# INVESTIGATION ON TICK-BORNE THEILERIOSIS IN CATTLE WITH PARTICULAR EMPHASIS TO EFFECT OF IMMUNIZATION WITH SOMATIC ANTIGEN OF BOOPHILUS ANNULATUS TICKS ON TRANSMISSION AND PREVALENCE OF THEILERIOSIS

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### **ABSTRACT**

A total number of 177 cattle of different ages, sex and breeds were parasitologically and clinically examined for theileriosis and tick infestation in different localities of Kaluobia governorate. Blood samples and lymph node biopsies were taken from animals to demonstrate Theileria sp. Ticks collected from the Theileria-injected animals identified and examined for the presence of Theileria sp. Clinical signs exhibited on infected animals were described and discussed.

The epidemiological data revealed that the prevalence of theileriosis was 24.86% and it was higher in Friesian breed than native ones. The highest prevalence occurred in the age group between 3-5 years, while the lowest appeared in the age group less than 1 year. The prevalence was higher in females (26.03%) than males (19.36%), El-Kanatir El-Khairia was the district with the highest infection (36.36%). Theileria infection was higher in B. annulatus ticks (68%) and lower in H. anatolicum excavatum (26%). Adult engarged ticks were highly infected with Theileria (85%) while larvae were the least infected (28%). Trials of treatment using buparvaquone and long acting oxyletracycline were studied and discussed. Immunization of calves with somatic antigen of B. annulatus ticks reduced the prevalence of theileriosis.

It is axiomatic to recommend that control of theileriosis among Egyptian animals rely upon two folds, firstly treatment of recently injected animals by using novel chemotherapeutic buparvaquone which is very effective in the treatment of clinical cases and secondly control of ticks through immunization of cattle with vaccine prepared from somatic antigen of tick.

### INTRODUCTION

The primary problem for cattle industry in tropical and subtropical regions is tick and tick-borne infestation. Tick infestation may cause weakness of animals affecting their profitable productivity especially milk and meat production and transmission of certain tick-borne diseases such as theileriosis, anaplasmosis and babesiosis.

Theileriosis is a tick-borne disease affecting cattle and caused by a species of protozoa of the genus Theileria. Theileriosis is of greatest importance and can be transmitted trans-stadially by ticks and even few numbers of ticks may cause fatal infestation. (Losos., 1986; Coetzer et al., 1994 and Radostits et al., 2000).

Theileriosis is a summer spreading disease since the rate of infection was higher than the other seasons of the year, this is simply because theileriosis is transmitted by ticks which breed and flourish in the summer (Sharma, 1980 and Radostits et al., 2000).

In Egypt, all environmental conditions offer the chance of theileria species to breed and flourish and lacks of a clear control system for facing theileriosis causes calamitous effect of the disease (EL-Refall and Michael., 1976).

Theileriosis is clinically characterized by fever, anorexia, decreased milk yield, anemia, lymphadenopathy, respiratory distress, diarrhea, jaundice and corneal opacity. In endemic areas, virtually all adult animals are infected but the case fatality rate is low. However, the recovered animals have long lasting immunity but remain a carriers (EL-Sawalhy., 1987; Abel-Kader1991 and Mourad, 1999).

Control of tick infestation and transmission of tick-borne diseases remains a challenge for the cattle industry. As with other tick-borne diseases, control of the tropical theileriosis principally involves vector control and treatment of diseased animals (**Pipano.**, 1981; **Hungerford.**, 1990 and Coetzer et al., 1994).

The traditional control methods of tick infestation include the use of chemical acaricides with partially successful results. This method of control has certain disadvantages such as the presence of acaricide residues in meat and milk and the development of chemical resistant tick strains. Alternative approaches for tick control such as the use of natural host resistance and development of vaccines to induce immune protection against tick infestations have been conducted (Wambura et al., 1998 and Del la Fuente et al., 2000).

The use of buparvaquone (butalex) as a specific drug for treatment of cattle theileriosis has been recommended by many investigators (Abdel-Kader., 1991, Singh et al., 1993; Gul et al., 1991; Patil et al., 1995 and Mourad., 1999).

