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Effect of external and blood parasites on fertility of German Shepherd dogs

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ABSTRACT

The present study aimed to investigate the effect of *Rhipicicephalus sanguineus* and *Babesia species* on the fertility of German shepherd dogs, 80 dogs (41 male and 39 female) were examined in veterinary clinics of Mansoura city and Sinbillawain, Dakahlia Governorate aged from 2-3 years during summer and autumn seasons 2021.Twenty-one dogs (26.5%) were infested with *Rhipicephalus sanguineus* (brown dog ticks) with different degree of infestation and higher prevalence in male dogs (29.2%) than female(23%). While 12 dogs (15%) showed signs of fever, anorexia, pale mucous membrane, weakness and increase in heart rate, examining of thin blood films indicated infection with *Babesia spp*. with percentage (17%) and (12.8%) in male and female dogs respectively. Biochemical laboratory examinations were done for estimation of testosterone hormone in male and progesterone hormone in female dogs in the estrus phase to detect the effect of external and blood parasites on their fertility. Laboratory findings of testosterone levels in male infested with *Rhipicephalus sanguineus* ranged from (2.01- 3.2 ng/ml), while others infected with ticks and *Babesia spp*. showed significant decrease in the testosterone levels in male (0.75 – 1.35 ng/ml). Testosterone levels in apparently healthy male dogs that free from ticks and *Babasia spp*. ranged from (3.04-4.1 ng/ml). Progesterone levels in bitches infested with *Rhipicephalus sanguineus* ranged from (2.5-3.4 ng/ml) and (0.2 -0.79 ng/ml) for bitches infested with ticks and *Babesia spp.,* while progesterone levels in bitches free from ticks and *Babesia spp*. were (2.9-3.4 ng/ml). These results indicate that infestation with ticks (*Rhipicephalus sanguineus*) only had little effect on the fertility of German Shepherd dogs but when more persistence of ticks with presence of babesiosis can affect their fertility.

Keywords: German Shepherd dog, external parasites, blood parasites, fertility of dog, testosterone and progesterone hormones

INTRODUCTION

Dogs are the best friend of man, also they can be trained to be helpers in many ways.

German Shepherd dogs are breed of medium to large-sized that important in military and police work that popular pet **(Benjamin et al., 2016).** Dogs, like all mammals are susceptible to different causes of diseases that affected by various types of external and internal parasites **(Gadahi et al., 2008).** Ticks are important external parasites that can transmit bacteria, viruses and protozoa during their blood sucking **(Beugent and Marie 2009)** and cause many problems as dermatitis, anemia and tick paralysis **(Greay et al., 2016).** The main dog tick-borne diseases are hepatozonosis, Ehrlichiosis and babesiosis that are transmitted by brown dog ticks **(Shaw et al., 2001).** Affected dogs by tick–borne pathogens suffer from loss of appetite, weight loss, pale mucus membrane, drowsiness, vomiting and high fever **(Dantas 2008).** Babesiosis is one of intra-erythrocytic protozoa which transmitted by bitting and release sporozoites through their salivary glands into blood stream of dog **(Solano et al., 2016)**, infected dogs suffer from wide range of clinical signs from sub clinical to serious or may be become chronic carrier **(Irwin 2009**). The two important *Babesia spp.,* of dog are *Babesia canis* and *B. gibsoni* that can be differentiated depending on their morphological distinct forms in the infected erythrocytes **(Solano et al., 2016)**, Phylogeny *Babesia canis* was reclassified into three sub-species: *B. canis canis, B. canis rossi, and B. canis vogeli*, then they were considered to be separate species **(Zahler et al., 1998; Matijatko et al., 2012).** Sexual puberty of dog that begins at 6 to 12 months of age but may delay in large breeds to 24 months of age (**Marthina et al., 2014)**, the canine reproductive cycle is unlike any other species, female dogs have unusual characteristic estrus cycle than other domestic animals (2 estrus cycle in the year), which divided into 4 phases as: proestrus, it lasts from 7-10 days in which blood flows, females attract males but not allow mounting, Estrus (Mating period) it lasts from 5-10 days, it is highly variable and can last up to 3 weeks **(Case 2005 and England 2013)** during which blood flow decreases and then stops, females attract and accept males, ovulation occurs during this time, unlike other mammals pre-ovulatory luteinization occurs in dogs and progesterone start to rise to about 2.5 ng/ml **(Case 2005 and Groppetti et al., 2015)**. Ovulation starts with a peak surge of L.H **(Case 2005),** during estrus about 2-3 days after L.H surge when progesterone concentration 4-10ng/ ml (**Concannon 2009),** if progesterone concentration is ≥ 5ng/ml is considered indicative of ovulation in breeding management **(England et al., 2009 and Scarlette 2016)**, diestrus: This period from 10-140 days after heat,

