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Original Article

An alternative combination therapy with metronidazole and doxycycline for babesiosis and theileriosis in stray dogs

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Abstract:

In Egypt, scare data explored blood protozoan parasites of stray dogs. Therefore, blood sample from 50 dogs were collected and microscopically examined for blood protozoan parasites by Giemsa-stained thin smear technique. Positive blood parasites cases (Babesiosis and Theileriosis) were blind randomly allocated into two groups to receive a combination of drugs. Group 1: Metronidazole (15mg/kg PO q 12h) with Doxycycline (5mg/kg PO q 12h) Group 2: Quinine (30mg/kg PO q 12H) with Clindamycin (25mg/kg PO q 12h) .The treatment period was seven days, but the first dose administration was given a double for each group. The hematological parameter (RBC; WBC; HCT; MCV; MCH; MCHC and platelets); liver enzyme and kidney function test of each sample were also assessed to follow up the effects of therapies. An overall prevalence of protozoan blood parasites was (33/50) 66%, the revealed species was Babesia spp. (25/33) 75.7% and Theileria spp. (8/33) 24.3%. Babesia gibsoni were the predominant Babesia spp. Physical examination revealed that all measured medical parameters of infected dogs were statistically significant compared to uninfected dogs except body weight and oral CRT (Capillary refill time) was statistically insignificant. The cured rate for group (1) was 56.2% and group (2) was 37.5% with statistically insignificant difference (x2=1.129, p=0.288). Still, the ROC curve values were higher among group (1) than group (2). Its values were (0.594 and 0.406, respectively). So, the combination of Metronidazole with Doxycycline is more suitable due to the area under curve nearest 0.6 while the value of area under curve for the combination of clindamycin with quinine in group (2) less than 0.5. Metronidazole combination therapy with doxycycline is more effective in improving blood parameters such as decreased liver enzymes, increased blood platelets and cure rate than combination therapy of quinine with clindamycin.

Keywords: Babesiosis, Theileriosis, stray dogs, treatment, prevalence

INTRODUCTION

Mostly, these blood parasites are diagnosed and identified by microscopic examination of peripheral blood or lymph node smears, based on their morphology of the erythrocytic or lymphocytic forms (piroplasm and schizont stages). Also, are diagnosed by serological methods **(Solano-Gallego and Baneth 2011).**

In addition, several medications, such as diminazene aceturate, imidocarb dipropionate, pentamidineis ethionate, trypan brown, primaquine and quinolinium sulfate, have been used to treat canine babesiosis and reduce the incidence of clinical symptoms and disease-related mortality **(Birkenheuer, et al. 2003; Wulansari, et al. 2003; Matsuu et al. 2008; Sakuma, et al.2009)** but no single drug has been found to be effective in eliminating babesiosis especially Babesia gibsoni **(An, et al. 2019).** For that, the lack of effectiveness of prior therapies has inspired research and implementation of novel protocols.Currently, the main novel protocols for treatment was combination therapy and it has shown good efficacy and several drug combinations have been recorded to be effective against canine blood parasites **(Irwin 2009; Solano-Gallego and Baneth 2011).**

The mechanisms of action of these drugs against Babesia sp. are largely unknown or not examined in depth and

Babesiosis and theileriosis are diseases caused by Babesia and Theileria hemoparasites, generally grouped under the designation of piroplasms, with global distribution and economic, veterinary and medical significance and transmitted by arthropods to dogs **(Uilenberg, 2006).** Moreover, these parasites have infected the erythrocytes of domestic and wild animals and humans that causing hemolytic anaemia accompanied by several clinical signs and symptoms, including fever, lethargy, anorexia, splenomegaly, and jaundice that may vary with the different species of Babesia and Theileria (Conrad et al., 1991; Irwin, 2009 and Gray et al. 2010). Mortality rates reported for malignant theileriosis can reach up to 73.0% (Taha et al. 2011). Also, with factors that determine the host's response to infection such as age, individual immune status, and the presence of concurrent diseases, **(Irwin, 2009).** The pathophysiology of detected Theileria species in dogs remains unknown **(Chomel, 2011)**

Canine babesiosis and theileriosis are serious and widespread tick-borne infectious diseases recognized as a dangerous emerging disease in dogs. Additionally, endemic occurrences of Babesia and Theileria spp. in Asia, Africa, Europe, North America and Australia have been recorded **(Matjila et al. 2004 and Matsuu et al. 2008).**

parasites among stray dogs in Alexandria governorate, Egypt. In addition to monitor the therapeutic efficacy of clindamycin in combination with quinine compared to those of metronidazole with deoxycycline against Babesia and Theileria species.

