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Molecular and Epidemiological Studies of Blood Parasites in Cattle and Buffaloes in New Valley Governorate, Egypt

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ABSTRACT: This study aimed to investigate the prevalence, risk factors, and molecular characterization of major tick-borne blood parasites affecting cattle and buffaloes in New Valley Governorate, Egypt. A total of 1530 animals (1450 cattle and 80 buffaloes) of different ages and sexes were clinically examined between April 2024 and July 2025. Blood samples were collected from 700 clinically suspected animals and examined using Giemsa-stained blood smears and polymerase chain reaction (PCR). Microscopic examination revealed infection rates of 15.62% for *Babesia* spp., 63.5% for *Theileria* spp., and 20.8% for *Anaplasma* spp. The highest prevalence was observed in animals aged>1–2 years and during the summer season. PCR confirmed the presence of *Babesia bigemina*, *Theileria annulata*, and *Anaplasma marginale*. Sequencing analysis showed high genetic similarity with international strains, and the sequences were deposited in GenBank. In conclusion, tick-borne hemoparasitic infections are highly prevalent in New Valley Governorate, highlighting the need for improved diagnostic surveillance and effective tick control state, the results emphasize the urgent need for enhanced diagnostic and control measures to reduce the burden of tick-borne haemoparasites in Egypt's New Valley Governorate. The high prevalence and genetic similarity to global strains highlight the risk of spread to other regions and the importance of integrated One Health strategies for tick-borne disease management.

KEYWORDS: Cattle; Buffaloes; Babesiosis; Theileriosis; Anaplasmosis; PCR

1. Introduction

Livestock is a crucial part of Egyptian agriculture, with cattle and buffaloes being the primary sources of milk and red meat, with rising living standards and growing awareness of the significance of animal proteins, the demand for livestock products has increased [1]. However, efficient livestock production is hindered by high-impact infectious diseases, particularly haemoparasitic infections transmitted by ticks [2]. Haemoparasites such as Babesia, Theileria, and Anaplasma are tick-borne pathogens that cause significant morbidity and mortality in cattle and buffaloes worldwide [3, 4] In Egypt, these infections are endemic and impose serious economic losses on the livestock industry [5, 6]. Morbidity rates can reach up to 90%, with mortality varying from 10% to over 50%, depending on factors such as parasite species, host breed, and environmental conditions [7]. Despite the known impact of these diseases, region-specific epidemiological data from the New Valley Governorate remain limited. Previous studies have largely focused on the Nile Delta and Upper Egypt, leaving a gap in understanding the prevalence, seasonal dynamics, and molecular characteristics of blood parasites in this arid, remote region [8, 9]. Moreover, most surveys rely on microscopy alone, which may underestimate prevalence and miss mixed infections. The clinical presentation of hemoparasitic diseases in ruminants is characterized by a complex array of symptoms that vary according to the stage of infection [10, 11]. In the New Valley environment, infected cattle often exhibit high-grade fever exceeding 40°C, accompanied by a sharp decline in physical activity [12]. One of the most common clinical markers for Theileriosis is the prominent swelling

of the lymphatic system, which can be palpated easily in the neck and flank areas [13]. For Babesiosis, the clinical picture is dominated by severe intravascular hemolysis, leading to the clinical observation of icteric (yellowish) mucous membranes [14]. In chronic cases, animals suffer from progressive emaciation and a significant drop in milk yield, which poses a threat to the farm's economy [15]. Furthermore, Anaplasmosis is clinically distinguished by the absence of hemoglobinuria despite the presence of severe anemia and digestive disturbances [16] Effective therapeutic management of hemoparasitic infections is fundamental for mitigating economic losses within the dairy and meat production sectors [17]. For the clinical management of babesiosis, particularly in cases of mixed infections, the administration of Diminazine aceturate is recommended as a potent alternative that facilitates rapid clinical recovery and restores animal vitality [18]. Furthermore, the strategic intervention using antibiotics from the Tetracycline family plays a critical role in controlling Anaplasma species and preventing the onset of secondary bacterial complications [19]. To achieve optimal therapeutic outcomes, specific anti-protozoal treatments should be integrated with immunomodulators and multivitamins; such supportive therapies stimulate the host's immune response and significantly reduce the risk of clinical relapse[20]. Therefore, this study aimed to investigate the prevalence, risk factors, and molecular characterization of major tick-borne blood parasites in cattle and buffaloes in the New Valley Governorate, using both microscopic and molecular diagnostic approaches.

2. Materials and Methods:

2.1. Ethical approval:

This research was conducted in accordance with the guidelines of the institutional review board of the faculty of veterinary medicine, New Valley University, Egypt (NVREC -02-1-5-2024-14).

2.2. Study area

The study were carried out during the period from April 2024 to July 2025 in five centers (Dakhla, Kharga, Farafra, Balat and Baris) in the New Valley Governorate, which is located in the southwest of Egypt in the Western Desert, Figure. 1 which located between 24.416667 and 29.616667 degrees east longitude, and 22.05 and 26.95 degrees north latitude. The climate is hot, dry desert climate with very little rainfall, temperatures are generally high, with an average annual temperature of 23 degrees Celsius. Humidity is low, averaging around 35.5% annually, and the area experiences abundant sunshine and is known for its clean, unpolluted air [21].

2.3. Studied Animals

A total of 1530 animals (1450 cattle and 80 buffaloes) of different ages and sexes were examined for signs of blood parasites, The examined cattle consisted of local Baladi and crossbreeds These animals were indigenous to the New Valley Governorate, having been born and raised in their respective localities (Dakhla, Kharga, Farafra, Balat, and Baris). and the epidemiological data of such animal is recorded, the distribution of examined animals according to locality and age are illustrated in Table 1

Table 1: Distribution of examined animals according to age and localities.

