Microscopic identification, Molecular and Phylogenetic Analysis of Babesia species in Buffalo from Slaughter House in Al-Najaf City of Iraq

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Abstract

Babesia is one of hemoprotozoan parasite transmitted by arthropod vectors which responsible for causing of Babesiosis disease in bovine worldwide. The present study was designed for Microscopic identification, Molecular, and Phylogenetic Analysis of Babesia species in Buffalo from Slaughter House in Al-Najaf City of Iraq . The study performed in three months of summer season (August into September 2017) and animals ages and sex was included in this study. The direct microscopic prevalence results were show highest prevalence of haemoprotozoa prevalence at *Babesia* sp. (45.74%). The prevalence of *Babesia* sp. related to animal sex, were show in male (43.48%) and female was (52.%), with non-significant differences at P value: (< 0.05). The Prevalence of *Babesia* sp. related to age were show (12.50%), (92.86%) and (30%) in young , adult and old age respectively with significant differences at P value: (< 0.05). The prevalence of *Babesia* sp. related to periods were show. (28.57%), (62.50%) and (42.86) in August , September and October respectively and with non-significant differences at P value: (< 0.05). Molecular study results were based on analysis of PCR products and DNA sequencing of ribosomal RNA genes in (*Babesia* sp., *Theileria* sp., and *Anaplasma* sp.) that show positive in Direct microscopic method by phylogenetic tree analysis (MEGA 6.0) and NCBI-BLAST Homology Sequence Identity to differentiation *Babesia* species typing . The molecular prevalence results

were show identified two *Babesia* species, high prevalence of *Babesia bovis* (38.30%) were closed related to NCBI-Blast *Babesia bovis* (HQ264126.1) with homology sequence identity (97-100%) and *Babesia bigemina* (7.45%) were closed related to NCBI-Blast *Babesia bigemina* (KU206291.1) with homology sequence identity (95-99%), then 43 *Babesia* species isolates were submitted into NCBI-Genbank and provided accession numbers (MH503811-MH503853). In conclusion, this study concluded that Phylogenetic tree and homology sequences identity was show accurate in differentiation of *Babesia* species. And these species can be isolated at high prevalence from local water buffalo from slaughter house in Al-Najaf city, of Iraq.

Key word: Babesia sp., PCR, Phylogenetic tree, Buffalo.

Introduction

Babesia is most important Haemoprotozoa parasites of cattle and buffaloes (Coetzer and Tustin, 2004). These parasites cause diseases characterized by infected of erythrocytes and caused severe anemia and highly losses in livestock industry throughout the world (Khan *et al*., 2014). *Babesia* protozoal parasites are tick borne protozoa and consider economically important disease because of direct losses of milk and meat production (El-Metenawy, 2000). However, their outbreak in exotic and crossbred cattle is mostly reported during the hot and humid months of the year (Wright, 1989). Babesiosis is recoded as the most ubiquitous and widespread haemoprotozoa in the world based on numbers and distribution of species in animals, other Haemoprotozoa has recorded as month wise infection of Theileriosis, Anaplasmosis and Trypnasomiasis in crossbred cattle and buffaloes" (Kamal *et al.,* 1994) (Sajid *et al*., 2014). Babesiosis is a tick-borne disease of cattle caused by the protozoan parasites of the genus *Babesia*. The principal species of Babesia that cause babesiosis in cattle and buffalo are: Babesia bovis, Babesia

bigemina and Babesia divergens (Salama and Gaabarya, 2007). *B. bovis and B. bigemina* which affected cattle, water buffalo (Bubalus bubalis) and African buffalo (Syncerus caffer). While B. divergens mostly affected cattle and reindeer (Rangifer tarandus) (Zahid *et al*., 2005). There are less survey studies about distribution of Babesia species in buffalo of Al-Najar city slaughter house. Therefore, our study designed to Microscopic identification, Molecular and, and Phylogenetic Analysis of Babesia species in Buffalo from Slaughter House in Al-Najaf City of Iraq at first time in Iraq.

Materials and Methods

Blood samples collections

A 94 blood samples were collected from buffalo in slaughter house in al Al-Najaf city by using anticoagulants tubes, from a period extended from August, 2017 into December 2017, the samples including different ages and both sex of buffalo, then the samples directly transport in ice box into laboratory to performing direct smear examination.

Direct Microscopic examination

Direct microscopic examination of blood samples were done by using Giemsa stain method **(Saal, 1964)** to preparation of thin and thick smears.

Blood DNA Extraction

Genomic DNA from blood samples were extracted by using gSYAN DNA mini kit extraction kit (Frozen Blood) Geneaid. USA, and done according to company instructions. The extraction method was don depend on the manufacturing instructions by using gram positive bacteria DNA Protocol extraction method by using (11 mg/ml) proteinase K.

Nanodrop: The extracted DNA was estimated by nanodrop device at 260/280nm, and then kept at deep freezer until used in PCR method.

Polymerase chain reaction (PCR)

PCR technique was performed for indirect detection blood borne *Babesia* sp. protozoa from blood of buffalo, the method was carried out according to method described by **(**Weerasooriya *et al* ,2016**), the** PCR primers that used in this study were design in this study by using NCBI Genbank data base and primer3 plus provided by (Macrogen company, Korea) as table (1):

Primers	Sequence		Amplicon
***Babesia* sp.**	**F**	**GGCCGTTCTTAGTTGGTGGA**	**357bp**
	R	**TGTGTACAAAGGGCAGGGAC**	

NCBI-Genbank: Babesia sp. (KF928959.1).