MATERIAIS AND METHODS

Study area

Fifty dogs of different ages and sexes were collected randomly from the center and west of Alexandria governorate, Egypt, and trapped in registered animal house with registration number 584813328 under control of Ministry of Supply and Internal Trade, Alexandria governorate. Dogs were physically examined (body weight, temperature, pulse, eye mucous membrane, oral capillary refill time CRT and respiratory rate)

Microscopic detection of blood parasites

Blood samples were collected from cephalic vein of 50 dogs and divided into two tubes: first with EDTA as anticoagulant aiming to perform complete blood count (CBC) analysis and the other gel blood tube to collect the clear serum to perform the liver and renal function tests before and after drugs administration. Moreover, the fresh blood samples were used for Giemsa stained thin smear before and after treatment, which microscopically examined under oil immersion lens for detection of blood protozoan parasites according to **Gadahi, et al. (2008).**

Treatment regimen

the reason for the added benefit of certain drug combinations, while evident in clinical trials, is not well understood **(Beugnet and Moreau 2015; Checa, et al. 2017).**

Among of these combination therapy is atovaquone-azithromycin combination, and although it has shown good efficacy, resistant of Babesia sp. have yet to be identified **(Jefferies, et al., 2007)**, and the combination therapy of clindamycin-doxycycline
metronidazole was used and stated to be successful against B. gibsoni and no resistance cases have been reported to date **(Sakuma, et al. 2009; An, et al. 2019 and Almendros, et al. 2020)**

Recently, clindamycin in combination with quinine was used extensively as an effective drug for malaria, toxoplasmosis and babesiosis. Clindamycin is a slow-acting antibiotic for parasites and fever clearance therapy when used as monotherapy, but when combined with quinine, the downside of clindamycin can be offset as both medications have different modes of action and quinine has a more rapid action **(Dorman, et al. 2000; Obonyo and Juma 2012).**

In Egypt, many studies of babesiosis and theileriosis in sheep, cattle and camels have been performed **(Taha, et al. 2018; Hassan, et al. 2017; Hussein, et al. 2017)** whereas, scare data were recorded for canine babesiosis and theileriosis, in particular with treatment, Therefore, the present study was conducted to assess the prevalence of blood protozoan

statistics were used to summarize the data. The prevalence of all animals was calculated for all data. Chi-square was used to assess the association of risk factors on the incidence of parasites. Moreover, T- Paired Samples Test for standard distribution quantities data. Z-Wilcoxon for non-parametric quantities data compares two related samples in analyses were performed using the "SPSS version 22" statistical. The significance level was $P<0.05$. Finely ROC curve was done to predict the degree of the cure rate of each group, and the cut of value was above 0.5. According to **Landau, et al; (2004)**

Ethical statement

All respective animal protocols were reviewed by state ethics commission and have been approved by competent authority (Ethical committee FWA No: 00018699 and IRB No: 00012098, faculty of veterinary medicine, Alexandria university, Egypt)

RESULTS

Out of 50 examined stray dogs, 33 dogs were found to be infected with blood parasites representing 66 % by thin blood film examination, as shown in Fig(3) . In 25 positive dogs (75.7 %), a single infection of Babesia spp. has been found higher than mixed infection Babesia and Theileria spp. 8 (24.3%), as shown in Fig (4). Babesia gibsoni were the predominant Babesia spp. was reported in this study.

Furthermore, the incidence of blood parasites according to sexes showed higher infection rate was recorded

Positive blood parasites cases (babesiosis and theileriosis) were allocated into one of the following two groups to receive either the combination drugs in each group for seven days, but first day of treatment the dosage was given double due to overcome the chronicity problem of infection and drugs resistance and then was given as the following a regime, according to Krause, et al. (2000)

Group 1: Metronidazole (Alexandria Pharmaceutical Company), in dose 15mg/kg PO q 12h with Doxycycline (Pfizer Pharmaceutical Company), in dose 5mg/kg PO q 12h

Group 2: Quinine (Khalil Pharmacy, Alexandria), 30mg/kg PO q 12h in dose with Clindamycin (Sigma Pharmaceutical Company) in dose 25mg/kg PO q 12h.

The drugs were enveloped inside part of an eviscerated chicken intestine to facilities administration of medications.

Cure assessment

Blood samples were collected on the tenth day from the beginning of drug administration due to the perfect therapeutic effect of oral dose at the third day after end drugs administration from all dogs to perform the CBC and biochemical analysis after treatment; then, a thin blood film was done from the treated dogs.

Statistical analysis

Raw data were entered into a Microsoft Excel spreadsheet, and descriptive

groups (1 and 2). Whereas, increased number of monocytes after post-treatment in groups (1 and 2) with high significant differences at level P≤0.05, as shown in Table (5).

