Journal of Animal and Veterinary Advances 3 (1): 45-51, 2004

Effect of Malaysian Zebu Cattle (Kedah-Kelantan) and their European Crosses on the Tick Biological Parameters Following Repeated Experimental Infestations

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Abstract: Four calves from each group of purebred Kedah-Kelantan (KK) cattle (Zebu); and halfbred, KK/Friesian were experimentally infested with 20,000 *B. microplus* larvae from first to fourth infestation at 2 months intervals. The level of tick resistance were assessed by the number of engorged ticks produced, weight of engorged ticks, eggs produced and the viability of the eggs. All animals developed a significant level of resistance in the subsequent infestation compared to the first infestation as manifested by a reduction in the above parameters. The average number of female ticks, weight of engorged female ticks per animal, the mean egg masses and hatchability of eggs was significantly lower ($P<0.05$) in purebred steers than their crosses form first to fourth infestation, indicating that purebred expressed a stronger acquired resistance to *B. microplus* ticks more readily than did their crosses.

Key words: Kedah-Kelantan (Zebu) cattle, Zebu x Frieaian cattle, *B. microplus*

Introduction

B. microplus is an important tick species infesting cattle in most cattle rearing countries particularly in tropical and subtropical regions of the world. Breeds of cattle differ in resistance to *B. microplus* and within breeds there are wide animal variations in susceptibility to tick infestation. Between breeds, the purebred Zebu (*Bos indicus*) cattle were relatively more innately resistance and acquired greater level of resistance than their crossbred animals, and also Zebus and its crosses were more innately resistant and acquired greater level of resistance than pure *Bos taurus* (European breed) cattle to the cattle tick, *B. microplus*, (Tatchell and Moorhouse, 1968; Seifert, 1971; O'Kelly and Spiers, 1976; Utech *et al.*, 1978; Utech and Wharton, 1982; Gomes *et al.*, 1989 and Lima *et al.*, 2000), and to other ticks species (Fivaz and Norval, 1990; Rechav *et al.*, 1990; Walker *et al.*, 1990; Latif *et al.*, 1991a and Wambura *et al.*, 1998). *Bos indicus, Bos taurs* and/or their crosses acquired significant levels of resistance after infestation to *B. microplus* (Lemos *et al.*, 1985; Miranpuri, 1989; Panda *et al.*, 1992 and 1993; Barriga *et al.*, 1993 and 1995; Hermans *et al.*, 1994; Mangold *et al.*, 1994; Melandez, *et al*; 1998; Bechara *et al.*, 2000; Jonsson *et al.*, 2000; Passos *et al.*, 2000 and Barre *et al.*, 2002) and to other ticks species (Gill, 1986; Chiera *et al.*, 1989 and de Castro *et al.*, 1989; Newson and Chiera, 1989; Walker *et al.*, 1990; Latif *et al.*, 1991b,c; Punyua *et al.*, 1991; Dipeolu *et al.*, 1992; Dossa *et al.*, 1996 and Sahibi *et al.*, 1998).

The most evident effect of host immunity on the ticks is premature detachment and reduced engorgement size. Increased mortality, decreased fecundity, and diminished hatching. The presence of innate resistance was suggested by (Riek, 1962; Barriga *et al.*, 1995 and Melendez *et al.*, 1998), but many previous studies have shown that the acquisition of resistance to ticks has an immunological basis (Brossard, 1976; Brown, 1985; Wikel and Whelen, 1986; Willadsen *et al.*, 1989; Inokuma *et al.*, 1993; Allen, 1994; Goncalves *et al.*, 1999; Bechara *et al.*, 2000; Jose *et al.*, 2000 and Passos *et al.*, 2000). The validity of the concept of innate resistance has been questioned. There have been several explanations proposed for the loss of ticks from cattle. Riek (1962) observed tick engulfed in serous exudates. Host grooming removes a number of ticks and this is most apparent on highly resistant animals (Bennett, 1969; Koudstaal *et al.*, 1978 and Schleger *et al.*, 1981)

In recent years resistance of ticks to insecticides has become of great concern in certain area of the world. Resistance of ticks to insecticides has helped stimulate interest in non-chemical methods of pest control. Resistance involving both selections for tick resistance among European breeds of cattle and crossbreeding with Zebu breeds has shown some promising results with ticks of genus *Boopbilus* (Hewetson, 1972; Turner and Short, 1972; Utech and Wharton, 1982; Miranpuri 1989; Panda *et al.*, 1993; Jonsson *et al.*, 2000 and Martins *et al.*, 2002).