when the bitch is either pregnant or in an arresting period by days 55-90, it is the end and the begin of anestrus **(Johnston et al., 2001),** anestrus: that is the resting period between diestrus and the next heat cycle in which in activity of ovaries (**Asmaa 2019**) lasts from 2-8 months depends on different factors (**Concannon 2011 and Walter et al., 2011)** with average 7 months **(Christie and Bell, 1971),** while male has no sexual cycle except responding to bitch in estrus at any time of the year and fertile when fully mature, testosterone concentration ranged from 4 -6 ng/ml in the intact dogs (**Depalatis et al., 1978).** Infertility problem is a common syndrome in bitches due to many causes while the most common cause is the mating, at incorrect time (**Asmaa 2019)**, in this study we noticed that there are other important causes may affect the fertility of the dogs as presence of external and blood parasites especially in German Shepherd dogs.

MATERIALS AND METHODS

Time and locations of examined animal

All animals procedures were performed by veterinarians. The study was conducted during summer and autumn of 2021. A totally 80 German shepherd dogs (41 male and 39 female) of age from 2 year to 3 years were introduced to veterinary clinics in Mansoura city and Sinbillawain veterinary administration, Dakahlia Governorate. All data about examined dogs were recorded as sex, age, presence or absence of ticks and clinical symptoms (body temperature, pulse, respiratory rate and the color of mucous membranes and color of urine)

Collection and preservation of ticks from hosts

Tick specimens were obtained from infested dogs during examination, the examined dog cast to the examination table or held in a crush then one half of the body specially predilection sites was fully searched, ticks were removed from host skin by good quality steel forceps with blunt points and serrated inner surfaces The forceps was used to grip the tick firmly over its scutum and mouthparts as closely to the host skin as possible, then pulled strongly and directly out from the skin. To preserve the ticks, it was placed directly into 70% alcohol **(Estrada-Peña et al., 2004)**, then the Preserved ticks were punctured with a fine needle (to evacuate contents of internal organs), heated in 10% sodium hydroxide in water bath for 15 minutes, washed several times with cold water, dehydrated by ascending concentrations of ethyl alcohol beginning from 70%, 90%, 95% and absolute

ethanol for 20 minutes each. Finally, dehydrated specimens were passed through clove oil, xylol and mounted in canada balsam **(Soulsby 1968)** to be identified according to **Taylor et al., 2007 and Bowman 2009.**

Blood samples

For measuring progesterone levels in blood serum, Blood samples were collected from suspected bitches at the 9^{th} day from the beginning of estrus. About 3 mL of blood were collected from examined bitches by cephalic venipuncture, placed into sterile vacuum tubes and centrifuged at 3500 rpm for 15 min to obtain serum sample. The same procedures were conducted in male dogs to measure testosterone hormone levels in blood serum then sent to laboratory at the same day of collection for measuring progesterone and testosterone hormones by chemiluminescence immunoassay analyzer (MINDARY-CL960i) as a quantitative assay then the results were expressed as ng/ml.

For thin blood films one drop of blood was obtained from ear vein of suspected dogs, spread gently on clean glass slide, air dried then fixed with methanol, stained by Giemsa-stain 10% and examined under light microscope with oil immersion lens for detection of intra-erythrocytic stages of the piroplasm according to **Jain 1986, Brown 1993 and Salem and Farag, 2014.**

Statistical analysis

Comparative analysis for Progesterone and testosterone data were performed by using Student's t-test. All differences were considered significant at $P \leq 0.05$.