PCR master mix preparation

PCR master mix was prepared by using (AccuPower PCR PreMix Kit) and this master mix done according to company instructions as following table:

PCR Master mix	Volume
DNA template	**5µl**
Forward primer (10pmol)	**1.5µl**
Reveres primer (10pmol)	**1.5µl**
PCR water	**12µl**
Total volume	**20µl**

After that, the PCR mix that revealed in table above placed in AccuPower PCR -PreMix that contain all other PCR components which needed to reaction such as (Taq DNA polymerase, dNTPs, 10 PCR buffer). Then, all the PCR tubes transferred into vortex centrifuge for 3 minutes. Then transferred into thermocycler (MJ-Mini BioRad. USA).

PCR Thermocycler Conditions

PCR thermocycler conditions were done by using convential PCR thermocycler system as following table:

PCR step	Temp.	Time	repeat
Initial Denaturation	**95C**	**5min**	**1**
Denaturation	**95C**	**30sec.**	**30 cycle**
Annealing	**58C**	**30sec**	
Extension	**72C**	**1 min**	
Final extension	**72C**	**5min**	**1**
Hold	**4C**	**Forever**	**-**

PCR product analysis: The PCR products (503bp) were examined by electrophoresis in a 1% agarose gel using 1X TBE buffer, stained with ethidium bromide, and investigation under UV transilluminator.

DNA sequencing method

DNA sequencing method was performed for species typing of positive *Babesia* sp. isolates by PCR technique. The genetic analysis done by phylogenetic tree analysis between local species isolates and NCBI-Blast submission local species. Then the identification species isolates were submitted into of NCBI-GenBank. The PCR ribosomal RNA genes positive products were sent to Macrogen Company in Korea in ice bag by DHL for performed the DNA sequencing by AB DNA sequencing system. The DNA sequencing analysis was conducted by using Molecular Evolutionary Genetics Analysis version 6.0. (Mega 6.0) and Multiple sequence alignment analysis of the partial ribosomal rRNA gene based ClustalW alignment analysis and The evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA method.

Results

4-1- Direct microscopic examination results:

4-1-1- Blood Smear results:

The microscopic examination includes direct identification of *Babesia* spp. from blood samples of buffalo by using Giemsa stain blood smear method. This method includes identification based on morphological characterization of haemoprotozoa, where , the *Babesia* sp. characterized by presence merozoites infective stage pear-like shaped which found inside RBCs and 1-1.5µm in long and 0.5- 1.0µm in wide and seen as pairs with obtuse angle. as show in figure (1).

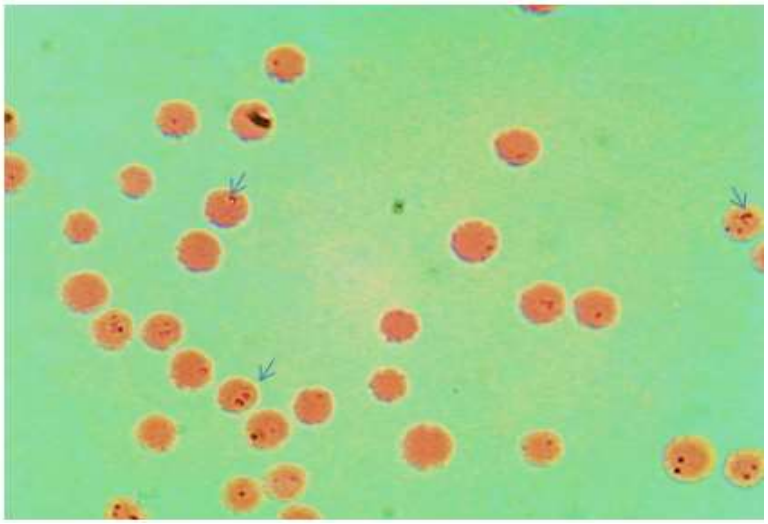

Figure (1). Thin blood smear microscopic image of Giemsa stain showing ring forms of intraerythrocytic trophozoites of *Babesia* spp.

Prevalence of Babesia sp. results:

Prevalence of *Babesia* sp. in blood samples of buffalo were studied according to limited period of time include only three months extend from (August into September 2017) and according to animals gender, as well as ages of animals. The study included collection of a 94 blood samples from buffalo in slaughter house in al Al-Najaf city. The *Babesia* sp. were show high prevalence at (45.74%). The present study includes

69 male and 25female buffalo blood samples and the prevalence and statistical analysis were show in following table:

Table (2): Prevalence of *Babesia* sp. related to sex

Gender	No. of tested samples	No. of positive *Babesia* sp.	Percent %
Male	69	30	43.48
Female	25	13	52.00
Total	94	43	**45.74**

Chi-square value (X2 :0.537) Non-significant at p value: 0.463

The Babesia sp. Prevalence and statistical analysis related to animals ages were show in following table:

Table (3): Prevalence of *Babesia* sp. related to age

Age	No. of tested samples	No. of positive *Babesia* sp	Percent %
Young	16	2	12.50
Adult	28	26	92.86
Old	50	15	30.00
Total	94	43	45.74

Chi-square value (X2 : 19.631) Significant at p value: 0.0001

The Babesia sp. Prevalence and statistical analysis related to study periods were show in table (4):

Table (4): Prevalence of *Babesia* sp. related to period

Periods	No. of tested samples	No. of positive *Babesia* sp	Percent %
August	14	4	28.57
September	24	15	62.50
October	56	24	42.86
Total	94	43	45.74

Chi-square value (X2 : 4.567) Non-Significant at p value: 0.102

Molecular Study results:

The molecular study results were included PCR technique for confirmative detection and DNA sequencing methods for Babesia species typing:

Polymerase Chain Reaction (PCR) results

The PCR technique was used for specific in direct confirmative detection of *Babesia* sp. from buffalo that only show positive in direct smear of microscopic examination method. This technique was depend on primers design of small subunit ribosomal genes in these parasites. The PCR results were show high specific and accurate confirmative detection which appeared at 357bp on 1% agarose gel electrophoresis. As show in figure (2)

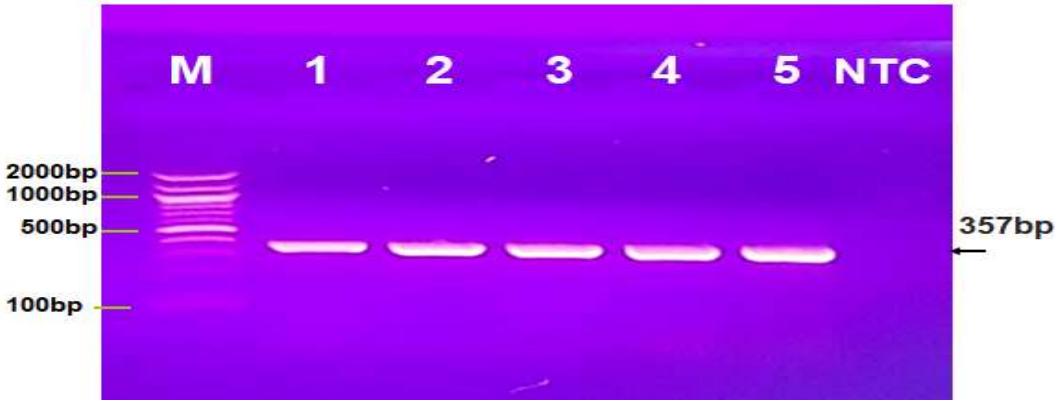

Figure (2): Electrophoresis of PCR reaction results for 18S_ribosomal RNA gene *Babesia* sp. of buffalo blood samples, using 1% agarose gel and DNA marker ladder (2000-100bp), Lane (1-4) positive PCR *Babesia* sp. from buffalo blood samples at 357bp PCR product size and lane (NTC) Non template negative control.

DNA Sequencing analysis results

DNA sequencing analysis results were includes Babesia species typing based on phylogenetic tree analysis and ClustalW alignment analysis by using (MEGA 6.0) between local species isolates and NCBI-Blast species recorded isolates. Then confirmative by NCBI-BLAST

Homology Sequence Identity. After that identified species isolates were deposited into of NCBI-GenBank to get Genbank accession number for each Babesia species isolates.

Molecular prevalence of *Babesia* sp. results

Table (5): The total prevalence of identified *Babesia* species isolates based on DNA sequencing analysis :

Babesia sp	No. of tested samples	No. of positive samples	Percent %
Babesia bovis	94	36	38.30
Babesia bigemina	94	7	7.45
Total	**94**	**43**	**45.74**