Age group	Localities					Total
	Kharga	Dakhla	Farafra	Balat	Baris	
One day to 6 month	515	50	5	20	250	**840**
6 month up to 1years	195	30	20	40	120	**405**
1 up to 2 years	80	20	30	30	40	**200**
2 up to 4 years	50	10	-	15	10	**85**
Total	840	110	55	105	420	**1530**

2.4. Clinical examination

The Clinical examination was carried out according to Ghareeb, El-Sify [13] on 1530 (1450 cattle and 80 buffaloes) for signs of blood parasite infection and all data for each case were recorded.

2.5. Blood samples

From all suspected clinically diseased animals, 700 blood samples were collected (650 cattle and 50 buffaloes), the blood samples were taken from jugular vein using vacutainer tube contain EDTA, and labeled and send to Lab.

2.6. Stained Blood film:

In the lab, 700 Thin blood films were prepared and preserved sent to the laboratory of infectious disease faculty of veterinary medicine immediately after taking the whole blood samples, allow these smears to dry by air then both samples were fixed by using methyl alcohol (methanol) for about 3-5min., allow them to dry by air after fixation step then stained Specify the concentration/dilution of Giemsa stain (diluted 1:10 with phosphate –buffered water at PH 7.2) for about 30 - 45 min. Dried by air and examined on Olympus microscope by using oil immersion lens at x1000 magnification [22].

2.7. Lymph node aspiration

10 lymph node aspirates were collected from enlarged superficial lymph nodes (prescapular and prefamural) in the sterile tube and needle after disinfections the site of introduce the needle, sample used for preparation of lymph smears immediately after collection, fixed with methanol and stained with Giemsa stain.

2.8. Identification by Polymerase chain reaction:

Nine blood samples from a stained samples were frozen in (-20c) and send to Animal Health Research Institute molecular biology lab for uniplex PCR identity and sequences Table 2

2.8.1. Extraction of DNA

According to QIAamp DNA mini kit instructions Preparation of PCR Master Mix for cPCR: according to Emerald Amp GT PCR master mix (Takara) Code No.RR310A kit as shown in Table 3

2.8.2. Cycling conditions of the primers during cPCR

Temperature and time conditions of the two primers during PCR are shown in Table 3

2.8.3. Agarose gel electrophoreses [26]

Electrophoresis grade agarose (1.5 g) was dissolved in 100 ml TBE buffer in a sterile flask, it was heated in microwave to dissolve all granules with agitation, and allowed to cool at 70 °C, then 0.5µg/ml ethidium bromide was added and mixed thoroughly, and the electrophoresis tank was filled with TBE buffer. Twenty µL of each uniplex PCR product samples, negative control and positive control were loaded to the gel. The power supply was 1-5 volts/cm of the tank length. The run was stopped after about 30 min and the gel was transferred to UV cabinet. The gel was photographed by a gel documentation system and the data was analyzed through computer software.

2.8.4. Sequencing and Phylogenetic Analysis:

A comparative analysis of sequences was performed using the CLUSTAL W multiple sequence alignment program, version 12.1 of MegAlign module of Lasergene DNA Star software Pairwise (Madison, Wisconsin, USA) which was designed by Thompson, Higgins [27] and Phylogenetic analyses were done using maximum likelihood, neighbor joining and maximum parsimony in MEGA7 [28]

2.9. Statistical analysis:

Data from clinical examination, microscopic blood films, and PCR were analyzed using SPSS version 26.The Chi-square test was used to analyze categorical data and assess associations between risk factors and infection status, while Fisher's Exact Test was applied when expected cell counts were less than 5. results were considered non-significant ($P > 0.05$), significant ($P < 0.05$), or highly significant ($P < 0.01$ or $P < 0.001$).

3. Results

3.1. Results of clinical examination:

Clinical examination (Table 5 and Figure. 1) of 1,530 animals between April 2024 and July 2025 showed that

Table 2: Oligonucleotide primers sequences.

Reference	Length of amplified product	Primer sequence (5'-3')	Target gene
Ganguly et al [23]	382 bp	ACAGGCGAAGAAGCAGACAT ATAAATGGGAACACGGTGGA	Anaplasma marginale msp5
Nourollahi-Fard et al.[24]	721 bp	GTAACCTTTAAAAACGT GTTACGAACATGGGTTT	Theileria annulata tams1
Salem and Farag, [25]	340 bp	GTCTTGTAATTGGAATGATGGTGAC ATGCCCCCAACCGTTCCTATTA	Babesia 18S rRNA

Table 3: PCR reaction mixture components

Component	Volume/reaction
Emerald Amp GT PCR mastermix (2x premix)	12.5 µL
PCR grade water	5.5 µL
Forward primer (20 pmol)	1 µL
Reverse primer (20 pmol)	1µL
Template DNA	5 µL
Total	**25** µL

700 animals (650 cattle and 50 buffalo) exhibited clinical signs of blood parasite infection, including fever, anemia, emaciation, jaundice, hemoglobinuria, increased heart and respiratory rates, lymph node enlargement, respiratory signs, ocular abnormalities, and varying degrees of tick infestation.

Statistical analysis of Table 6 demonstrated a highly significant difference in the prevalence of clinical disease among different localities in both cattle and buffalo ($P < 0.01$). The highest infection rate in cattle was recorded in Dakhla (85%), while a 100% prevalence was observed among examined buffalo in Farafra and Baris. Additionally, a statistically significant difference was found between the overall prevalence in buffalo (60%) and cattle (44.82%) ($P = 0.0075$), indicating that buffalo may be more susceptible to or more exposed to clinical infection under the study conditions.

Table 7 data shows a highly significant association ($P < 0.001$) between age and the prevalence of clinical disease in both cattle and buffalo. In cattle, the highest prevalence was recorded in animals aged >1 to 2 years, while the lowest prevalence occurred in the > 2 to 4 years age group. In contrast, buffalo showed the highest infection rate in the > 2 to 4 years age group. These findings indicate that age is an important risk factor affecting disease susceptibility and clinical manifestation in the examined animals.