Regarding, by the kidney and liver function tests in groups (1 and 2), the kidney functions (uric acid and creatinine) were in normal ranges. However, statistically significant differences were noticed at level P≤0.05 between pre and post-treatment, as shown in Table (6). Furthermore, the liver enzymes were improved with the combined medication in-group (1) more than group (2), which noticed the significant differences at level P≤0.05, as shown in Table (7)

On the other hand, the cure rate among the treatment groups of dogs, the group (1) was more effective than the group (2), (56.2% and 37.5%) respectively, as shown in Table (8). The area under the curve of predicate the efficacy of drug recorded the combination therapy of Metronidazole and doxycycline was more extended compare with combination therapy of quinine and clindamycin, as shown in Table (9), and Fig (5).

Regarding, by the kidney and liver function tests in groups (1 and 2), the kidney functions (uric acid and creatinine) were in normal ranges. However, statistically significant differences were noticed at level P≤0.05 between pre and post-treatment, as shown in Table (6). Furthermore, the liver enzymes were among female as compared to male animals but based on the X2- Pearson Chi-Square was found non-significant different (P>0.05). On the hand, the medical examination of the eye mucus membrane was reported 48.5% of dogs was abnormal and with significant differences at level ≤0.05, as shown in Table (1)

Regarding, from Table (2) the clinical findings of examined dogs, the body temperature, pulse and the respiratory rate were higher in infected dogs than non-infected dogs (≈39.4^{o}C, ≈88 and ≈32 respectively) with high significant differences at level ≤0.05 .

After that, the complete blood pictures of the treatment groups of infected dogs were recorded depending on the treatment group. Based on the width of the blood cells, no difference was found between group (1 and 2) of RDW_SD, RDW_CV, PDW and MPV. Whereas, the t- Paired Samples Test z-Wilcoxon showed highly significant differences between pre / post-treatment in both groups (1 and 2) as shown in Table (3). On the other hand, for blood contents (Hematology parameters), Table (4) showed the effect of combined medications in groups (1 and 2) post-treatment on decrease number of WBCs with slightly significant differences at level ≤0.05. Moreover, post-treatment in-group (1) showed the increase of the mean corpuscular volume (MCV) and platelets with highly significant differences at level ≤0.05. Concerning the white blood cells, it was observed decreased number of lymphocytes and eosinophil cells post-treatment in

(1) was more effective than the group (2), (56.2% and 37.5%) respectively, as shown in Table (8). The area under the curve of predicate the efficacy of drug recorded the combination therapy of Metronidazole and doxycycline was more extended compare with combination therapy of quinine and clindamycin, as shown in Table (9), and Fig (3).

improved with the combined medication in-group (1) more than group (2), which noticed the significant differences at level P≤0.05, as shown in Table (7).

On the other hand, the cure rate among the treatment groups of dogs, the group

Table (1): Prevalence of blood parasites infection in stray dogs according to sexes a qualitative clinical examination.

Clinical examination		**Study group 50 dogs**				**Significant**	
		Infected (33)		**Non-infected (17)**		**X2**	**P**
		No	%	No	%		
Sexes	**Male**	16	48.5	12	70.6	2.225	0.136
	Female	17	51.5	5	29.4		
Eye mucous membrane	**Normal**	17	51.5	14	82.4	4.529	0.033*
	Abnormal	16	48.5	3	17.6		

X2- Pearson Chi-Square *-significant difference at level ≤0.05

Table (2): Parameters of the clinical examination of dogs.

Clinical examination	Study group 50 dogs		Significant	
	Infected (33)	Non-infected(17)		
	Mean± S.D.	Mean± S.D.	t/z test	P- value
Body weight	16.424±3.2527	17.941±2.1278	-1.736[t]	0.089
Temperature	39.364±0.9137	38.288±0.7680	4.151[t]	**0.000***
Pulse	87.65±5.338	79.55±8.419	-3.231[z]	**0.001***
Oral CRT	2.12±.781	1.88±.697	-1.076[z]	0.282
Respiratory rate	31.79±4.068	21.82±2.099	-5.171[z]	**0.000***

t- Independent Samples Test (Levene's Test) z- Mann-Whitney U *-significant difference at level ≤0.05