RESULTS

Clinical signs of affected dogs

There was different degree of *Rhipicephalus Sanguineus* infestation on the bodies of the dogs. Ticks were mostly found on ears, neck, trunk and axilla(figure 1). Infested dogs with ticks suffered from skin irritation and itching, while clinical signs of infected dog with Babesia spp. ranged from slight rise of temperature above 39 °C in early stage of infection to higher temperature reaches 41.5°C when taken from rectum, dullness, weakness, anorexia, dyspnea,increased plus rate, pale mucus membran and dark brown urine due to blood hemolysis (figure 2)

Figure (1) presence of ticks on the dog.

Figure (2) red urine of infested dog with babesia species.

Investigation of external and blood parasites examined dog:

Prevalence of tick infestation recorded in this study was (26.5%) with higher percentage in male dog (29.2%) than female (23%), while prevalence of *Babesia spp*. was (15%) recording higher percentage in male (17%) than female (12.8%) (Table 1).

Table (1) Prevalence of ticks (*Ripicephalus sanguineus*) infestation and *Babesia spp*. Infection among examined dogs in relation to gender.

Gender	No. Examined dogs	No. Infested with ticks	Percentage	No. Infected with Babesia spp.	Percentage
Male	41	12	29.2%	7	17%
Female	39	9	23%	5	12.8%
Total	80	21	26.5%	12	15 %

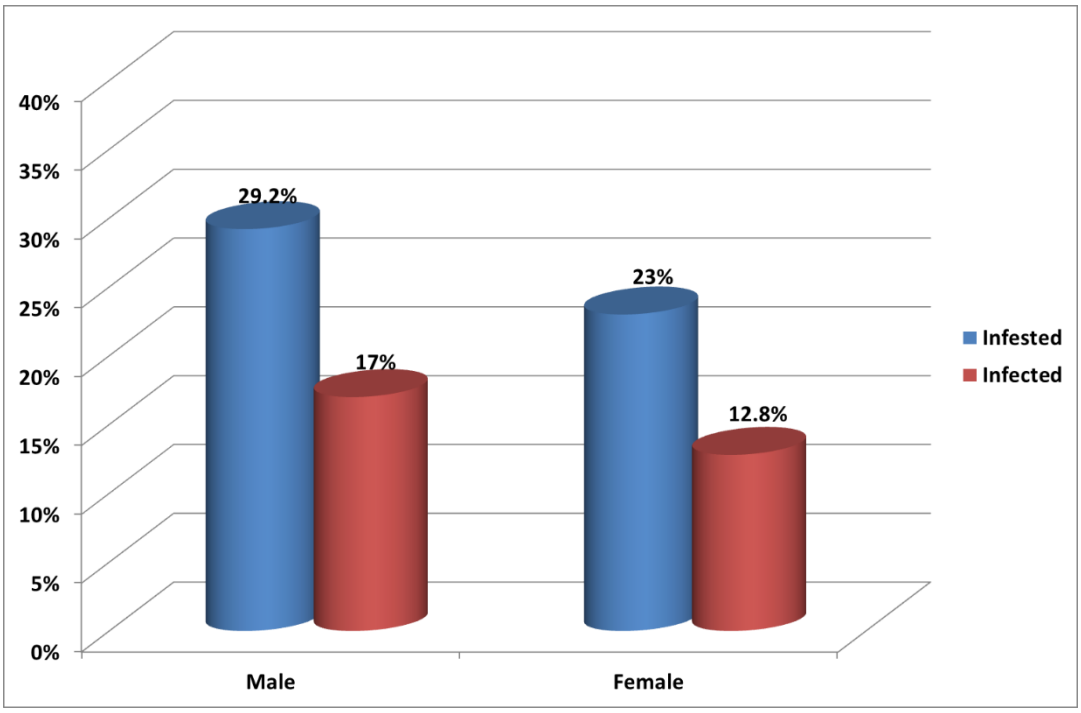


Figure (3) Prevalence of ticks and *Babesia spp*. infection among examined dogs related to gender**.**

Morphological structure of collected ticks

Rhipicephalus sanguineus female was identified according to **Estrada-Peña et al., 2004**. It was small in size dark yellow to dark brown in color when fresh. when examined from dorsal view under dissecting microscope after being preserved and mounted, these structures can be seen 1- Basis capituli lateral angles are sharp 2- Porose areas separation is broad 3- Palp pedicels are short, 4- Cervical fields are large and straight. 5-Scutum posterior margin is distinctly concave curve posterior to the eyes 6- Eyes are slightly convex. 7-Scutum is slightly dark in color (Figure 4, A). From ventral view genital aperture was seen with posterior lips have a broad U shape, while *Rhipicephalus sanguineus* male 1-Coxae 1 anterior spurs are not visible dorsally, Cervical fields depression is not apparent as Cervical fields texture has no wrinkles and has 2-slightly convex eyes, 3-Spiracle areas have sparse setae. 4- Posterior grooves are distinct (deep with wrinkled texture) (Figure 4, B). From ventral adanal plates were seen narrow and trapezoid, accessory adanal plates were large.