Chi-square value (X2 : 25.35) Significant at p value: 0.0

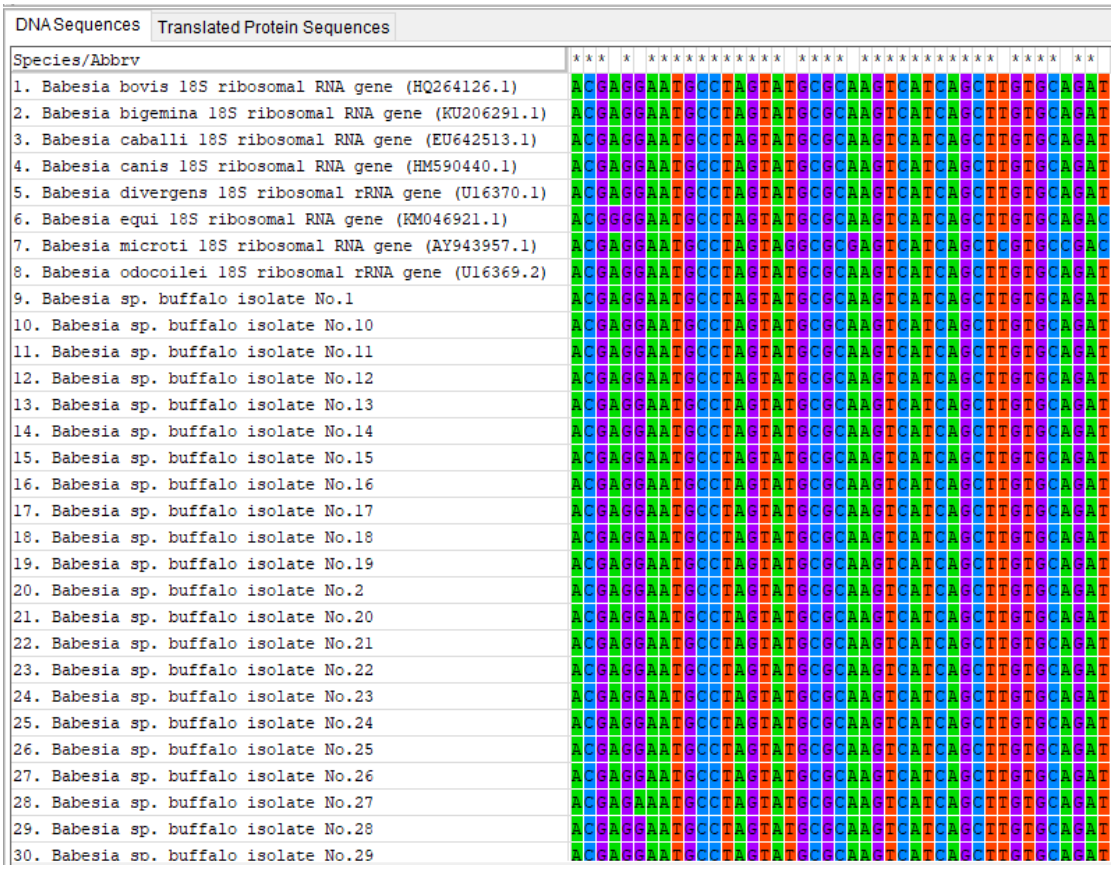

Figure (3): Multiple sequence alignment analysis of the 18S ribosomal rRNA gene partial sequence in local *Babesia* sp. buffalo isolates (No.1-No43) that aligned with different NCBI-Blast *Babesia* species based ClustalW alignment analysis by using (MEGA 6.0). The image show the multiple alignment analysis similarity (*) and differences in 18S ribosomal rRNA gene nucleotide sequences.

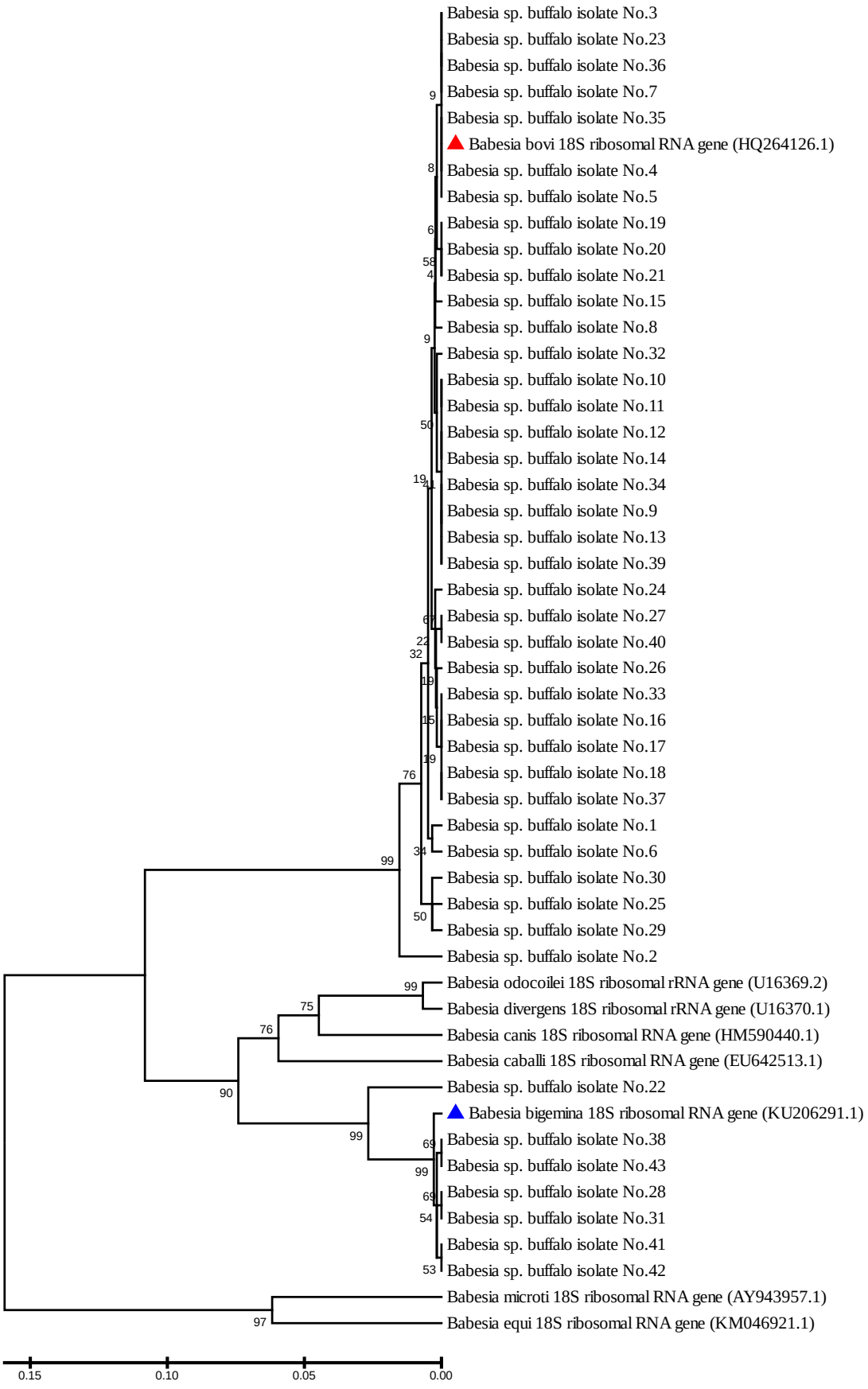
Babesia sp. buffalo isolate No.3
Babesia sp. buffalo isolate No.23
Babesia sp. buffalo isolate No.36
Babesia sp. buffalo isolate No.7
Babesia sp. buffalo isolate No.35
Babesia bovi 18S ribosomal RNA gene (HQ264126.1)
Babesia sp. buffalo isolate No.4
Babesia sp. buffalo isolate No.5
Babesia sp. buffalo isolate No.19
Babesia sp. buffalo isolate No.20
Babesia sp. buffalo isolate No.21
Babesia sp. buffalo isolate No.15
Babesia sp. buffalo isolate No.8
Babesia sp. buffalo isolate No.32
Babesia sp. buffalo isolate No.10
Babesia sp. buffalo isolate No.11
Babesia sp. buffalo isolate No.12
Babesia sp. buffalo isolate No.14
Babesia sp. buffalo isolate No.34
Babesia sp. buffalo isolate No.9
Babesia sp. buffalo isolate No.13
Babesia sp. buffalo isolate No.39
Babesia sp. buffalo isolate No.24
Babesia sp. buffalo isolate No.27
Babesia sp. buffalo isolate No.40
Babesia sp. buffalo isolate No.26
Babesia sp. buffalo isolate No.33
Babesia sp. buffalo isolate No.16
Babesia sp. buffalo isolate No.17
Babesia sp. buffalo isolate No.18
Babesia sp. buffalo isolate No.37
Babesia sp. buffalo isolate No.1
Babesia sp. buffalo isolate No.6
Babesia sp. buffalo isolate No.30
Babesia sp. buffalo isolate No.25
Babesia sp. buffalo isolate No.29
Babesia sp. buffalo isolate No.2
Babesia odocoilei 18S ribosomal rRNA gene (U16369.2)
Babesia divergens 18S ribosomal rRNA gene (U16370.1)
Babesia canis 18S ribosomal RNA gene (HM590440.1)
Babesia caballi 18S ribosomal RNA gene (EU642513.1)
Babesia sp. buffalo isolate No.22
Babesia bigemina 18S ribosomal RNA gene (KU206291.1)
Babesia sp. buffalo isolate No.38
Babesia sp. buffalo isolate No.43
Babesia sp. buffalo isolate No.28
Babesia sp. buffalo isolate No.31
Babesia sp. buffalo isolate No.41
Babesia sp. buffalo isolate No.42
Babesia microti 18S ribosomal RNA gene (AY943957.1)
Babesia equi 18S ribosomal RNA gene (KM046921.1)
0.15
0.10
0.05
0.00