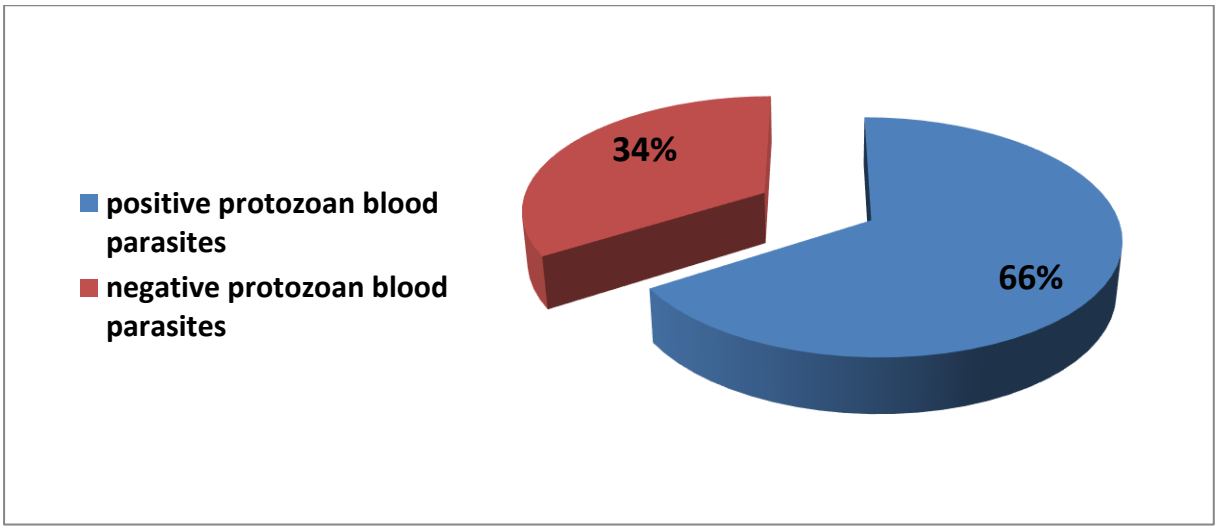

Fig (1): Prevalence of blood parasites in the examined dogs.

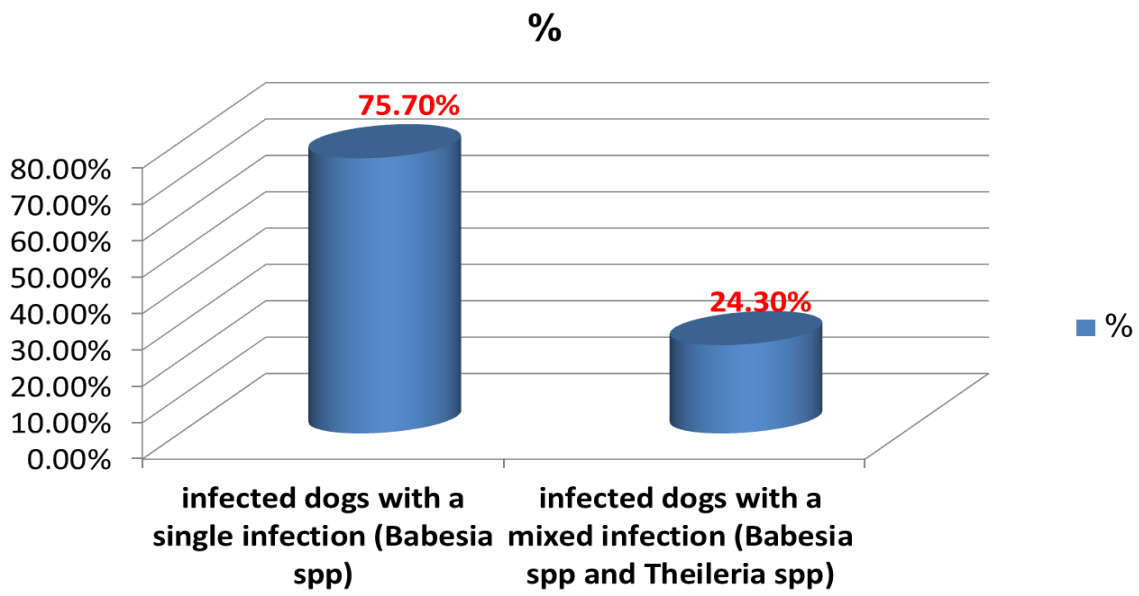

Fig (2): Prevalence of single and mixed infection of blood parasites among dogs.

Table (3): The width of cells based of infected dogs.

