2nd Sci. Congr. of Egypt. Soc. Anim. Mang., 29-31 Oct.(2007):5-23

# IMMUNIZATION OF RABBITS AND CAMELS AGAINST *HYALOMMA dromedarii* TICKS USING WHOLE ADULT TICK ANTIGEN

By

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## SUMMARY

Ten rabbits of 2 months old, as well as ten young camels aged between 8-10 months old were used to evaluate the immunizing effects of whole adult *Hyalomma dromedarii* antigen under laboratory and field conditions. A vaccine was prepared from male and female of such ticks, used for vaccination of rabbits and camels. Animals within each species were allocated into two groups each consisting of five animals. The first experiment was done under laboratory conditions in which five rabbits administered with two immunization, the first one was given subcutaneously at the beginning of the experiment and the second was given 12 days later. At the same time, rabbits of control group were injected with phosphate buffer saline (PBS) plus adjuvant. All rabbits in the two groups were challenged with starved adult tick *Hyalomma dromedarii* 7 days after the second injection. The second experiment was done under field conditions where five young apparently healthy camels given two immunization, the first was administered subcutaneously at the beginning of the experiment and the second dose was injected three weeks later. At the same time another five appearently healthy young camels were injected with PBS plus adjuvant. The results in the first experiment revealed that there was reduction in number and weight of ticks that fed on all immunized rabbits. Also the number of oviposit female ticks, number of eggs laid per tick and the percentage of egg laid per tick and the percentage of egg hatchability were reduced. Concerning the results of the second experiment, it was noticed that the vaccination of animals resulted in 40.04%, 76.91% and 85.56% reduction in mean values of tick weight, oviposition and egg hatchability, respectively.

## INTRODUCTION

Camels were placed in most desert countries as they constitute the main animals of the carven which connected the old world, so it is called ship of desert in most Arabian countries. Accordingly camel breeding received high attention as the majority of the farmers depend upon its products like meat, leather and milk

and as well as transportation of their crops. The most abundant species of ticks on the camels were *Rhipicephalus pulchellus, Hyalomma dromedraii, Amblyomma gemma* and *Ablyomma variegatum* **(Zeleke and Bekele, 2004)**

Camels usually exposed to infestation by ticks which may not only of direct cause of certain diseases, but also it adversely affect the general condition and predispose to other diseases such as thaileriasis, babesiasis and anaplasomosis **(Yeoman and Walker, 1967; Kettle 1995, Higgins, 1984 )** . Ixodid ticks, are blood sucker arthropods cause debility and anaemic condition, irritation, traumatic injuries and even death in camels **(Steward, 1950).**

The control of tick still depends mainly on intensive use of acaricides. However, these traditional control methods have been only partially successful and the parasite continue to result in significant losses for animals **(Del La Fuente, et al., 1999).** Also chemical control become increasingly difficult due to the emergence of acaricides resistant strains of ticks as well as pollution of the environment and toxicity to man and animals **(Allen and Humphryes, 1979 and Roulston et al., 1981)**. Alternative measures using biological control such as pasture spilling and tick resistant animals species can reduce tick population **(Wharton and Norris, 1980).**

Enhancement of host resistance by immunization against ixodid ticks would constitute a novel and major advance in control. Some success with vaccination have been achieved the ticks *Dermcenter andersoni* by **Allen and Humphreys (1979),** *Amblyomma americanum* by **McGwan et al., (1981),** *Rhipicephalus appendiculatus* by **Abd EL-Aal et al. (2001)** and *Hyalomma dromedarii* by **Ramadan (1997)** and *Hylamma anatolicum anatolicum* by **Singh and Ghosh**

**(2003).** So it is needed to carry out some immunization experiments against the most common ticks in Libya and production of specific vaccine against such ticks.

## MATERIALS AND METHODS

### I-Ticks:

Ticks were collected from infested camels at different areas in Wady EL-Hyat Governorate, Libya. The collected ticks were poured into Petri disches, separated from foreign materials, spread on filter papers to absorb excess liquid and finally placed on clean filter paper on the stages of stereobinocular microscope (**Hoogstraal, 1965**). *Hyalomma dromedarii* ticks were isolated and collected in clean dry test tubes for antigen preparation.