Substantial effort is now being placed towards the development of recombinant vaccines against blood parasites to replace the current method of vaccination with live attenuated strains (Callow, 1977). This method of vaccination has been successful for several years, but an increase, in the failure rate of the B. bovis vaccine was recently reported. It was suggested that these failures were due to changes in the composition of the parasite population through the selection of strains that evade the immune response induced by the vaccination (Bock et al., 1992).

Early experiments with immunization of cattle by antigens derived from ticks showed an effect on the transmission of babesiosis in vaccinated herds (Rodriguez et al., 1995c, De la Fuente et al., 1995 and Vanegas et al., 1995). Cattle immunized with Anaplasma marginale derived from tick cent culture did not display clinical anaplasmosis and this antigen showed promise for use as antigen in development of a new killed vaccine for anaplasmosis (Kocan et al., 2001).

Under field conditions, the transmission of babesiasis but not the anaplasmosis decreased after vaccination with the tick vaccine Gavac TM. The incidence of diseased animals caused by infestations with Babesia spp. was reduced after vaccination (De la Fuente et al., 1998).

This study most es three main parts, the first is investigation of cattle theileriosis in Kaluobia governorate, the second part includes therapeutic trials of infected animals and the third part deals with the effect of immunization of cattle with somatic antigen of B, annulatus ticks on the transmission and prevalence of theileriosis.

### MATERIAL AND METHODS

### 1- Investigation of cattle theilerlosis in Kaluobia governorate:

- A- Examination of investigated cattle: A total number of 177 cattle of different ages, sex and breeds were parasitologically and clinically examined for theileriosis and tick infestation during a period extended from April 2000 to March 2001 in different localities of Kaluobia governorate.
- B- Blood samples: Blood films prepared from blood taken from ear or tall veins were stained with Gleinsa stain to demonstrate Theileria sp (Coles. 1986).
- C- Lymph node biopsies. It were taken from cases manifesting peripheral lymphadenopathy and used for preparation of smears which stained with Giemsa stain (Coles, 1986).
- D- Examination of ticks for Thellena infection: Ticks collected from Theileria-infected animals are identified and examined for the presence of Theileria (Ochanda et al., 1996).

2- Therapeutic trials: 44 diseased animals were allocated into two groups, the first one treated specifically by bupar/aquone with recommended dose of 5 mg/kg, b/w (single dose of Butalex, I/M., Schering-Plough Animal Health); while the second group treated with long acting oxytetracyclines (20mg/kg, b/w, 1/V., Pfizer co., USA, 3 doses with 2 days intervals), the recurrent cases in both groups were taken the same regimens of therapy within one week of initial therapy, this beside supportive and symptomatic therapy (Losos., 1986; Sharma and Mishra., 1990 and Radostits et al., 2000).

# 3- Effect of immunization with tick antigen on prevalence of theilerlosis:

- A-Preparation of the tick antigen: Ticks used for preparation of the somatic antigen proved to be free from Theilerta infection. The somatic antigen was derived from B. annulatus ticks following the technique described by **Johnston et al.**, (1986). Adult ticks were picked up free of hair and washed several times in phosphate buffered saline (PBS) and then homogenized using a blender. The resultant supernatant was filtered to remove the tick enticles and then centrifuged at 5000 rpin for one hour. The protein concentration of the tick antigen was estimated by using the method described by **Bradford** (1976).
- B- Experimental design: A total number of 60 native calves were used to study the effect of immunization of animals with adult tick somatic antigen on the transmission and prevalence of thelleriosis. Animals were assigned into two groups; the first was the vaccinated and the second was the control, each group consisting of 30 animals. The vaccinated group received 1 mg of the antigen per animal (S/C), while the control one was injected in the same route with phosphate buffered saline. Three weeks postimmunization, all animals were challenged with infected larvae of B. annulatus ticks (5000/arvae/animal). The effect of vaccination on tick burden and fertility of ticks were determined (Khalaf-Allah and EL-Akabouy., 1996). As well, the effect of immunization on the prevalence of theileriosis was estimated before and after immunization with the tick antigen (De la Fuente et al., 1998). The experimental animals exposed to close clinical and parasitological investigation for 4 months post vaccination to evaluate the efficiency of the vaccine.

