



Short Communication

Efficacy of Whole Crude Extract of Hard Ticks in the Treatment and Control of Tick Infestation in Livestock

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ABSTRACT

Ticks infestation is the most common constraint to the livestock production in terms of drop in meat and milk production, decline in hide worth, physical damage to the skin of animals and susceptibility to infestation of tick borne parasitic infections. Conventionally acaricides are used in the treatment of tick infestation in livestock but potentially satisfactory alternative technique is the immunological control against ticks infestation. Therefore, this study was planned to investigate the effect of crude extract of hard ticks and its application as a vaccine against hard ticks of the cattle. For this purpose, ticks were collected from cattle and crude extract of ticks was prepared using standard procedures *i.e.* mixed with formalin, antibiotic and vegetable oil to prepare the vaccine. This vaccine was administered thrice (at weekly interval) in 15 cattle @ 1ml/50 Kg of the body weight of each animal accordingly. Fifteen cohort cattle of different age groups served as controls, administered with the standard acaricidal drug, ivermectin. At the end of each week post administration of this oil based whole crude larval extract, the number of hard ticks was counted with naked eye and the efficacy of this crude tick vaccine was determined in terms of reduction in tick number. Control of ticks and the clinical efficacy of the prepared vaccine were determined on the basis of number of ticks infesting the vaccinated and control group over 01-month period, post vaccination. The results revealed that physically the number of ticks were significantly reduced ($P < 0.05\%$) using crude larval extract vaccine (78.4%) than standard acaricidal drugs, ivermectin (63.24%) being conventionally used.

Out of total 899 species of ticks, 713 species belong to Ixodidae family (hard ticks), 185 species to family Argasidae (soft ticks) and one species to Nuttalliellidae (monotypic). Hard ticks nourish on their hosts for extended period ranging from days to weeks, depending upon various elements like species of infesting tick, life stage and nature of the host. Cuticle of the Ixodidae ticks can expand to accommodate the enormous volume of blood ingested by ticks (Iqbal *et al.*, 2006), increase in size and

may reach 200-600 times of their unfed body weights. While, Argasidae exhibit supernatural resistance to starvation and can survive for years without blood meal. Cuticle of Argasidae also expand but it lacks the capability to grow for accommodation of large volume of ingested blood meal and their weights may increase 5-10 times of their unfed body weights.

Economically ticks are considered to be the most important pests of ruminants and other domestic animals in tropical and subtropical regions. They serve as vectors, transmitting various infecting microbes like protozoan (*Babesia* and *Theileria*), rickettsiae (*Anaplasma* and *Ehrlichia*), bacteria (*Brucella*, *Listeria*, *Pasteurella* and

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Authors' Contribution

AK, NA, MSK, SU and AZ conducted the research trial. KS and KM analyzed the data. AT, MIM, MKS, AUR and HM helped in formatting the manuscript.

Key words

Acaricides, Crude larval extracts, Ticks vaccine, Formalin, Vegetable oil

Staphylococcus), viruses (Congo virus) and spirochaetes. Blood of the hosts is the only food for ticks. Ticks burden results in stunted growth of calves and young animals, increased nutritional requirement of the animals to meet the requirements of parasites, weakness and debility in animals and disease transmission by the ticks. Though, economic importance of ticks is subjected to the diseases transmitted by them. However, financial losses are also associated with degrading the skin and hide quality. Prevalence of common tick species of domestic animals including *Amblyomma*, *Hyalomma* and *Rhipicephalus* as 3.05, 36.65 and 16.88%, respectively. Most of the animals are infested with more than one genus of the ticks simultaneously (Muhammad *et al.*, 2008).

Hyalomma species are responsible for the transmission of blood parasites primarily *Theileria*. Therefore, in order to control and eliminate the ticks infestations, various techniques have been applied which include uses of acaricidal drugs. But the excessive use of acaricides resulted in drug resistance and enhanced the treatment cost. A potential alternative to this is the use of oil based *Hyalomma* tick vaccine. Various experiments have been conducted on inoculation of ticks vaccine in cattle (Ali *et al.*, 2009). Immunological control of ticks gave a convincing alternative technique with significant efficacy and no harmful impacts (Patarroyo *et al.*, 2002).