### II- Antigen Preparation:

Mixture of male and female *Hyalomma dromedarii* antigen was prepared according to method described by **Mongi et al. (1986)** as follow: Two hundreds partially fed adult females plus three hundred partially fed males of *Hyalomma dromedarii* ticks were washed in distilled water and surface sterilized by immersion in 70% ethanol. Then ticks allowed to dry at room temperature and then homogenized in 5ml phosphate buffer saline (PBS) pH 7.4 containing 60µg sodium penicillin plus 100µg neomycin sulphate per mL. then sonicated for 30 seconds in an ice bath. The suspension obtained was filtered by clean dry filter paper in Buchner's funnel to remove the tick cuticle, and then centrifuged at 5000 r/m for 5 minutes. The protein concentration of the supernatant was estimated with the method of **Lowery et al., (1952)** or **Millers (1958)**. The supernatant fluid was stored at -20 °C until used.

**III. Vaccine preparation:**

The vaccine was prepared by mixing antigen of *Hyalomma dromedarii* with Freund's incomplete adjuvant at a ratio of 1:1 **(Wikel, 1981, Das et al. 1993 and El-Kelesh , 1999).**

## IV. Experimental design:

**IV.I Experiment I**

The experiment was carried out to study the effect of a mixed soluble extract vaccine produced from both males and females *Hyalomma dromedarii* on the biology of adult ticks.

**IV. 1.1 Animals :**

Ten apparently healthy rabbits (free from external and internal parasites) of 2 months old were used. The rabbits were kept in clean dry hygienic cages and feed on balanced ration and supplied with clean source of water during the period of the experiment. The rabbits were divided into two groups each of 5 animals.

### IV.1.2 Vaccination:

All rabbits in the first group were inoculated subcutaneously by 0.1mL of vaccine containing 10µg protein. A boster dose was given 12 days later. The second groups received phosphate buffer saline (PBS) plus adjuvant and Kept as a control **(Ramadan 1997).**

### IV. 1.3. Challenge

The nymphs of *Hyalomma dromedarii* were collected and kept in incubator at 27C° and 90% relative humidity until moulting. The moulted adult *Hyalomma dromedarii* ticks were kept under starvation for 3 days, then used for challenge.

Each animal of both groups was challenged with 16 starved adult *Hyalomma dromedarii* at 7 days after the booster dose by using ear bag method (160 starved adult *Hyalomma dromedarii* for all animals in the two groups). The ticks were allowed to feed until engorgement as described by **Allen and Humphries (1979).** The attachment period of challenged ticks were recorded. The number of attached unfed, partially fed and fully engorged ticks as well as the number of dead ticks were counted. The dropped female ticks were collected counted, weight and kept at 27°C and 90% R.H. for oviposition. The number of eggs laid by each female as well as the rate of hatchability and fecundity for each group were recorded.

## IV.2. Experiment II

### IV.2.1 Animals:

Ten apparently healthy young camels about 8-10 months old with no previous exposure to tick infestation were used in the study. Animals were housed in clean place until the onset of vaccine injection, after which they were turned out to the pasture to acquire natural tick infestation. Animals were allocated into two group, each consisting of 5 young camels.

### IV.2.2 Vaccination:

The vaccination protocol involved two subcutaneous immunization with 1mg of soluble protein antigen plus Freund's Incomplete adjuvant. The second vaccination was given 3 weeks after first one. The second group (control group) received PBS plus adjuvant.

### IV.2.3. Tick fecundity:

The number of adult ticks attached to the right side of both vaccinated and control animals were counted before immunization and every week thereafter and multiplied by two to get the total number of ticks **(Nolan et al., 1981).** One

hundred ticks, from vaccinated camels along with an equivalent number of ticks from control camels were collected and weighed. The female ticks were incubated in clean dry test tubes at 27°and 90% R.H. to monitor oviposition and hatching of larvae. After about two weeks when egg laying was completed individual egg masses were placed in test tubes plugged with a gauze to monitor egg hatching **(Kalaf-All and EL-Akabawy 1996).**

**Table (1):** Effect of vaccination on attachment, engorgement and body weight of *Hyalomma dromedarii* ticks in rabbits

| Treatment | Means No. of challenged ticks | | No. of attached ticks | %of attachment | Period of attachment | Unfed ticks | | | | Partially fed ticks | | | | Fully engorged female ticks | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Number | | Weight (mg) | | Number | | Weight (mg) | | Number | | Weight (mg) | |
| | M | F | | | | T | % | Total | Mean | T | % | T | Mean | T | % | Total | mean |
| Vaccinated group | 40 | 40 | 48 | 60 | 2.12 days | 17 | 35.41 | 452 | 26.59 | 25 | 52.08 | 2120 | 84.8 | 6 | 12.5 | 917 | 152.8 |
| Control group | 40 | 40 | 71 | 88.75 | 2-9 days | 3 | 4.22 | 87 | 29.0 | 50 | 70.42 | 8117 | 162.3 | 18 | 25.3 | 4420 | 245.6 |

