SUCCESS AND FECUNDITY OF *AMBLYOMMA AMERICANUM* (L.) ON BRAHMAN, HEREFORD, AND BRAHMAN X HEREFORD CROSSBRED HEIFERS

by

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CHAPTER I

INTRODUCTION

Lone star ticks, Amblyomma americanum (L.), are major pests of domestic livestock and wildlife throughout much of the southeastern and southwestern United States, and particularly in the Ozark region (Drummond 1967, Hair and Howell 1970). Recommendations for control of ticks on cattle are based on topical application of acaricides. Because of problems associated with topical application of acaricides in areas such as the Ozark region, control of ticks on cattle is often difficult and costly. Additional labor is required to gather animals in areas where heavy vegetation occurs and because of the short residual of the acaricides currently in use, animals must be dipped or sprayed every 10-14 days to prevent tick cycling. Thus, increased vegetation and availability of suitable microhabitat found in the Ozark area are not only conducive to increased survival of the lone star tick but also contributes to the cost of cattle production in this area.

Integrated approaches to the control of ticks on cattle that emphasize basic ecological principles and preservation of environmental quality are needed. One such approach, that of utilizing resistance to tick attack in different breeds of cattle, has been extensively used in tropical and to some extent, subtropical areas of Australia to supplement chemical control of Boophilus microplus (Canestrini), the

southern cattle tick (Hewetson 1968, Johnston and Haydock 1969, Utech et al. 1978 (a,b), Wharton et al. 1969, Wilkinson 1962). Reduced usage of acaricides with less handling of livestock could be accomplished by using resistant animals. Furthermore, a reduction in the use of acaricides would contribute to reducing the possibility of environmental contamination and minimizing the probability of the development of an acaricide-resistant strain of ticks.

Studies conducted in the United States have recently demonstrated differences in the susceptibility of various breeds of cattle to lone star ticks. Strother et al. (1974) in a laboratory study found differences in the susceptibility to adult lone star ticks and reduced fecundity of females engorging on various breeds of cattle. These workers demonstrated that Brahman and Brahman x Hereford crossbred animals were more resistant to adult lone star ticks than purebred Hereford. Stacey et al. (unpublished data) when studying the effects of lone star ticks on weight gain and blood composition of drylot Brahman and Hereford steers, found Brahman supported fewer numbers of adult lone star ticks than Hereford. No published information is available that compares the level of infestation of lone star ticks on various breeds of cattle on native pasture and no information is available in the literature on the success and viability of lone star tick larvae and nymphs after feeding on various breeds of cattle.

Although Brahman cattle are more resistant to lone star ticks (Strother et al. 1974), they are often not as acceptable as English breeds by commercial beef producers because carcass quality is slower to obtain, they have a longer recycling period after calving, and they are frequently difficult to manage. Since Brahman and English breeds of

cattle have different evolutionary backgrounds, improvements in carcass quality and increases in productivity due to hybrid vigor when cross-breeding is practiced should be expected. Considerable interest among beef cattle producers in this direction is being generated as a result of the acceptance by cattle feeders of cattle that contain some Brahman breeding. Because of these facts, studies comparing the success and fecundity of lone star ticks on Brahman x Hereford crossbred and Hereford animals would be practical and timely.

Reported in this manuscript are a series of studies designed to determine the success and fecundity of lone star ticks on Brahman, Hereford, and Brahman x Hereford crossbred heifers under both woodlot-pasture and laboratory conditions. The overall objective of these studies was to investigate the potential use of Brahman crossbreeding for managing lone star tick populations. Also reported are observations on the host response to repeated feeding by ticks and attempts to demonstrate serologically circulating antibodies to tick oral secretions.

CHAPTER II

A COMPARISON OF AMBLYOMMA AMERICANUM (L.) ON BRAHMAN AND HEREFORD HEIFERS

Lone star ticks, Amblyomma americanum (L.), are abundant and important pests of cattle in wooded pastures of the Ozark region. Although many ranchers in the region practice some chemical control efforts against this pest on cattle, control is minimal and tick numbers on treated herds are frequently great (Hair and Howell 1970). Conventional methods of dipping or spraying with acaricides for tick control are difficult and often costly because of the increased labor of gathering animals. In addition, short residual of most pesticides now in use require that application be made every 10-14 days to prevent tick cycling. The overall objective of this and related studies was to investigate the potential use of Brahman (zebu) crossbreeding for managing ticks.

Although tick resistance in cattle has been extensively studied in Australia (Utech et al. 1978) the only published work in the U.S. on lone star ticks is that of Strother et al. (1974) who evaluated resistance by Brahman, Hereford, and Brahman x Hereford crossbred steers under laboratory conditions. They reported that Brahman and Brahman x Hereford crossbred steers were more resistant to lone star ticks and produced fewer replete females with lighter mean ($\overline{X}$) wts than Hereford. They also noted that hatchability of eggs from females feeding on

Brahman steers was significantly less than the hatchability of eggs from ticks repleting from the other test animals.

This study was designed to compare lone star tick populations on purebred Brahman and Hereford breeds under field conditions. In addition, the effects of the 2 breeds of cattle were measured on the biotic potential of A. americanum.

Materials and Methods

Ten Hereford heifers, avg ca. 175 kg, and 10 Brahman heifers, avg ca. 178 kg, were placed in a 125 ha woodlot pasture in eastern Oklahoma. About 0.75 kg/head of 20% natural protein pellets were given every 2-4 days to facilitate handling animals on pasture. The entire left side of each animal was selected as a sample area for adult ticks; both sexes were counted and females were categorized as to engorgement stage. Engorgement stage was estimated visually for female ticks using the following system: flat ticks (<100 mg), stage A -- a female tick exhibiting only slight distension of the body (ca. 100-200 mg), stage B -- moderately distended (ca. 200-400 mg), stage C -- fully distended (>400 mg). This rating system was utilized since it was impractical under field conditions to remove and weigh all ticks.

The left ear and a 150±1 mm^2 area on the brisket of each animal were selected as sampling areas for nymphs and larvae. Total numbers of nymphs and larvae and estimated stage of engorgement were recorded. Engorgement of larvae and nymphs was estimated visually as either engorged (larvae >0.45 mg, nymphs >4.0 mg) or unengorged (larvae <0.45 mg, nymphs <4.0 mg).

Under field conditions, observations on the biotic potential of lone star ticks was impractical. Therefore, when available, 10 adult female ticks at engorgement stage C were removed from each animal weekly and placed in 0.47 liter paper ice cream cartons, cooled with wet ice, and transported to the laboratory where they were weighed individually on a Voland^{R} analytical balance to the nearest 0.1 mg and placed in preweighed 29.5 ml plastic oviposition cups. The cups containing the female ticks were transferred to oviposition chambers at 27°±1°C, 90-98% RH, and a light:dark regimen of 14:10. After 22 days, egg masses were weighed, returned to the chambers, allowed to hatch, and % eclosion was recorded.

If present, 10 larvae and nymphs/animal were removed and weighed individually. These were placed in rearing chambers under the conditions described for females. After 45-50 days postmolt, the molted nymphs and adults were weighed.