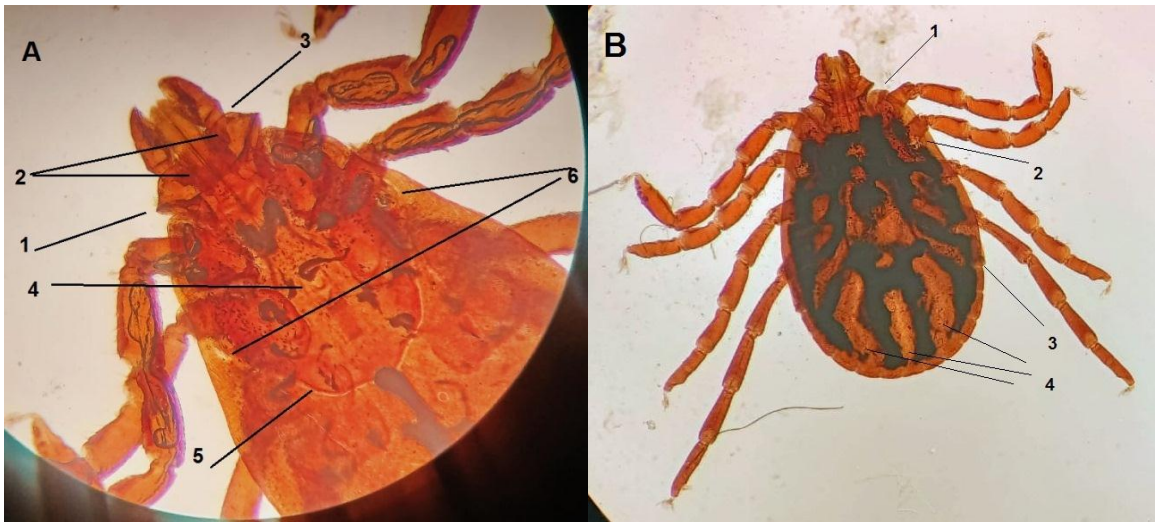


Figure (4) *Rhipicephalus sanguineus* adult female (A) and male (B)

Parasitological identification of blood films

Microscopical examination of stained blood films under oil immersion lens (x 100) showed various types of intra-erythrocytic merozoites *Babesia spp.* from marginal to pyriform shapes inside the RBC were identified according to **Soulsby 1968** (Figure 5).