The present study was undertaken to evaluate and compare the acquisition of resistance of purebred and their crosses to infestation with 20,000 *B. microplus* larvae. Host resistance was measured by comparing between the purebred and their crosses, the percentage of female ticks that engorged, the mean weights of engorged female *B. microplus*, the average weights of egg masses, and the percent hatch of the eggs.

Materials and Methods

Collection of Engorged Ticks: Engorged female cattle ticks, *B. microplus*, were collected from Sahiwal X Friesian in University Putra Malaysia, Dairy Unit in Selangor. Engorged ticks were collected, gently washed with water and dried with soft paper tissue, without damaging the ticks. They were kept in glass vials and covered with fine meshed cloth secured with rubber bands, and maintained in an incubator at 28°C and about 95% relative humidity. The eggs were separated from the replete female ticks two weeks after oviposition commenced, mixed gently and weighed in 1 gram lots, placed in plastic tubes, capped with fine meshed cloth and secured by rubber bands. Each lot (1 g) of eggs was expected to yield approximately 20,000 larvae (Riek, 1962). Larvae that hatched after three weeks were used for experimental infestations.

Breeding Larvae: Two cross-bred (Sahiwal X Friesian) male calves, about 6 months old were experimentally infested with laboratory culture larvae. These two animals were routinely used to harvest ticks. A dose of 16 mg (2 mg/ml) dexamethasone was injected intramuscularly daily for 5 days to each animal during subsequent infestations. Engorged adult ticks were collected from the infested animals processed as mentioned above for larval production.

Experimental Studies

Experimental Cattle: The experimental animals were healthy steers comprising four purebred Kedah-Kelantan (KK), Zebus, and four halfbred (50% KK and 50% Friesian). These animals were previously exposed to the cattle tick, *B. microplus*, as they had been grazing the infested pasture since birth. The experimental steers were kept individually in stalls and fed with palm kernel cake and supplemented with Signal grass. At the first infestation, the steers were estimated to be 8 months old and 100 to 125 kg.

Tick Infestation: The experimental steers were infested on four occasions at interval of about two months between each infestation. For each infestation, about 20,000 larvae obtained from the experimentally infested animals were applied with a soft brush along the animal's midline from the scapular region extending to the base of the tail. To prevent self-grooming, the animals were restrained for 24 hours after larval application for larvae to move freely without interference.

Engorged Female Ticks

Count and Weight of Ticks: The numbers and weight of engorged females from each animal were recorded daily and the mean numbers and weight of all ticks engorged on individual animal was calculated according the technique described by Amin-Babjee and Riek (1986).

Measurement of Weight of Eggs: The masses of eggs produced by engorged ticks from each animal were mixed, weight in the same manner as described by Amin-Babjee and Riek (1986).

Measurement of Larval Production from 1 g of Eggs: The counting of larvae was done two weeks after hatching (active larvae) according to the technique described by Amin-Babjee and Riek (1986).

Statistical Analysis of Data: Results were expressed as mean ± standard deviation. The statistical difference between the tick biological parameters of the purebred group and that of their crossbred was calculated by using Student's *t*-test.

Results

The average numbers of engorged female ticks, their mean weights, the mean egg masses produced by each engorged tick and the mean number of larvae produced per gram of eggs for each breed are summarized in Table 1.

Number of Engorged Female Ticks: The mean yield of replete female ticks from purebred KK was significantly lower ($P<0.05$) than the mean yield from their crosses from first to fourth infestation (Table 1). Purebred yield significantly lower engorged ticks even in first infestation. In all animals yield of replete female ticks declined from the first to subsequent infestations. Based on the yield of replete females, these data indicate that crossbreds were considered less resistance than the purebred. However, all cattle in this study acquired a relatively high level of resistance to cattle ticks. Examination of the cattle (especially purebred) in this study revealed that many of the female ticks that did not feed to repletion died and appeared to shrivel up in place on the cattle at various ages and levels of engorgement. These responses of the animals to the ticks were apparently immunological. The female ticks yield from the purebred in the first and subsequent infestations were abnormal in color and shape, some of the ticks were small in size and pale yellow in color indicating that these ticks were not fully engorged as compared to those dropped from their crosses. Also the duration of engorged ticks that dropped from the purebred was longer (2-3 days) than those dropped from their crosses for all infestation. Purebred carried relatively few tick even during the first infestation. None of the genotypes in subsequent infestations produced as many ticks as during the first infestation.

