Sys Rev Pharm 2020;11(12):1345-1349
A multifaceted review journal in the field of pharmacy

STUDY OF EFFECTES THE OUTER INSECTES AS PARASITES ON SOME OF HEMATOLOGICAL CHANGES OF CATTLE IN NANAVA CITY

Thabet Mudheher Khalaf[1], Torken Ahmed Hama Hasan[2], Harith Ahmed Mustafa[3]

[1]Department of Biology ,College of Education for women, University of Tikrit
[2]Department of Physics , College of Education tuzkurmatu , University of Tikrit
[3]Department of Biology, College of Education, University of Samarra, Salah-Aldin , Iraq
Mohammed.alsad3@gmail.com
turkan.azm@tu.edu.iq

Abstract
Ticks constitute the second vector group that transmit the major number of pathogens to humans and play a role primary for animals in the process of diseases transmission. Some ectoparasites that affect in cattle this infestation could result in skin damage, blood loss and severe anemia. It can induced the production of CBC counts (Total WBC, differential WBC, Total RBC, HCT, HGB, MCV, MCH, MCHC) and Fe, Mg tests. That study was done detect the hematology tests using mindray instrument and detect serum test (Fe, Mg, Ferritin) with kit from company equipped. They production of these tests with infection and comparison with the healthy animals (control). Significant difference in the (differential WBC, MCV, MCH, MCHC, Fe, Mg, Ferritin). and no significant difference in the (T WBC, RBC, HCT, HGB). The result showed significant differences in the MCV, MCH and MCHC (84.01 ± 10.35 , 89.58 ± 18.88) , (26.58 ± 1.50 , 29.73 ± 1.15) and (30.78 ± 1.25 , 35.86 ± 6.53), and The level in Fe, Mg and ferritin (5.60 ± 1.10 , 10.79 ± 4.01) , (0.54 ± 0.09 , 0.71 ± 0.02) and (8. 12 ± 2.35, 0.3 ± 0.48) in the patients' groups were significant differences when compared with control groups (respectively). But result no showed significant differences in the White blood cells count (5.90 ± 0.12 , 7.76 ± 0.20), RBCs (5.21 ± 0.48 , 4.89 ± 0.77) , HCT (41.83 ± 10.78, 46.65 ± 11.56) and HGB (11.96 ± 3.43 , 13.81 ± 2.98) when compared between patients groups with control groups (respectively).

Keywords: Insectes as parasites, hematological changes, cattle in Nanava City

INTRODUCTION

The presence of a parasite may be detrimental indifferent or beneficial to the host. Parasites are grouped according to location as ectoparasites and endoparasites, ectoparasite include those organisms (e. g. ticks, lice, mites, flies) living (obligate or facultative) on the surface of the host where they derive food, shelter and other basic needs to survive (Adamu et al., 2018). One aspect of animal health is the prevalence of ectoparasite and the diseases they transmit (Byford et al., 1992). Some ectoparasites that affect sheeps goats, dogs, cattle and fowls Include ticks, lice, mite, flies and fleas, this infestation could result in skin damage (Emmanuel et al., 2017). Insect and mite pest activity results in lowered milk production levels and reduced feed conversion efficiency. It exposes cattle to pathogenic microorganisms and causes blood loss and hide damage (Onu & Shiferaw, 2012 ; Rutz & Geden, 2009). All parasites causes skin damage, blood loss and severe anemia, moreover they are important vectors of protozoan, bacterial, viral and rickettsial diseases (Parola & Raoult, 2011). Ticks are ectoparasites widespread globally and its eco epidemiology are closely related to the environmental conditions. They are obligatory hematophagous ectoparasites and responsible as vectors or reservoirs at the transmission of pathogenic fungi, protozoa, viruses, rickettsia and others bacteria during their feeding process on the hosts. Ticks constitute the second vector group that transmit the major number of pathogens to humans and play a role primary for animals in the process of diseases transmission (Brites-Neto, et al., 2015). Phylogenetic studies based on morphological analysis and mitochondrial ribosomal DNA sequencing demonstrated that ticks belong to suborders of Arachnida. These ectoparasites belong to the Phylum Arthropoda, Class Arachnida, Subclass Acari, Order Parasitiformes and Suborder Ixodida, sharing the Order Parasitiformes with the suborders Holothyrida, Mesostigmata (commonly known as mites) and Opilioacarida (Barros-Battesti et al., 2006). A reduction in haematocrit and haematological values are reported in infested cattle as compared to those calves which were free from tick infestation. An increase in number of eosinophils and lymphocytes in ticks infested cattle was reported by some workers, however, a decrease in neutrophils and monocytes in tick-infested cattle was also reported by other researcher. Lower Hb, TEC and marked leucocytosis is due to anaemia because of the blood sucking ability of parasites and hemorrhage (Kaur et al., 2017). Hematological and sero-