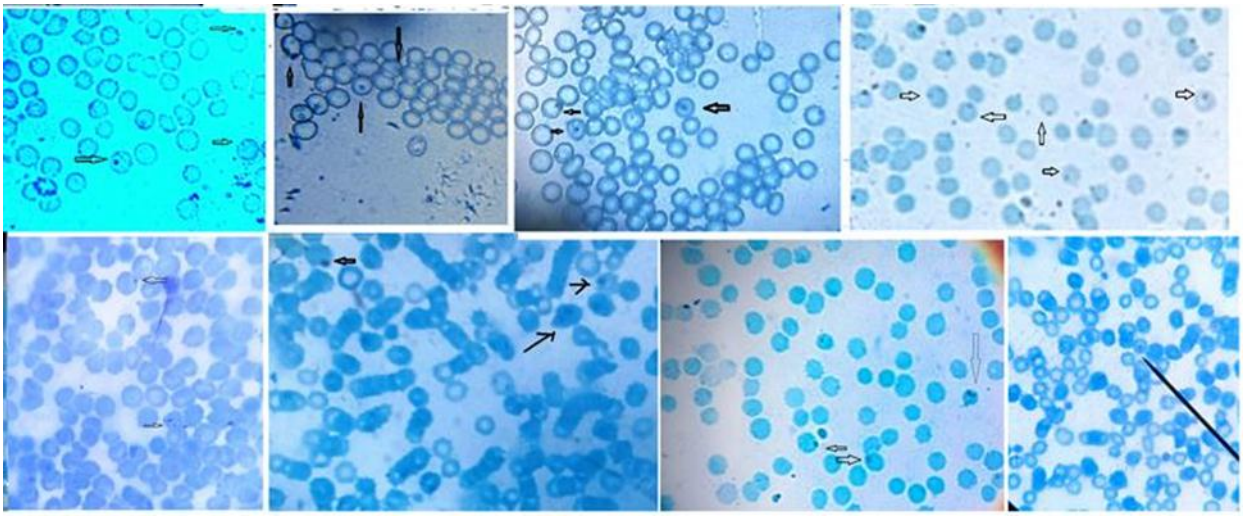


Figure (5): *Babesia spp.* in blood films of infected dog with oil lens x100.

Biochemical laboratory examination

Estimation of testosterone hormone in male dogs

In male dogs that were infested with ticks, testosterone levels were ranged from (2.01-3.2 ng/ml), in male dogs infested with both

ticks and infected with *Babesia spp.* the testosterone levels were ranged from (0.75 – 1.35 ng/ml) but testosterone levels in male dog free of ticks and *Babesia spp*. were ranged from (3.04-4.1 ng/ml) table (2). Which indicate that there was significant decreased in testosterone levels in male dogs infested with ticks only but when it is complicated with *Babesia spp.* infection, there were highly significant decrease in testosterone levels.

Estimation of progesterone hormone in bitches:

For bitches infested with ticks, progesterone levels at the 9th day of estrus phase ranged from (2.5 - 3.4 ng/ml), while bitches (infested with both ticks and infected with *Babesia spp*. progesterone levels at the 9th day of estrus phase ranged from (0.20 - 0.79 ng/ml) while in bitches free from ticks and *Babesia spp.* progesterone levels at the 9th day of estrus phase were ranged from (2.9-3.4 ng/ml) it shown in table (3). Which indicates that there were non-significant decreases in progesterone levels in bitches infested with ticks only but when it is complicated with *Babesia spp*. infection , there were highly significant decrease in progesterone levels than that free of ticks and *Babesia spp.*

Table (2) Testosterone levels among infested dogs with ticks *(Ripicephalus sanguineus*), infected with *Babesia spp*. & male dog (free from ticks and *Babesia spp*.)

Case no.	Testosterone level ng/ml (infested with ticks only)	Testosterone level ng/ml (infected with *Babesia spp*.)	Testosterone level ng/ml in male dog (free from ticks and *Babesia spp.)*
1	2.9	1.1	4.1
2	2.01	1.2	3.9
3	3.1	0.75	3.30
4	2.4	0.90	3.04
5	3.2	1.35	3.80
6	----------	1.3	3.40
7	----------	1.10	3.2

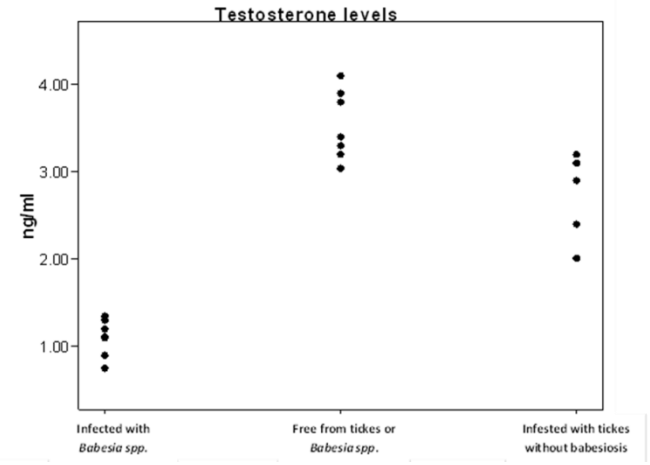
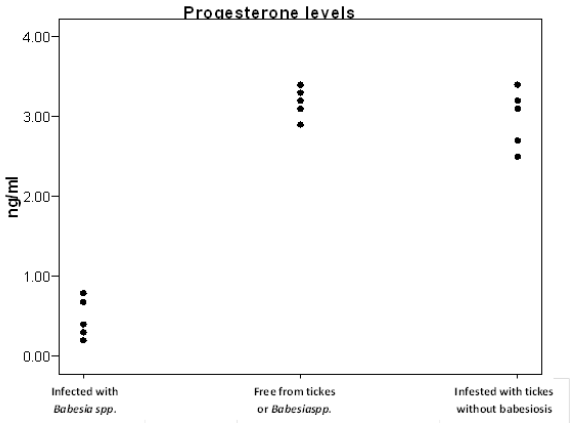