Figure (3): Phylogenetic tree analysis based on the partial sequence of 18S ribosomal rRNA gene in local *Babesia* species buffalo isolates (No.1-No.43) that used for *Theileria* species genetic identification. The evolutionary distances were computed using the Maximum Composite Likelihood method by phylogenetic tree UPGMA method (MEGA 6.0 version). The local *Babesia* sp. isolates (No.1-No.21, No.23-No.27, No.29-No.30, No.32-No.37, and No.39-No.40) were show closed related to NCBI-Blast *Babesia bovis* (HQ264126.1) and The local *Babesia* sp. isolates (No.22, No.28, No.31, No.38, No.41, No.42, and No.43) were show closed related to NCBI-Blast *Babesia bigemina* (KU206291.1) whereas, other the NCBI-Blast *Babesia* species were show different and out of tree.

Table (4-12): NCBI-BLAST Homology Sequence Identity between local *Babesia* sp. buffalo isolates and closed related to NCBI-Blast *Babesia bovis* (HQ264126.1) and *Babesia bigemina* (KU206291.1):

Local *Babesia* sp. isolate No.	**Genbank Accession number**	**NCBI-BLAST Homology Sequence Identity**	
		Identical *Babesia* sp.	**Identity (%)**
Babesia sp. No.1	MH503811	*Babesia bovis*	**99%**
Babesia sp. No.2	MH503812	*Babesia bovis*	**97%**
Babesia sp. No.3	MH503813	*Babesia bovis*	**100%**
Babesia sp. No.4	MH503814	*Babesia bovis*	**100%**
Babesia sp. No.5	MH503815	*Babesia bovis*	**100%**
Babesia sp. No.6	MH503816	*Babesia bovis*	**99%**
Babesia sp. No.7	MH503817	*Babesia bovis*	**100%**
Babesia sp. No.8	MH503818	*Babesia bovis*	**99%**
Babesia sp. No.9	MH503819	*Babesia bovis*	**99%**
Babesia sp. No.10	MH503820	*Babesia bovis*	**99%**
Babesia sp. No.11	MH503821	*Babesia bovis*	**99%**
Babesia sp. No.12	MH503822	*Babesia bovis*	**99%**
Babesia sp. No.13	MH503823	*Babesia bovis*	**99%**
Babesia sp. No.14	MH503824	*Babesia bovis*	**99%**
Babesia sp. No.15	MH503825	*Babesia bovis*	**99%**
Babesia sp. No.16	MH503826	*Babesia bovis*	**99%**
Babesia sp. No.17	MH503827	*Babesia bovis*	**99%**
Babesia sp. No.18	MH503828	*Babesia bovis*	**99%**
Babesia sp. No.19	MH503829	*Babesia bovis*	**99%**
Babesia sp. No.20	MH503830	*Babesia bovis*	**99%**
Babesia sp. No.21	MH503831	*Babesia bovis*	**99%**
Babesia sp. No.22	MH503832	*Babesia bigemina*	**95%**
Babesia sp. No.23	MH503833	*Babesia bovis*	**99%**
Babesia sp. No.24	MH503834	*Babesia bovis*	**99%**
Babesia sp. No.25	MH503835	*Babesia bovis*	**99%**
Babesia sp. No.26	MH503836	*Babesia bovis*	**99%**