Figure. 2 showing Prevalence of clinically diseased animals in cattle and buffalo according to age

Table 9 shows that 480 out of 700 diseased animals (68.6%) were positive on stained blood film examination. Theileria was the most prevalent parasite (63.5%), followed by Anaplasma (20.8%) and Babesia (15.62%). Theileria was most common in cattle (64.4%), whereas buffalo showed the highest prevalence of Anaplasmosis (33.3%).

Table 9 shows the prevalence of blood parasites in cattle by age using Giemsa-stained blood films. Theileria was the most common parasite (43.5%), followed by Anaplasma (14%) and Babesia (10.7%). The prevalence of Babesia and Theileria was highly age-dependent ($P < 0.001$): Theileria was highest in the youngest cattle (<6 months, 62.9%) and declined with age, while Babesia increased with age, peaking in cattle over 2 years (50%). Anaplasma prevalence showed no significant age association ($P = 0.542$). These results indicate that young cattle are more susceptible to Theileria, whereas Babesia risk rises with age. Table 10 shows the prevalence of blood parasites in cattle by locality using Giemsa-stained blood films. Theileria was most prevalent in Kharga (51.7%) and Baris (47.2%), while Babesia was highest in Farafra (40% in cattle, 50% in buffalo). Anaplasma infection was most frequent in Kharga (17.2%). Statistical analysis revealed a highly significant geographical variation in parasite prevalence ($P < 0.01$) and a significant association of Anaplasma with locality ($P = 0.0028$), suggesting that local environmental factors and livestock management influence the distribution of blood parasites.

Table 4: Cycling conditions of the primersduringcPCR.

Parameter	*A. marginale* (msp5)	*T. annulata* (tams1)	*Babesia* spp. (18S rRNA)
Initial denaturation	94°C, 5 min	94°C, 5 min	94°C, 5 min
Denaturation (per cycle)	94°C, 30 s	94°C, 30 s	94°C, 30 s
Annealing	53°C, 40 s	55°C, 40 s	56°C, 40 s
Extension	72°C, 40 s	72°C, 45 s	72°C, 40 s
Number of cycles	35	35	35
Final extension	72°C, 10 min	72°C, 10 min	72°C, 10 min

Note: Annealing temperatures are gene-specific. Extension times are suitable for amplicons < 800 bp.

Table 5: Result of clinical examination of cattle and buffaloes for signs of blood parasite infestation.

Clinical Signs	No. of Animals	Percentage (%)
Total animals examined	1530	–
Fever	1250	81.70
Enlargement of lymph node	950	62.09
Anorexia	400	26.14
Conjunctivitis	100	6.53
Hemoglobinuria	400	26.14
Corneal opacity	50	3.26
Jaundice (icterus)	300	19.60
Ticks infestation	1100	71.90
Respiratory manifestation	700	45.75

Table 6: Prevalence of Disease in Cattle and Buffaloes According to Locality

Locality	Total Examined Animals		No. of Diseased Animals (%)	
	Cattle	Buffalo	Cattle	Buffalo
Kharga	800	30	290 (36.25%)	10 (33%)
Dakhla	100	10	85 (85%)	5 (50%)
Farafra	50	5	25 (50%)	5 (100%)
Balat	100	15	70 (70%)	10 (60%)
Baris	400	20	180 (45%)	20 (100%)
Total	**1450**	**80**	**650 (44.82%)**	**50 (60%)**

3.2. Results of PCR:

Molecular characterization reveled 9 sample examine by specific primer to babesia ,theileria ,anaplasma revaled that 4 positive babesia ,3 positive Theileria and 3 positive anaplasma and sequence reveled accession number On gene bank . PV254897 (T. annulata Egnv-2), PV248787 (B. bigemina egnv-1) , The reference sequences used for comparison included AF214824 and AF214823 for T. annulata, and DQ785311, OL583949, and X59604 for B. bigemina."

Table 7: Prevalence of clinically disease animals in cattle according to age:

Age Group	Examined		Diseased Animals			
	Cattle	Buffalo	Cattle		Buffalo	
			No.	%	No.	%
One day to 6 months	840	810	310	38.0%	10	33.33%
6 months to 1 year	405	385	180	46.7%	20	20.0%
1 to 2 years	200	180	140	77.7%	10	50.0%
2 to 4 years	85	75	20	26.6%	10	100.0%
Total	**1530**	**1450**	**650**	**44.82%**	**50**	**62.5%**

Table 8: Prevalence of blood parasites infection in cattle using Giemsa stained blood film

