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Acute Hemotropic Mycoplasmosis Of Newborn Calves In Basrah, Iraq

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Abstract *: The present study was conducted on forty five (45) local calve breeds aged (2-13) days old of both sexes, carried out at Basrah governorate, Iraq.Thirty five (35) local calve breeds had been showing signs of Panting , Lateral recumbency and unable to stand, Petechial hemorrhages on sclera and conjunctival membranes, Pale or icteric mucous membranes, Different hemorrhagic patches on the skin, Enlargement of superficial lymph nodes and Hemoglobinurea with passing of coffee like urine. Ten, healthy local calve breeds of the same ages and sexes considered as controls. Complete clinical examinations have been applied to all animals. Moreover, all animals were checked out and found free from all ectoparasite infestations. Hemomycoplasma weynonii were detected by Giemsa stained blood smears as the organism appears with different shape such as coccoid or rod, Furthermore, it might found individually or in chains, infect the erythrocyte cell wall, In addition, the molecular method, PCR test was used to confirm the diagnosis, which show a strong band. In accordance with the hematological analysis, Results indicated a significant decrease in TRBc, Hb concentration and PCV which reflect the Macrocytic hypochromic type of anemia, Moreover, a significant increase has been encountered in total leukocyte count due to a significant increase in absolute lymphocyte count. On the other hand clotting factor indices, indicated a significant difference, as results reveal a significant decrease in the total platelet count of infected newborn calves than in controls. Moreover, results indicated a significant increase of platelet volume and platelet distribution width. However, clotting time, prothrobmin time and activated partial thromboplastine time was also increased significantly in diseased calves than in controls. Furthermore, results for acute phase response indicated a significant decrease of haptoglobin values and fibrinogen time in infected local calves breeds with Mycoplasma weynoni compared with controls.*
Keywords: *Hemomycoplasma weynonii, newborn calves, Basrah, Iraq*

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I. Introduction

Bovine Hemotropic mycoplasmosis is an infectious disease caused by *Hemomycoplasma weynoni* (Constable *et al*, 2017). The organism thought to be rickettsial parasites (formerly classified as Eperythrozoon) cause an infectious anemia in different mammalian species and their effects might terminate with severe weakness, apathy and death of diseased animal (Fard *et al* ,2014). The disease always manifested by fever, anorexia, anemia, lethargy, rough coat, decreased milk production in lactating animals, enlargement of superficial lymph nodes and weight loss (Jarad and Alsaad,2016).

The disease is highly prevalent in different parts of the world, Since it detected in Africa, southern Europe, Central Asia and some others where (Messick, 2004., Adresi and Saki, 2009., Al-Badrani and Rhaymah, 2012). Moreover, it was also distributed in most regions of Iraq (Basima and Baraa, 2016., Jarad and Alsaad, 2016., Sahey *et al*, 2016., Kshash, 2017). However, the disease had a considerable economic impact on livestock production due to the high morbidities and mortalities, beside its real zoonotic impact (Yang *et al*, 2000., Neimark *et al*, 2002., Bosnic *et al*, 2010., Fard *et al*, 2014).

Intrauterine transmission of *Hemomycoplasma wenyonii* seems to be diagnosed, and might be accidental, However, it was reported in cattle and the fetus may be infected with Hemomycoplasma parasites at any stage, Furthermore, in enzootic areas, it may cause abortions (Fisher and Zinkl, 1996., Fard *et al* , 2014., Girotto-Soares *et al* ,2016).

Intrauterine infection with *Hemomycoplasma wenyonii* is strongly suggested and the infection appears to have taken place prenatal (Almy *et al*, 2006., Hornok *et al*, 2011). One indication of a possible incidence of newborn calves Hemomycoplasmosis infection, As, the causative organisms were detected in blood smears from thirty seven animals (37) out of seventy nine (97) cows (at two farms) expected to calve within 35 days. Similarly, 29 of 66 calves born to the above cows also exhibited *Hemomycoplasma wenyonii* infection on day 1 to day 17 postpartum, Furthermore, all diseased newborn calves are free from ectoparasites, as the course of the study is in the winter months, when the activity of the ticks is reduced to the minimum.

It has been documented that molecular evidence is provided for the first time on the transplacental transmission of bovine Hemomycoplasmosis. (Hoelzle *et al*,2010., Hornok *et al* , 2011) Moreover (Girotto-

Soares *et al*, 2016), added that the results of his study were proof that the organism *Hemomycoplasma wenyonii* parasite can be transferred via the placenta.

