

# A Comparative Study of The Acquired Resistance to The Tick Species *Rhipicephalus Appendiculatus*, *Rhipicephalus Evertsi Evertsi* and *Amblyomma Hebraeum* in Crossbred (Saanen X South African Indigenous Goats) Goats

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**Abstract.** The development of acquired resistance in crossbred (Saanen X South African Indigenous goat) goats against adult *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi evertsi* and *Amblyomma hebraeum* tick infestations was investigated. The rise in gamma globulin levels, decreased number and mass of engorged female ticks towards the end of detachment period after three consecutive infestations on the crossbred goats, were the main indicators of the development of resistance. The results indicated that the crossbred goats that acquired resistance to one species of tick also possess a certain amount of resistance to the other tick species (cross resistance). Even though all the three tick species exhibited immunogenicity, however *Rhipicephalus evertsi evertsi* is more immunogenic when compared with *Rhipicephalus appendiculatus* and *Amblyomma hebraeum*. Although a certain degree of innate resistance was observed in crossbred goats, this was lower than the South African Indigenous goats and Saanen goats. As crossbred goats possess the potential to develop an acquired resistance however, it might be possible to improve this Breed through selective breeding.

**Keywords:** *Rhipicephalus appendiculatus*; *Rhipicephalus evertsi evertsi* ; *Amblyomma hebraeum* ; tick infestation; immune response; Saanen goats; South African Indigenous goats; crossbred goats; engorged female; immunogenicity.

## 1. Introduction

The main purpose of this project was to provide a better goat breed to small-scale farmers that should exhibit better productivity and milk yield than the presently used South African Indigenous goats. The crossbred (Saanen X South African Indigenous goat) goat bred developed at the Medical University of Southern Africa (MEDUNSA) showed an improved productivity and milk yield compared to the South African Indigenous goats. Preliminary results of productivity of these goats in terms of fertility, kidding rate, and milk production have been reported [1]; and subsequently more fully [2]. It is desirable that such a crossbred goat should also have the potential to develop a meaningful degree of resistance to tick infestation.

## 2. Literature survey

Ticks are the most economically important haematophagous ectoparasites of domestic animals worldwide, both because of the damage they cause during feeding and as vectors of numerous viral, bacterial and protozoal diseases [3]. Ticks are the most common external parasites of economic importance in the small holder farming areas of the southern region of Africa [4]. Four tick species are of major economic importance as vectors of diseases that affect domestic cattle in Southern Africa. These are *Amblyomma*

hebraeum, the vector of Ehrlichia (Cowdria) ruminantium, the cause of heartwater in cattle, sheep, goats and certain wild ruminant species [5]; Rhipicephalus appendiculatus, the vector of Theileria parva, the cause of theileriosis in cattle [6][7]; and Rhipicephalus evertsi evertsi, which is a vector of Babesia caballi and Theileria equi, the cause of piroplasmiasis in horses [8]. The tick has a preference for horses in all its stages of development, but is also a common parasite of cattle, goats and sheep [9]. A fourth tick, Rhipicephalus (Boophilus) decoloratus, the vector of Babesia bigemina, is the cause of babesiosis or African redwater in cattle [11].

In ticks, the salivary glands are important sites for producing antigens [12][13][14]. Therefore, after the first contact of these ectoparasites with some hosts, the hosts develop resistance [12][15]. Many studies have been carried out to verify the acquisition of resistance by hosts when they are immunized by successive infestations [17]-[20]. In Africa, tick-borne diseases are considered to be the most important animal disease problems [21]. Natural resistance to a number of vectors and vector-borne microorganisms exists in ruminants and this varies with breed [22].

Tick resistance might vary between breeds with the species of infesting tick [23]. It is known that in many subtropical and semi-arid environments in Africa, indigenous dual-purpose breeds are highly resistant to ticks, resulting in low infestation rates [24]. So, it is therefore of utmost importance to check whether the crossbred goats (Saanen X South African Indigenous goats) have the potential to develop a meaningful degree of resistance against adult Amblyomma hebraeum, Rhipicephalus appendiculatus and Rhipicephalus evertsi evertsi tick infestations.

### **3. Materials and Method**

#### **3.1 Goats**

The protocol of this study was approved by the Animal Ethics Committee - MEDUNSA campus of University of Limpopo. Sixteen month old naive healthy crossbred goats (Saanen X South African indigenous goats) were kept in individual metabolic cages and fed a Total Mixed Ration (Goat diet consisted hay, macro and micro nutrients, minerals, vitamins and buffer) [1].

#### **3.2 Ticks**

Flat females and males of the one of the following species - Rhipicephalus appendiculatus , Rhipicephalus evertsi evertsi , Amblyomma hebraeum .