## RESULTS AND DISCUSSION

Theileriosis are a group of tick born diseases of cattle, water buffalo, sheep and goats, caused by species of protozoa in the genus Theileria. It is of greatest importance in veterinary medicine (Losos., 1986).

Examination of blood smears and lymph node biopsies prepared from diseased animals re-

vealed Theileria annulata piroplasms in erythrocytes and shizonts in lymphocytes of lymph node. Such findings were similar to those reported by **EL-Sawalhy.**, (1987) and **Mourad**, [1999]. The Giemsa staining method is considered to be the most suitable one for demonstrating parasites in smears (Coetzer et al., 1994).

On clinical investigation, the diseased animals showed tick infestation with fever, depression, decrease of appetite, respiratory distress, conjunctivitis with ocular discharges, characteristic enlargement of superficial lymph nodes namely prescapular and prefemoral lymph nodes, anemie or letric mucous membranes and emaciation. The observed symptoms were similar to those described by Shastri et al., (1982); EL-Sawalhy., 1987; Abdel-Kader., (1991); EL-Sayed and Rady., (1999) and Mourad, (1999) and Omey (2002). The lymphadenopathy usually related to the multiplication of theileria by schizogony in the lymphoid tissues, respiratory distress were due to pulmonary edema while progressive anemia and jaundice might related to hemolysis and appetite depression. These findings coincided with those of Coetzer et al., (1994).

The results shown in Table (1) pointed out that the prevalence of theileriosis was higher in Friestan breed (33.33%), followed by the cross-bred cattle (24.42%) while the native breed showed the lowest infection (21.88%). The increased susceptibility of Friestan breed may be attributed to the absence of premunition against the egyptian type of theileria. Such results nearly similar to that recorded by **Fadzil and Ragavon.**, (1986) and Abdel-Kader., (1991). Also table (1) cleared that the total prevalence of theileriosis as 24.86%. This finding was higher than that previously recorded by **Basslouny.**, (1980) and El-Sawalhy., (1987) but lower than that found by **Hassanin.**, (1984).

The results presented in Table (2) revealed that the highest infestation of cattle with theileriosis occurred in the age group between 1- <3 years where 40.9% of the total animals examined was positive for theileriosis. Friesian breed was the highest breed infested in this group and that between 3-5 years where the infection rate was 50% and 33.3%, respectively. The prevalence of infection was lower in animals lying in the age group less than one year where the infection rate was 9.4% for the total examined animals. Infection in animals lying in the age group more than 5 years were relatively lowered where the infection rate was 21.1% for the total animals examined in this age group.

The low susceptibility of calves below one year age might contributed to a state of premunition, also the frequent exposure to the disease create this phenomena in animals more than five years age resulting in the lower susceptibility. This result was in accordance to that pointed by Tutushin., (1981); Anandan and Lalitha., (1984) and Radostits et al., (2000). Moreover, Losos., (1986) reported that, young cattle are more resistant to theileriosis in areas where the dis-

ease is enzootic. The prevalence of theileriosis among female cattle was higher (26.03%) than in males (19.36%), as in table (3).

Concerning the geographical distribution of thelleriosis infection in different localities of Qaluobia governorate, the results displayed in Table (4) revealed that cattle in El-Qanatir El-Khayria
showed the highest infection (36.36%), this was followed by cattle in Tukh (35.29%) and the low
est infection was observed in cattle in Benha (12.5%). This variation may contributed to the tick
population in each locality which may be differed and/or favored according to the environmental
conditions and management systems as reported by **Habela et al.**, (1999). The current results
were nearly similar to that discussed by **EL-Sawalhy.**, (1987) and **Abdel-Kader.**, (1991).