Table 1: Engorgement and Oviposition Rates of *B. microplus* following experimental infestation in cattle

Genotype	Infestation	No. Engorged tick	Wt. Engorged tick (mg)	Wt. Egg/tick (mg)	No. hatched larvae (per/g)
KKKK	1	43.75 ± 10.56^b	148.5 ± 2.65^b	72.00 ± 0.82^b	12830.00 ± 766.77^b
	2	23.00 ± 5.600^b	143.5 ± 1.29^b	68.50 ± 1.29^b	11705.00 ± 438.13^b
	3	14.00 ± 2.580^b	140.0 ± 0.82^b	67.00 ± 1.83^b	10265.00 ± 617.69^b
	4	5.000 ± 1.830^b	135.75 ± 0.96^b	65.00 ± 1.41^b	8291.250 ± 915.89^b
KKFF	1	366.75 ± 87.60^a	160.5 ± 1.29^a	81.25 ± 1.71^a	16230.00 ± 343.76^a
	2	200.25 ± 53.68^a	153.5 ± 1.29^a	75.75 ± 1.71^a	14211.50 ± 621.87^a
	3	132.50 ± 46.09^a	149.0 ± 1.41^a	72.50 ± 1.00^a	12240.00 ± 661.34^a
	4	84.750 ± 5.910^a	146.0 ± 1.41^a	70.25 ± 0.50^a	10269.75 ± 299.51^a

All values are expressed as mean and ± standard deviation
KKKK = purebred Kedah-Kelantan (KK) cattle
KKFF = crossbred (50% KK X 50% Friesian)
Between genotype, means with different superscripts are significantly different ($P<0.05$)

Weights of Engorged Female Ticks: The average weights of replete females for breeds and infestations are presented in Table (1). The mean weight of ticks dropped from purebred was significantly lighter ($P<0.05$) than those dropped from their crosses. In all genotypes the average weight of engorged female ticks were lower in subsequent infestations compared to first infestation. Yield of weight of replete female ticks declined from the first infestation to the subsequent infestation. Trends observed in this study on the reduction in average yield and weight of replete female ticks indicate that acquisition of resistance in all animals.

Weight of Eggs Produced by Engorged Female Ticks: The mean weight of eggs masses laid by one engorged female tick was significantly lighter ($P<0.05$) in purebred than their crosses from the first to fourth infestations. The average weight of egg masses laid by one engorged female tick dropped form all animals were lighter in the subsequent infestation than the first infestation. The color of eggs laid by ticks dropped from purebred were yellowish to light brown suggesting that the ticks were not normal, and the size of eggs produced by each tick were smaller, while the eggs laid by female ticks that dropped from their crosses were dark brown in color and the size of eggs laid by each tick was larger than that dropped by purebred.

Mean Number of Larvae Hatched from 1 g of Eggs: Data on the hatchability of eggs laid by replete female ticks from the 2 groups of cattle are shown in Table (1). The difference between the hatchability of eggs from females dropped by purebred steers as compared to their crosses was significant ($P<0.05$).
The mean numbers of larvae produced from 1 g of eggs were significantly lower ($P<0.05$) in purebred than their crosses from first to fourth infestations. The hatchability of eggs from purebred steers was considerably less than that of their crosses. In all animals, the viability of egg masses were declined form first to subsequent infestations.

Discussion

This experiment has demonstrated the resistance of purebred and their crosses to the endemic cattle tick (*B. microplus*). The purebred and their crosses were experimentally infested with 20,000 larvae of *B. microplus* per animal from first to fourth infestation. All genotypes showed acquisition of resistant in subsequent infestations. The purebred produced significantly ($P<0.05$) fewer ticks than their crosses (Table 1). Similarly, some previous observations and a preliminary study have shown that the purebred in this country was highly resistance to cattle tick, *B. microplus*, infestations (Amin-Babjee and Yaccob, 1992).
Differences in the tick biological parameters between purebred *Bos indicus* and purebred *Bos taurus;* purebred *Bos indicus* and their crosses; *Bos indicus* crossbred and purebred *Bos taurus* have been detected for example, *Bos indicus* cattle develop higher level of resistance to *B. microplus* than *Bos taurus* and/or their crossbred animals, as noted by lower number of replete female ticks dropped from cattle that were more resistance to *B. microplus* (Riek, 1962; Tatchell, 1969; Hewetson, 1971; Seiferet, 1971;O'Kelly and Spiers, 1976; Utech *et al.*, 1978; Utech and Wharton, 1982; Gomes *et al.*, 1989 and Lim *et al.*, 2000). Also purebred *Bos indicus* cattle develop greater level of resistance (few ticks production) than *Bos taurus* and/or their crossbred animals to other tick species (Strother *et al.*, 1974; George *et al.*, 1985; Fivaz and Norval, 1990; Rechav *et al.*, 1990; Latif *et al.*, 1991a and Wamburn *et al.*, 1998). This agrees with the results of the present study.
Also, several researchers used Zebu, European cattle or their crosses in experimental infestation with cattle tick, *B. microplus*, showed all cattle acquired resistance after single experimental infestation and resulted in reduction in tick yields (Lemose *et al.*, 1985; Amin-Babjee and Riek, 1986; Miranpuri, 1989; Barriga, 1993 and1995; Hermans *et al.*, 1994; Magnold *et al.*, 1994; Melendez *et al.*, 1998; Jasson *et al.*, 2000; Passos *et al.*, 2000 and