Babesia sp. No.27	MH503837	*Babesia bovis*	**99%**
Babesia sp. No.28	MH503838	*Babesia bigemina*	**99%**
Babesia sp. No.29	MH503839	*Babesia bovis*	**99%**
Babesia sp. No.30	MH503840	*Babesia bovis*	**99%**
Babesia sp. No.31	MH503841	*Babesia bigemina*	**99%**
Babesia sp. No.32	MH503842	*Babesia bovis*	**99%**
Babesia sp. No.33	MH503843	*Babesia bovis*	**99%**
Babesia sp. No.34	MH503844	*Babesia bovis*	**99%**
Babesia sp. No.35	MH503845	*Babesia bovis*	**100%**
Babesia sp. No.36	MH503846	*Babesia bovis*	**100%**
Babesia sp. No.37	MH503847	*Babesia bovis*	**99%**
Babesia sp. No.38	MH503848	*Babesia bigemina*	**99%**
Babesia sp. No.39	MH503849	*Babesia bovis*	**99%**
Babesia sp. No.40	MH503850	*Babesia bovis*	**99%**
Babesia sp. No.41	MH503851	*Babesia bigemina*	**99%**
Babesia sp. No.42	MH503852	*Babesia bigemina*	**99%**
Babesia sp. No.43	MH503853	*Babesia bigemina*	**99%**

Discussion

The present study was designed to identification of species *Babesia* species by direct microscopic and molecular methods from blood samples of buffaloes at first time in Iraq. The study performed in three months of summer season (August into September 2017) due to high distribution of transmitted vectors in this time of year, as well as ages and sex of animals was included in this study. The direct microscopic identification was performed by Giemsa stain method. This method includes identification of haemoprotozoa based on morphological characterization when used in thin and thick blood smears method and it has been considered to be the standard technique for routine diagnosis, because itis still cheapest and fastest methods (Demessie and Derso, 2015). The morphologic characteristics observed on microscopic examination of blood smears do not allow differentiation between Babesia species so that the molecular identification is the best of choose.

The present study was recorded highest prevalence of haemoprotozoa at (45.74%). Study in Egypt by (Hazem *et al*.,2014).

Their results are agreement with our observation, who recorded a higher prevalence of Babesiosis in buffaloes than in cattle and higher prevalence of Theileriosis in cattle than in buffaloes by blood films examination and revealed that, the infection rate with Babesiosis was (22.47%) of cattle and (51.28%) of buffaloes, while the infection rate of Theileriosis was (14.61%) of cattle and (7.69%) of buffaloes. The presence of ticks or history of tick infestation to the animal was associated with the presence of babesiosis and the high incidence of babesiosis in domestic cattle and buffalos, it is possibly due to tick infestation, was more distribution in buffaloes during summer season lead to risk factors associated with higher prevalence of Babesiosis (Mohammed *et al*., 2016). The present was show the high prevalence of *Babesia* sp. female at (52.%) than male was (43.48%) but non-significant differences at p value: (0.463). this prevalence was agreement with the report of (Khattak *et al*, 2012) who observed females buffalo more susceptible to *Babesia* sp. at (34.06 %) than males at (22.70%). Overall prevalence of haemoprotozoan parasite in female buffalo was higher than male, this higher prevalence in female population may be due to hormonal disturbances which pretense it to weakened immune system. Our results were prevalence of *Babesia* sp. was (12.50%), (92.86%) and (30%) in young , adult and old age respectively and with significant differences at p value: (0.0001). This present prevalence was supports by (Hazem *et al*., 2014) who revealed that, adult animals (36/60) (60%) were more infected by Babesiosis as compared with calves (24/60) (40%). The increase of *Babesia* infection with increasing animal age, was mainly due to the postponed infection caused by restriction of calf movement by keeping them indoors of farmers (Rubaire-Akiiki, 2004).

The present study was performed in this time of year, because most of the animals exposed to haemoprotozoa during summer months may be due

to high abundance of vectors in these seasons of the year (Bhatnagar *et al*., 2015) and (Radostits *et al*., 2007). The prevalence of *Babesia* sp. (28.57%), (62.50%) and (42.86) in August , September and October respectively and with non-significant differences at p value: (0.102). Our finding was agreement with (Maharana *et al*. 2016) who revered that risk of babesiosis was significantly higher in summer season followed by rainy compared to winter season and reported high prevalence babesiosis in buffaloes. The higher infection of *Babesia* sp. in summer season may be explained by the highest abundance of the ticks in this months and this observation agreed with that previously reported by (Alim *et al*., 2011).

The present study was identified two *Babesia* species, *Babesia bovis* and *Babesia bigemina* based on phylogenetic tree analysis and homology sequence identity from local *Babesia* sp. isolates. Our results were show high prevalence of *Babesia bovis* (38.30%) were closed related to NCBI-Blast *Babesia bovis* (HQ264126.1) with homology sequence identity (97-100%) followed *Babesia bigemina* (7.45%) were closed related to NCBI-Blast *Babesia bigemina* (KU206291.1) with homology sequence identity (95-99%) as show in DNA sequencing analysis figures and tables (4-8) (4-8), with significant differences at p value: (0.0). less studies about the molecular identification of *Babesia bigemina* and *Babesia bovis* in water buffalos in Iraq, but recent study in Wasit province, of Iraq was consistence with our finding by (Alkefari *et al*., 2017) who recorded the incidence of *Babesia bigemina* in apparently healthy buffaloes using of three different diagnostic assays and their results show revealed (11.73%) positive animals by PCR. Another study was agreement with our results by (Aaiz and Sabbar.2016) who recorded high prevalence of *Babesia bovis* (47.91 %) in alive and slaughtered cattle from different areas and abattoir of Al-Qadisiyah province of Iraq. The high prevalence of *Babesia bovis* in buffalo of our study may be due

to most buffalo were appear to be bearing the infection predominantly as a carrier hosts.