#### **3.3 Method**

Three groups of eight naive crossbred goats were each infested three times with equal numbers of flat females and flat males of one of the three tick species and the engorged females were collected upon repletion. To simulate the natural situation, the goats were reinfested as soon as the engorged females of the previous infestation had all been collected.

#### **3.4 Infestation procedure**

The goats were kept individually in metabolic cages for the duration of each trial. A patch of hair was removed from the mid back of each animal and cotton sleeves were glued around the shaven area 24 hours prior to the release of ticks into the sleeves. During infestation, perspex collars (Rechav, pers.com) were fixed around the neck of goats to restrain them from grooming. Grooming by the goats was again limited by chaining to either side of the experimental cage, which allowed sufficient space for limited movement necessary for the goats to eat, drink and lie down. At the end of each infestation, on a daily basis the engorged females were collected and the mass of each tick was recorded.

#### **3.5 Blood samples**

Blood samples for quantitative serum analysis were taken from each experimental goat prior to each infestation and at the end of each infestation. Blood samples from a control group of eight naive animals of existing crossbred goats were taken and analysed as control. Blood samples were

collected from the jugular vein of goats, in vac-u-test sterile evacuated blood collection tubes, at the time of each infestation and again towards the end of detachment period (Rechav,pers com). The samples were allowed to clot at room temperature before the serum was retrieved for serological analysis.

### 3.6 Eletrophoresis and serum proteins

The serological analysis was performed on the day of collection by the Department of Chemical Pathology, MEDUNSA. Serum protein - eletrophoresis was done on agorse gel using the Paragon High Resolution Eletrophoresis kit (Beckman instruments, Fullerton, California U.S.A.) with a barbital buffer, PH 8.8 (0.15 ionic strength). The membranes were scanned with an Appraise densitometer (Beckman instruments) and were separated into albumin  $\alpha$ 1,  $\alpha$ 2,  $\beta$  and  $\gamma$  globulin fractions.

## 4. Result

### 4.1 Rhipicephalus appendiculatus

Development of acquired resistance in crossbred goats (Saanen X South African Indigenous goat) against Rhipicephalus appendiculatus tick infestation

#### Gamma globulin levels:

When crossbred goats were infested with adult Rhipicephalus appendiculatus ticks, the mean gamma globulin levels increased significantly after each infestation when compared to the uninfested control animals as shown in (Table 1).

**Table1: Serum gamma globulin levels (g/l) in crossbred goats after three consecutive infestations by adult *Rhipicephalus appendiculatus* ticks**

Groups	Pre infestation Mean $\pm$ SD	1st Post infestation Mean $\pm$ SD	2nd Post infestation Mean $\pm$ SD	3rd Post infestation Mean $\pm$ SD	p-value
Control (n=8)	17.4 $\pm$ 3.5	16.5 $\pm$ 3.2	16.5 $\pm$ 3	16.2 $\pm$ 3	p<0.90
Test group (n=8)	17.4 $\pm$ 3.9	20.7 $\pm$ 3.2	21.1 $\pm$ 3.0	22.2 $\pm$ 3.2	p<0.05

### 4.2 Rhipicephalus evertsi evertsi

Development of acquired resistance in crossbred goats (Saanen X South African Indigenous goat) against, Rhipicephalus evertsi evertsi tick infestation.

#### Gamma globulin levels:

The mean serum gamma globulin levels increased progressively in the test group after each infestation by Rhipicephalus evertsi evertsi ticks when compared to the uninfested control animals, as shown in (Table-2).

**Table 2: Serum gamma globulin levels (g/l) in crossbred goats after three consecutive infestations by adult *Rhipicephalus evertsi evertsi* ticks**

Groups	Pre infestation Mean $\pm$ SD	1st Post infestation Mean $\pm$ SD	2nd Post infestation Mean $\pm$ SD	3rd Post infestation Mean $\pm$ SD	p-value
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Control (n=8)	16.6±1.37	16±1.05	14.1±1.59	15.3±2.23	0.04
Test group (n=8)	17.7±2.5	23±3.21	27±6.17	26.9±6.77	0.002

### 4.3 *Amblyomma hebraeum*

Development of acquired resistance in crossbred goats (Saanen X South African Indigenous goat) against adult *Amblyomma hebraeum* tick infestation.

#### Gamma globulin levels:

The mean serum gamma globulin levels increased progressively in the test group after each infestation by *Amblyomma hebraeum* ticks when compared to the uninfested control animals, as shown in (Table 3).

