



EXPERIMENTAL VACCINATION OF SHEEP AGAINST HARD TICKS (IXODIDAE) USING WHOLE CRUDE LARVAE EXTRACT OF *HYALOMMA ANATOLICUM ANATOLICUM* IN SULAIMANI GOVERNORATE-IRAQ

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ABSTRACT: The present study was carried out at October to December months 2011, unfed larval of *Hyalomma anatolicum anatolicum* were used for preparation of larval antigen for sheep (local breed) were inoculated subcutaneously (s/c) with 0.1 ml (8.2 mg/ml) in five successive doses of weekly interval. In each week end, five ml of blood was drawn directly from jugular vein for evaluation of gamma immunoglobulin (IgG) response. In this study there was that the highly correlation differences ($r^2 = 0.976$, $p < 0.01$) between all inoculation (immunization) weekly intervals with control sheep. Immunoglobulin-G level concentration in fifth week 2761.5 mg/dl was highly significant differences when comparison with others level values 1448.1 mg/dl, 1847.8 mg/dl in and 2097.7 mg/dl of other weeks 1st, 2nd and 3rd week's immunization respectively, while showed no significant differences in immunoglobulin-G concentration with fourth inoculation sheep 2703.3 mg/dl. In this study observes that the increasment of immunization sheep percentage between each weekly immunization when compared with the control group, and revealed a highly resistance when exposed to challenge against of larval infestation in site of ear-pinnae.

Keywords: Sheep, Engorged *Hyalomma a. anatolicum*, Radial immunodiffusion plates, IgG RID.

INTRODUCTION

The infestation with ticks can cause vast losses in farm animal's production, due to tick borne disease, tick and physical damage as well as to huge financial losses due to tick control [1]. Immunological control of ticks constitutes an important alternative and would satisfy sustainable requirements in terms of target species specificity, absence of residual effect and long-lived immunity [2] and play an important role in preventing transmission of tick-borne organisms [3]. For example Crimean-Congo haemorrhage fever (CCHF) virus was isolated in three patients in Iraq [4]. The development of anti-tick vaccines is an area of research with considerable potential to minimize acaricidal hazards, resistance and residues and environment pollution [5]. Their use however, has had limited efficacy in the reduction of tick infestations and is often accompanied by serious drawbacks including selection of acaricide-resistant ticks and environmental contamination [6]. These acaricides are toxic and costly, and tick have developed resistance to all major chemical groups used for their control, in addition the residues of these chemical may persist in food and the environment [7]. Effective control measures against tick borne disease are best achieved through combination of tick control, prevention of disease through vaccination and treatment [8]. The present study has been carried out to determine the protective efficiency of *Hyalomma anatolicum anatolicum* whole crude larval extract derived antigens in local sheep and challenged with it.

MATERIALS AND METHODS

Tick and sheep sampling:

Ticks were collected and isolated from 100 local breed sheep (Karadi) from various flock in sulaimani governorate, the fully engorged female ixodid ticks were counted and identified, based on morphological features according to [9], which using a dissecting microscope (Dissecting microscope, Motic-Education, China).

Rearing and breeding of hard tick *Hyalomma a. anatolicum* under laboratory condition.

In vitro engorged female of *Hyalomma a. anatolicum* were collected and isolated from in sterile glass with bijou bottle and covered by muslin cloth and incubated at $26 \pm 1.9^\circ\text{C}$, kept in desiccator jars with relative humidity (RH%) $86 \pm 1.6\%$ (using saturated solution of potassium chloride for humidity control) both temperature and humidity measured by (Humidity-Temperature meter, France). When the larvae became sclerotized and hardening. The larvae were placed into a deep freezer at -20°C for preparation of antigens for immunization.

Preparation of Larval antigen.

Larval antigen of *Hyalomma anatolicum anatolicum* preparation and described by [10], that unfed larvae (5 g) placed onto pre-sterilized Petri dishes containing an ice-cold 0.15 M phosphate buffered saline (PBS), PH 7.2, washed thrice in PBS, air-dried and then wrapped with aluminum foil papers, and homogenized when containing $1.0 \mu\text{m/l}$ disodium ethylene diamine tetra acetic acid, the homogenate and sonicated at 4°C for 10 min, the suspensions were filtered free of cuticle and debris with a double layered muslin cloth into a sterile beaker. Washing by PBS and centrifuged (speed at 10000 rpm for 10 min three times. 5ml of PBS was added to the precipitate protein and kept in sterile glass vial at -20°C until further use [11].