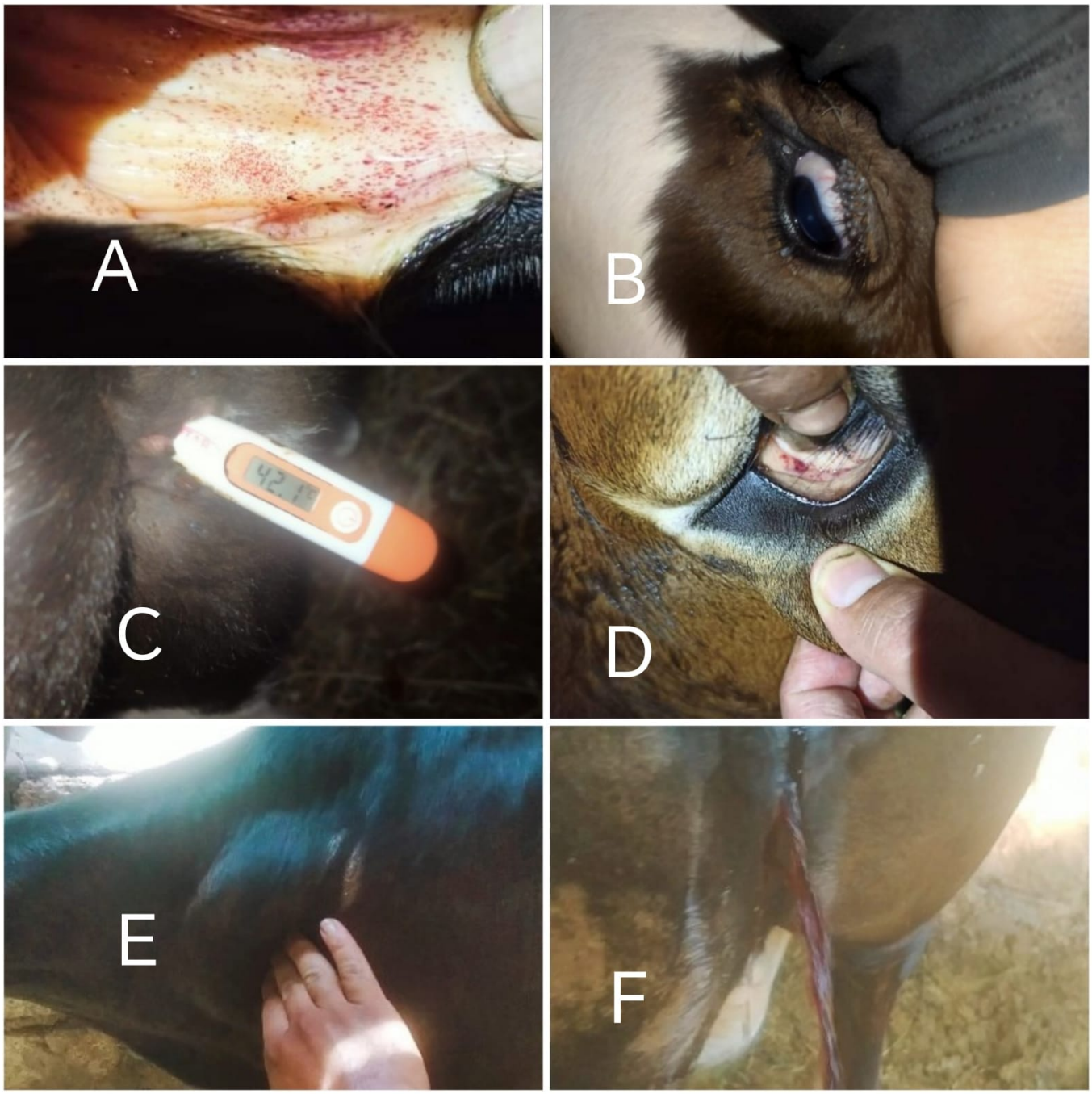
Species	Total	Positive Blood Film		Babesia		Theileria		Anaplasmosis	
	Examined	No.	%	No.	%	No.	%	No.	%
Cattle	650	450	69.23	70	15.5	290	64.4	90	20.0
Buffalo	50	30	60.00	5	16.6	15	50.0	10	33.3
Total	**700**	**480**	**68.6**	**75**	**15.62**	**305**	**63.5**	**100**	**20.8**

N.B * mixed infection of Theileria and Anaplasma ** mixed infection of Babesia and Anaplasma

3.3. Phylogenetic analysis:

Figure. 7: Multiple sequence alignment of the 18S rRNA gene sequences. " this figure illustrates the sequence alignment of the Babesia engv-1 isolate (marked with •) compared against 11 reference sequences of various Babesia species retrieved from GenBank. The alignment highlights the conserved regions and specific nucleotide substitutions that distinguish the egnv-1 isolate. The analysis was performed to establish the genetic relationship and percentage of identity between our local isolate and global strains, providing a basis for the subsequent phylogenetic tree construction using the Maximum Likelihood method. Figure. 9 shows a phylogenetic tree constructed using the Maximum Likelihood method based on partial 18S rRNA

Figure 1: different clinical signs associated with blood parasite infections in



cattle: (A) Petechial hemorrhage in a case of babesiosis, (B) Heavy tick infestation, (C) Measurement of high body temperature, (D) Congestion of mucous membranes, (E) Enlargement of the prescapular lymph node, (F) Hemoglobinuria in a case of babesiosis

gene sequences. The tree compares local isolates (•) with reference Babesia strains from GenBank. Bootstrap values at the nodes indicate statistical confidence. The isolate PV248787 (B. bigemina egnv-1) clusters closely within the B. bigemina group with high bootstrap support, confirming strong genetic similarity to known B. bigemina strains .

Figure. 6 Multiple sequence alignment of 18S rRNA gene for T. annulata Egnv-2. 'The alignment displays the partial 18S rRNA gene sequences of the PV254897

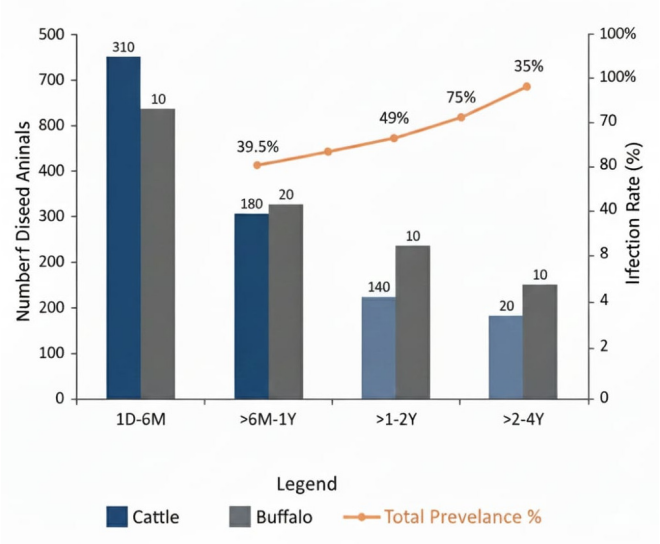


Figure 2: prevalence of clinically diseased animals in cattle/buffaloes according to age

Table 9: Prevalence of Babesia, Theileria and Anaplasma infections in cattle and buffalo according to Age usind Giemsa stained blood film.