It was found from the results shown in Table (5) that out of 177 examined animals, 132 were infested with ticks (74.57%). Boophilus annulatus was the most abundant tick spp. among the identified ticks eollected from thetleria-infested cattle (61.58%), followed by R appendiculatus (9.04%), whereas H. anatolicum, excavatum was the least abundant tick spp. (1.13%). The results presented in Table (6) revealed that B. annulatus was the common tick species infected with thetleria (68%), followed by Hyalomma anatolicum anatolicum (52%), Rhipicephalus appendiculatus (48.5%) and the least tick species found infected was Hyalomma anatolicum excavatum (26%), Such findings nearly similar to that mentioned by **Abdel-Kader.**, (1991); Mourad., (1999); Habels et al., (1999) and EL-Kammah et al., (2001), in this respect Pipano et al., (1982) reported that a single tick is capable of transmitting a fatal infection.

The data recorded in Table (7) showed that prevalence of theileriosis was higher in the engorged adult ticks where 85% of examined ticks were infected, whereas larvae were the least tick stages found positive for theileriosis as only 28% of examined larvae were infected. These results agreed with those reported by Young et al., (1995) and Sherriff and Gettinby., (1996) who recorded that adult fed ticks were highly infected with Theileria parva than the nymphs and the mean abundance of T. parva-infected salivary glands of infected ticks was higher in female ticks than in males.

Regarding the chemotherapy of the infected animals (Table 8), animals group treated by buparvaquone was clinically cured in a percent of 86.4% and showed recurrence in 13.6% of treated animals without emergency slaughtered cases while the group treated by oxytetracycline was clinically cured in a percent of 63.6% and showed recurrence in 22.7% of treated animals with emergency slaughtered cases.

The recurrent cases which occurred within one week of initial therapy treated as regimens of initial therapy, the first group successfully treated by second dose of buparvaquone while the recurrent cases in second group, only five cases clinically cured (22.7%) and three cases emer-

gency slaughtered (13.6%). These results were in agreement with that obtained by many workers as **Dahar et al.**, (1986); **Singh et al.**, (1993); **Patil et al.**, (1995), **Mourad.**, (1999) and **Radostits et al.**, (2000). The availability of a chemotherapeutic means of controlling theileriosis by buparvaquone proved, but there are two constraints against the widespread use of medication, firstly the drug is too expensive and a rapid accurate diagnosis is required for effective therapy. A two dose regimen of buparvaquone was most effective and should be recommended in therapy of theileriosis (**Dolan et al.**, 1992).

Immunization of cattle with the somatic tick antigen derived from B. annulatus was significantly (P<0.001) effective in reducing the mean tick burden in vaccinated animals (203.48  $\pm$  24.51) as compared to controls (695.73  $\pm$  72.90) with a 70.75% reduction in the mean tick count (Table 9). The immunization results in abnormal feeding of the ticks, which characterized by their white to pale-yellow colour instead of the dark-grey colour of normally fed ticks. This attributed to their inability to gain access to the blood vessels owing to the host immunological reaction at the bite sites, consequently, the ticks fed on extravascular fluid devoid of red blood cells resulting in their white to pale-yellow colour (**Sran et al.**, **1996**).

It was evident from Table (10) that immunization had a considerable effect on the fertility of ticks. The mean number of eggs laid per tick as well as the mean number of eggs hatched were significantly (P<0.001) lowered in vaccinated animals as compared to controls. The current data nearly similar to that obtained by **Rodriguez et al.**, (1995 a & b) who mentioned that the effect of vaccine on Boophilus tick infestations is influential and vaccination of cattle with vaccine controls the tick numbers in successive generations. Also **Ghosh and Khan.**, (1999) recovered abnormally fed larvae and nymphs with significant rejection of larvae and nymphs from calves immunized against Hyalonima anatolicum a vector of tropical theilcirosis using larval antigen.

The results presented in Table (11) showed that immunization of calves with somatic antigen of B, annulatus ticks reduced the theileriosis where 9 out of 30 animals was positive before immunization as compared to non-vaccinated controls. This figure was reduced from 9 before immunization to be 5 after immunization with 44.44% reduction in theileria infection. This result documented the efficacy of immunization of cattle with tick antigen as a means of control of tick-borne theileriosis. The obtained results were nearly agreeable to that reported previously (Khalaf-Allah and EL-Akabouy., 1996; Canales et al., 1997 and De la Fuente et al., 1998). Moreover Mahoney et al., (1981) found that in a farm with instability for tick-borne diseases, a decrease in the number of ticks will further reduce the inoculation rate, thus lowering the number of clinical cases. FAO, (1984) reported that, the decrease in the number of clinical cases after immunization suggests that the vaccine acts on the larval stages of Boophilus microplus ticks and particularly on larvae three days after their attachment to the host.