Bianchi *et al.*, 2003). Also, several researchers used Zebu, European cattle and/or their crosses in experimental infestation with other tick species. All cattle acquired resistance after single experimental infestation and resulted in significant reduction in tick yields (Gill, 1986; Chiera *et al.*, 1989; de Castro *et al.*, 1989; Newson *et al.*, 1989; Fivaz and Norval, 1990; Rechav *et al.*, 1990; Walker *et al.*, 1990; Latif *et al.*, 1991b,c; Dipeolu *et al.*, 1992; Dossa *et al.*, 1996 and Sahibi *et al.*, 1998).

In contrast, there are some reports, however, that pure European cattle experimentally infested with various tick species, did not express significant level of resistance after repeated experimental exposures (Brown *et al.*, 1984; George *et al.*, 1985 and Fivaz *et al.*, 1991). Although resistance is mainly associated with Zebu (*Bos indicus*) breeds, studies carried out in the Gambia show a higher resistance to tick and tick-borne disease in N'dama (*Bos taurus*) than Gobra (*Bos indicus*) cattle (Mottioli and Dempfle 1995).

Only few infested larvae were able to feed to repletion from the first to fourth infestation in purebred animals. This low survival rate (from infestation to repletion) can be due to partially to grooming activities such as licking and rubbing (Riek, 1962; Bennett, 1969; Koudstaal *et al.*, 1978 and Schleger *et al.*, 1981). Riek, (1962) and Bennett (1969) reported that hosts grooming activity was an important factor in reduction of tick burdens, and animals restricted from grooming yield increase numbers of engorged ticks. The grooming responses were developed only in animals which had acquired a high level of resistance to *B. microplus*. Antigenic material isolated from tick larvae caused an immediate hypersensitivity reaction in the skin of cattle with prior tick exposure (Willadsen and Williams, 1976) and the intensity of the reaction could be correlated with the level of host resistance (Willadsen *et al.*, 1977). Irritation of animals of high resistance might result from degranulation of mast cells and the subsequent reactions in the host skin (Schleger *et al.*, 1976). Examination of cattle, specially purebred, in this study revealed that many of the female ticks that did not feed to repletion died and appeared shrived up in place on the cattle at various ages and level of engorgement. These responses of the animals to the ticks were apparently immunological.

Data of the present study also showed that differences in the weight of replete females between breeds were highly significant. The replete *B. microplus* female dropped by crossbred steers were significantly heavier ($P<0.05$) that replete female ticks dropped by purebred steers (Table 1). This agrees with results of other researchers (Riek, 1962; Tatchell, 1969; Hewetson, 1971 and Wagland, 1975) who noted that a lighter mean weight of replete female ticks dropped from cattle that were more resistance to *B. microplus*. Riek (1962) reported that replete *B. microplus* females dropped by *Bos taurus* steers were significantly heavier than replete female tick dropped by *Bos indicus* steers. Heweston (1971) observed that purebred and crossbred Sahiwal cattle dropped *B. microplus* similar size when first infested, but as resistance developed, tick dropped by purebred were significantly lighter ($P<0.05$) than those dropped by crossbred. Wagland, (1975). Reported that the mean weight of engorged female *B microplus* dropped from Brahman cattle were significantly lighter than that dropped from Shorthorn cattle from first to fourth infestation, also the mean weight of ticks were lighter in subsequent infestation compare to primary infestation in both genotypes Also as resistance developed, the weight of replete females became progressively less in subsequent infestations in all animals as compared to first infestation.