Age Group	Species	Total Examined	Babesia No.	Babesia %	Theileria No.	Theileria %	Anaplasma No.	Anaplasma %
One day to 6 months	Cattle	310	0	0.0	195	62.9	35	11.0
	Buffalo	10	0	0.0	5	50.0	0	0.0
>6 months up to 1 year	Cattle	180	20	11.1	70	38.8	20	11.1
	Buffalo	20	0	0.0	5	14.0	5	25.0
>1 up to 2 years	Cattle	140	40	28.5	25	17.8	30	21.0
	Buffalo	10	5	50.0	5	50.0	0	0.0
>2 up to 4 years	Cattle	20	10	50.0	0	0.0	5	25.0
	Buffalo	10	0	0.0	0	0.0	5	50.0
Total		**700**	**75**	**10.7**	**305**	**43.5**	**100**	**14.0**

Table 10: Prevalence of Babesia, Theileria and Anaplasma infections in cattle and buffalo according to localities using giemsa stained blood film

Locality	Species	Diseased animals	Positive Blood film					
			Babesia		Theileria		Anaplasma	
			No	%	No	%	No	%
Kharga	Cattle	290	30	10.3	150	51.7	50	17.2
	Buffalo	10	5	50	5	50	5	50
Dakhla	Cattle	85	15	17.6	30	35.3	10	11.7
	Buffalo	5	0	0	0	0	0	0
Farafra	Cattle	25	10	40	5	20	5	20
	Buffalo	5	0	0	0	0	0	0
Balat	Cattle	70	5	7.1	20	28.5	5	7.1
	Buffalo	10	0	0	0	0	0	0
Baris	Cattle	180	10	5.5	85	47.2	20	11.1
	Buffalo	20	0	0	10	50	5	25
Total		**700**	**75**	**10.7**	**305**	**43.5**	**100**	**14.2**

Egnv-2 isolate alongside various Theileria annulata reference strains. Dots represent nucleotide identity with the top reference sequence (Arish1), while letters indicate specific substitutions. The alignment demonstrates a high degree of sequence conservation, with only minor single nucleotide polymorphisms (SNPs) observed, confirming the stability of this genetic marker in the Egyptian isolate .

Figure. 8 presents a phylogenetic tree constructed using the Maximum Likelihood method, illustrating the evolutionary relationship of the local isolate PV254897 (T. annulata Egnv-2) within the genus Theileria. The isolate (marked with a blue dot) clusters firmly within the T. annulata clade, forming a distinct sub-cluster with high bootstrap support (98%) alongside reference strains from GenBank (e.g., AF214824, AF214823). The tree shows that Egnv-2 is genetically distinct from other Theileria species, confirming it as a typical T. annulata strain circulating in the region.

4. Discussion

Blood parasites represent some of the most important pathogenic protozoan infections affecting livestock, particularly bovines [29]. Among them, Theileriosis is considered one of the most detrimental constraints on livestock production in Egypt. The New Valley Governorate showed the highest prevalence of T. annulata infection in the country, likely due to its arid climate, which favors the ecology of specific tick vectors, and extensive communal grazing systems, which increase animal exposure to infected ticks [30]. In this study, 1,530 animals (1,450 cattle and 80 buffalo) were examined, with 700 animals (45.75%) showing clinical signs of disease, including fever, anorexia, and conjunctival changes [3]. Among these, 480 animals (68.6%) tested positive for blood parasites on microscopic examination, a prevalence higher than that reported in China (43.48%) (7). Theileria spp. was the most prevalent parasite, infecting 305 animals (63.5%), consistent with findings in Iraq (69.43%) [28] and higher than other reports [31]. These geographical differences in prevalence likely reflect variations in local temperature, humidity, tick life cycles, and vector control measures [32,