Numbers of ticks on the cattle and estimated stage of engorgement were used as criteria to compare the 2 breeds and mean wt of removed ticks, mean egg mass wt, postmolt wt, % molt, and % hatch of eggs laid were used to establish breed effect on tick biotic potential. Data collected were analyzed statistically as a split-plot in time with main units (heifers of both breeds) in a completely randomized design, and dates served as subunits. For each criterion, treatment means at each date between breeds and date means within each breed were compared by the LSD test (5% level).

Results and Discussion

Table 1 presents the degree of adult infestation of lone star ticks on Brahman and Hereford heifers. Significant differences ($P<0.001$) between breeds were found and Hereford heifers supported ca. 3 times more ticks than Brahman heifers. Of the estimated percentage female engorgement, ticks at engorgement stage A made up the majority in both breeds. For Hereford animals, ticks at engorgement stage A averaged 32.7%, at stage B, 9.8%, and at stage C, 5.2%. The percentage female engorgement on Brahman measured engorgement stage A at 52.6%, at stage B, 8.5%, and at stage C, 3.1%.

We observed a similar trend in nymphal and larval tick counts as was apparent with adults on the 2 cattle breeds (Table 1). Although no significant differences were evident between breeds except for the nymphs observed attaching to the animal's ears ($P<0.001$), Brahman heifers carried fewer numbers of immature ticks. Of larvae and nymphs recorded on Hereford heifers an average of 55.8% of the larvae and 45.3% of the nymphs were engorged. For Brahman heifers, an average of 69.2% of the larvae and 38.7% of the nymphs were engorged.

Resistance to ticks is influenced by animal breed (Hewetson 1972) and is a combination of the host's immunological responses (both innate and acquired) and behavioral responses, i.e., grooming (Arthur 1973). We observed that Brahman animals tended to carry fewer ticks of all stages than Herefords. Our data agree with American (Strother et al. 1974, Stacey et al. 1978, Williams et al. 1977) and Austrailian (O'Kelly and Spiers 1976, Wagland 1975, 1978 a,b) workers who reported that Brahman cattle support fewer ticks than other breeds.

Table 1.--Infestation of lone star ticks on woodlot-pastured Brahman and Hereford heifers in eastern Oklahoma, 1977.[a]

	Adults ($\bar{X}$ ± SE)[b]				Immatures ($\bar{X}$ ± SE)[c]			
	Male[d]		Female[d]		Nymphs		Larvae	
Date	Hereford	Brahman	Hereford	Brahman	Hereford	Brahman	Hereford	Brahman
June 3	101.9±13.3a	33.0±3.4a	111.0±13.5a	30.5±6.1a	39.3±6.3a	8.9±2.1a	0.0	0.0
June 10	58.8±9.4b	18.1±3.0b	61.2±7.2b	18.1±2.2b	29.4±5.8a	11.4±3.5a	0.0	0.0
June 17	36.0±4.9c	8.4±2.0b	43.5±5.7c	9.5±2.1b	28.3±5.3a	5.2±1.5a	0.0	0.0
June 24	21.1±3.4d	4.0±0.7b	33.9±4.6c	8.4±1.1b	9.3±1.3b	4.6±1.2a	0.0	0.0
July 1	11.7±2.4e	2.9±0.4b	22.6±3.3d	5.0±0.8b	11.4±3.6b	4.4±1.2a	13.1±5.9b	14.1±6.3ab
July 8	3.9±0.5e	1.5±0.5b	8.3±1.1e	2.3±0.3b	8.4±4.7b	2.2±1.0a	28.6±7.0a	20.1±8.7a
July 15	1.8±0.6e	0.5±0.2b	4.5±1.2e	0.8±0.5b	2.7±1.1b	1.6±0.7a	17.3±6.7n	12.8±3.7b
July 22	0.6±0.3e	0.1±0.1b	2.4±0.7e	0.1±0.1b	6.4±3.6b	1.0±0.4a	12.6±6.1b	11.5±3.8b
July 29	0.3±0.2e	0.2±0.1b	0.6±0.2e	0.3±0.1b	1.4±0.5b	0.8±0.5a	17.4+5.9b	17.3±6.2ab
Aug. 5	0.1±0.1e	0.0b	0.9±0.3e	0.1±0.1b	2.8±0.8b	1.1±0.6a	39.8±13.7c	18.8±6.7ab

[a]Date means within each breed followed by the same letter are not significantly different at the 1% level (LSD).

[b]Total counts of adults are all ticks attached to one side and represent 50% of total attached/head. There were 10 animals of each breed.

[c]Total immature counts are sum of ear and brisket counts/animal.

[d]Breed means over dates were significantly different ($P<0.001$).

The data in Table 2 are measures of the reproductive potential for female ticks collected from Brahman and Hereford animals. Significant differences ($P<0.005$) were observed between breeds for female wt and egg mass wt. Females collected from Brahman weighed an avg of 380.6 mg and laid an avg of 216.5 mg of eggs and females collected from Hereford weighed an avg of 442.9 mg and laid an avg of 256.5 mg of eggs. There were some significant differences between dates for egg mass wt and female wt (see Table 2 for date mean differences by LSD, 5% level). For % hatch, there was a decline over dates in both breeds but there were no significant differences between breeds.

Table 3 shows mean wt, postmolt wt, and % molt of immature ticks collected from Brahman and Hereford heifers, and these are further measures of their biological potential. Average wt of larvae and nymphs collected from the 2 breeds were significantly different ($P<0.025$) with ticks collected from Brahman heifers weighing less, 0.64 mg for larvae and 5.44 mg for nymphs, than from Hereford heifers, 0.72 mg for larvae and 7.10 mg for nymphs. Postmolt wts of larvae and nymphs collected from the 2 breeds were also significantly different ($P<0.025$); avg postmolt wts for larvae and nymphs were 0.24 and 2.74 mg and 0.20 and 2.15 mg for Hereford and Brahman heifers, respectively. Differences in % molt of larvae and nymphs between the 2 breeds were not significant but fewer immatures collected from Brahman heifers molted.

Data in Tables 2 and 3 assume importance when the total effect of these 2 cattle breeds on the biotic potential of this tick species is considered. Brahman and Hereford breeds successfully reduced the population of ticks available for future infestation by suppressing the

Table 2.--Effects of woodlot-pastured Brahman and Hereford heifers on reproduction in female lone star ticks.[a]

Date	Mean wt (mg)[b]		Egg Mass wt (mg)[b]		% Hatch	
	Hereford	Brahman	Hereford	Brahman	Hereford	Brahman
June 3	435.6ab	395.8a	261.7a	239.2a	91.5a	94.1a
June 10	452.6ab	371.3ab	269.1a	196.1b	95.2a	92.8a
June 17	433.8b	314.2c	255.4a	156.8bc	97.9a	59.6b
June 24	440.8ab	343.5bc	235.9a	151.8c	64.5b	51.0b
July 1	465.2ab	--	238.4a	--	55.4b	--
July 8	478.7a	360.0ab	237.6a	134.7c	41.5c	30.1c
Mean	442.9	380.6	256.5	216.5	74.3	65.5
SE	±15.9	±15.9	±23.5	±23.5	±3.5	±3.5

[a]Date means within each breed followed by the same letter are not significantly different at the 5% level (LSD).