**Table 3: Serum gamma globulin levels (g/l) in crossbred goats after three consecutive infestations by adult *Amblyomma hebraeum* ticks**

Groups	Pre infestation Mean ±SD	1st Post infestation Mean ±SD	2nd Post infestation Mean ±SD	3 <sup>rd</sup> Post infestation Mean ±SD	p-value
Control (n=8)	16.6±2.3	17.5±2.3	16.4±2	16.3±2	0.6
Test group (n=8)	16.7±3.3	19.4±4.7	21.6±4.8	22.2±3.5	0.05

## 5. Comparison between Tick species

### 5.1 Gamma globulin levels

Comparison between the increase of gamma globulin levels (g/l) in the serum of experimental crossbred goats before and after three consecutive infestations of *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi evertsi* and *Amblyomma hebraeum* adult ticks are shown in Figure-1.

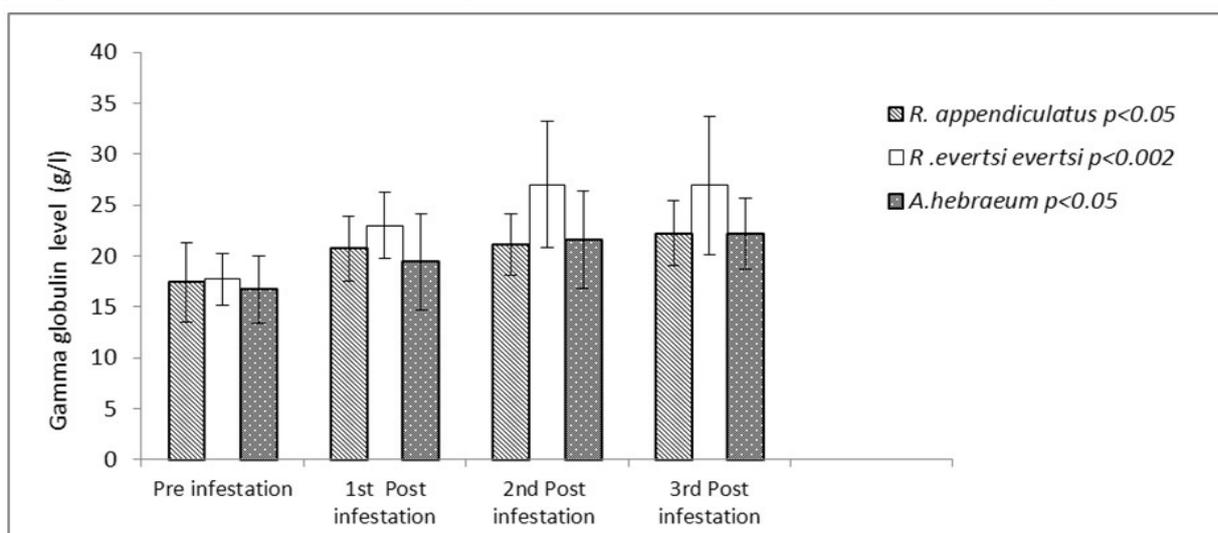


Fig: 1 Increase in Gamma globulin levels (g/l) in the serum of crossbred goats before and after three consecutive infestations of *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi evertsi* and *Amblyomma hebraeum* adult ticks.

The gamma globulin level increased to a great extent during *Rhipicephalus evertsi evertsi* infestation ( $F=6.01$ ;  $P<0.002$ ). Even though all the three tick species exhibited immunogenicity, *Rhipicephalus evertsi evertsi* seems to be more immunogenic when compared with *Rhipicephalus appendiculatus* ( $F=2.95$ ;  $p<0.05$ ) and *Amblyomma hebraeum* ( $F=2.86$ ;  $p<0.05$ ). A statistically significant trend ( $F=2.92$ ;  $p<0.05$ ) was also observed between tick species.

## 5.2 Engorged female tick mass

The decline in mean engorged mass of female *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi evertsi* and *Amblyomma hebraeum* that completed their blood meal on the experimental crossbred goats are shown in Figure-2.