Table (3) Progesterone levels among infested bitches with ticks (*Ripicephalus sanguineus*), infected with *Babesia spp*. & bitches (free from ticks and *Babesia spp.).*

Case no.	Progesterone level in ng/ml (infested bitches with ticks only)	Progesterone level in ng/ml (infected bitches with *Babesia spp.)*	Progesterone level in ng/ml (bitches free from ticks and *Babesia spp.)*
1	2.7	0.79	3.4
2	2.5	0.20	2.9
3	3.1	0.4	3.1
4	3.2	0.68	3.2
5	3.4	0.30	3.3



DISCUSSION

Rhipicephalus sanguineus (brown dog tick) was the detected species in our study, it is adapted in temperate, tropical and subtropical regions so more common in Mediterranean areas **(Moraes-Filho et al., 2011)** and dogs are the main hosts beside other animal hosts **(Labruna 2004 and Walker 2000)**. The prevalence of infestation was 26.5% that nearly to that reported by **Ayodhya 2014** but higher percentage were recorded by **Ciuca et al., 2021**, while **Chandra et al., 2019 and EL- Neshwy et al., 2020** recorded lower percentage, concerning with the morpholological structure was similar to that described by **Hmoon et al., 2018, Jain and Jain 2006 and EL- Neshwy et al., 2020.**

Canine babesiosis is host specific and caused by different species of genus Babesia that have different mode of transmission, clinical investigations, treatment and prognosis **(Solano et al., 2016)**, clinically the infected dogs showed pale mucus membranes, fever, anorexia and red urine. The same result was reported by **Islam et al., 2017,** the detected incidence in our study during summer and autumn was 15%, the nearly percentage was recorded by **Costa et al., 2015, EL-Neshwy et al., 2020 and Obeta et al., 2020,** while lower percentage recorded by **Abdel-Rahman et al., 2015, Badawi and Youseif 2020 and Duth et al., 2004.** However higher incidence was reported by **Bhattacharjee and Sarmah 2013 and Andersson et al., 2017**. The variation in prevalence may be due to breed of dogs, immune status, season and the country under the study,

Male Germain Shapered dogs (17%) were more affected than females (12.8%), sex of dog may affect on presence of disease as the hormonal status and temperament of male may influence the infection due to less care given them by owners this result is in agreement with **Davitkov et al., 2015, Nalubamba et al., 2015, Mahalingaiah et al., 2017, Badawi and Youeif 2020., El-Neshwy et al., 2020, Obeta et al., 2020 and Ciuca et al., 2021,** other reports recorded that male dogs were more exposure to ticks infestation due to desiring to roam in search of mates but female received more management from their owners for monetary gains from their puppies **(Daniel et al., 2016)** while other studies recorded that the prevalence of female higher than male, and caused that female more lazy especially during nursing the off springs which increase the chance of ticks infestation (**Gadahi et al., 2008, Okubanjo et al., 2013 and Opara et al., 2017)** but **Amuta et al., 2010 and Amirtpal et al., 2014** reported that the gender of the host wasn't affect on the incidence of babesiosis.

Concerning the effect of external and blood parasites on fertility of German Shepherd dogs, there is little or nothing has been said about the impact of ticks and blood parasites on the fertility of dogs (**Anna and Stawomir 2020**). So we tried to study to the effect of ticks infestation and *Babesia spp.* infection on the testosterone and progesterone hormones levels in male and female German Shepherd dogs respectively. Because testosterone can increase sexual behavior (increasing libido and matting) **Eisenegger et al., 2016** so its levels has great affects on fertility, in our study male dogs infested with ticks only without babesiosis showed significant decrease in testosterone hormone levels from that was recorded in group free from ticks and babesiosis, the same result was recorded by **Muller et al., 2013,** as they reported that tick infestation lowered the plasma testosterone levels and had a bad effects on the health status on the other hand we noticed that male dogs infested with ticks and babesiosis recorded highly significant decrease in testosterone levels than that was recorded in group free from ticks and babesiosis, these results could be explained by **Ubah et al., 2019** who reported that canine babesiosis was the most infertility risk factor in male dogs that cause testicular necrosis in the chronic cases, reduction of the blood flow causing testicular necrosis and impairing the process of spermatogenesis, also **Leisewitz et al., 2019** noticed that babesiosis infection in dogs caused inflammation effects and elevation of testicular temperature with impairment of testicular function leading to infertility also **Eichenberger et al., 2016** reported that there are