CBC analysis		Intervention drugs					
		Treatment group 1 (16 cases)			Treatment group 2 (16 cases)		
		Pre	Post	%	Pre	Post	%
RDW_SD	mean± SD	34.8±5.66	14.46±1.01		33.36±4.2	15.69±5.09	
	t	-15.591			-3.303ᶻ		
	p	**0.000***		-58.44	**0.001***		-52.96
RDW_CV	mean± SD	15.53±2.11	2.18±0.9		16.48±1.53	3.82±4.8	
	t	-3.527ᶻ			-3.303ᶻ		
	p	**0.000***		-85.96	**0.001***		-76.82
PDW	mean± SD	9.73±5.02	49.36±4.73		10.98±4.81	46.54±14.01	
	t	-3.527ᶻ			9.327		
	p	**0.000***		403.29	**0.000***		323.86
MPV	mean± S.D.	9.84±0.5	10.19±0.3		10.3±1.12	10.75±1.07	
	t	1.944			1.135		
	p	0.071		7.21	0.274		4.36

- One multiple infected (*Babesia* & *Theileria*) case died the second day of treatment related to group 2, so it was excluded from this study.
- t- Paired Samples Test z-Wilcoxon (non-parametric compare two related samples) *-significant difference at level ≤0.05.
- RDW =,Red cell distribution width **RDW-CV = Coefficient of VariationRDW-SD = Standard Deviation** PDW =Platelet Distribution Width MPV =mean platelet volume.

Table (4): Number and the content of cells among groups (1 and 2) of the infected dogs.

CBC analysis		Study groups					
		Treatment group 1 (16 cases)			Treatment group 2 (16 cases)		
		Pre	Post	%	Pre	Post	%
WBC	mean± SD	17.54±4.36	13.21±2.94	-	15.70±4.72	12.26±3.15	-
	t	-3.459			-2.878		
	p	**0.003***		24.68	**0.011***		21.91
RBC	mean± SD	6.79±1.28	6.88±0.73		7.48±0.55	7.37±0.76	
	t	0.342			-0.418		
	p	0.737		1.32	0.682		-1.47
HGB	mean± SD	15.13±2.87	15.05±1.36		16.56±1.17	16.30±1.74	
	t	-0.143			-0.486		
	p	0.888		-0.52	0.634		-1.57
HCT	mean± SD	47.45±8.13	50.19±4.63		51.30±3.73	52.63±5.15	
	t	1.523			1.33		
	p	0.149		5.77	0.203		2.59
MCV	mean± SD	70.09±4.24	73.15±2.01		68.70±4.49	52.35±30.24	
	t	3.65			-1.956		-
	p	**0.002***		4.36	0.069		23.79
MCH	mean± SD	22.25±0.92	21.91±1.05		22.15±0.91	22.10±0.44	
	t	-1.44			-0.224		
	p	0.17		-1.52	0.826		-0.22
MCHC	mean± SD	31.84±1.34	29.9±1.23		32.36±1.91	30.93±1.69	
	z	-6.19			-2.278		
	p	**0.000***		-6.09	**0.038***		-4.41
Platelets	mean± SD	271.50±167.85	410.31±98.15		348.69±148.52	310.94±133.11	
	t	3.393			-0.846		-
	p	**0.004***		51.12	0.411		10.82

- One multiple infected (Babesia&Theileria) case died the second day of treatment related to group 2, so it was excluded from this study. t- Paired Samples Test z-Wilcoxon (non-parametric compare two related samples) *-significant difference at level ≤0.05

Table (5): Complete blood picture among groups (1 and 2) of the infected dogs.

CBC analysis		Intervention drugs					
		Treatment group 1 (16 cases)			Treatment group 2 (16 cases)		
		Pre	Post	%	Pre	Post	%
Neutrophil%	mean± SD	24.9±5.14	25.6±13.1		27.31±10.21	24.7±2.72	
	t	-1.141^{z}			-0.895		
	p	0.254		2.81	0.385		-9.55
lymphocytes %	mean± SD	68.06±1.09	11.88±4.39		68.21±11.93	18.28±20.41	
	t	-35.627			-3.303^{z}		
	p	**0.000***		-82.54	**0.001***		-73.2
Monophil %	mean± SD	6.6±3.43	46.41±15.99		4.1±3.27	47.29±19.78	
	t	9.723			-3.303^{z}		
	p	**0.000***		603.18	**0.001***		1053.41
Eosinophil%	mean± SD	7.63±3.93	0.12±0.19		4.63±3.14	0.1±0.18	
	t	-3.527^{z}			-3.31^{z}		
	p	**0.000***		-98.42	**0.001***		-97.84
Basophil%	mean± SD	0.14±0.13	0.093±0.05		0.12±0.06	0.14±0.072	
	t	-1.221^{z}			-1.732^{z}		
	p	0.222		-35.57	0.083		16.66

- t- Paired Samples Test z-Wilcoxon (non-parametric compare two related samples) *-significant difference at level ≤0.05

Table (6): Kidney function tests of the infected dogs.

kidney function test		Intervention drugs					
		Treatment group 1 (16 cases)			Treatment group 2 (16 cases)		
		Pre	Post	%	Pre	Post	%
Uric acid	mean± SD	1.41±0.3	2.18±0.45	**54.6**	1.28±0.15	1.72±.43	**34.37**
	t	4.707			4.534		
	p	**0.000***			**0.000***		
Creatinine	mean± SD	0.82±0.15	1.3±0.09	**58.53**	0.82±0.12	0.95±0.11	**15.85**
	t	9.711			3.183		
	p	**0.000***			**0.006***		

- t- Paired Samples Test z-Wilcoxon (non-parametric compare two related samples) *-significant difference at level ≤0.05

Table (7): Liver function tests of the infected dogs.