To date almost little or maybe nothing is known about intrauterine Hemomycoplasmosis in newborn Iraqi calves, Therefore, In the current work, infection of newborn calves with *Hemomycoplasma wenyonii* was identified in Basarh, Iraq, with evaluation of hemogram, clotting factor indicese and acute phase response.

II. Materials and methods

Animals:

The study was conducted on 45 newborn local Iraqi calve breeds carried out at Basrah governorate, Iraq. Thirty five (35) local calve breeds 2-13 days old and from both sexes show signs of severe anemia, panting, Petechial hemorrhage was detected on sclera and a different sizes of hemorrhagic skin patches was also detected on the skin. Ten, clinically healthy local Iraqi calve breeds of same ages considered as controls. Complete clinical examinations have been applied to all animals. Moreover, all animals were checked out and found, free from all types of ectoparasite infestations .

Samples :

Ten milliliters (10 mL) of blood were drained from each newborn calf by a jugular venipuncture rout. Two and half (2.5 mL) milliliter of blood mixed with EDTA used to determine the Total erythrocyte count (RBCs), Hemoglobin concentration (HB), packed cell volume (PCV), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin concentration (MCHC), Platelet count , mean platelet volume, platelet distribution width, and total leukocyte count (TLC) on an automatic full digital cell counter (Beckman, USA). Giemsa-stained blood smears were used for differential leukocyte counts, according to (Weiss and Wardrop, 2010). Another 2.5 mL of blood mixed with trisodium citrate (with the ratio of 9:1) was used to determine Fibrinogen time, prothrombine time and activated partial thromboplastine time (Biolabo / France). Clotting time was also estimated according to (Bush, 1975). Serum haptoglobin concentrations were assayed for evaluation of haptoglobin according to manufacture instructions from (Haptoglobin Elisa method, Biotechnology co -china).

For exclusion of the infection with other blood parasites, lymph smears were prepared and examined according to (Washburn *et al*, 2007).

The primary diagnosis of *Hemomycoplasma wenyonii* was done by microscopical examination of Giemsa stained blood smears and was confirmed by using PCR techniques.

***PCR assay and molecular methods*:**

For the PCR assay, the blood was stored in EDTA tube until assayed. DNA was extracted from 200µl of blood using a commercial kit (Bioingentech Genomic DNA Purification Kit, BioInGentch, Chile). According to manufacture protocol of (*Mycoplasma wenyonii* Detection Kit, BioInGentch,Chile) that used for the specific amplification of a region (180-basepair) of 16S RNA gen from *Mycoplasma wenyonii*, The kit consists of the following components that are enough for amplification of genomic DNA, such as, *Mycoplasma wenyonii* Pre-mixture, PCR internal control, *Mycoplasma wenyonii* PCR Positive control, DNase/Ranse free water, PCR negative control, mineral oil solution and brig™molecular weight marker. Preparation of the *Mycoplasma wenyonii* PCR mixture was done according to the protocol of the same detection kit.The amplification condition was included, one cycle Initial Denaturation at 94ċ for 2 minutes, 30 cycles of denaturation at 94ċ, annealing at 57ċ and extension at 72ċ for 30 second, final extension one cycle at 72ċ for 5 minutes using an automatic cycler. Amplification products were electrophoresed on 1.5% agarose gels. Gels were stained with ethidium bromide and examined with ultraviolet illumination. Bands seen at the expected location (180- bp).

Statistical analysis:

Statistical variations between diseased and healthy newborn calves was analyzed using the student t-test, (Leech *et al*, 2007).

III. Results

Diseased newborn calves show Panting (94.2%), Lateral recumbency and unable to stand (85.7%), Petechial hemorrhage on the sclera and conjunctival membranes (77.14%), (Fig: 1), Pale and or icteric mucous membranes (71.4%), Different hemorrhagic patches on the skin (51.4%), (Fig: 2), Enlargement of superficial lymph nodes (48.5%) and Hemoglobinurea (25.71%) with passing of coffee like urine. Table.1. Moreover, Clinical examinations of diseased calves show a significant increase ($P<0.05$) of body temperature, respiratory and heart rate, However, a significant increase ($P<0.05$) in capillary refilling time was also encountered in diseased calves than in controls. Table 2.

Hemomycoplasma weynonii was detected by Giemsa stained blood smears as the organism appears with different shape such as coccoid or rod, Furthermore, it might found individually or it arranged in chains,

and infect the erythrocyte cell wall. Fig: 3. In addition, the molecular method, PCR test was used to confirm the diagnosis, Fig: 4.