Ticks vaccines have been made available commercially in early 1990's for the treatment and control of tick infestations in cattle like Tick Guard™ in Australia and Gavac™ in America were developed from midgut recombinant protein (BM₈₆) of *Rhipicephalus microplus*. The efficacy of BM₈₆ proteins based vaccines is due to positive correlation of antigen specific antibodies and reduction in number of ticks infesting the host. Mechanism of immunization involves antigen-antibody interaction that interferes with the biological functions of ticks and ultimately reducing weight, number and fertility of engorged female ticks. As a result of this, prevalence of ticks and tick borne protozoal infections can be controlled. Immunization trials with BM₈₆ successfully reduced the incidence of babesiosis and ticks infestation rate in cattle herds (Merino *et al.*, 2013).

The purpose of the study was to use the oil based ticks vaccine, as an alternative approach to acaricidal drugs, against the ticks infestation in livestock to prevent its hazards in dairy enterprise and harms associated with the use of acaricidal drugs. The use of ticks vaccine is a simple and more effective tick control strategy for farmers and practitioners.

Materials and methods

The present study was designed for experimental

vaccination of cattle against hard ticks using their whole crude larval extract. Thirty cattle of different age groups were randomly selected for the study and were divided into two equal groups comprising 15 in each group. Group I was vaccinated, while group II served as control was administered with ivermectin which is used as conventional drug. Ticks were collected from cattle of various herds in district Faisalabad. The fully engorged female ixodid ticks imbibed huge amount of blood, were counted and identified on the basis of morphological features using stereomicroscope, based on the taxonomic and structural differences of the species, different instars and sexes as described by Walker *et al.* (2003). Five to seven partially engorged hard ticks collected from each infested cattle and were chilled at 4°C for 30 min and washed with 70% alcohol. Then ticks were rinsed with normal saline three times to remove the debris. The rinsed ticks were ground in ice-cold 0.15M PBS (pH 7.2) at the rate of 10 ticks per ml, and filtered through 4 layers of muslin cloth. The resulting crude tick extract was centrifuged and the supernatant was stored at -40°C. The total protein content of the supernatant was estimated by the kit method (Ali *et al.*, 2009; Ghosh *et al.*, 1998; Mustafa, 2012). The vaccine was prepared using 1% formalin, 5% antibiotic (oxytetracycline, 100mg) and 40% vegetable oil in the ticks extract in this collected supernatant. Animals of Group I were vaccinated with whole crude tick extract vaccine via subcutaneous route @1ml/50 Kg body weight of the animal, in 3 successive doses at weekly interval. Group II served as control and was administered with standard acaricidal drug (ivermectin).

At the end of each week post administration of whole crude tick extract vaccine, the number of hard ticks was counted with naked eye and the efficacy of the vaccine was determined in terms of reduction in number of infesting ticks. Weight of the engorged ticks was calculated to determine the amount of blood sucked from the hosts accordingly. Control of ticks was determined on the basis of number of ticks infesting the vaccinated and control group over period of 01 month, post vaccination. Moreover, immunoglobulin G (IgG) level was detected in the animals on weekly basis using ELISA technique.

Results and discussion

Table I shows the number of ticks counted at the end of each week on different parts of the body of the animals like udder, thighs, ear etc. Group A was administered with whole crude tick extract vaccine and Group B was treated with acaricidal drug (ivermectin) in 3 successive doses at weekly interval.

The results of the current study revealed that whole crude tick extract vaccine was more effective in the control of ticks compared to standard acaricidal drug ivermectin.

Table I. Ticks collected from different sites of experimental cattle before and after vaccination.

Group	No. of Animals	No. of adult ticks collected		Ticks collected from thighs		Ticks collected from ears		Ticks collected from udder		Mean	
		Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac	Pre-vac	Post-vac
Group A	15	123	27	45	12	58	8	20	7	41±3.86	9 ±0.23
Group B	15	117	43	38	18	63	14	16	11	39±2.97	14.33±2.1
Total	30	240	70	83	30	121	22	36	18		

Pre-vac, before vaccination; Post-vac, after vaccination. A, administered with crude ticks extract; B, treated with ivermectin.