biochemical alterations are the indicators of severity of disease and are considered to be good tools for the diagnosis, prognosis for effective therapy (Nazifi, 2010). Anemia is classified as normocytic when erythrocytes that have a normal size or volume (normal MCV); macrocytic, when the MCV is high; microcytic, when the MCV is low; normochromic, when erythrocytes containing the normal amount of hemoglobin (normal MCHC), hypochromic, when the MCHC is abnormally low; and hyperchromic, when the MCHC is abnormally high (Pfaffle et al., 2009). The macrocytic hypochromic anemia observed in cattle with babesiosis could be attributed to intravascular haemolysis of red blood cells (Kaur et al., 2017). The normocytic normochromic anemia may be attributed to the toxic metabolites which have a suppressing effect on the bone marrow and interfere with the process of erythropoeisis (Ibrahim et al., 2009). Keeping in view the importance of blood profile of cattle as an indicator of its good health and diseased condition, the present study is designed to investigate the effect of tick infestation on the hematological parameters of naturally infected calves in the selected area (Kaur et al., 2017). ÷Some of animal health is the prevalence of ectoparasite and the diseases they transmit.. r and other basic needs to survive.

2. MATERIALS AND METHODS

2.1 STUDY ANIMALS

2.2 METHODOLOGY

Five ml venous blood sample was collection from each infection cattle, 2 ml of the blood was collected to ethylenediamine tetra-acetic acid (EDTA) for estimation of the various haematological parameters (CBC complete blood counts). Also collection 3 ml in plain tube and put to centerfuge to take serum for ferriten, TSB and Fe^{+2} estimation.

2.2.1 TOTAL ERYTHROCYTE COUNT (TEC) (MILLIONS PER ML)

Total erythrocytes count (TEC) (RBCs) were estimated by hemocytometer, the red cells diluted using isotonic Hayem's solution.

2.2.2 TOTAL LEUKOCYTE COUNT (TLC) (THOUSANDS PER ML)

Total leucocytes count (TLC) (WBCs), were estimated by hemocytometer using Turke's solution as diluting fluid.

2.2.3 HAEMOGLOBIN LEVEL (MG/L00 ML)

The hemoglobin concentration (Hb) was estimated by the acid hematin method.

2.2.4 DIFFERENTIAL LEUKOCYTES COUNT (DLC) (%)

It is the differential count of different leucocytes *viz*., Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils. All are expressed in percentage (%).

2.2.5 PACKED CELL VOLUME (PCV)

The percentage of packed red cells in a given volume of blood after centrifugation is known as hematocrit. The packed cell volume (PCV), was estimated by Microhematocrit method using a capillary hematocrit tube approximately 7.5 cm in length and having a bore about 1 mm centrifuged in a special speed centrifuge (Micro-Hematocrit, Taiwan).

2.2.6 MEAN CORPUSCULAR/CELL VOLUME (MCV) (FL)

The mean cell volume is a measure of the volume of the average red cell in a sample. It is expressed in femtoliters (fl). It is calculated by the following formula:

MCV (fl) = PCV x 10 / RBCs count

2.23.7 MEAN CORPUSCULAR HAEMOGLOBIN (MCH) (PG)

It is a measurement of the average amount of haemoglobin per cell. It is expressed in picograms per cell (pg/cell) and calculated by using the following formula:

MCH (pg) = Hb x 10 / RBCs

2.2.8 MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC) (%)

It is a measure of the average concentration of haemoglobin per red blood cell. It is expressed in grams per deciliter (g/dL). It is calculated by the following formula :

MCHC(%) = Hb/ PCV x 100

2-2-9 FE LEVEL

Set up three tubes and proceed as follows:

	Blank	Unknown	Calibrator
Reagent 1	0.8 ml	0.8 ml	0.8 ml
Serum	-	0.1 ml	-
Calibrator	-	-	0.1 ml
Deionized water	0.1 ml	-	-

Mix and measure the absorbance of the unknown and calibrator against water at 560 nm (540-580), obtaining the absorbance A_1.