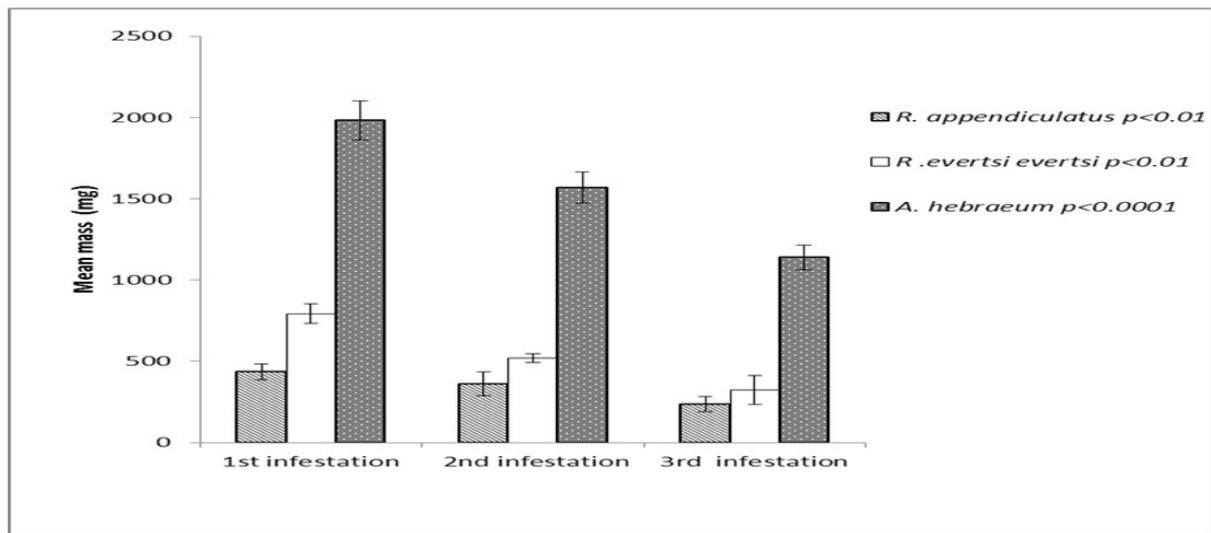


Fig: 2 Mean engorged mass ( $\pm$ SD) of female *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi evertsi* and *Amblyomma hebraeum* collected from crossbred goats after each of three successive infestations.

The mean engorged mass of female *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi evertsi* and *Amblyomma hebraeum* declined progressively from first infestation to last infestation as well as there was significant species differences in engorged female tick mass ( $F=62.51$ ;  $p<0.0001$ ). Significant differences in engorged female tick mass were found between *Rhipicephalus appendiculatus* and *Rhipicephalus evertsi evertsi* tick species ( $p<0.01$ ) and also between *Rhipicephalus appendiculatus* and *Amblyomma hebraeum* tick species ( $p<0.01$ ). Again significant statistical difference in engorged female tick mass was noted between *Rhipicephalus evertsi evertsi* and *Amblyomma hebraeum* tick species ( $p<0.01$ ) during three consecutive infestations.

## 5.3 Numbers of females completing the blood meal

The decline in numbers of *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi evertsi* and *Amblyomma hebraeum* females that completed their blood meal on the experimental crossbred goats shown in Figure-3.

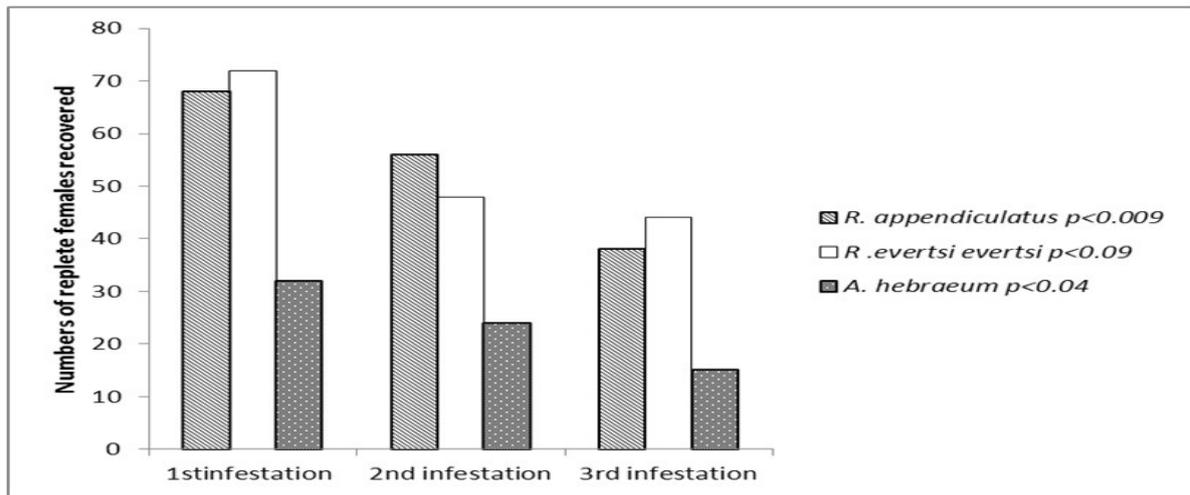


Fig: 3 Replete female numbers recovered from crossbred goats during three consecutive infestations of *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi evertsi* and *Amblyomma hebraeum* adult ticks.