Liver enzymes		Intervention drugs					
		Treatment group 1 (16 cases)			Treatment group 2 (16 cases)		
		Pre	Post	%	Pre	Post	%
AST(SGOT)	mean± SD	71.06±32.18	54±16.34		58.56±13.82	51.31±9.73	
	t	-2.583			-1.647		
	p	**0.021***		-23.78	0.12		-12.38
ALT(SGPT)	mean± SD	52.06±12.8	41.31±13.83		82.75±64.034	76.88±71.2	
	t	-2.165			-0.662^{z}		
	p	**0.047***		-20.64	0.508		-7.09
ALP	mean± SD	56.69±20.53	45.5±18.05		55.62±31.14	46.5±21.21	
	t	-2.374			-0.824		
	p	**0.031***		-19.73	0.423		-16.39

- t- Paired Samples Test *-significant difference at level ≤0.05

Table (8): Cure rate of the infected dogs.

Drugs outcome						
Blood film examination	**Group 1 (16 cases)**		**Group 2 (16 cases)**		**X2**	**p**
	No.	**%**	**No.**	**%**		
Cured	9	56.2	6	37.5		
Non-cured	7	43.8	10	62.5	**1.129**	**0.288**
Total	16	100	16	100		

- X2-Pearson Chi-Square *-significant difference at level ≤0.05.

Table (9): The area under the curve to predicate the efficacy of drug used in two different studies groups.

Test result variable (s)	Area	S.E	Asymptotic Sig	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
. Group 1 (doxycycline +metronidazole)	.594	.102	.365	.394	.794
. Group 2 (clindamycin +quinine)	.406	.102	.365	.206	.606

- The test result variable(s): group1 (doxycycline+ Metronidazole), group2 (clindamycin +quinine has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.
- a. Under the nonparametric assumption
- b. Null hypothesis: true area = 0.5

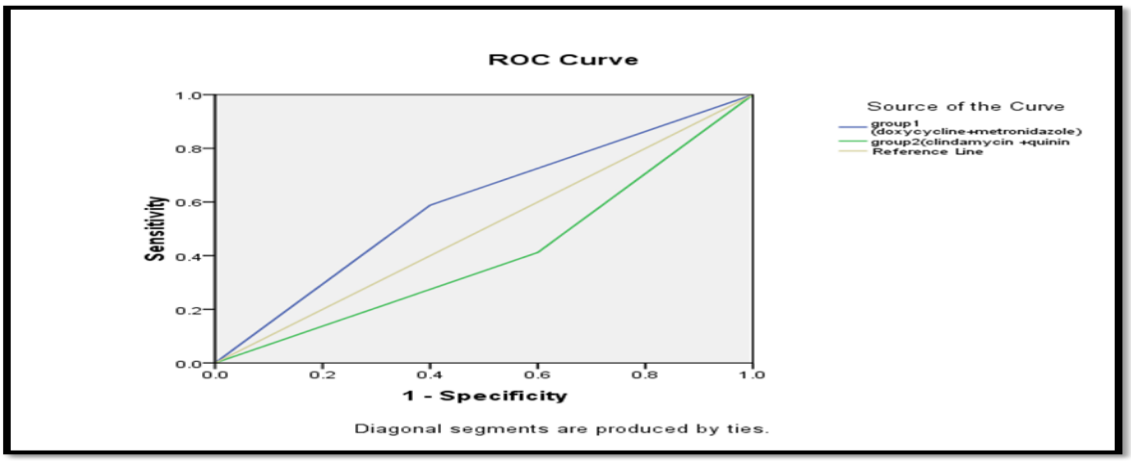

Fig (3): The area under the curve to predicate the efficacy of treatment groups

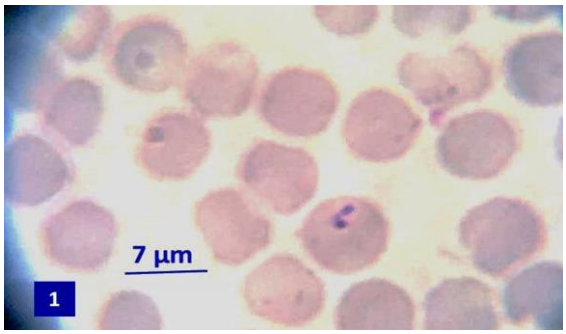

Fig (4) Intraerythrocytic Babesia gibsoni in canine red blood cells; light microscopy -Giemsa stain, 100×.