	Blank	Unknown	Calibrator
Reagent 2	0.2 ml	0.2 ml	0.2 ml

Mix, incubate at 37º c for 5 minutes and measure the absorbance of the unknown and calibrator against blank at 560 nm or green filter (540-580), obtaining the absorbance A_2.

2-2-10 MAGNESIUM LEVEL

	Blank	Standard	Sample
Standard sample	-	10 µl	10 µl
Working reagen	1 ml	1 ml	1 ml

Mix and read the optical density (Wavelength 520 nm) after incubation for 5 minutes (20-25 ºc) at room temperature stability of the colour: 1 hour.

Mg = sample / stander × 0.082

2-2-11

1- Preparation of the standards ($S_0, S_1, S_2, S_3, S_4, S_5$)

The standards are ready to use and are calibrated against WHO 1st is ferritin 80/602 and have the following concentrations:

S_5	S_4	S_3	S_2	S_1	S_0	
1000	400	100	20	5	0	Ng/mL

For sample with concentration greater than 1000ng/ml dilute the sample with zero standard S_0 Once open, the standards are stable 6 months at 2 C- 8 C

2- PREPARATION OF WASH SOLUTION

Dilute the content of each vial of the wash solution concentrate (10X) with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 diluted ratio. The diluted wash solution is stable for 30 days at 2 C – 8 C. In concentrated wash solution is possible to observe the presence of crystals; In this case mix at room

temperature until the complete dissolution of crystals; for greater accuracy, dilute the whole bottle of concentrated wash solution to 500mL, taking care to transfer completely the crystals are completely dissolved.

3- PREPARATION OF THE SAMPLE

Ferritin determination should be done in human serum or plasma. Specimen can be stored at 2 C – 8 C for a shot time(max five days).For longer storage the specimen should be frozen at 20 C. Avoid repeated freezing and thawing. The Control is use.

4- PROCEDURE

Allow all reagents to reach room temperature (22 C-20 C) for at least 30 minutes. At the end of the assay. Store immediately the reagents at 2 C-8 C: avid long exposure to room temperature.

Unused coated microwell strips should be released securely securely in the foil pouch containing desiccant and stored at 2 C-8 C.

To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.

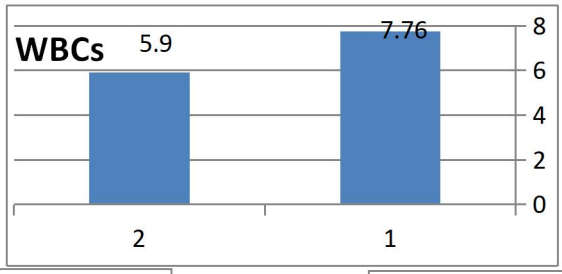
As it is necessary to pertorm the determination in duplicate in order to improve accuracy of the lest results, Prepare two wells for each point of the calibration curve (S_0-S_5), two for each control, two for each sample, one for blank.

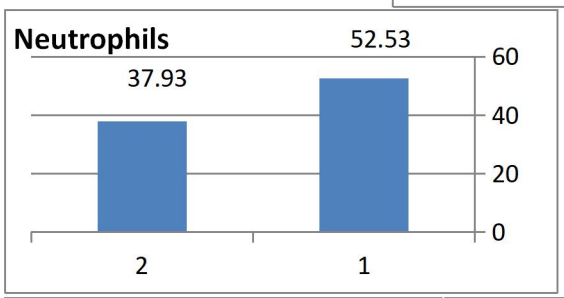
2.4 STATISTICAL ANALYSIS

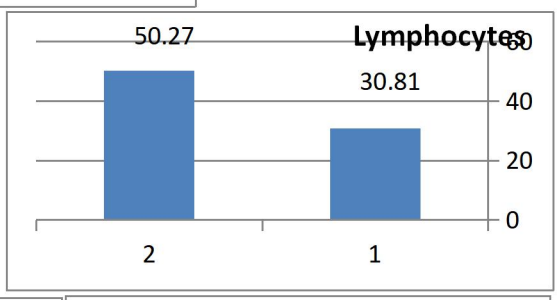
The student Duncan test ($P < 0.05$) was done to show significant differences in haematological parameters between infested and non-infested Cattle.