Although vaccination has proved to be effective in controlling tick populations and tick-borne diseases in the field, extensive studies involving tick damage and the correlation between the antibody titers elicited due to immunization and the physiological status of animals have not been reported. These studies, together with economic evaluation of vaccination are required to support the application of the vaccine (**De is Fuente et al., 1998**).

Theileriosis poses a persistent threat to eattle in Egypt and it have severe depressant effects on the immune system; therefore, it is recommended that any vaccinations must be delayed until the animals have recovered. It is axiomatic to recommend that, control of theileriosis among Egyptian animals rely upon two folds, firstly treatment of recently infected animals with bupar-vaquone and secondly control of ticks through immunization using vaccine prepared from the highest prevalent tick population in the locality.

Table (1): Prevalence of theileriosis among the different breeds of examined cattle

Cattle breed	Prevalence of theileriosis				
	No. of animals examined	No. of +ve	Prevalence %		
Friesian	2.7	9	33,33		
Cress-bred*	86	21	24,42		
Native	64	14_	21.88		
Total	177	44	24.86		

<sup>\*</sup> Friesian x Native

Table (2): Prevalence of theilerisois among the different age groups of examined animals

Cattle breed					A	ge gro	up of	cattle				
	< 011	e yea	ľ	1 -<	3 yea	rs	3 - 5	years	years	More	tha	in 5
•	No.	+ve	%	Na.	+ve	%	No.	+ve	%	No.	+ve	%
Friesian		_		4	2	50,0	3		33.3	20	6	30
Cross- bred*	25	2	8.0	20	9	45.0	19	5	26.3	22	5	22.7
Native	7	1	14.3	20	7	35,0	22	5	22.7	15	1	6.7
Total	32	3	9.4	44	18_	40.9	44	11	25	57	12	21.1

<sup>\*</sup> Friesian x Native

Table (3): Prevalence of theilerisois in males and females examined animals.

Sex	Prevalence of theileriosis in animals					
	No. examined	No. +ve	% +ve			
Females	146	38	26.03			
Males	31	6	19.36			

Table (4): Prevalence of theileriosis infection in different localities of Qaluobia governorate

Locality	Prevalence of theileriosis in cattle				
	No. examined	No. +ve	% +ve		
Shibin El-Qanatir	28	8	28 57		
El-Gabal Asfer	41	7	17.07		
Tukh	17	6	35.29		
Qalioub	34	8	23,53		
Benha	24	3	12.50		
El-Qanatir El-Khairia	33	12	36.36		

Table (5): Different tick species isolated from theileriosis-infested animals.

Prevalence of tick species on infested animals				
No. examined	No. infested	%		
	109	61.58		
1	16	9.04		
1	5	2.82		
'	2	1.13		
177	132	74.57		
	Prevalence of t	No. examined No. infested 109 16 5 2		

Table (6): Prevalence of theileriosis in examined ticks collected from infested animals.

Tick species	Prevalence of theileriosis in examined ticks			
	No. examined	No. +ve	% +ve	
Boophilus annulatus	300	204	68.0	
Rhipicephalus appendiculatus	200	97	48.5	
Hvalomma anatolicum anatolicum	100	52	52.0	
Hyalomma anatolicum excavatum	50	13	26.0	
Total	650	366	56.3	

Table (7): Prevalence of theileriosis in various tick stages collected from infested animals.

Tick stages	Prevalence of thieleriosis in various tick stages				
	No. examined	No. +ve	a/u +∧6		
Larvae	50	14	28		
Nymphs	50	21	42		
Adult unfed	100	60	60		
Adult engorged	100	85	85		

Table (8): Effect of treatment of infected animals by buparvaquone and long acting oxtytetracycline.