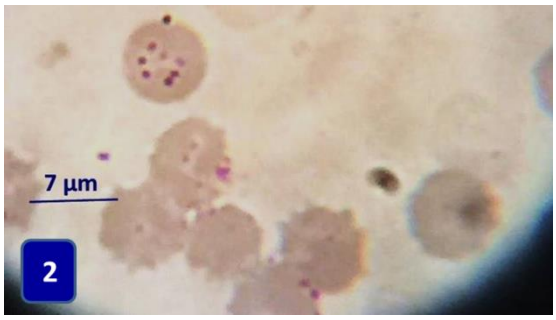

Fig (5) Intraerythrocytic multiplication of Theileria .spp in canine red blood cells; light microscopy -Giemsa stain, 100×.

However, **Veneziano, et al. (2018)** in southern Italy, have also reported male-biased blood parasites infection prevalence, this distinction was due to the males more used in hunting than females, at variance the stray dogs .

Moreover, in the current study, high temperatures, heart rhythms with fast respiratory rates and abnormal eye mucous membrane around 48.5% were reported in infected stray dogs and corresponding with the study by **Suarez, et al. (2001)** in Spain and **Badawi and Yousif (2020)** in Iraq. Furthermore, in the present study, clinical signs included pale mucus membranes, illness, and hemolysis. In contrast, malnutrition, anaemia, and anorexia were considered symptoms of a chronic course of the disease.

Anaemia was diagnosed on the basis of the full blood count, and this means that simple physical observation might not be enough to diagnose anaemia, and white blood cells were higher than normal levels. Although, the number of WBCs has been documented in other studies, it is variable and may not be a reliable parameter in the diagnosis of blood parasites **(Ybañezet al. 2017)**.

DISCUSSION

Canine piroplasmosis is a disease that is transmitted worldwide and caused by Theileria and Babesia spp., affecting different dog species. It considers the prevalent issue that adversely affects the health and productivity of dogs in Egypt. However, little is known about the epidemiology of piroplasmosis in this region **(Omer, et al. 2012).** The prevalence rate of blood parasites under this study was around 66%, higher than the previous study in Iraq (5.1%) by **Badawi and Yousif (2020).** Also, higher than those in Italy in hunting dogs (14.1%) as shown by **Veneziano, et al. (2018)** where were infected with one or more blood parasites. Otherwise, this study showing 75.7% of single infection by Babesia spp. and 24.3% of mixed infection by both Theileria and Babesia spp. and corresponding that with **De Caprariis, et al. (2011)** in Italy .

With respect to the sexes of stray dogs, this study indicates that it could be a risk factor for infection with blood parasites since the incidence of infection in females was higher than males (51.5%, 48.5%), respectively.

hepatotoxicity and hematuria in dogs. On the same harmony clindamycin administration may induce acute kidney injury **(Subedi, et al. 2018)**

Nowadays, for the treatment of B.gibsoni, imidocarb dipropionate and diminazene aceturate are considered ineffective. The current treatment of choice for B. gibsoni infection is the combination of atovaquone and azithromycin treatment, although suppressing parasites replication by this combination may not be associated with parasites clearance **(Kirk, et al. 2017)**

Furthermore, Babesia spp. that infected dogs are vulnerable to dissimilar drugs and react differently to drugs. Therefore, necessary to treat infections with appropriate anti-protozoal drugs or their combinations and to consider the limitations of the medication **(Solano-Gallego and Baneth, 2011)**

In addition, in this study the combination of clindamycin with quinine did not demonstrate the required improvement for babesiosis and theileriosis could be due to the most effective chemotherapeutic regimen, clindamycin plus quinine for malaria, in cases of severe infection with babesiosis and theileriosis, sometimes ineffective. So, in conjunction with effective antimicrobial treatment, the rapid establishment of a whole-blood exchange transfusion may be lifesaving **(Dorman, et al. 2000)**

In order to identify alternative therapies against canine babesiosis and theileriosis infection, the synergistic

In comparison, there was no noticeable difference in the RBC count, Hb estimation, and Neutrophil that agree with findings by **(Sudhakara et al. 2016)**