In accordance with the hematological analysis, Results indicated a significant decrease ($P<0.05$) in TRBc, Hb concentration and PCV which reflect the Macrocytic hypochromic type of anemia, Moreover, a significant increase ($P<0.05$) have been encountered in total leukocyte count due to a significant increase ($P<0.05$) in absolute lymphocyte count. On the other hand clotting factor indices indicated a significant difference, as results reveal a significant decrease ($P<0.05$) in the total platelet count in infected newborn calves than in controls, Moreover, results indicated a significant increase ($P<0.05$) of platelet volume and platelet distribution width. However, clotting time, prothrobmin time and activated thromboplastine time was also increased significantly ($P<0.05$), in diseased calves than in controls, Table 3. Furthermore, results for acute phase response indicated a significant decrease ($P<0.05$) of haptoglobin values and fibrinogen time in infected local calves breeds with *Mycoplasma weynoni* compared with controls, Table 4.

IV. Discussion

Acute Hemomycoplasmosis was diagnosed in newborn calves of the Basrah governorate at age ranged between 2-13 days old, Since, the diagnosis was confirmed by Giemsa stain blood smears and PCR technique. This is the first report of transplacental infection in case of bovine Hemomycoplasmosis and the first molecular confirmation, Same results are in supporting data mention by (Hornok *et al*, 2011., Girotto-Soares *et al*, 2016). In the present study neonatal PCR-positivity of calves to bovine should have been a consequence of transplacental infection of the fetus, since other post-parturient routes (including colostral/galactogenic, vector-borne infection) can be discounted, Moreover, Vertical transmission was suggested and proven for only very few other hemotropic Mycoplasma spp, with some controversy in the literature (Messick, 2004., Constable *et al*, 2017). The present results also raise the possibility that intrauterine infection may be influenced by the genotype, cluster together with *Hemomycoplasma wenyonii* based on 16S rRNA phylogeny (Messick, 2004., Hoelzle *et al*, 2010). In this study the manifestation of transplacental infection apparently did not depend on bacterial load, as reflected by copy numbers and age of mother cows.

It have been mentioned that regarding the epidemiology of hemomycoplasmosis , In general, routes of infection are incompletely characterized and transplacental transmission was suggested and verified for only a few species (Almy *et al*, 2006 ., Girotto-Soares *et al*, 2016), Furthermore, Transplacental transmission of the causative agent has been reported with no known natural exposure to the tick or other biological vector in areas where transmitter vectors may be present(Neimark *et al* , 2002 ., Hornok *et al* ,2011). It had been added that carrier cows with Hemomycoplasmosis can transmit *Hemomycoplasma weynoni* to their calves and such calves might born at term can have a massive and increased parasitaemia, On the other hand, (Constable *et al* ,2017) mention that although anti hemomycoplasma drugs (specilly the tetracylines group) can suppress the organism but do not eliminate completely, Therefore carrier cows may transmit the organism to their offspring and it is assumed that once the hemomycoplasma crosses the placenta and infects the fetus, the result is either terminated with an abortion or a calf born with neonatal hemomycoplasmosis . Nevertheless, It could not prove yet that transplacental transmission of *Hemomycoplasma wenyonii* results from abnormal placentation as the placental damage might allow the maternal and fetal blood to mix, or that reverse erythroblastosis fetalis may occur, allowing the hemomycoplasma to cross the placenta, (Fard *et al*, 2014., Girotto-Soares et al, 2016). In contrast. Others, like (Hornok *et al*, 2011) suggest that hemomycoplasma transmission can also occur during pregnancies where the placentation is normal.

Diseases newborn calves show different clinical manifestations which are mentioned by others (Messick, 2004., Fard *et al*, 2014., Jarad and Alsaad, 2016., Constable *et al*, 2017). Moreover, On giemsa stained blood smears the organism appear with different shape, small coccoid or rod structures and it could be arranged as a singular or in chains pattern on the erythrocyte cell membranes of infected RBCs of diseased calves, same result were detected by (Basima and and Baraa, 2016 ., Sahey *et al*, 2016), Furthermore, In the current study, all blood samples belong to diseased calves were apply with molecular analysis (PCR) to confirm the presence of *Mycoplasma wenyonii*, and the results show a strong bands on this technique, However, this finding is found agreed with the results that obtained by(Adresi and Saki, 2009 ., Wang *et al* , 2009 ., Hornok *et al* , 2011 ., Jarad and Alsaad, 2016).