[b]Breed means over dates were significantly different ($P<0.005$). There were 10 animals of each breed.

Table 3.--Mean wt, postmolt wt, and % molt of immature lone star ticks collected from woodlot-pastured Brahman and Hereford heifers, 1977.[a]

	Nymphs						Larvae					
	Mean wt (mg)[b]		Postmolt wt (mg)[b]		% Molt		Mean wt (mg)[b]		Postmolt wt (mg)[b]		% Molt	
Date	H[c]	B[c]	H[c]	B	H	B	H	B	H	B	H	B
June 17	8.64a	8.33a	3.16a	3.11a	100a	90a	--	--	--	--	--	--
July 8	7.48ab	--	2.74b	--	100a	--	--	--	--	--	--	--
July 15	6.65bc	4.64b	2.66b	1.83b	91.6a	100a	0.78a	0.62a	0.25a	0.20a	94.1a	87.2a
July 22	5.39c	4.87b	2.17b	1.99b	100a	66.6b	0.58b	--	0.20a	--	85.7a	--
July 29	7.89ab	4.19b	3.14ab	1.95b	90.9a	33.3c	0.73a	0.65a	0.24a	0.19a	69.8b	50.0b
Aug. 5	6.52bc	5.16b	2.58bc	1.89b	91.3a	68.8b	0.77a	0.64a	0.23a	0.20a	88.9a	50.0b
Mean	7.10	5.44	2.74	2.15	95.6	71.7	0.72	0.64	0.24	0.20	84.6	62.4
± SE	0.42	0.42	0.17	0.17	20.7	20.7	0.03	0.03	0.02	0.02	18.7	18.7

[a]Date means within each breed followed by the same letter are not significantly different at the 5% level (LSD).

[b]Breed means over dates were significantly different ($P<0.025$).

[c]Letters represent breed, H = Hereford and B = Brahman. There were 10 animals of each breed.

--No immatures were available on that date from that breed.

tick's biotic potential, thus affecting the survival of this species after engorgement and detachment from its host. However, the effect of the Hereford breed on the biotic potential of lone star ticks was not at a level as great as occurred with Brahman and it occurred later in the season.

CHAPTER III

FECUNDITY AND SUCCESS OF *AMBLYOMMA AMERICANUM* (L.) ON WOODLOT-PASTURED HEREFORD AND BRAHMAN X HEREFORD CROSSBRED (BRAFORD) HEIFERS

The lone star tick, *Amblyomma americanum* (L.), is frequently found abundant on cattle in wooded pastures of the Ozark region. Recommendations for control of this tick species on cattle are based on topical application of acaricides and because of short residual, dipping or spraying must be done every 10-14 days to prevent cycling of ticks. Thus, cattle production is often costly, especially in this region where heavy vegetation dictates the necessity of additional labor to gather animals. In addition, the increased vegetation and availability of suitable microhabitat found in this area favor the survival of the tick after engorgement and detachment from its host (Semtner et al. 1971, Patrick and Hair 1978, 1979).

Recent studies in the U.S. suggest ticks cause economic losses to the cattle industry and show differences in yield of ticks from various breeds of cattle. Williams et al. (1977, 1978) when studying the effects of *Amblyomma maculatum* Koch on drylot and native pastured Hereford steers, found reductions in yield of ticks over time on the infested animals. Similarly, when the effects of *A. maculatum* infestations were compared on drylot Hereford and Brahman steers, Stacey et al. (1978) reported Brahman were less affected and supported

fewer numbers of ticks than Hereford. Strother et al. (1974) has also reported differences in various breeds of cattle to infestations with A. americanum.

More recently, Garris et al. (1979) found that when purebred Brahman cattle were compared to Hereford on native woodlot pasture, Brahman produce fewer numbers of larvae, nymphs, and adults of the lone star tick. These workers also reported that the biotic potential of ticks collected from Brahman was less than that for ticks collected from Hereford.

Straightbred Brahman cattle are frequently not as acceptable by commercial beef producers as English breeds because carcass quality is slower to obtain, they are sometimes difficult to manage, and they have a longer recycling period after calving. Improvements in carcass quality and a dramatic increase in the productive life over purebred Brahman can be achieved through crossbreeding. Reported here are results of a study to determine the fecundity and success of A. americanum on woodlot-pastured Hereford and Braford (Brahman x Hereford Crossbred) heifers.

Materials and Methods

Experimental procedures were essentially the same as described in Chapter II. Briefly, 10 Hereford heifers, average ca. 193 kg, and 10 Braford heifers, average ca. 219 kg, were placed in a 125 ha woodlot-pasture in eastern Oklahoma. Animals were kept on pasture from 8 May until 10 August.

Both sexes of adult lone star ticks attached to the left side of each animal were counted weekly and the females categorized as to stage

of engorgement. Total larvae and nymphs attached to the left ear and a 150±1 mm^2 area on the brisket and around the anus/animal were counted weekly and degree of engorgement recorded.

When available, 10 replete larvae, nymphs, and adult females (stage C)/animal were removed, transported to the laboratory, weighed individually, and placed in rearing chambers at 27°±1°C, 90-98% RH, and under a 14:10 hr photoperiod.