T = TOTAL = Male F = Female

Table (2): Effect of vaccination on the biology of *Hyalomma dromedarii* ticks

| Treatment / Parameters | Vaccinated rabbits | Non vaccinated rabbits |
|---|---|---|
| Number of attached males | 21(52.5%) | 38(95%) |
| Number of attached females | 27(67.5%) | 33(82.5%) |
| Number of oviposit females | 4 | 22 |
| %of oviposit females | 10% | 55% |
| Pre-oviposition period (days) | 10-15 | 6-9 |
| Oviposition period (days) | 10-12 | 12-15 |
| Average number of eggs | 380-1121 | 2112-7150 |
| Means number of eggs/batch | 912±217.3 | 4811.3±451.2 |
| Number of hatched egg batches | 1 | 18 |
| %of hatchability | 25% | 81.82% |
| Number of dead ticks | 32 | 9 |
| Mortality % | 40 | 11.25 |

Number of challenged ticks = 40 males +40 females *Hyalomma dromedarii* /group.

**Tables (3):** Mean number of *Hyalomma dromedarii* ticks on vaccinated and non-vaccinated young camels.

| Treatment | Average number of *Hyalomma dromedarii* ticks | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Weeks after vaccination | | | | | | | |
| | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 |
| Vaccinated group | - | 121.2±17.5 | 98.5± 12.5 | 56.2±11.5 | 27.4±8.4 | 14.5±2.5 | 8.5±1.2 | 6.2±1.5 |
| Control group | - | 165.3±22.3 | 195.2±18.5 | 235.5±25.2 | 361.2±31.5 | 418±57.5 | 352.5±40.2 | 287.5±51.6 |

**Table (4):** Effect of vaccination on tick weight, number and hatchability of eggs laid from *Hyalomma dromedarii* ticks fed on vaccinated and control camels.

| Treatment | Tick weight (mg) | | Number of eggs | | Number o\f hatched eggs | |
|---|---|---|---|---|---|---|
| | Mean ± S.E | reduction % in tick weight | Mean ± S.E | reduction % in egg laying | Mean ± S.E | reduction % in hatchability |
| Vaccinated group | 141.2±31.5[a] | 40.04 | 718.5±62.2[a] | 76.91 | 362.5±37.3[a] | 85.56 |
| Control group | 235.5±25.3[b] | | 3112.5±87.5[b] | | 2510.5±77.4[b] | |

Columns with unlike superscript are significantly ($P<0.05$) differ

## RESULTS

The results displayed in Table (1) indicated that the percentage of attached ticks was 60% and 88.75% in the vaccinated and control groups, respectively. The period of attachment was prolonged in vaccinated rabbits (12 days) compared to control rabbits (9 days) added to that , there was a reduction in weight of ticks especially engorged female ticks on vaccinated rabbits (152.8mg) compared to control rabbits (245.6mg).

Concerning the effect of vaccination on biology of *Hyalomma dromedarii* ticks, the results present in Table (2) showed that , there was a reduction in the number of oviposit female ticks that challenged on vaccinated rabbits (10%) compared with non-vaccinated rabbits (55%). The preoviposition period for such female ticks was prolonged in vaccinated rabbits (10-15 days) than in non-vaccinated rabbits (6-9 days). The oviposition period of ticks fed on immunized rabbits was short, as well as the egg number of each batch was greatly reduced. The average number of eggs produced by female ticks on vaccinated rabbits was greatly reduced (380-1121 eggs) compared with controls (2112-7150 eggs).

From aforementioned Table (2) , the results also revealed that only one egg batch from ticks, collected from immunized rabbits was hatched (25%) compared to 18 egg batches of ticks collected from control rabbits (81.82%). There was high mortality percentage among ticks challenged on vaccinated rabbits (40%) compared to those tick challenged on control rabbits (11.25%).

Table (3) revealed that at the time of vaccination (Day 0), there was no ticks on both vaccinated and control camels. Three weeks post-vaccination the means

tick burden was 121.2±17.5 and 165.3 ±22.3per animal in vaccinated and control animals, respectively. Six weeks Post vaccination, the tick count began to decrease in vaccinated animals (98.5), while it could be increased in control animals. At the end of the experiment (21 weeks, after immunization) the mean count was 6.2 and 287.5 per animal in vaccinated and control camels, respectively.