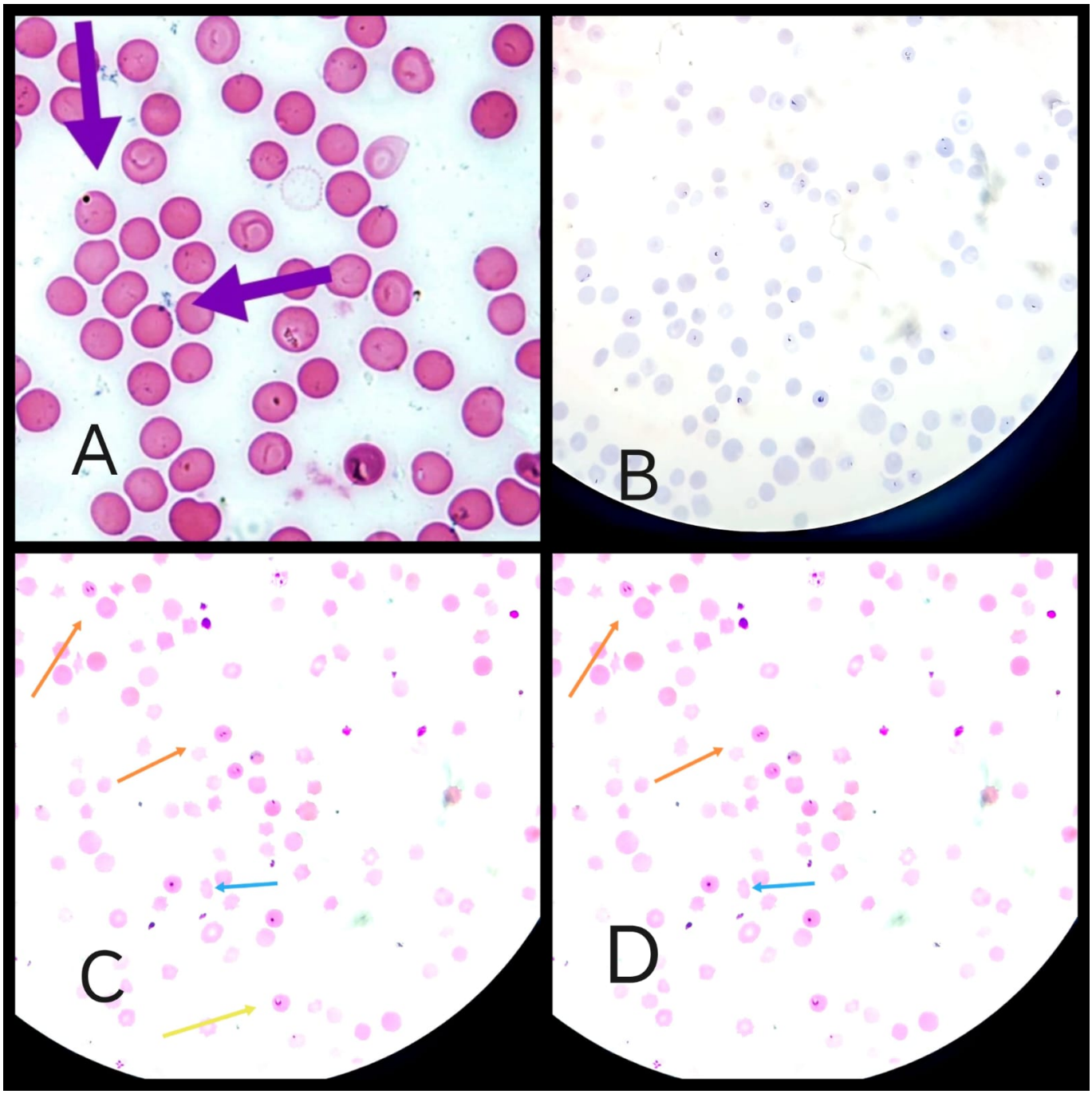


Figure 3: Stained blood film with Giemsa stain under microscope (x100) showing A-Anaplasma; B-Theileria; C-mixed babesia and anaplasma; D-Babesia

33]. In the New Valley Governorate, prevalence rates of theileria ranged between 38.65% and 42.8% across different localities. Babesia spp. infections were less common (15.62%), supporting previous observations that Theileria infections tend to be more prevalent than Babesia in bovine populations [32]. Age was a significant risk factor: the highest clinical prevalence (75%) was observed in cattle aged >1 to 2 years, whereas Theileria was most frequent in young calves (<6 months), suggesting early-age exposure, potentially via transovarial or transstadial transmission by ticks [33]. Conversely, Babesia prevalence increased with age, peaking at 50% in cattle aged >2 to 4 years, in agreement with earlier findings that animals younger than one year have lower Babesia infection rates than older

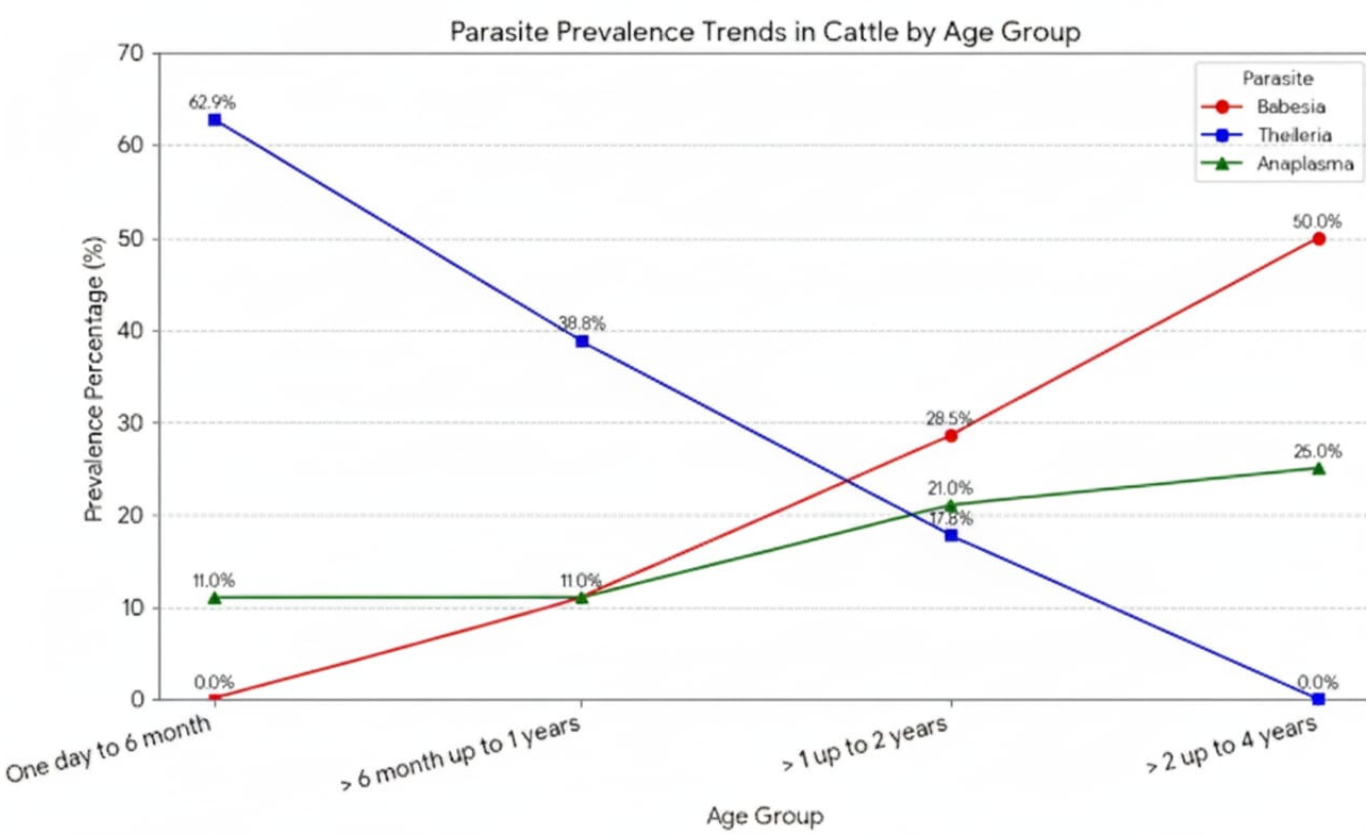


Figure 4: showing Prevalence of Babesia, Theileria and Anaplasma infections in Cattle and buffalo according to Age usind giemsa stained blood film