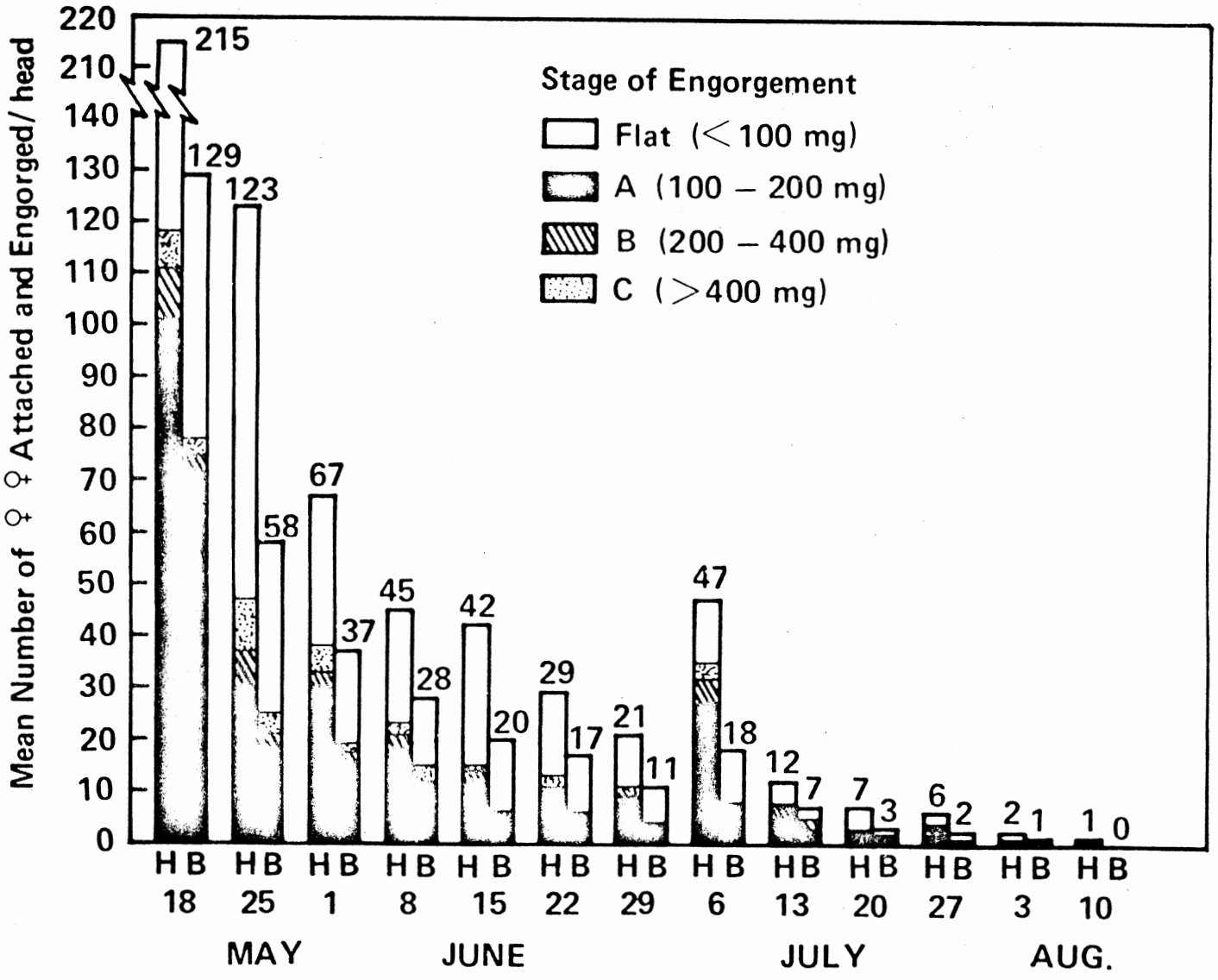
Data were collected from adult females on egg mass wt and % egg hatch, and from immatures on % molt and postmolt dry wt. Dry wts were determined as follows: molted nymphs and adults were placed in pre-weighed glass test tubes, heated at 100°C for 24 hrs, weighed, and dry wts calculated.

This study was designed as a split-plot with heifers on both breeds serving as main units and dates as subunits. Tick counts and estimated stage of engorgement, mean wt of collected ticks, mean egg mass wt, % egg hatch, % molt, and postmolt dry wt were the criteria used to evaluate breed effect on tick fecundity and success.

Results and Discussion

The patterns of adult female infestation and engorgement are presented in Fig. 1. Significant differences ($P<0.001$) between breeds were found with Hereford heifers carrying more than twice as many female ticks as Braford heifers. Garris et al. (1979) reported that Brahman heifers carried ca. 1/3 as many female lone star ticks as did Hereford heifers. Counts of females on both breeds were recorded at high levels at the beginning of the study but decreased over time.

Fig. 1. Mean number and estimated stage of engorgement of female (♀) lone star ticks on the left side of woodlot-pastured Braford (B) and Hereford (H) heifers in eastern Oklahoma, 1978.


Mean Number of ♀ ♀ Attached and Engorged/ head
220
210
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Stage of Engorgement
Flat (< 100 mg)
A (100 – 200 mg)
B (200 – 400 mg)
C (>400 mg)
215
129
123
58
67
37
45
28
42
20
29
17
21
11
47
18
12
7
7
3
6
2
2
1
1
0
HB 18
HB 25
HB 1
HB 8
HB 15
HB 22
HB 29
HB 6
HB 13
HB 20
HB 27
HB 3
HB 10
MAY
JUNE
JULY
AUG.

The engorgement behavior of female lone star ticks found on the 2 breeds was not significantly different but biological trends were evident. On 25 May and again on 6 July, a large proportion of the female ticks engorging on Hereford were observed at the estimated stages B and C (Fig. 1). There was an increase in the number of female ticks observed at engorgement stages B and C on Braford heifers on 25 May, but no increase was observed on 6 July. Thus, when compared to Hereford, Braford were able to prevent females from engorging to a stage that could be visually distinguished from unengorged (flat) or A stage ticks.

The infestation patterns observed for males were similar to females. Significant differences ($P<0.001$) were found between breeds with Hereford heifers carrying twice as many throughout the study as did Braford heifers.

Patterns of infestation and estimated stage of engorgement of nymphs and larvae counted on both breeds are presented in Fig. 2. Nymphal counts and estimated stage of engorgement on both breeds were similar. Peak engorgement of nymphs occurred in late May and early June on both breeds. A second peak in nymphal counts and estimated stage of engorgement was also observed in early August and may represent activity by newly molted nymphs since larvae began feeding in early July. Larval counts and estimated stage of engorgement were significantly different ($P<0.05$) between breeds. Braford heifers consistently carried less than Hereford heifers, and of those counted on Braford fewer were recorded as engorged.

Table 4 shows the effects of woodlot-pastured Braford and Hereford heifers on female lone star tick reproduction. Significant differences

Fig. 2. Mean number of flat and replete larvae and nymphs on woodlot-pastured Braford (B) and Hereford (H) heifers in eastern Oklahoma, 1978. Means are sum of ear and 150±1 mm^2 areas on the brisket and around the anus/animal.