Amin-Babjee and Riek, (1986) and Passos *et al.* (2000) reported that pure European cattle in experimental infestation with *B. microplus,* all cattle acquired resistance after a single experimental exposure and resulted in a significant reduction in average tick engorgement. Latif *et al.* (1991a) reported that engorged female ticks dropped from East African Zebu cattle were significantly lighter ($P<0.05$) than those dropped from Friesian steers. Similarly, several workers used pure Zebu, European or their crosses in experimental infestation with other tick species. All cattle acquired resistance after a single experimental exposure and resulted in a significant reduction in average tick engorgement weight (Walker *et al.*, 1990; Latif *et al.*, 1991b,c; Dipeolu *et al.*, 1992 and Sahibi *et al.*, 1998). Based on yield of replete females, these data indicate that crossbred were considered less resistance than purebred cattle

In contrary of the results of the present study, pure Zebus, pure European and/or their crosses experimentally infested with other tick species, acquired resistance after a single experimental exposure, with no significant effect in weight of replete female ticks dropped from resistant cattle in subsequent infestations (Strother *et al.*, 1974; Geoge *et al.*, 1985; Norval *et al.*, 1988 and Fivaz *et al.*, 1991). Strother *et al.*, (1974) and George *et al.*, (1985) reported that differences in the weight of replete female tick (*A. americanum*) between the purebred and crossbred *Bos indicus* calves were no significant

Data from the present study showed that the average weight of egg masses laid by replete female ticks dropped from purebred were significantly lighter ($P<0.05$) than that laid by ticks dropped from crossbred (Table 1) Similarly, Reik (1962) concluded in a general terms that the mean egg mass production was greater for *B. microplus* ticks which obtained a blood meal from purebred *Bos taurus*, as compared with purebred and crossbred *Bos indicus*. The mean weights of egg-mass laid by engorged female *B. microplus* ticks dropped from Brahman cattle were significantly lighter than those dropped from Shorthorn cattle from primary to fourth infestation, also the mean weight of egg-mass were significantly lighter in fourth infestation compared to first infestation in both genotypes (Wagland, 1978). Pure Zebus, pure European and their crosses experimentally infested with various tick species acquired resistance after single experimental exposure and resulted in significant reduction in egg-mass weight in subsequent infestations (Utech and Wharton, 1982; Gill, 1986; Momin *et al.*, 1991; Sahibi *et al.*, 1998 and Passos *et al.*, 2000). this is consistent with the results of the current study.

In contrary, Hewetson, (1971) and Amin-Babjeeb and Riek, (1986) reported that although all animal acquired resistance after single infestation with *B. microplus* larvae, the egg-laying capacity of female ticks dropped from resistant animals was not affected. Also Strother *et al.*, (1974) and George *et al.*, (1985) reported that both purebred and crossbred *Bos indicus* calves acquired resistance following infestations with adult *A. americanum*, no statistical significant differences in the oviposition data between breeds.

Data on the hatchability of eggs laid by replete female ticks from the two groups of cattle are shown in Table (1). The difference between the hatchability of eggs from females dropped by purebred steers as compared to their crossbred was highly significant ($P<0.05$) from first to fourth infestation. The hatchability of eggs from purebred steers was considerably less than crossbred.

However, in contrast to the result of our study, several authors used pure Zebu, pure European and their crosses experimentally infested with *B. microplus* showed that all cattle acquired significant level of resistance after a single experimental infestation, there were no significant effect on the viability of eggs of ticks dropped from these animals (Amin-Babjee and Riek, 1986; Panda *et al.*, 1992). Hewetson (1971) reported that there was no significant differences in the number of *B. microplus* egg-produced during first and second infestations of purebred and crossbred *Bos indicus* cattle. During the third and fourth infestations, significant fewer ticks engorged on any of these animals regardless of genetic composition. This indicated a similar pattern of resistance acquisition. Similarly, pure Zebu, pure European and their crosses experimentally infested with other tick species, acquired resistance after a single experimental exposure and resulted in a significant reduction in the viability of eggs laid by female ticks (Strother *et al.*, 1974 and Momin *et al.*, 1991). Strother *et al.* (1974) reported that the difference between the hatchability of eggs from females (*A. americanum*) dropped by *Bos indicus* steers as compared to *Bos taurus* or *Bos indicus* crossbred animals was highly significant

Trends observed in this study on the reduction in average yield and weight of replete female ticks as well as reduction in mass egg production and hatchability of eggs indicate that a program of crossbreeding Zebu with European breeds of cattle may be effectively utilized as a non-chemical approach to tick control in areas heavily infested with cattle tick.

Acknowledgement

This study was supported by University Putra Malaysia and Malaysian Technical Cooperation program for a Ph.D degree (parasitology) in Faculty of Veterinary Medicine and Animals Science, Malaysia.

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