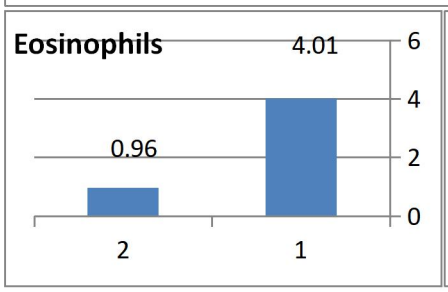
The results showed significant differences in the Neutrophils percentage, Lymphocytes percentage, Monocytes percentage, Eosinophils percentage and Basophils percentage when compared between patients groups with control groups. The percentage of Neutrophils (37.93 ± 0.07 , 52.53 ± 0.76) and Eosinophils percentage (0.96 ± 0.06 , 4.01 ± 0.68) and basophils percentage (0.60 ± 0.33 , 0.78 ± 0.04) in patients groups were lower compared with control groups (respectively), and they show high significant in lymphocytes percentage and monocytes percentage (50.27 ± 1.42 , 30.81 ± 5.12) , (5.417 ± 0.77, 0.08 ± 0.91) in patients groups were lower compared with control groups (respectively). But result no showed significant differences in the White blood cells count (5.90 ± 0.12 , 7.76 ± 0.20) when compared between patients groups with control groups.

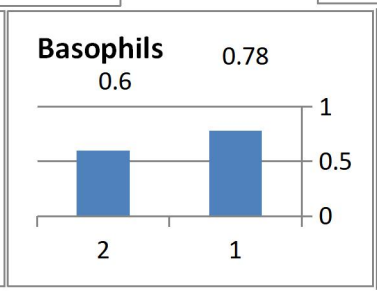
In red blood cell counts, They are no show significant differences in the RBCs, HCT and HGB (5.21 ± 0.48 , 4.89 ± 0.77) , (41.83 ± 10.78, 46.65 ± 11.56) and (11.96 ± 3.43 , 13.81 ± 2.98) when compared between patients groups with control groups (respectively). But result showed significant differences in the MCV, MCH and MCHC (84.01 ± 10.35 , 89.58 ± 18.88) , (26.58 ± 1.50 , 29.73 ± 1.15) and (30.78 ± 1.25 , 35.86 ± 6.53) in patients groups were lower compared with control groups (respectively).