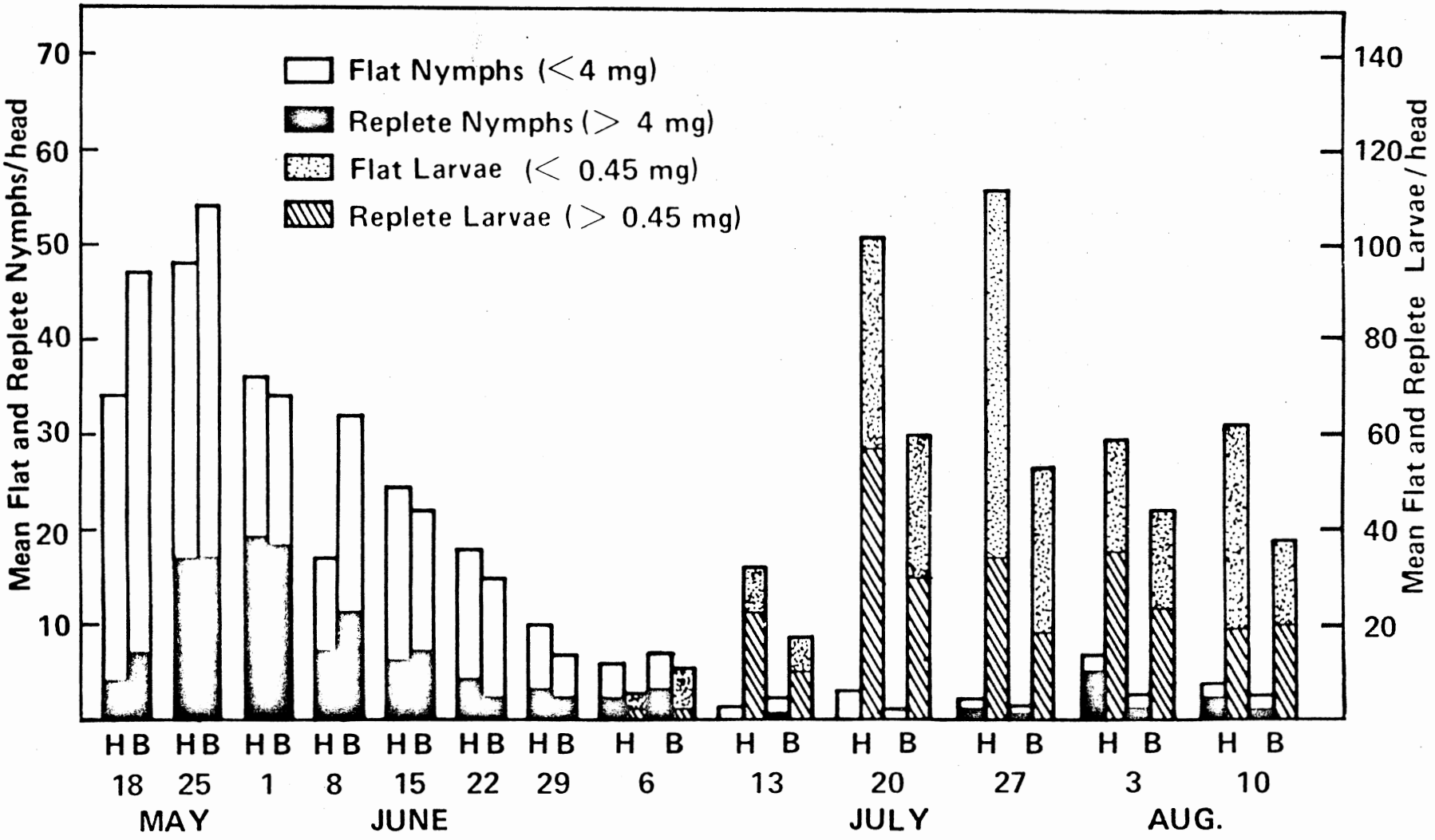

Flat Nymphs (<4 mg)
Replete Nymphs (> 4 mg)
Flat Larvae (< 0.45 mg)
Replete Larvae (> 0.45 mg)
Mean Flat and Replete Nymphs/head
Mean Flat and Replete Larvae/head
70
60
50
40
30
20
10
140
120
100
80
60
40
20
H B H B H B H B H B H B H B H B H B H B H B H B H B
18 25 1 8 15 22 29 6 13 20 27 3 10
MAY
JUNE
JULY
AUG.

Table 4.--Effect of woodlot-pastured Braford and Hereford heifers on female reproduction in lone star ticks.[a]

Date[c]	Mean wt (mg)[b]		Egg Mass wt (mg)[b]		% Hatch[b]	
	Hereford	Braford	Hereford	Braford	Hereford	Braford
May 18	518.0	447.9	235.5	176.0	40.8	32.5
May 25	473.6	424.0	226.8	228.6	35.6	41.6
June 1	423.3	404.1	242.2	235.5	85.4	88.9
June 8	414.9	336.8	249.9	185.2	91.4	90.6
June 15	464.2	389.7	235.2	152.4	39.1	31.3
June 22	390.5	384.5	176.9	163.1	4.2	8.0
June 29	381.7	297.5	166.6	162.4	9.6	26.4
Mean	453.6	411.0	228.4	199.6	50.2	48.2
± SE	10.7	14.1	6.3	8.3	1.6	2.2

[a]The interaction of breed and date was significant ($P<0.05$) for % hatch but not for egg mass wt or mean wt ($P>0.10$).

[b]Breed effects were significant for mean wt ($P<0.01$) and egg mass wt ($P<0.05$) but not for % hatch ($P>0.50$).

[c]Date represents date females collected.

between breeds were found for female wt ($P<0.01$), and egg mass wt ($P<0.05$). Garris and Hair (unpublished data) in another study with Braford and Hereford heifers artificially infested with known numbers of lone star ticks, also found significant differences between breeds for female wt and egg mass wt. Thus, the evidence indicates that the Braford breed has an effect on the ticks ability to take in blood thus, causing a reduction in wt when compared to female ticks feeding on Hereford. It is well established that the wt of a female tick is positively correlated with the number of eggs produced and thus, the egg mass wt (Drummond et al. 1971). Since significant differences between breeds were also found for egg mass wt, use of Braford in a tick management program should reduce future populations of ticks.

There were no significant differences between breeds for % hatch of eggs. The interaction of breed and date were not significant for any of the parameters measured except the % egg hatch. Thus, breed differences were constant over dates for female wt and egg mass wt. For the % egg hatch, the most marked effect occurred during the period of 8 to 22 June, at which time the % hatch of eggs laid by females collected from Braford had declined to an average of 8% compared to 4.2% for eggs laid by females from Hereford. Beyond this period, an increase in % hatch occurred for both breeds.

Table 5 show mean wt and % molt of nymphs and dry postmolt wt of adults molting from nymphs collected from Braford and Hereford heifers. Significant differences were found for nymphal wt, % molt, and dry postmolt wt for males ($P<0.05$) collected from Braford. There were no significant differences found between breeds for dry postmolt wt of females indicating a possible biological response by the tick to insure

Table 5.--Effect of woodlot-pastured Braford and Hereford heifers on nymphal lone star tick success.[a]

Date[c]	Mean wt (mg)[b]		% Molt[b]		Dry Postmolt wt (mg)[b]			
	Hereford	Braford	Hereford	Braford	Hereford		Braford	
					Females	Males	Females	Males
May 18	7.24	6.01	51.7	48.4	1.31	0.83	--	0.76
May 25	6.07	6.07	80.4	57.9	1.33	0.75	1.35	0.70
June 1	6.71	6.42	87.8	83.9	1.40	0.83	1.35	0.71
June 8	5.90	4.92	87.7	79.7	1.07	0.69	1.02	0.59
June 15	6.91	6.42	72.2	70.7	1.47	0.79	1.33	0.62
June 22	6.14	5.41	0	0	--	--	--	--
June 29	6.88	5.59	75.0	66.7	1.39	0.68	0.57	--
Mean	6.54	5.89	79.3	71.1	1.34	0.78	1.16	0.79
± SE	0.22	0.25	3.0	3.3	0.1	0.1	0.1	0.1

[a]The interaction of breed and date was not significant ($P>0.50$) for mean wt, % molt, or dry postmolt wt.

[b]Breed effects were significant for mean wt, % molt, and dry postmolt wt of males ($P<0.05$) but not for dry postmolt wt of females ($P>0.10$).