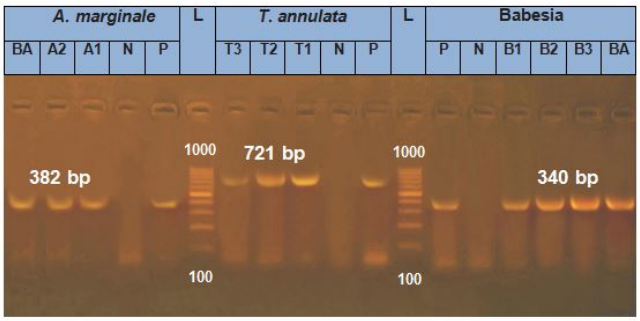


Figure 5: Agarose gel electrophoresis for multiplex polymerase chain reaction (PCR) assay showing the detection of Theileria annulata , Babesia bigemina and Anaplasma marginale. The expected band size for T.annulata 721 bp, the expected band size for Babesia bigemina 340bp and A.marginale is 382bp.

animals [34]. Seasonal trends were also evident. The highest number of clinical cases occurred in summer (270 cases out of 550 examined), coinciding with peak Theileria prevalence in cattle (57.6%), reflecting the seasonal activity and abundance of tick vectors [29]. Geographical variation significantly influenced disease prevalence. Dakhla recorded the highest clinical prevalence (85%), while Kharga had the highest Theileria prevalence on Giemsa-stained blood films (51.7%). Molecular confirmation using PCR on a subset of samples validated the presence of A. marginale, T. annulata, and Babesia spp., identifying specifically Babesia bigemina and Theileria annulata. PCR demonstrated 100% positivity in comparison to microscopic examination, emphasizing its value as a sensitive and accurate diagnostic tool, particularly for low-level infections or precise species identification. Sequencing of these isolates further confirmed their molecular identity and allowed for phylogenetic analysis, with results registered in GenBank.

Conclusion:

This study demonstrates a high prevalence of blood parasites, especially Theileria and Babesia, in cattle and buffalo in the New Valley Governorate. Age, season,

Figure 6: Multiple sequence alignment of 18S rRNA gene for T. annulata Egnv-2

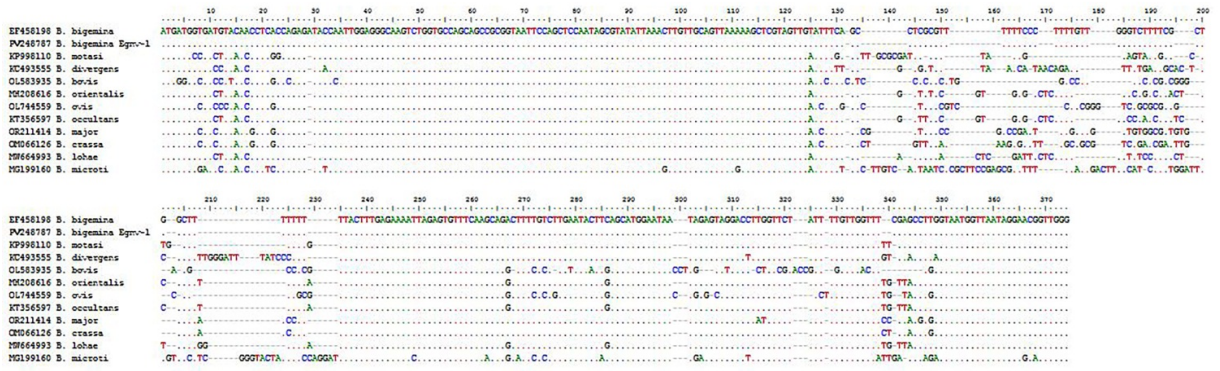


Figure 7: Phylogenetic analysis

locality, and management practices were identified as key risk factors, and molecular diagnostics proved valuable for accurate detection. The findings support the development of targeted tick and parasite control strategies to reduce the impact of these infections.