The obtained data in Table (4) showed that the mean weight of adult ticks collected from immunized camels (141.2±31.50 mg) was significantly lower than that of ticks collected from control camels (235.5 ± 25.3 mg) and the reduction percentage in the mean tick weight was 40.04%. The overall mean number of eggs laid was 718.5 ± 62.2 and 3112.5 ± 87.50 eggs per tick collected from vaccinated and control camels, respectively with a reduction% of 76.91% in egg laying capacity. The overall mean number of larvae that hatched from eggs of ticks collected from vaccinated camels was 362.5 ± 37.3 as compared to 2510.5 ± 77.4 larvae hatched from eggs of ticks collected from control camels. The reduction % in egg hatchability was 85.56%.

**DISCUSSION**

Immunization of rabbits using whole tick extract prepared from both males and females *Hyalomma dromedarii* could induce protective immunity against such ticks. The degree of immunity produced was reasonable and measured by a reduction in number ad weight of challenged ticks and increased the attachment period of ticks on vaccinated rabbits compared to control rabbits. The obtained results were in accordance with that recorded by **Fatma Abd El-Halim (1991)** and **Ramadan (1997)** who found that the attachment percentage of *Hyalomma dromedarii* was reduced , while the attaching and

feeding period were prolonged when fed on immunized rabbits against such ticks. Similar results were recorded by **Binta et al. (1985), El-kelesh (1999)** and **Abd EL-Aal et al. (2001)** who found that females ticks *Rhipicephalus appendiculatu* feeding on immunized rabbits against such ticks take longer time to engorge than on non-vaccinated rabbits. Rabbits immunized with homogenates of unfed larvae and nymphs of the tick *Rhipicephalus sanguineus* developed significant level of protective immunity to infestation with all the stages of the species. The acquisition of resistance was based on feeding and developmental performance parameters (rejection, moulting percentage and feeding period) of larvae and nymphs (**Tripathi et al. 1998**). Added to that **Wikel (1979)** attributed that to the effect of basophiles and homocytotrophic antibodies of skin which were responsible for prevention of tick feeding on resistant hosts.

The same data revealed that there were a decrease in the number and weight of fully engorged tick that fed on vaccinated rabbits. Nearly similar results were recorded by **Fatma Abd El-Halim (1991)** and **Ramadan (1997)** who found that the weight of engorged females *Hyalomma dromedarii* was reduced when fed on immunized rabbit against such ticks. Added to that **Ngau and Nyindo (1987)** and **Nyindo et al.(1989)** found a reduction in the engorgement weight of *Rhipicephalus appendiculatus* that fed on repeatedly infested rabbits by such ticks.

Concerning the effect of vaccination on the biology of *Hyalomma dromedarii*, the obtained data revealed a reduction in the number of oviposit females, egg output and oviposition period , while the pre-oviposition period was prolonged , in addition, low hatchability % and high mortality % among ticks

engorged from vaccinated rabbits. About this point. **Fatma Abd EL-Halim (1991)** and **Ramadan (1997)** and **Kumar & Kumar (1995)** recorded that preoviposition period of adult female *Hyalomma dromedarii* was prolonged while oviposition period was reduced when fed on immunized rabbits against such ticks and also a reduction in hatching % of larvae from eggs. Also, **Allen and Humphreys (1979)** found that *Dermacentor andersoni* females produced few or no eggs when allowed to fed for two weeks on guinea pigs injected intradermally with antigenic extract of midgut and reproductive organs or from all internal organs of the ticks and the larvae failed to hatch from eggs that were laid. Added to that **Binta et al. (1985)** and **Abd EL-Aal et al.(2001)** recorded that hatching of eggs from females *Rhipicephalus appendiculatus* that fed on immunized rabbits against such ticks was lower than control ticks. **Moreover, Andreotti et al. (2002)** and **Jittapalpong et al. (2004)** found that cows immunized against *Boophilus microplus* showed a decrease in tick oviposition, egg mass weight engorged female tick weight and reduction in the mean tick count as compared to control animals.

**Agbede and Kemp (1986)** attributed those findings to gut damage and the digest cells were sloughed off leaving the basal lamina. Such damage allowed host leukocytes to enter haemocael and attack tick muscles, malpighian tubules and blocking synthesis of vitellogenesis by gut cells.

The results reported under field conditions, show that immunization of young camels against *Hyalomma dromedarii* ticks, is feasible. The degree of immunity produced was reasonable and measured by a reduction in tick population and tick weight. Theses obtained data confirm the results of **Abd EL-Aal et al. (2001)** who found that calves immunized with whole adult tick

extract of *Rhipicephalus appendiculatus* developed significance levels of protective immunity to infestation with adult ticks of this species and the mean engorged weight of the female ticks feeding on vaccinated animals was reduced by 39.69% compared to that of female ticks feeding on control calves. In addition cattle immunized with extract derived from adult *Boophilus microplus* could be develop protective immunity against such ticks (**Kemp, et al. 1986; Opdebeeck et al. 1988 and Panda et al. 1993**). Similar results were obtained by **Johnston et al. (1986)** who found that injection of cattle with crude adult Boophilus ticks reduced the tick population on vaccinated cattle by 70% as compared to that of controls. **Added to that Sharma et al. (2001), Das et al. (2000), Das et al. (2005), Ghosh et al. (2005) and Singh & Ghosh (2003)** found that animals immunized against *Hyalomma anatolicum anatolicum* showed a significant decrease in number of ticks as compared to control animals.