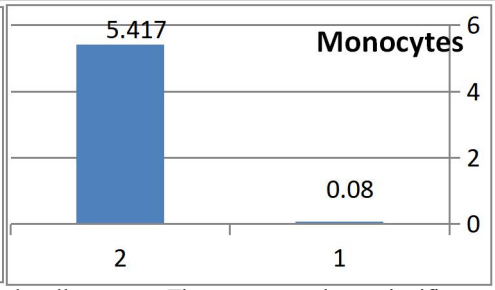












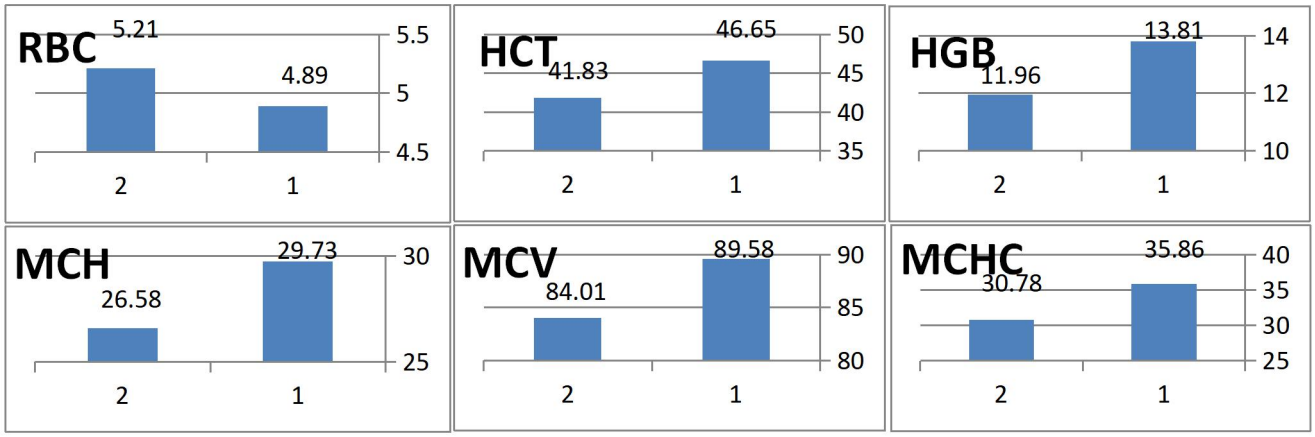
In red blood cell counts, They are no show significant differences in the RBCs, HCT and HGB (5.21 ± 0.48 , 4.89 ± 0.77) , (41.83 ± 10.78, 46.65 ± 11.56) and (11.96 ± 3.43 , 13.81 ± 2.98) when compared between patients groups with control groups (respectively). But result showed significant differences in the MCV, MCH and MCHC (84.01 ± 10.35 , 89.58 ± 18.88) , (26.58 ± 1.50 , 29.73 ± 1.15) and (30.78 ± 1.25 , 35.86 ± 6.53) in patients groups were lower compared with control groups (respectively).

4- RESULT

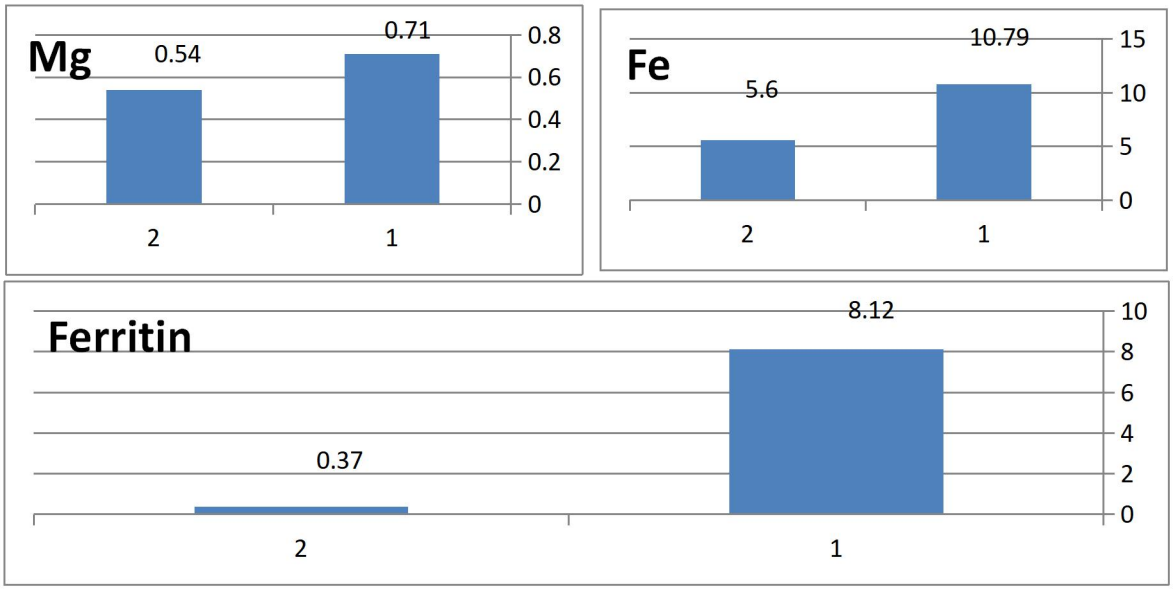
MCHC g/dl	MCH Pg	MCV fL	HGB g/dl	HCT %	RBC Mm^3/c	Groups
35.86 ± 6.53	29.73 ± 1.15	89.58 ± 18.88	13.81 ± 2.98	46.65 ± 11.56	4.89 ± 0.77	Control group
30.78 ± 1.25*	26.58 ± 1.50*	84.01 ± 10.35*	11.96 ± 3.43	41.83 ± 10.78	5.21 ± 0.48	Patients group