Hematological analysis of diseased calves compared with controls reveal sever anemia with macrocytic and hypochromic type, same results are also indicated by (Tagawa *et al* , 2010 ., Felder *et al* 2011., Girotto-Soares *et al* , 2016) In addition a significant diffrence indicated in clooting factor indicese of infected calves which might suggest the clear disturbances in clotting system of diseased animal with imbalanced regulation may lead to hypo or hyper coagulation, terminated with the initiation of disseminated intravascular coagulation, (Bick, 2003 and Pantanowitz, 2003).

The results of the current study indicated a significant decrease in values of acute phase response as a significant decrease in both haptoglobin and fibrinogen was encountered, same results was also mentioned by

(Murata *et al*, 2004 ., Jain *et al*, 2011., Korman *et al*, 2012), Since, haptoglobin will binds the free hemoglobin loose from erythrocytes with high affinity, Therefore, will prohibit its oxidative functions (Cary *et al*, 2009). In addition, (Tecles *et al*, 2005), added that haptoglobin levels will be decreased in hemolytic anemia in the process of binding hemoglobin, because haptoglobin insulate the iron within hemoglobin, Therby, preventing *Hemomycoplasma wenyonii* from utilizing this iron for the benefit.

Figures and Tables

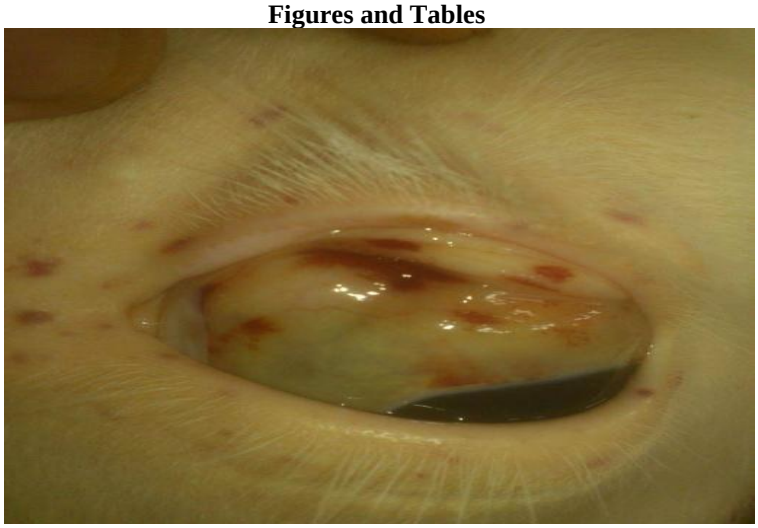

Fig 1 : *Petechial hemorrhage on sclera and conjunctival membranes*

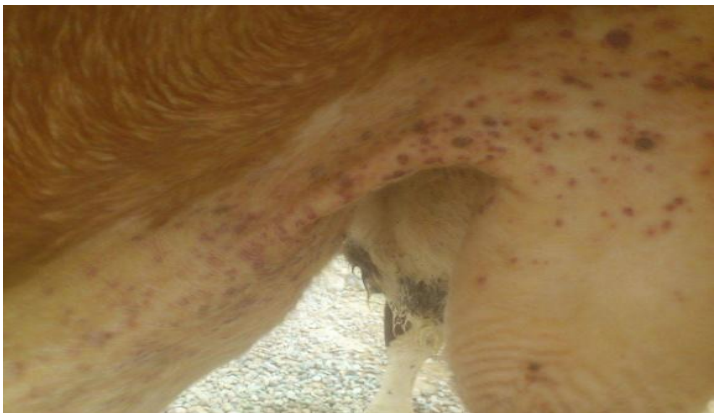

Fig 2 : Different hemorrhagic patches on the skin

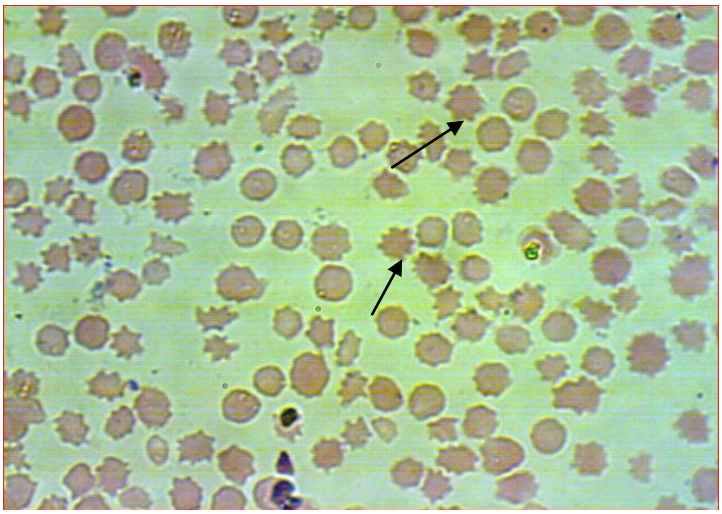

Fig 3 : *Hemomycoplasma weynonii infect RBC cell wall of newborn calves*

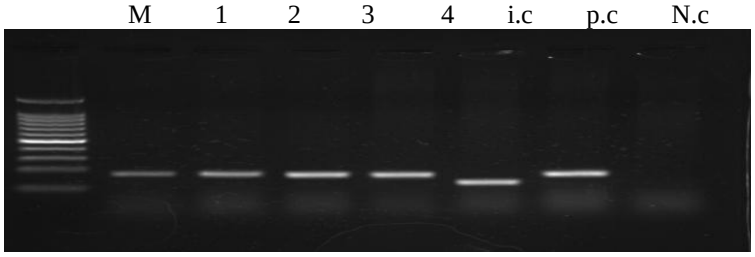

Fig 4: Amplification of Mycoplasma wenyonii genomic DNA by PCR

Lane M: BrigTM Molecular Weight Marker (1000-bp)
Lane 1,2,3 and 4: Mycoplasma wenyonii Positive samples(180-bp)
Lane I.C.: Internal control(140-bp)
Lane P: Positive control(180-bp)
Lane N: Negative control

Table 1: Clinical signs of diseased calves with acute Hemomycoplasmosis

Clinical signs	**Diseased calves n=35**	**%**
Panting	33	94.2
Lateral recumbency and unable to stand	30	85.7
