. DOI: 10.1023/a:1022054522725
- Parra, M., X. Liu, S.C. Derrick, A. Yang, J. Tian, K. Kolibab, S. Kumar and S. L. Morris (2013). Molecular analysis of non-specific protection against murine malaria induced by BCG vaccination. PLoS One 8(7): e66115. DOI: 10.1371/journal.pone.0066115
- Parson, W., K. Pegoraro, H. Niederstatter, M. Foger and M. Steinlechner (2000). Species identification by means of the cytochrome b gene. Inter. J. Leg. Medi. 114: 23-28. DOI: 10.1007/s004140000134

- Prajapati A., P.Prajapati, A.Patel, P. Chauhan, B. Das, S. Raval, A. Suthar, T. Sutaria, R.K. Chaudhari, P. Patel, V. Chauhan and R. Patel (2023). Molecular identification and genetic characterization of *Theileria* and *Anaplasma* infection in sheep and goat of North Gujrat, India. *Parasitol. Res.*122:1427–1433. <https://doi.org/10.1007/s00436-023-07848-w>
- Radostitis, O.M., C.C. Gay, K.W. Hinchcliff and P.D. Constable (2010). *Text book of Veterinary Medicine*. Saunders, USA: 1034-1051.
- Singh, S.K., U. Dimri, M.C. Sharma, D. Swarup, M. Kumar and R. Tiwary (2012). *Psoroptes cuniculi* induced oxidative imbalance in rabbits and its alleviation by using vitamins A, D 3, E, and H as adjunctive remedial. *Trop. Anim. Health Prod.* 44(1): 43-48. DOI: 10.1007/s11250-011-9884-3
- Soulsby, E. (1982). In: *Helminths Arthropods and Protozoa of Domestic Animals*, 7th Ed. Baillire Tindall and Cassell Ltd. London. [https://doi.org/10.1016/0035-9203\(84\)90110-X](https://doi.org/10.1016/0035-9203(84)90110-X)
- Selim A, Weir W, Khater H (2022) Prevalence and risk factors associated with tropical theileriosis in Egyptian dairy cattle, *Veterinary World*, 15(4): 919-924. doi: 10.14202/vetworld.2022.919-924
- Temiz, M., N. Altug and N. Yuksek (2014). Relationship between degree of anemia and blood gases in cattle with theileriosis. *Turk J. Vet. Anim. Sci.* 38(1): 82-87. <https://doi.org/10.3906/vet-1305-75>.