Basophils%	Eosinophils%	Monocytes%	Lymphocytes%	Neutrophils%	WBCMm³/c	Groups
0.78 ± 0.04	4.01 ± 0.68	0.08 ± 0.91	30.81 ± 5.12	52.53 ± 0.76	7.76 ± 0.20	Control group
0.60 ± 0.33*	0.96 ± 0.06*	5.417 ± 0.77*	50.27 ± 1.42*	37.93 ± 0.07 *	5.90 ± 0.12	Patients group

The level in Fe, Mg and ferritin (5.60 ± 1.10 , 10.79 ± 4.01) , (0.54 ± 0.09 , 0.71 ± 0.02) and (8. 12 ± 2.35 , 0.3 ± 0.48) in the patientsgroups were lower significant differences when compared with control groups (respectively).



Ferritin Ng/ ml	Mg Mmol/L	Fe Mmol/L	Groups
8.12 ± 2.35	0.71 ± 0.02	10.79 ± 4.01	Control group
0.37 ± 0.48*	0.54 ± 0.09*	5.60 ± 1.10*	Patients group



DISCUSSION

Ectoparasitic infestation by ticks in other livestock has also shown alterations in the blood parameters (Kumar., 2010). This may be attributed to the normocytic normochromic anaemia in the infested cattle of the study area. The reduction in the RBC counts occurred due to blood sucking by the ticks as well as due to destruction of RBC's by the protozoan parasites transmitted by ticks. Lower Hb and TEC are due to anaemia because of blood sucking ability of parasites and haemorrhage (Solsby, 1982). The results in the present study support the findings of other study (Rajendran & Hafeez, 2003 ; Maske, 1993) who have also reported lowered TEC along with lowered Hb. In the present study, Total Leucocyte Count (TLC) of the naturally infested cattle were found to be lower significantly (P<0.05) when compared to non-infested cattle. A significant decrease in TLC is also reported by other researchers in their respective studies (Ahmed et al., 2006). However, a significantly higher (P<0.05) lymphocytes and Monocyte count were found in infested cattle than non-infested, which may be due to inflammation caused by tick bite which leads to migration of white blood cells as a response toward the tick bite. Neutrophils couns in non-infested cattle was observed to be significantly higher (P<0.05) than in the infested cattle. The changes in haematological parameters as well as reduction in live weight gain due to tick infestation in calves have also been reported. It is also a fact that poor nutrition produces a fall in haematocrit and haemoglobin levels in any animal including cattle (Vatsya et al., 2008). Ticks are known for their ability to ingest large amounts of blood from their host, reaching more than a hundred times their unfed body weight. The numerous bioactive molecules in theirsaliva allow them to evade the host's immune and hemostatic mechanisms, which is important for successful attachment and feeding (Francischetti et al., 2010). However, they also must cope with potentially toxic molecules in the host blood, including

iron. Ferritin is an iron-storage protein involved in iron homeostasis in most living organisms. The physiological importance of ferritin in bloodfeeding and reproduction of the ticks (Hajdusek et al., 2009).