Worldwide distribution prevalence of *Babesia bigemina* and *Babesia bovis* in cattle and buffalo were show disagreement with our finding, study in the northeastern region of Thailand, by (Terkawi *et al*., 2011) who reveal that PCR prevalence of B. *bovis* and B. *bigemina* was (11.2%) and (3.6%) respectively. Study in Egypt by (Ibrahim *et al*., 2013) who recorded prevalence of *Babesia bigemina* and *Babesia bovis* in cattle and water buffalos by Molecular and serological methods were 10.42% and 4.17% by PCR and 15.63% and 11.46% by ELISA, respectively. Another Study in Vietnam by (Yan *et al*., 2014) who recorded prevalence of *Babesia bigemina* and *Babesia bovis* in cattle and water buffalos by Molecular and serological methods were 23.3% and 0% by PCR, 37.2% and 9.3% by ELISA and 27.9% and 18.6% by IFAT, respectively., respectively.

In conclusion, this study concluded that Phylogenetic tree and homology sequences identity was show accurate in differentiation of *Babesia* species. And these species can be isolated at high prevalence from local water buffalo from slaughter house in Al-Najaf city, of Iraq.

Reference:

Aaiz, N. N., and Sabbar, K. H. (2016). Molecular detection of *Babesia bovis* in cattle in Al-Qadisiyah province. Iraqi Journal of Veterinary Sciences. 40(2):155-158.

Alim MA, Das S, Roy K, Masuduzzaman M, Sikder S, Hassan MM‹ et al. (2012). Prevalence of hemoprotozoan diseases in cattle population of Chittagong division, Bangladesh. Pak. Vet. J. 32: 221-224.

Alkefari, O., AAJ. Al-Gharban, H., & H. Ahmed, T. (2017). Microscopic, serological and molecular detection of *Babesia bigemina* in buffaloes (*Bubalus bubalis*) in Wasit province, Iraq. AL-Qadisiyah Journal Of Veterinary Medicine Sciences, 16(1), 123-130.

Bhatnagar, C.S., Bhardawaj, B, Sharma, D.K. and Meena, S.K. (2015). Incidence of Haemoprotozoan diseases in cattle in Southern Rajasthan, India. Int. J. Curr. Microbiol. App. Sci. 4: 509-514.

Coetzer J.A.W. & Tustin R.C. Eds. (2004). Infectious Diseases of Livestock, 2nd Edition. Oxford University Press.

Demessie, Y. and Derso, S. (2015). Tick Borne Hemoparasitic Diseases of Ruminants: A Review. Advance in Biological Research, 9(4): 210-224.

El-Metenawy T.U., (2000). Prevalence of blood parasites among cattle at the central area of Saudi Arabia. Vet. Parasitol. 87: 231-236.

Hazem, M., El Moghazy; Ebied, M.H.; Mohamed G. Abdelwahab; Amr Abdel Aziz El-Sayed. (2014). Epidemiological Studies on Bovine Babesiosis & Theileriosis in Qalubia

Governorate. Benfa Veterinary Medical Journal.27 (1):36-48.

Ibrahim, H.M., Adjou Moumounib, P.F., Mohammed-Gebaa, K., Sheira, S.K., Hashemc, S.Y., Caob, S., Terkawib, M.A., Kamyingkirdb, K., Nishikawab, Y., Suzukib, H., and Xuanb, X. (2013). Molecular and serological prevalence of *Babesia* ***bigemina*** and ***Babesia bovis*** in cattle and water buffalos under small-scale dairy farming in Beheira and Faiyum Provinces, Egypt. Vet. Parasitol., 198: 187-192.

Kamal M. AlSaad. Clinical, hematological and biochemical studies of anaplasmosis in local buffalos breed in Mosul. (1994). Iraqi. Vet. Med. Vol(1) No 18.

Khan M. Q., A. Zahoor1 , M. Jahangir and M. Ashraf Mirza (2004). Prevalence of blood parasites in cattle and buffaloes. Pakistan Vet. J., 24(4).

Khattak R.M., Rabib M., Khan Z., Ishaq M., Hameed H., Taqddus A., Faryal M., et al., (2012). A comparison of two different techniques for the detection of blood parasite, *Theileria annulata*, in cattle from two districts in Khyber Pukhtoon Khwa Province (Pakistan). Parasite, 19 (1): 91-95.

Maharana BR, Kumar B, Prasad A, Patbandha TK, Sudhakar NR , et al. (2016). Prevalence and assessment of risk factors for haemoprotozoan infections in cattle and buffaloes of South-West Gujarat, India. Indian J Anim Res 50: 733-739.

Mohammed, K., Tukur, S.M., Watanabe, M., ABD-RANI, P.A., Lau, S.F., Shettima, Y.M., & Watanabe, M. (2016). Factors Influencing the Prevalence and Distribution of Ticks and Tick-borne Pathogens among Domestic Animals in

Malaysia. Pertanika Journal of Scholarly Research Reviews, 2 (2): 12-22.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W., and Constable, P.D.. (2007). Veterinary medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats, 10 edn. Elsevier, Philadelphia, pp: 1110-1489., 1522-1532.

Rubaire-Akiiki, C., Okello-Onen, J., Nasinyama, G.W., Vaarst, M. Kabagambe, E.K., Mwayi, W. Musunga, D., Wandukwa, W. (2004). The prevalence of serum antibodies tick-borne infections in Mbale district,Uganda: The effect of agroecological zone, grazing management and age of cattle. J Ins Sci, 4(8): 1-8.

SaaL J.R. (1964). Giemsa Stain for the Diagnosis of Bovine Babesiosis. I. Staining Properties of Commercial Samples and Their Component Dyes. J Protozool. 1964 Nov;11:573-82.