**Morevover, Kalaf-Allah and EL-Akabawy (1996)** injected cross –bred calves with crude adult *Boophilus annulatus* ticks , and found that 73.9 reduction in number of adult female ticks, compared to 36.87% in control group. Added to that **Frisch (1999)** mentioned that a vaccine against *Boophilus microplus* confer partial long-term control but has little immediate effect on tick burdens. The reduction in tick weight collected from vaccinated animals than those collected from non-vaccinated ones, indicate that some immune reactions had been developed by the host which cause damage to ticks gut, and this damage prevents the ticks from feeding properly.

Immunization of young camels with whole adult tick extracts, significantly reduced the reproductive capacity as demonstrated by the reduction of the

average number of eggs laid and their hatchability. Similar results obtained by **Panda et al. (1993)** and **Abd EL-Aal et al. (2001) and Banergee et al. (2003)** who found that calves immunized with adult tick antigen rejected more adults and the attached ticks had poor reproductive performance. In general, vaccination can lower tick fertility and a consequence the number of developing ticks is reduced and thus the tick population might be effectively controlled.

For conclusion the whole adult tick extracts prepared from *Hyalomma dromedarii* ticks can be used in the experimental induction of immunity to such ticks. Also the antigen has potential as a vaccine in endemic areas under field nditions and consequently reduce the economic losses due to tick infestation the lowest level.

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# تحصين الأرانب والجمال ضد قراد الهيالوما دروميدراي باستخدام مولدات المضاد المستخلص من القراد البالغ

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أجريت هذه الدراسة على عدد عشرة أرانب وعشرة جمال سليمة صحياً وخالية من الإصابة بالقراد ، وذلك لدراسة تأثير تحصين هذه الحيوانات بمولدات المضاد المستخلص من خليط من ذكور وأناث القراد البالغ الهيالوما دروميدراي . تم تقسيم الأرانب والجمال إلى مجموعتين كل مجموعة تتكون من عدد خمسة حيوانات .

تم أجراء التجربة الأولى على الأرانب تحت الظروف المعملية حيث تم حقن حيوانات المجموعة الأولى مرتين بمولدات المضاد المستخلص من القراد حيث كانت الجرعة الأولى عند بداية التجربة عن طريق الحقن تحت الجلد وكانت الجرعة الثانية بعد مرور 12 يوم من الجرعة الأولى ، وفى نفس الوقت تم حقن المجموعة الضابطة من الأرانب بمحلول الملح الفوسفاتى المنظم. تم أجراء تجربة التحدى ضد القراد على جميع الأرانب فى المجموعتين بعد مرور 7 أيام من الجرعة الثانية وذلك عن طريق العدوى باستخدام القراد البالغ الجائع.

أجريت التجربة الثانية على الجمال الصغيرة تحت الظروف الحقلية حيث تم حقن حيوانات المجموعة الأولى مرتين بمولدات المضاد حيث أعطيت الجرعة الأولى عند بداية التجربة ، أما الجرعة الثانية فكانت بعد مرور ثلاثة أسابيع من الجرعة الأولى، وفى نفس الوقت تم حقن حيوانات المجموعة الضابطة بمحلول الملح الفوسفاتى المنظم ثم اعيدت الحيوانات فى المجموعتين إلى المناطق الموبوؤه بالقراد.

أثبتت النتائج الموضحة فى هذه الدراسة أن تحصين الأرانب بمولدات المضاد المستخلص من القراد البالغ قد أدى إلى تقليل عدد، ووزن القراد المتواجد على الحيوانات المحصنة كما أن عدد إناث القراد التى تبيض قد قل وان عدد بيض هذا القراد ونسبة الفقس قد تناقص بدرجة كبيرة مقارنة بالنتائج المبينة على المجموعة الضابطة.

أما نتائج التجربة الثانية فقد أوضحت أن تحصين الجمال بمولدات المضاد المستخلصة من القراد البالغ أدت إلى تقليل متوسطات وزن القراد ، عدد البيض ونسبة الفقس على الحيوانات المحصنة مقارنة بالمجموعة الضابطة.