Even though the replete female numbers recovered from crossbred goats during three consecutive infestations of *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi evertsi* and *Amblyomma hebraeum* adult ticks declined between infestations, however the difference in engorged female tick numbers between tick species was not significant ( $F=1.98$   $p<0.16$ ).

## 6. Discussion

Host resistance to ticks is frequently acquired both naturally and artificially through repetitive infestations, and expressed by significant reductions in the tick yield, engorgement weight [25]. These immune responses affect feeding, reproduction and survival of the ticks [26][27][28]. In the development of host resistance, antibody responses have been shown to be important [29][30][31]. An increase in the level of the protein globulins in the serum of the host indicates the development of acquired humoral immune response [32][33]. Rise in gamma-globulin level was observed in crossbred goats while they were infested with all three tick species. When crossbred goats were infested with *Rhipicephalus appendiculatus*, the difference in gamma globulin levels were significant during three consecutive infestations as well as between the pre infestation and the third post infestation indicating that three successive infestations of *Rhipicephalus appendiculatus* on crossbred goats elicited a strong acquired immune response. The difference in gamma globulin levels were extremely significant during three consecutive infestations of *Rhipicephalus evertsi evertsi* on crossbred goats indicative of very strong acquired immune response when compared with *Rhipicephalus appendiculatus* infestations. *Amblyomma hebraeum* infestation on crossbred goats also exhibited rise in mean gamma globulin levels implying that three successive infestations of *Amblyomma hebraeum* elicited a strong acquired immune response. Even though all the three tick species exhibited immunogenicity, *Rhipicephalus evertsi evertsi* is more immunogenic when compared with *Rhipicephalus appendiculatus* and *Amblyomma hebraeum* and the difference was statistically significant between tick species.

The results also indicated that the crossbred goats resistant to one species of tick were also resistant to other tick species. Hlatshwayo & Szabó (2004) pointed out that one-way cross-reactivity of *A. hebraeum* with *A. cajennense*, suggesting that *A. hebraeum* is more immunogenic than *A. cajennense*. Our current finding showed that even though all the three tick species exhibited immunogenicity, however *Rhipicephalus evertsi evertsi* seems to be more immunogenic than *Amblyomma hebraeum* and *Rhipicephalus appendiculatus*. However this finding agreed with the previous findings of Hlatshwayo et al (2004) that *Amblyomma hebraeum* is also immunogenic along with *Rhipicephalus appendiculatus* and *Rhipicephalus evertsi evertsi*.

Resistance to ticks differs with the breed (Clarke, Els, Heller-Haupt, Rechav & Varma 1989; de Castro & Newson 1993) and with the species of ticks (de Castro 1991). As only few females complete their blood meal successfully on an immune host (Brown 1985), the number of ticks recovered were also used as indicative of an immune response. The mean engorgement mass was found to be a consistently reliable parameter indicative of the effect of an immune response on tick feeding success (Heller-Haupt, Varma & Langi 1981). Decreased number and mass of engorged ticks towards the end of detachment period after three consecutive infestations also indicated the development of acquired resistance in these crossbred goats. The mean engorged mass of female *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi evertsi* and *Amblyomma hebraeum* declined progressively from first infestation to last infestation as well as the difference was significant between tick species. The numbers of engorged *Rhipicephalus appendiculatus*, *Rhipicephalus evertsi evertsi* and *Amblyomma hebraeum* females recovered from crossbred goats after three subsequent infestations showed decrease in tick yield between the first and last infestation.

Although a certain degree of innate resistance was observed in crossbred goats, the pre-infestation gamma globulin level of crossbred goats seems to be lower ( $\pm 17.0$  g/l) when compared with the pre-infestation gamma globulin levels of Saanen and South African Indigenous goats (Gopalraj, Clarke & Donkin 2013). The crossbred goats used for this investigation was 50% South African Indigenous and 50% Saanen mix. Since South African Indigenous goats got a very good potential to develop acquired resistance against tick infestation [40], may be 75% South African Indigenous and 25% Saanen cross can be able to develop higher degree of acquired resistance against tick infestation.

## 7. Conclusion

The results indicated that the crossbred goats that acquired resistance to one species of tick also possess a certain amount of resistance to the other tick species (cross resistance). Although a certain degree of innate resistance was observed in the crossbred goats, this was lower when compared with Saanen and South African Indigenous goats [40]. As crossbred goats possess the potential to develop acquired resistance however, it might be possible to improve this Breed through selective breeding.

## 8. Acknowledgement

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