REFERANCES

1. Adamu, M. O. ; Onawo, A. S. & Adebayo, F. O. (2018). An Investigation of the Ectoparasites of Slaughtered Cattle at Keffi Abattoir in Nassarawa State of Nigeria, Sub-Sahara Africa. International Journal of Animal Science and Technology, 2(1): 18-22.
2. Ahmad, N. ; Hashmi, H. A. ; Khan, S. A. (2006). Study on ticks and haemoparasitic diseases of local cattle in Malakand agency. Journal of Animal and Plant Science. 16:3- 4.
3. Barros-Battesti, D.M., Arzua, M. and Bechara, G.H. (2006) Carrapatos de Importância Médico-Veterinária da Região Neotropical: Um guia ilustrado para identificação de espécies. Vox/ICTTD-3/Butantan, São Paulo.
4. Brites-Neto, J. ; Duarte, K. M. R. ; Martins, T. F. (2015). Tick-borne infections in human and animal population worldwide. *Veterinary World 8*(3):301-315.
5. Byford, R. L. ; Craig, M. E. & Crosby, B. L. (1992). A review of ectoparasites and their effect on cattle production. *J ANIM SCI*, 70:597-602.
6. development. Proc Natl Acad Sci U S A 106: 1033–1038
7. Emmanuel, O. I. ; Anyebe, A. A. ; Agbo, O. E. ; Odeh, U. P. ; Love, O. & Agogo, I. M. (2017). A Preliminary Survey of Ectoparasites and Their Predilection Sites on Some Livestock Sold in Wadata Market, Makurdi, Nigeria. American Journal of Entomology, 1(1): 11-15.
8. Francischetti, I. M. B. ; Sa-Nunes, A. ; Mans, B. J. ; Santos, I. M. & Ribeiro, J. M. C. (2010). Therole of saliva in tick feeding. Front Biosci 14: 2051–2088.
9. Hajdusek O, Sojka D, Kopacek P, Buresova V, Franta Z, et al. (2009)
10. Hajdusek, O. ; Sojka, D. ; Kopacek, P. ; Buresova, V. & Franta, Z. (2009). Knockdown of proteins involved in iron metabolism limits tick reproduction and development. Proc Natl Acad Sci U S A 106: 1033–1038.
11. Ibrahim, A. K. ; Behairy, A. M. ; Mahran, K. A. ; Awad, W. (2009). Clinical and laboratory diagnosis of piroplasmids in naturally infected cattle in Egypt. Journal of Egyptian Veterinary and Medical Association. 69(2):105- 203.
12. Kaur, D. ; Jaiswal, K. & Mishra, S. (2017). Effect of tick infestation on haematological parameters of calves. Journal of Entomology and Zoology Studies; 5(4): 107-111.
13. Knockdown of proteins involved in iron metabolism limits tick reproduction and
14. Kumar, A. ; Vihan, V. S. ; Sadhna, Sharma, H. N. (2010). Haematological and biochemical effects of tick infestation in common Indian goat. Advances in Bioresearch. 1:163-168.
15. Maske, D. K. (1993). Journal of Veterinary Parasitology. 7(2):130-131.
16. Nazifi, S. (2010). Diagnostic values of acute phase proteins in Iranian indigenous cattle infected with *Theileria annulata*. Veterinarski arhiv, 80:205-214.
17. Onu, S. H. and Shiferaw, T. Z. (2012). Prevalence of ectoparasite infestations of cattle in Bench Maji Zone, Southwest Ethiopia. *Veterinary World,* 6(6): 291-294.
18. Parola, P. & Raoult, D. (2011). Ticks and tick borne bacterial diseases in humans; an emerging infectious threat Infect Dis, 15(6): 897-928.
19. Pfaffle, M. ; Petney, T. ; Elgas, M. ; Skuballa, J. & Tarasschewski, H. (1009). Tick-induced blood loss leads to regenerative anemia in the European hedgehog (*Erinaceus europaeus*). Parasitology, 136(4):443-452.
20. Rajendran, C. & Hafeez, M. D. (2003). Haemato-biochemical changes and efficacy of different acaricides in cross bred animals. Indian Journal of Animal Science. 73(5):481-483.
21. *Rutz*, D. A. & *Geden*, C. J. (2009). Pest Management Recommendations for Dairy Cattle. *Department of Entomology, Cornell University, and Charles W. Pitts, Department of Entomology, Penn State.*
22. Soulsby, E. J. L. (1982). Helminths, Arthropodes and Protozoa of domesticated animals, 7th edition the ELBS and Baillieve, Tindal cass LTD, London. 766.
23. Vatsya, S. ; Kumar, R. R. ; Yadav, C. L. ; Garg, R. (2008). Effect of the control of one-host cattle tick Boophilus microplus on the growth and haematological parameters of calves. Journal of Parasitic Diseases. 32(1):64-67.