On the other hand, in the current study, the effect of curative efficacy of the combination therapy on the infected dogs was used by the combination of doxycycline and metronidazole as group (1) and the combination of clindamycin and quinine as group (2). Moreover, these findings on infected dogs were described the combination doxycycline and metronidazole was more suitable, and efficacy than clindamycin and quinine and the cured rate was higher than that drug which used on group (2). During a few days of treatment, after using the doxycycline and metronidazole combination, the dogs demonstrated clinical and haematological progress and improved by the end of the therapeutic regimen. Metronidazole is a compound of nitroimidazole widely used as an anti-trichomonal agent. While metronidazole has been reported to be used as part of combination therapy, it has also been reported that doxycycline hydrochloride exhibits activity against blood parasites **(Losson, et al. 1989; Suzuki, et al. 2007).** However, in the present work, doxycycline with metronidazole increased uric acid and creatinine levels of the treated dogs. Similar finding was reported by **(Giguère, et al. 2013).** The authors mentioned that tetracyclines may induce fatal nephrotoxicity in dogs. Moreover, **Plumb (1999)** reported that metronidazole may induce

effects of combinations of pharmaceutical products should be further examined.

CONCLUSION

In the first time, the high prevalence rate of blood parasites (babesiosis and theileriosis) among stray dogs in Alexandria governorate, Egypt was investigated and based on the findings, metronidazole and doxycycline combination therapy is more effective than quinine and clindamycin combination therapy, particularly for the cure rate and improvement of blood parameters, and could be used as a new therapeutic alternative.

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Conflict of interest

Authors declare there was no conflict of interest.

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الملخص العربى

علاج تركيبي بديل من ميترونيدازول ودوكسيسيكلين لداء البابيزيا والثَّيْلَريَّا في الكلاب الضالة

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نظرا لندره البيانات عن إصابات الكلاب الضالة بطفيليات الدم في مصر. فقد تم تجميع50 عينة دم من الكلاب الضاله وفحصها مجهريًا بحثًا عن طفيليات الدم بإستخدام تقنية المسحات الرقيقة المصبوغة بـ جيمسا. وقد تم تقسيم الكلاب المصابة بطفيليات الدم إلى مجموعتين لتلقي توليفة من الأدوية. **المجموعة (1):** تم تجريعها بمركب ميترونيدازول (15 مجم / كجم) مع دوكسيسيكلين (5 مجم / كجم). **المجموعة (2):** كوانين (30 مجم / كجم) مع كليناميسين (25 مجم / كجم)علما بان تم اعطاء جميع الادويه عن طريق الفم كل 12 ساعه لمده سبعة أيام، ولكن تم مضاعفة الجرعة الأولى وقت تجريعها. وتم أيضًا تقييم معدلات قياسات الدم (كريات الدم الحمراء RBC وكريات الدم البيضاء WBC، معدل الهيماتوكريت (HCT)، متوسط حجم كريات الدم (MCV)، متوسط الهيموجلوبين بكريات الدم (MCH)، متوسط تركيز الهيموجلوبين بكريات الدم (MCHC) بالإضافة الي معدل الصفائح الدموية.

بلغ معدل الإنتشار الإجمالي لطفيليات الدم (50/33) 66٪، اما بالنسبه لنواع طفيليات الدم فكانت كالتالى بابيزيا بنسبة 75.7٪ و ثيليريا بنسبة 24.3٪. وقد وجد أن بابيزيا جيبسونى هى النوع السائد. وقد أظهر الفحص البدني أن جميع المعايير الطبية التي تم قياسها للكلاب المصابة كانت ذات دلالة إحصائية مقارنة بالكلاب غير المصابة باستثناء وزن الجسم وزمن إعادة الملء الشعري (CRT)، حيث كانت غير ذات دلالة إحصائية. كان معدل الشفاء للمجموعة (1) 56.2٪ والمجموعة (2) كان 37.5٪ مع إختلاف غير معنوي إحصائياً (x2 = 1.129، P = 0.288). ومع ذلك ، كانت قيم منحنى خصائص المستقبل التشغيلي ROC في المجموعة (1) أعلى من المجموعة (2)، حيث كانت القيمه (0.594 و 0.406 في كلتا المجموعتين على التوالي).

لذا، فقد وجد الباحثين أن توليفة ميترونيدازول مع دوكسيسيكلين أكثر ملاءمة نظرًا للمنطقة الواقعة تحت المنحنى الأقرب 0.6 بينما قيمة المنطقة تحت المنحنى لتوليفة كليندامايسين مع كينين في المجموعة (2) أقل من 0.5.

ومن ذلك يعتبر العلاج بكل من مركبات ميترونيدازول مع دوكسيسيكلين أكثر فعالية في تحسين مقاييس الدم مثل انخفاض إنزيمات الكبد وزيادة الصفائح الدموية ومعدل الشفاء عن العلاج بكل من مركب كينين مع كليندامايسين.