Salama, A. O, and Gaabarya, M. A. (2007). Clinical, haematological and therapeutic studies on tropical theileriosis in water buffaloes (Bubalus bubalis) in Egypt. Vet.Parasitol. 2007. 146(3-4): 337-340

Terkawi M.A., Huyena N.X., Shinuoa C., Inpankaewb T., Maklonc K., Aboulailaa M., Uenoa A., Gooa Y.K., Yokoyamaa N., Jittapalapong S., Xuana X., Igarashi I. (2011). Molecular and serological prevalence of *Babesia bovis* and *Babesia bigemina* in water buffaloes in the northeast region of Thailand. Vet. Parasitol. 178: 201-207.

Weerasooriya G., Sivakumar T., Lan D. T. B., Long P. T., Takemae H., Igarashi I., Inoue N., Yokoyama N. (2016). Epidemiology of bovine hemoprotozoa parasites in cattle

and water buffalo in Vietnam. J. Vet. Med. Sci. 78: 1361–1367.

Wright, I. G., (1989). Veterinary Protozoan and Haemoparasite Vaccines. CRC press Inc. Bola Raton, Florida, USA.

Yan Li, Yuzi Luo, Shinuo Cao, Mohamad Alaa Terkawi, Dinh Thi Bich Lan, Phung Thang Long, Longzheng Yu, Mo Zhou, Haiyan Gong, Houshuang Zhang, et al. (2014). Molecular and seroepidemiological survey of *Babesia bovis* and *Babesia bigemina* infections in cattle and water buffaloes in the central region of Vietnam.Trop Biomed. 31(3): 406–413.

Zahid IA, M Latif and KB Baloch, (2005). Incidence and treatment of theileriosis and babesiosis. Pak Vet J, 25: 137-139.

التشخيص المجهري والتحليل الجزيئي والشجرة الوراثية لأنواع طفيلي البابيزيا في الجاموس من مجزرة مدينة النجف في العراق

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الخلاصة

طفيلي البابيزيا هو من الطفيليات أواليه المحمولة بالدم تنتقل بواسطة الحشرات مفصلية الارجل ومسؤولة عن وحدوث اصابات حادة كمرض في الابقار في جميع أنحاء العالم. صممت الدراسة الحالية للتحري عن طفيلي البابيزيا من نماذج دم الجاموس للمرة الاولى في العراق. باستخدام طرق الفحص المجهري المباشر والطرق الجزيئية كافحص البي سي ار وتسلسل تتابع الحمض النووي). انجزت الدراسة في مدة ثلاثة اشهر في موسم الصيف خلال (شهر أب الى شهر تشرين الاول لسنة ٢٠١٧م). كذلك تضمنت الدراسة علاقة عمر الحيوان وجنسه. اظهرت نتائج نسب الفحص المجهري المباشر وجود نسبة انتشار عالية لطفيلي انواع البابيزيا بنسبة (٤٥,٧٤%) وكانت علاقة نسبة انتشار الطفيليات مع الجنس في طفيلي البابيزيا في الذكور بنسبة (٤٣,٤٨%) وفي الاناث بنسبة (٥٢%)، ونسبة طفيلي انواع الثيلريا في الذكور بنسبة (٢٨,٩٩%) وفي الاناث بنسبة (٣٢%). اظهرت نتائج الدراسة حسب العمر نسبة انتشار طفيلي البابيزيا بنسبة (١٢,٥٠%) في الصغيرة و(٩٢,٨٦%) في البالغة و (٣٠%) في الحيوانات الكبيرة مع وجود فروقات معنوية بنسبة احتمال اقل من (٠,٠٥). اظهرت نتائج الدراسة حسب الفترة نسبة انتشار طفيلي البابيزيا بنسبة (٢٨,٥٧%) في شهر اب و(٦٢,٥٠%) في شهر أيلول و (٤٢,٨٦%) في شهر تشرين الاول مع عدم وجود فروقات معنوية بنسبة احتمال اقل من (٠,٠٥). اعتمدت نتائج الدراسة الجزيئية على تحليل ناتج تفاعل البي سي ار والفحص تسلسل تتابعات الحمض النووي لجينات الاريبوسومية في طفيلي انواع البابيزيا التي ظهرت موجبة بطرية الفحص المجهري المباشر وباستخدام برنامج تحليل الشجرة الوراثية والمطابقة التتابعات لتفريق انواع طفيلي البابيزيا. واظهرت نتائج التحليل الجزيئي وجود نوعيين لطفيلي البابيزيا وهي طفيلي البابيزيا بوفس بنسبة (٣٨,٣٠%) بعلاقة جينية متقاربة لطفيلي طفيلي البابيزيا بوفس المسجل (HQ264126.1) مع نسبة تطابق بلغت (٩٧-١٠٠%). وطفيلي البابيزيا بايجيمنا بنسبة (٧,٤٥%). بعلاقة جينية متقاربة لطفيلي طفيلي البابيزيا بايجيمنا المسجل (KU206291.1) مع نسبة تطابق بلغت (٩٥-٩٩%). بعد ذلك ٤٣ نوع من انواع البابيزيا المفرقة سجلت غي موقع بنك الجينات للحصول على ارقام تسجيلية من (MH503811-

(MH503853 . في الاستنتاج ، استنتجت الدراسة بان تحليل الشجرة الوراثية وفحص التطابق بين القواعد النيتروجينة يعطي تفريق دقيق لانواع طفيلي البابيزيا والتي يمكن ان تعزل بنسب عالية من جاموس الماء المحلية في محافظة النجف والتي المحتمل يؤدي الى حدوث تفشي لمرض الثيلريا في المواشي والجاموس.