[c]Dates represent date nymphs collected.

survival since female wt is associated with future egg production (Koch and Hair 1975, Drummond et al. 1971). Breed effect on nymphal success was most evident on 22 June when no nymphs collected from either breed molted. Mean wt of nymphs and dry postmolt wt of females from nymphs collected from Braford animals decreased until 29 June when an increase occurred. There was also no significant interaction between breed effect and date, indicating that effect of breed on nymphal success was constant over time.

The effect of Braford and Hereford heifers on larval survival is presented in Table 6. Significant differences between breeds were found for larval wt ($P<0.05$). No differences were found for % molt or dry postmolt wt. There was also no significant interaction of breed and date.

The data presented in Fig. 1 and Tables 4-6 assume importance when the total impact of breeds on the success and fecundity of lone star ticks is considered. When compared to Hereford, Braford were less susceptible to tick attack and had a greater reduced effect on the survival of lone star ticks after engorgement and detachment from its host. Thus, a Brahman crossbreeding program should successfully limit build up of future populations of ticks and thus provide an effective, economical, and supplemental approach to chemical control of lone star ticks with minimum handling of animals.

Table 6.--Effect of woodlot-pastured Braford and Hereford heifers on larval lone star tick success.[a]

Date[c]	Mean wt (mg)[b]		% Molt[b]		Dry Postmolt wt (mg)[b]	
	Hereford	Braford	Hereford	Braford	Hereford	Braford
July 6	0.599	0.428	90.0	55.4	0.046	0.034
July 13	0.473	0.433	91.6	82.2	0.067	0.053
July 20	0.537	0.506	75.7	75.7	0.098	0.092
July 27	0.480	0.342	88.2	85.9	0.104	0.083
Aug. 3	0.608	0.572	89.3	90.9	0.099	0.094
Aug. 10	0.755	0.721	87.3	86.1	0.152	0.126
Mean	0.565	0.496	86.1	82.2	0.094	0.079
± SE	0.02	0.02	19.4	21.9	0.005	0.005

[a]The interaction of breed and date was not significant ($P>0.50$) for mean wt, % molt or dry postmolt wt.

[b]Breed effects were significant for mean wt ($P<0.05$) but not for % molt and dry postmolt wt ($P>0.16$).

[c]Dates represent date larvae collected.

CHAPTER IV

BREED EFFECT ON THE SUCCESS AND FECUNDITY OF *AMBLYOMMA AMERICANUM* (L.) AFTER REPEATED INFESTATIONS

It is reported that Brahman cattle (Zebu) and their crosses are more resistant to tick infestation than English cattle (Utech et al. 1978 (a,b), Strother et al. 1974, Norval 1975, Garris et al. 1979). It is also established that within a breed individuals show different levels of resistance (Utech et al. 1978 (a), Seifert 1971). Selection for resistance in different breeds of cattle to *Boophilus microplus* (Canestrini) has been effectively used to supplement chemical control on cattle in Australia (Utech et al. 1978 (a,b), Franklin et al. 1976). Until recently, no reported attempt has been made to utilize Brahman crossbreeding to supplement chemical control measures for managing economically important ticks in the U.S. It was the purpose of this and related studies to investigate the potential use of Brahman crossbreeding for managing populations of *Amblyomma americanum* (L.), the lone star tick, on cattle.

Knowledge of the effect of breed of cattle on the biotic potential of all developmental stages of a 3-host tick species is vital before management strategies against it can be evaluated. Norval (1978) found after repeated infestations with immature *Amblyomma hebraeum* Koch that

rabbits and sheep were unable to develop resistance and speculated that the inability of a host to acquire an effective level of resistance would allow ticks to utilize a wide host range and thus, insure survival. Sutherst et al. (1979) while studying resistance in cattle to Haemaphysalis longicornis Neumann found differences in numbers of larvae, nymphs, and females maturing on previously exposed cattle, indicating that these cattle have an effect on the survival of each instar of the tick. Evidence from a previous study (Chapter III) suggested that Hereford and Braford (Brahman x Hereford crossbred) heifers have an effect on the survival of each instar of A. americanum. This study was designed to determine the success and fecundity of lone star ticks on Braford and Hereford heifers under controlled laboratory conditions. In this report, success is measured by the number of ticks surviving to the next developmental stage.

Materials and Methods

Experimental Animals

Seven purebred Hereford heifers, average ca. 205 kg, and 7 Braford heifers, average ca. 232 kg, were purchased through local cattle markets, held in drylot, and fed, free choice, a standard ration to achieve an average daily gain of 0.68 kg. The ration formulation used was essentially the same as described by Williams et al. (1977) and was fed to all animals so that the effect of breed of cattle on the biotic potential of the ticks could be measured under a relatively controlled nutritional plane. Fresh water was available at all times.

After a 2 week acclimation period in drylot each animal was weighed. Five animals of each breed were randomly selected from drylot and placed

in specially constructed individual stalls containing head stanchions. Four animals of each breed were then randomly selected and designated as the treatemnt (Trt) group, the remaining 2 were designated as controls (Cnt). All animals were fed, free choice, the standard ration throughout the remainder of the study.

Rabbits were used as hosts to rear larvae and sheep were used as hosts for nymph and adult ticks used in bovine challenges. Larvae and adults were reared on the respective hosts as described by Patrick and Hair (1975). Nymphs were reared on sheep in organdy cloth cells that were attached with contact cement to a shaven area on the hosts' side. The organdy cloth allowed for ventilation of the cells so that build up of moisture and waste materials would not interfere with the engorgement process of the nymphs. Rearing nymphs on sheep also allowed for greater production of adults needed for artificial infestation of bovine. Unfed adults were maintained in the laboratory in rearing chambers until needed for bovine challenge.

Artificial Infestation of Bovine Host

It was assumed that a ratio of ∿ 1:1 males - females existed. After a 1 week acclimation period in the head stanchions, each animal in the Trt group was infested with 5,000 adult ticks weekly for 9 weeks by placing the ticks along the backline of the stanchioned animals. Controls were not infested but were later challenged with Trt as described below so that the effects of both previously unexposed and exposed cattle on the biotic potential of confined ticks could be compared. New Cnts replaced old ones before each challenge. At weekly intervals, counts were made to determine the number of nonconfined adult ticks attaching to

the stanchioned animals.

Assessment of Tick Biological Potential and Survival

At intervals of 3 weeks during the 9 week study, organdy cloth cells as described for the rearing of nymphs on sheep were attached to experimental bovine with contact cement near the withers of each Trt and Cnt animal. Care was taken not to place new organdy cloth cells over old ones or over attachment sites of nonconfined adults. Two hundred larvae and 100 nymphs were placed in separate organdy cloth cells on each animal. Observations were made on the number of larvae and nymphs attached and feeding 48-72 hrs post-infestation.

Cloth stockinette for confining adult ticks (Patrick and Hair, 1975) was also placed on each animal at 3 week intervals. Sixty adults, 30 males and 30 females, were placed in each cell and the number attached and feeding recorded 5-7 days post-infestation.