Treated animals Type of treatment Recurrent Clinical cured cases Recurrent cases and cases and cured emergency slaughtered T. No. of No.of % No % % No treated cured 86.4 First group(treated by 22 19 3 13.6 0.0 0.0 buparvaquone) Second group (treated 22 14 63.6 22.7 3 13,6 by long acting oxytetracycline)

Table (9): Effect of immunization of calves with somatic antigen on the population dynamics of B. annulatus ticks.

Treatment	Mean tick count ( S. E.)	Reduction % in tick count
Vaccinated	$203.48 \pm 24.51^a$	
animals		70.75
Controls	695.73 ± 72.90b	

Data in columns with unlike superscripts indicate significant difference (P<0.001)

Table (10): Effect of immunization of cattle with somatic antigen on fertility of

female adult engorged B. annulatus ticks.

	congot god an antimora	PED CICIES,		
Statement	Mean No. of eggs laid / tick (± S. E.)	in egg laying	Mean No. of eggs hatched / tick (± S. E.)	
Vaccinated animals	$263.82 \pm 48.01^{2}$	72.93	$168.43 \pm 34.60^{a}$	80,48
Controls	974.56 ± 139 52b	_	862 91 ± 162 93b	

Data in columns with unlike superscripts indicate significant difference (P<0.001)

Table (11): Effect of immunization of calves with somatic antigen of B. annulatus

ticks on prevalence of theileriosis.

Treatment	Prevalence of theileriosis							
	Befor	Before immunization 4 months post immunization					Reduction % in prevalence	
	No.	+ve	%	No.	+ve	%		
Immunized	30	9	30.00	30	5	10.00	44.44	
Controls	30	11	36.67	30	13	43.33		

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# اللة ص العربي

بحث عن مرض الثاليريا في الماشية وبالأخص توكيد تأثير التحصين باللقاح المحضر من الأنتجين الخلوى لقراد البوفيلس على إنتقال ومعدل الإصابة بالثاليريا

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مرض الثاليريا من الأمراض الطفيلية التي تؤثر على إنتاجهة الماشية في مصر لذا فقد إستهدف هذا البحث محاولة التعرف على وبانية المرض والصورة الإكلينيكية لدني مناطق مختلفة محافظة القليوبية بالإضافة إلى المقارنة العلاجية بين إستخدام ألاوكسي تبتراسيكلين والبيوتالكس وكذا محاولة تحضير لقاح من الأنتجين الخلوي لقراد البوفيلس أنبولاتس ودراسة مدى فاعليته على إنتقال ومعدل الإسابة بالثاليريا، تم عمل مسح شامل لعدد ١٧٧ من الماشية المختلفة في الأعمار والسلالات والأجناس بواسطة الذحص الإكلينيكي والطفيلي لعينات الدم والغدد الليمفاوية السطحية المنضخمة وذلك على مدار عام كامل (إبريل ٢٠٠٠ مارس ٢٠٠١) ووجد الآتي : ١- كان أهم الأعراض الإكلينيكية هو إرتفاع في درجة الحرارة ووجود إحتقان والتهابات في العين وكذلك تضخم في الغدد الليمفاوية السطحية بدرجات متفاوتة كعا أظهرت بعض الحيوانات أعراض تنفسية ونقص في الشهيبة وهزال شديد بالإضافة إلى وجود أنيميا واصفرار في بعض الأغشية المخاطبة. ٢- النسبة الكلية للإصابة كانت (٢٤ر٢٤٪) وكان معدل الإصابة في الأبقار الفريزيان كانت أعلى (٣٣ر٣٣٪) عنها في الأبقار الخليط (١٥ر٤٤٪) والمحلية (٨٨ر٢١٪). نسبة الإصابة كانت منخفضة نسبباً في الحيوانات الصغيرة الأقل من عام (٤ر٩٪) بسما إرتفعت في الحيوانات ذات العمر من سنة إلى ما أقل من ثلاث سنوات (٩٠٤٪) ثم عباودت الانخفاض في المسرانات ذات الأعبمار من ثلاث إلى خمس سنوات (٢٥٪) والحيوانات التي جاوزت الخمس سنوات (١ر٢١٪) هذا وقيد وجد أن نسبة الإصابة في الاناث والذكور كانت ٢٦٠٠٣٪ و ٢٦ر ١٩ ٪ على الدرالي. ٢- وجد أن أعلى معدل للإصليدين المناطق المختلفة لمحافظة القليوبية كانت في القناطر الخبرية وسجلت ٣٦ر٣٦٪ بينما كانت أقلها في بنها وسجلت ١٢٥٠٪ وكانت نسبة الإصابة بقراد البوفيلس أنبولانس هو أعلى معدل لإصابة الحيوانات بالأنواع المختلفة من القراد وسجل ٥٨ر٦١٪، عند الفحص الطفيلي لأنواع القراد المختلفة لطفيل الشابلريا وجد أن أعلى نسبة إصابة كانت في قراد البوفيلس أنبولاتس (٦٨٪). الفحص الطفيلي للأطوار المختلفة للقراد تبين أن أعلى معدل إصابة كانت في القراد اليافع المتغذى (٨٥٪). ٣- عند مقارنة علاج الحيوانات المصابة بكلا من البيوتالكس والأوكسي تتراسيكلين وجد أن المجموعة المعالجة بالدواء الأول قد تم شفاء ١٩ حيوان (٤,٨٦٪) شفاءاً كاملاً بيد أنه حدثت إنتكاسة مرضية أخرى في ثلاث حيوانات (١٣٦٦٪) تم علاجهم وشفائهم عند تكرار العلاج مرة أخرى، بينما الحيوانات المعالجة بالعقار التّاني قد تم شفاء ١٤ حيوان (٢ر٦٣٪) وحدثت إنتكاسه في