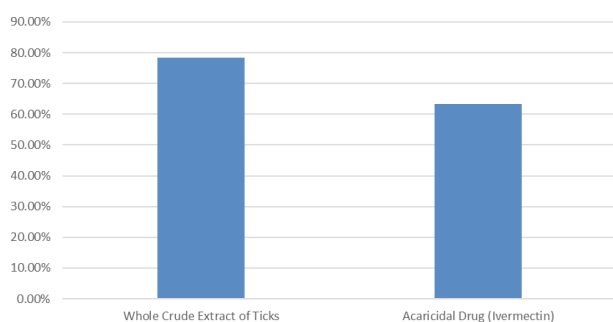


Fig. 1. Clinical efficacy of whole crude extract of hard ticks for the treatment and control of tick infestation in cattle.

In group A, 78.4% ticks were significantly ($P < 0.05$) eliminated from different parts of the animals and in Group B the recovery/ticks elimination rate was 63.24% from the infested animals (Fig. 1). Similar studies were conducted on sheep in which *Hyalomma anatolicum anatolicum* were used for larval antigen preparation and administered via subcutaneous route @ 0.1ml and sheep were found resistant to ticks infestation (Mustafa, 2012). It was also reported from a study that antigen from midgut of *Hyalomma anatolicum anatolicum* was administered in rabbits and that maximum resistance was reported against tick infestation (*H. a. anatolicum*) in the immunized rabbits (Madani et al., 2008).

The hematological parameters of the cattle infested with ticks were determined and compared to the healthy cattle free from ticks (Table II). A similar study also revealed that the hematological parameters were significantly affected ($P < 0.001$) in calves, due to tick infestations (Kaur et al., 2017).

The IgG level was determined at the end of each week and it showed significant increase i.e. 1590, 1698, 1838, 2055 and 2140 mg/dl of IgG level in the blood. At the end of 5th week, IgG level as maximum showing maximum resistant against tick infestation in cattle.

It is further added that no doubt ticks are ectoparasites, but ticks attach to the body of the host and sucks the blood that contain antibodies. A study was conducted on the similar concept of anti tick vaccine, whereby the efficacy

of the Bm86 midgut antigen and the cytosolic Subolesin (SUB) antigen was evaluated in vitro (Trentelman et al., 2019) and it was concluded that *R. australis* larval feeding was significantly reduced. Similarly, Salp 15 antibodies were also considered effective against ticks infestations. In another study it was observed that hosts developed immune response to tick salivary proteins following repeated tick exposure that impaired tick and pathogen viability, including cutaneous inflammation and resulted in itch and an increased awareness of infesting ticks (Walker et al., 2003; Wikel, 1996).

Table II. Comparison of hematological parameters of ticks infested and healthy cattle.

Parameters	Healthy	Infected
Red blood cells x 10 ¹² /l	4.8	2.06
Hematocrit (l/l)	0.26	0.13
Hemoglobin (g/dl)	7.5	3.7
Mean cell volume (fl)	54.0	61
Mean cell hemoglobin concentration (g/dl)	30	29.36
WBCs (x10 ⁹ /l)	11.7	17
Neutrophils (x10 ⁹ /l)	4.79	6.46
Lymphocytes (x10 ⁹ /l)	7.19	0.34
Platelets (x10 ⁹ /l)	310	115
Myeloid: erythroid ratio	2.47	-
Myeloblasts (in marrow)	2.6	-

Conclusion

From the present study, it has been concluded that whole crude tick extract vaccine is more effective than ivermectin in the treatment and control of ticks infestation in cattle, with better recovery rate and no harmful impacts. Moreover, the ivermectin is effective only for 2-3 weeks while crude tick vaccine is effective for long period.

Statement of conflict of interest

The authors have declared no conflict of interest.

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