After repletion of experimental ticks began, larvae, nymphs, and females were collected every 24 hrs, weighed, and randomly separated into 2 groups. One group was placed in environmentally controlled rearing chambers at 27°±1°C, 90-98% RH, and 14:10 hr photoperiod. Observations were made on the amount of eggs laid and the % hatch of eggs of females, and % molt and dry postmolt wt of immatures placed in the chambers. Postmolt dry wts were determined as described in Chapter III 45-50 days postmolt. The second group of ticks harvested was used to determine the volume of blood imbibed. Because few larvae were available from Braford, no determinations were made on the volume of blood ingested by larvae. The technique used was as described by Koch et al. (1974) and Sauer and

Hair (1972) and consisted of homogenizing individual females and nymphs in a 0.1M NaOH solution. After homogenization, 0.8 g sodium dithionite ($Na_2S_2O_2$) and 1 ml of pyridine/6 ml of NaOH were mixed to a total volume of 6 ml/nymph and 180 ml/female, the mixture centrifuged at 2000 rpm for 5 min, poured into a cuvette, and read spectrophotometrically with a spectronic 20 colorimeter at 525 mμ against a blank of 0.1M NaOH, pyridine, and $Na_2S_2O_2$ in the same proportion as the test solution. The volume of blood was determined by comparing the optical densities of the homogenates against known amounts of host blood. Blood samples were periodically drawn from the experimental cattle and optical densities determined.

Statistical Analysis

This study was designed as a randomized split-plot with heifers of both breeds serving as main units (Trt groups) and challenges as subunits. Differences between the effects of previously exposed and naive animals were biologically compared; where feasible t-tests were calculated.

Results and Discussion

Assessment of Tick Biological Potential and Survival

Larvae. Table 7 shows biological potential of larvae of the lone star tick after having fed on previously exposed and unexposed Hereford and Braford heifers. There were significant differences ($P<0.01$, F-test) between the breed Trt groups for attachment and engorgement success of the larvae confined in cells. Of the number placed in the organdy cloth cells, averaging the 3 challenges, 3.1% of the larvae on the Trt Braford

Table 7.--Biological potential of larvae of Amblyomma americanum (L.) after feeding on previously exposed (Treatment) and naive (Control) Hereford and Brahman x Hereford Crossbred (Braford) heifers.

	1st Challenge[a]				2nd Challenge[a]				3rd Challenge[a]			
	Hereford		Braford		Hereford		Braford		Hereford		Braford	
	TrT[b]	CnT[b]	TrT	CnT	TrT	CnT	TrT	CnT	TrT	CnT	TrT	CnT
Average number attached[c] (48-72 hrs Post-Infestation)	97	148[e]	10	3[f]	56	32[f]	23	5[f]	147	178[e]	40	89[e]
Engorgement period (days)[df]	4	4	4	4	4	5	4	5	3	4	3	4
Number repleting[ce]	64	54	4	3	20	26	4	8	120	174	30	96
Average repletion wt (mg)[e]	1.0[d]	1.4	1.0[d]	1.8	0.8[c]	1.0	0.4[c]	0.8	0.8[d]	1.1	0.9[d]	0.9
% Molt[df]	91	64	84	100	83	70	55	50	66	92	73	40
Average dry-post molt wt (mg)[df]	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.1	0.2
% Survival[g]	29.2	17.4	1.7	1.5	8.3	9.1	1.0	2.0	39.1	79.9	11.2	19

[a]200 larvae were placed in organdy cloth cells on all animals at 3 week intervals.

[b]Treatment (TrT) heifers, 4 of each breed, were infested with 5,000 adult lone star ticks/week for 9 weeks. Controls (CnT), 1 of each breed, were infested only with ticks confined in cells. New CnTs replaced old ones at each challenge.

[c]Treatment means were significantly different ($P<0.01$, F-test) between breeds.

[d]Treatment means were not significantly different ($P>0.10$, F-test) between breeds.

[e]Control means were significantly different ($P<0.05$, T-test) from TrT means within each breed.

[f]Control means were not significantly different ($P>0.10$, T-test) from TrT means within each breed.

[g]% Survival = Number repleting/200 (total) x % molt.

were attached at 48-72 hrs post-infestation as compared to 12.5% for Hereford. Similarly, 8.5% of the total number of larvae placed in the cells on Hereford engorged to repletion compared to only 1.6% for Braford. Differences between the effect of Cnt and Trt animals on the average number of larvae attached and repleting at each challenge suggest that individuals within a breed show different levels of susceptibility to infestation. This is in agreement with Utech et al. (1978a), Wagland (1975, 1978a,b), and Seifert (1971) who found considerable variations to infestation with Boophilus microplus (Canestrini) larvae within the same breed.

There were no differences between breed Trt groups for length of the engorgement period but there were biological differences between the Trt and Cnt animals at challenges 2 and 3. Larvae were observed to attach and detach at different sites within the cloth cells on the animals. Movement of larvae was also indicated by the increased number repleting on the Cnt Braford when compared to the number attached at 48-72 hrs post-infestation. During the first 48 hrs on the host, larvae of B. microplus were also found to frequently attach and detach on highly resistant animals (Kemp et al. 1976). These workers also suggested that the sequence of attaching-detaching for short periods could be a defense reaction to avoid detrimental effects of the host, e.g. removal by grooming or death by drowning from exudate secretions. The lower yield of larvae from the Trt animals suggests that fewer favorable attachment sites were available within the cells on the Trt animals. Exudate secretions were observed to form hardened crust-like areas within the cells on 3 of the Trt Braford and 1 of the Trt Hereford. This thin crust over the skin may have prevented the larvae from attaching and thus, may have

caused their death either from dessication or drowning. Larvae of B. microplus were observed to die from dessication 24 hrs post-infestation if prevented from feeding while on the host (Kemp et al. 1976).

Although no significant differences were found for the % molt, dry postmolt wt or repletion wt of larvae, some biological trends were apparent. There were no differences between the 2 breed Trt groups during the first challenge for larval repletion wt but there were differences between the Trt groups in the second and third challenges. The average repletion wt for larvae recovered from the treated Hereford during the second challenge was about twice that for larvae recovered from Braford. However, by the third challenge period, the repletion wt of larvae recovered from the Braford Trt group was greater than that for larvae recovered from the Hereford Trt group but not significant ($P>0.10$, T-test).

Breed effect on the survival of the larvae was greatest during the second challenge period when only 8.3 and 1% of the larvae placed in the cells survived on the Hereford and Braford Trt animals, respectively. A comparison of the survival of larvae feeding on previously exposed (Trt) and unexposed (Cnt) hosts suggests that resistance may not have developed. In contrast, Strother et al. (1974) was able to show resistance to lone star ticks in various breeds of cattle. However, throughout the study Hereford heifers were more susceptible to larval infestation and thus, the yield of ticks greater than from Braford.

Nymphs. The biological potential of nymphs confined in cells on Hereford and Braford heifers repeatedly infested with adults is shown in Table 8. Considerably fewer nymphs successfully attached and repleted on the Braford Trt heifers than on the Hereford Trt heifers. Averaging

Table 8.--Biological potential of nymphs of Amblyomma americanum (L.) after feeding on previously exposed (Treatment) and naive (Control) Hereford and Brahman x Hereford Crossbred (Braford) heifers.