Petechial hemorrhage on sclera and conjunctivae	27	77.14
Pale and /or icteric mucous membranes	25	71.4
Different hemorrhagic patches on the skin	18	51.4
Enlargement of superficial lymph nodes	17	48.5
Hemoglobinurea	9	25.71

Table 2: Body temperature, Respiratory and Heart rate, Capillary refilling time of infected newborn calves and controls.

Parameters	**Controls n=10**	**Diseased calves n=35**
Body temperature C°	38.46 ± 0.77	41.2 ±1.46 *
Respiratory rate / min	22.72 ±4.54	93.3 ±12.4*
Heart rate / min	103. ±7.43	147.8 ±15.7 *
Capillary refilling time	1.24 ± 0.44	5.71 ± 0.72*

Values are mean ± standard error of mean. * (P<0.05).

Table 3: Hemogram of infected newborn calves and controls

Parameters	**Controls n=10**	**Diseased calves n=35**
TRBC $\times 10^6$	7.63±0.84	4.73±1.21*
HB g/dl	13.38±1.83	6.34±3.15*
PCV %	33.76±3.85	24.33±5.21*
MCV /fl	45.09±6.44	51.43±3.56*
MCHC /dl	39.63±7.22	26.05±5.71*
TLC $\times 10^3$	11.158± 1.36	15.811±2.56*
Lymphocytes /Absolutes	4986 ± 266.23	9872 ± 722.11*
Nutrophiles /Absolutes	4988 ± 456.78	4786.146 ± 457.81
Monocytes /Absolutes	598 ± 367	559 ± 320
Eosinophiles /Absolutes	492 ± 22	491 ± 25
Basophiles /Absolutes	94±57	92±66
Total platelet counts $\times 10^3$	455 ± 65	188 ± 88*
Platelet volume /fl	12.342 ± 4.56	17.451 ± 3.73 *
Platelet distribution width	13.417 ± 2.862	21.167 ± 5.88 *
Clotting time / mint	2.32 ± 1.342	5.34 ± 1.56 *
Prothrombin time /Sec	15.17 ± 3.54	33.45 ± 5.13 *
Activated partial thromboplastin time /Sec	49.36±5.27	72.67± 12.54 *

Values are mean ± standard error of mean ,* (P<0.05).

Table 4: Acute phase response of infected newborn calves and controls

Parameters	Controls n= 10	Diseased calves n =35
Haptoglobin g/dl	0.021± 0.011	0.009 ± 0.002*
Fibrinogen time / Sec	28.51± 3.38	15.34 ± 7.27*

Values are mean ± standard error of mean. * (P<0.05).

V. Conclusions

It has been concluded that Acute Hemotropic mycoplasmosis of newborn calves in Basrah, Iraq was detected which might lead to high economic losses, Therefore, periodic examination of adult and pregnant cows should advise .

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