Determination of total protein concentration of the antigen:

Total protein concentration of larval antigen was determined by modified Lowry's method, Bovine serum albumin (BSA) 1.0mg/ml of distilled water was used as a standard solution with an extinction coefficient of 0.670 at a wavelength of 280nm [12]. Employing UV/ Visible spectrophotometer. Standard protein curve was constructed, using different concentrations of bovine serum albumin (BSA) standard solution as form (50, 100, 200, 400, 600,800 $\mu\text{g/ml}$) versus the absorbance of (0.08, 0.2, 0.33, 0.55, 0.76, 0.90) at 280 nm. Then (5 μl of antigen + 995 μl of distilled water) were measured. The absorbance of both antigens was plotted into the standard curve to determine protein concentration of each antigen.

Experimental sheep

Ten local breed sheep (Karadi) were used for immunization. Blood smears of sheep were examined (Giemsa stain) for presence of any haemoparasites and serum samples were tested for determined immunoglobulin type-G concentration in control (pre-immunization) and immunized sheep when inoculated subcutaneously S/C using a sterile spring and needle of 23 gauge with 0.1 ml (8.2 mg/ml) whole crude larval extract mixed with vegetable oil in five successive doses at weekly intervals.

Determination of the IgG protein:

Determination of the IgG protein (Shaheed Hadi Consultation Clinic), using radial immunodiffusion plate (Radial immunodiffusion plates, IgG RID, Italy) which contained specific antiserum in agarose gel. Radial immunodiffusion was based on the diffusion of antibody from a circular well radial into a homogeneous gel containing specific antiserum IgG. A circle of precipitated antigen and antibody forms, and continues to grow until equilibrium is reached. After 48 hr of incubation, the precipitating diameter of control and samples were measured and conversion with (IgG-RID plate kits).

Challenge larval infestation

Unfed larva of *Hyalomma a. anatolicum* were placed (unfed larvae of *Hyalomma a. anatolicum*) were placed on and clipped and shaved ear-pinnae of immunized (immunized) and control (pre-immunized) sheep, and put inside tubular ear-bags (muslin cloth) (10 x 20) cm and fixed at the base of each ear by thread (cotton) after 48h changes in each ear of sheep detected for attachment and feeding.

Statistically analysis

The data were analysed according to the analysis of variance as a general test and the comparison between the means conducted by least significant difference LDC at the significant level of ($\alpha = 0.01$) and Linear regression.

RESULTS

Statistically concluded of immunoglobulin type G for the W5 (2761,5) mg/dl shows highly statistically correlation ($r^2 = 0.976$, $p < 0.01$) between the control treatment (1119.5) mg/dl and all other immunized (1448.1 mg/dl in 1st, 1847.8 mg/dl in 2nd, 2097.7 mg/dl in 3rd, 2703.3 mg/dl in 4th, while founded not significant change in the level of immunoglobulin-G in fourth when compared with fifth group immunized sheep, may be the immunity system tolerance after fourth immunized, thus suggested that the inoculation is a best appropriate dose using for immuno-response and shows the comparism between each immunized sheep and control(pre-immunized) shows the increasment of immunization percentage of 29%, 65%, 87%, 141% and 146% for the 1st, 2nd, 3rd, 4th and 5th immunization respectively.this calculated during the results in Table (1) and Figure(1).

Table 1: Mean of immunoglobulin-G concentration between the control (pre-immunized) with immunized sheep.

Treatment		Mean of Immunoglobulin concentration- mg/dl
Pre-immunized sheep (control)		1119.5e
Immunized Sheep/weekly	1 st week	1448.1d
	2 nd week	1847.8c
	3 rd week	2097.7b
	4 th week	2703.3a
	5 th week	2761.5a
LSD 0.01		124.3