عدد ٥ حيرانات (٧٢٧٪) وتم شفاء أربع حيوانات منهم فقط عند تكرار العلاج لمرة أخرى بينما تم ذبح أربع حيوانات (٢٦٣١٪) ذبحاً إضطرارياً. ٤- تم تحضير لقاح من الأنتجين الخلوى لقراد البونيلس أنبولاتس وتم حقن هذا اللقاح في عدد ثلاثون عجلاً بجرعة ١مجم/عجل تحت جلا الرقبة هذا بالإضافة إلى ثلاثون عجل أخرى كمجموعة ضابطة غير محصنة. بعد ثلاث أسابيع من التحصين تم عمل عدوى إصطناعية وذلك باستخدام عدد ١٠٠٠ يرقة مصابة بطفيل الشابلريا / حيوان وقد تم وضع الحيوانات تحت الملاحظة لمدة الدراسة (٤ شهور) وتعرضت هذه الحيوانات للفحص الإكلينيكي والطفيلي طول مدة الدراسة. أظهرت النتائج إلى أن متوسط العدد الكلي لقراد البوفيلس أنيولاتس كان أقل في المجموعة الضابطة بنسبة ٥٧٠٠٪ وكان معدل وضع القراد للبيض وكذا فقسه كان أقل في المجموعة الضابطة بنسبة على التوالي، أيضاً وجد أن معدل الإصابة بطفيل الثاليريا في المجموعة أنل عنها في المجموعة الضابطة بنسبة ١٤٤٤٪.

النتائج النهائية لهذا البحث تشير إلى أن السيطرة والتحكم في موض الثاليريا يجب أن يتم في محورين أساسين أولهما علاج الحيوانات المصابة بعقار البيوتالكس حيث أثبت كفاءة عالية في العلاج وثانيهما هو نصنيف الغراد الناقل للمرض في المنطقة المويزه بالمرض ومحاولة عمل لقاح منه إلا أنه بالرغم من أن اللقاح المحضر من البوفيلس أنيولاتس أثبت كفاءة في إنخفاض متوسط العدد الكلي للإصابة بالقراد وكذا معدل وضع وفقس البيض للقراد بالإضافة إلى إنخفاض معدل الإصابة بالشايليا في الحيوانات المحصنة عنها في المجموعة الضابطة إلا أنه بنصح بعمل دراسات المحتقبلية متقدمة على مستوى محافظات مصر حتى يمكن تطبيق واستخدام ذلك في الحقل البيطري.