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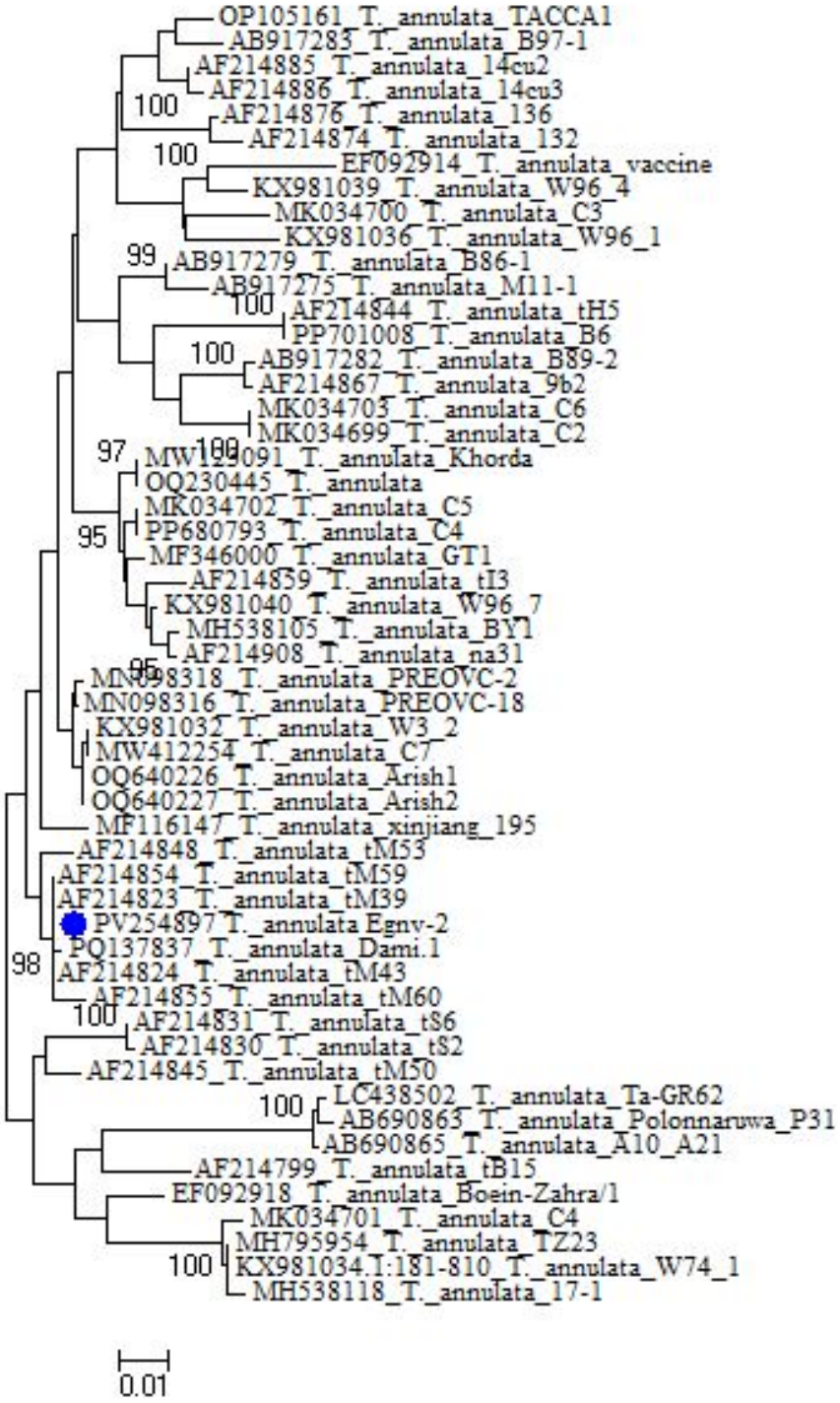

Figure 8: phylogenetic tree constructed using the Maximum Likelihood method

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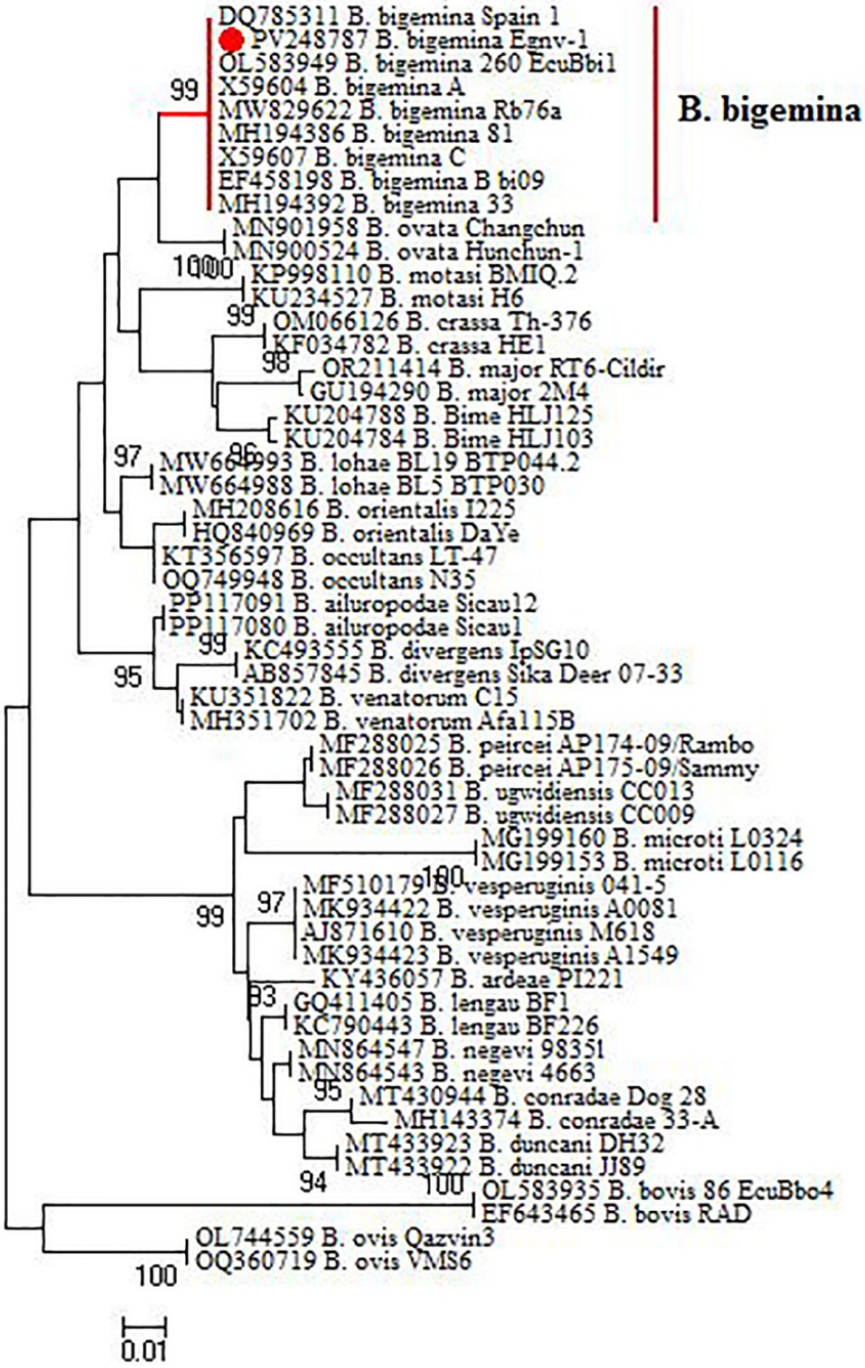

Figure 9: Phylogenetic tree

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