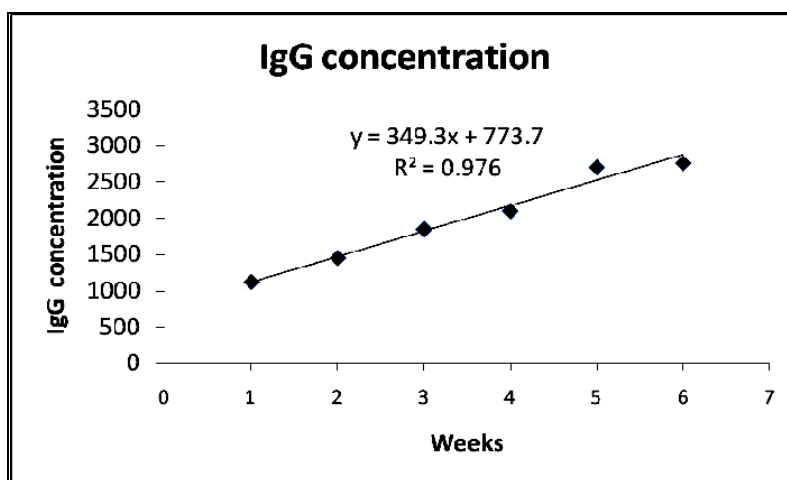


Figure 1: Relationship curve between immunoglobulin-G concentration and weekly intervals immunization (five inoculations). ($r^2 = 0.976$, $p < 0.01$).

Larval challenge

In this study showed that the attachment and feeding behaviour of larvae on the ear skin in immunized and control sheep, there no skin reactions were noted at the tick feeding sites in the animals in the control group but small papules, the exudation, dying at site of attachment and high number of larvae dropped in the ear-bags in immunized sheep, were noted after 48h from the fifth immunization.

DISSCUSION

In Sulaimani governorate *Hyalomma anatolicum anatolicum* is the most common Hyalomma ticks [13], the predominant of Hyalomma may be due to their ability to tolerate the dry and harsh environment with little hiding places [14]. Compared with acaricides, vaccine offer sustained action, freedom from residues and specificity and they may be cheaper than chemical agents. It may also be argued that resistance to vaccine is less likely to develop than with acaricides and if it did occur, its effects may be circumvented in ways that are impossible [15].

In this study the highest level of immunoglobulin shows fifth immunization but no significant differences observed with fourth immunization sheep, that meaning the immunity of sheep may be tolerance. And shows highly correlation between all immunized and control sheep. [13] calculated that the IgG highly significant differences in immunized when comparism with pre-immunized rabbit. [16] observed increased antibody level in calves at one week and reached in a peak at eight weeks post infection, then decreased in nine weeks, this due to larval body antigen. [17] Showed significant elevation in the antibody titers in the sera of the immunized rabbits compared with control. Most of the larvae feeding on a resistant animal tend to detach after a short time. They may re-attach briefly at other sites before leaving the host or dying [18]. Gamma globulins are an inexpensive source of IgG with only trace amounts of other immunoglobulin [19]. Since the immunity of midgut antigens of *H. anatolicum anatolicum* has been proved in Iran [20]. [21] calculated that the immunized Cattle with Haa86 and challenged with larvae, and adult tick females of Hyalomma ticks. The percentage rejection of ticks' on immunized animals higher than control animals. The reduction of number of female ticks, mean weight of eggs, and efficacy of antigen. [13] revealed that the immunized rabbit show strongest larval challenge when inoculated three weekly intervals with larval whole crude extract.

In this present, the challenge of larvae infestation with *Hyalomma a. anatolicum* in both experimental sheep (control and immunized sheep) using whole crude larval extract, In post-immunized sheep showed congestion, papules and odema observed in the site of the infestation, this may be due to cellular infiltration at the site of attachment and the individual engorged, abnormal fed-rejected and high number of larvae was dead on to ear-bags. In pre-immunized (control) sheep showed the high number of engorged larvae still sucking blood. The abnormal rejection of larvae may be due to their inability to gain entrance to blood vessels as a result of the host immunological reactions. [22] found that after the third infestation, the number of engorged larvae in rabbits was significantly reduced due to the development of immune response of the challenged animals. [23] found protective effect against tick infestation using purified 37 kDa larval antigens and demonstrated that the larvae of *Hyalomma a. anatolicum* are an important source of biological material for isolation of protective antigens. Only the larval extract of *Hyalomma a. anatolicum* showed significant protection against ticks challenge in immunized rabbits. This may be due to the presence of higher immunoprotective antigen concentrations in larval extract [24].

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