alterations in the serum biochemical profile of dogs infected with babesiosis.

Progesterone hormone in bitches is very important in reproduction, it is easy to be tested and used to estimate when the LH surge occurred, Progesterone level is baseline prior to the LH surge, it starts to elevate at the time of the LH surge, and keep rising, by time bitches ovulate is in the 4-8 ng/ml range as it was reported by **Angelika 2008**. In our present study bitchs infested with ticks only without infection with babesiosis showed non-significant decrease in serum progesterone levels than that was recorded in group free from ticks and babesiosis, which indicate the little effect of ticks infestation on its fertility, that is may be due to low infestation or good care and rapid eradication of ticks, while in bitches infested with ticks and infected with babesiosis showed highly significant decrease in serum progesterone levels than that was recorded in group free from ticks and babesiosis which indicate the high effect of babesiosis infection on its fertility, this may be due to general bad condition of infected bitches and alterations in the serum biochemical profile as it was reported by **Eichenberger et al., 2016.**

Conclusion:

Infestation with ticks (*Rhipicephalus sanguineus*) only has no or little effect on the fertility in German Shepherd dogs, while ticks associated with babesiosis can affect testosterone levels in male and progesterone levels in bitches so we pointed to infertility of dogs due to infection with external and blood parasites, it is the first time in Egypt to study the effect of ticks and *Babesia spp.* on fertility of German Shepherd dog. Additional research is needed to confirm findings.

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الملخص العربى

تأثير الطفيليات الخارجية و طفيليات الدم على الخصوبه في كلاب الراعي الألماني

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تهدف الدراسة الحالية لمعرفة تأثير الإصابة بالقراد والبابيزيا على الخصوبه في كلاب الراعي الالماني، تم فحص 80 كلب(39اناث و41 ذكور) في عيادات الحيوانات الاليفه بمديرية الطب البيطري بمحافظة الدقهلية فى مدينة المنصورة و السنبلاوين و الاعمار من سنتين الي ثلاث سنوات في صيف و خريف سنة 2021 و تبين إصابة 21 كلب (27.5%) بالقراد من نوع ربيسيفالس سانجويمس بدرجات إصابة مختلفة و كانت نسبة الإصابة في الذكور أعلى من الإناث حيث بلغت(29.2%) في الذكور و (23%) في الإناث. أيضا تبين إصابة 12 كلب (15%) بأعراض البابيزيا وهى الحمى ،النهجان ،اعراض الانيميا وشحوب الأغشية المخاطية والضعف والوهن والتأكد من الاصابات بفحص افلام شرائح الدم بالميكرسكوب و بلغت الإصابة (17%)في الذكور و (12.8%) في الاناث. بينما الفحص الكيميائي لعينات الدم لقياس مستوي هرمون التستسترون و البروجسترون لبيان تأثير الإصابة بالقراد والبابيزيا على الخصوبه أوضح أن هرمون التستسترون في الذكور المصابه بالقراد تراوح (2.1-3.2 ng/ml) و لكن تراوحت من (0.75-1.35 ng/ml) في الذكور المصابه بكل من القراد و البابيزيا ولكن (3.04-4.1 ng/ml)في الذكور الغير مصابه بأي من القراد أو البابيزيا. اما مستويات البروجسترون في الاناث المصابة بالقراد فقد تراوحت(2.5-3.4 ng/ml) و(0.20-0.79ng/ml) في الإناث المصابة بالقراد والبابيزيا، اما لغير المصابه تراوحت بين (2.9-3.4 ng/ml). هذا يوضح التأثير الضعيف للقراد على الخصوبه أما في حالة الإصابة بالقراد مع االبابيزيا فإنها تأثر على خصوبتها.