	1st Challenge[a]				2nd Challenge[a]				3rd Challenge[a]			
	Hereford		Braford		Hereford		Braford		Hereford		Braford	
	TrT[b]	CnT[b]	TrT	CnT	TrT	CnT	TrT	CnT	TrT	CnT	TrT	CnT
Average Number attached[ce] (48-72 hrs Post-Infestation)	75	81	34	68	91	88	57	59	74	91	54	87
Engorgement period (days)[df]	4	5	4	5	5	5	5	5	5	5	4	4
Number repleting[ce]	68	86	22	40	74	81	26	28	65	46	49	95
Average repletion wt (mg)	8.5[d]	10.3[e]	8.6[d]	7.8[f]	8.9[c]	9.2[e]	6.8[c]	6.9[f]	7.7[d]	8.3[e]	7.6[d]	10.3[e]
Volume of blood imbibed (µl)[df]	14.6	8.3	17.0	2.7	4.9	8.6	2.2	--	9.1	8.7	10.8	12.1
% Molt[c]	60	28[e]	75	52[e]	59	76[e]	92	83[f]	78	93[e]	90	61[e]
Average dry-post molt wt (mg)[d] of female from nymphs	1.7	1.2[f]	1.3	1.5[f]	1.8	2.2[e]	1.5	1.8[f]	1.7	1.9[f]	1.5	2.2[e]
% Survival[g]	41	24	16	21	43	61	24	23	50	43	44	58

[a] 100 nymphs were placed in organdy cloth cells on all animals at 3 week intervals.

[b] Treatment (TrT) heifers, 4 of each breed, were infested with 5,000 adult lone star ticks/week for 9 weeks. Controls (CnT), 1 of each breed, were infested only with ticks confined in cells. New CnTs replaced old ones at each challenge.

[c] Treatment means were significantly different ($P<0.5$, F-test) between breeds.

[d] Treatment means were not significantly different ($P>0.20$, F-test) between breeds.

[e] Control means were significantly different ($P<0.05$, T-test) from TrT means within each breed.

[f] Control means were not significantly different ($P>0.10$, T-test) from TrT means within each breed.

[g] % Survival = Number repleting/100 (total) x % molt.

the 3 challenge periods, 20% of the nymphs placed in the cloth cells attached and 17.2% repleted on the Trt Hereford animals as compared to 12.1% attaching and 8% repleting on Trt Braford. Differences in the number attached and repleting on the animals also indicates, as with the larvae, some movement of nymphs occurred within the cloth cells. There were no significant differences between the Trt groups of each breed or between Trt and Cnt within the same breed for the engorgement period. However, there were some differences between breeds for the average repletion wt and % molt.

There were no differences between the Trt groups of each breed for the average repletion wt until the second challenge when nymphs repleting on the Hereford Trt group were heavier than nymphs repleting on Braford. However, by the third challenge period the average repletion wt for nymphs from both breeds was similar but biological differences were found for the volume of blood ingested. Data for the volume of blood ingested suggest that nymphs repleting on the Trt Braford imbibed less red blood cells than nymphs repleting on the Trt Hereford. Furthermore, a large proportion of the nymphs repleting on the Trt Braford during the second and third challenges were light in color, e.g. white or clear. Koch et al. (1974) and Sauer and Hair (1972) reported that immature stages of Amblyomma species are sometimes light in color and concluded that this was due to their ingestion of non-blood tissue or fluids.

Differences in the ingested blood meal by nymphs on the 2 breeds and the 2 Trt groups affected the final molted wt of the nymphs. Since the number of eggs laid is positively correlated with the engorged wt of female lone star ticks (Drummond et al. 1971), and since the unfed wt is directly proportional to the engorged wt, those unfed females that

are larger have a greater reproductive potential in nature (Koch and Hair 1975). The average dry postmolt wts for females molting from nymphs recovered from Trt Braford were lighter than those recovered from Trt Hereford. There were significant ($P<0.05$, T-test) differences between Cnt and Trt animals within a breed for the dry postmolt wts (see Table 8).

The % molt of nymphs recovered from the Trt Braford was significantly ($P<0.05$, F-test) greater than that for nymphs from Hereford. However, 44.9% of the total number of nymphs placed in cells on the treated Hereford survived comapred to 27.9% surviving on the treated Braford. Thus, the host effect on the nymphs was similar to that observed for larvae.

Females. Table 9 shows the success and fecundity of female lone star ticks confined in cells on previously exposed and previously unexposed bovine hosts. Although no significant differences between breed Trt groups were found for the average number of females attached, the engorgement period, or % hatch of eggs laid, some biological and behavioral trends were apparent. As observed with both the larvae and nymphs, some movement of females occurred within the stockinette cells. The volume of blood ingested by females repleting on Trt Hereford was significantly ($P<0.05$, T-test) greater than the volume of blood ingested by females repleting on Trt Braford for the second and third challenges but not the first. Thus, the evidence suggest that a host response may have developed and may have affect on the volume of blood imbibed by females. Although no differences were found between the breed Trt groups for % hatch, a decline in the % hatch occurred during the second challenge period for both breeds and Trt groups. Similar results were reported in Chapter III and by Strother et al. (1974) who found no difference between

Table 9.--Reproductive potential of female Amblyomma americanum (L.) after feeding on previously exposed (Treatment) and naive (Control) Hereford and Brahman x Hereford Crossbred (Braford) heifers.

	1st Challenge[a]				2nd Challenge[a]				3rd Challenge[a]			
	Hereford		Braford		Hereford		Braford		Hereford		Braford	
	TrT[b]	CnT[b]	TrT	CnT	TrT	CnT	TrT	CnT	TrT	CnT	TrT	CnT
Average number attached[d] (5-7 Days Post-Infestation	26	23[f]	21	7	28	27[f]	24	29[f]	26	25[f]	23	27[f]
Engorgement period (days)[df]	12	12	11	12	11	11	10	10	10	12	10	12
Average number repleting[c]	26	23[f]	8	3[f]	29	10[e]	18	18[f]	30	30[e]	14	11[e]
Average repletion wt (mg)[ce]	355	396	246	319	442	353	297	261	485	436	358	399
Volume of blood imbibed (µl)	362[d]	386[e]	355[d]	--	548[c]	690[e]	318[c]	430[e]	614[c]	631[f]	454[c]	405[f]
Average egg mass wt (mg)[c]	194	192[f]	118	101[f]	256	152[e]	153	132[e]	268	214[f]	205	252[e]
% Hatch [de]	56	64	59	82	50	58	49	52	72	46	65	68
% Survival[g]	15	15	4	3	14	6	9	9	22	14	9	8

[a]60 adults, 30 males and 30 females, were placed in cloth stockinette cells on all animals at 3 week intervals.

[b]Treatment (TrT) heifers, 4 of each breed, were infested with 5,000 adult lone star ticks/week for 9 weeks. Controls (CnT), 1 of each breed, were infested only with ticks confined in cells. New CnTs replaced old ones at each challenge.

[c]Treatment means were significantly different ($P<0.01$, F-test) between breeds.

[d]Treatment means were not significantly different ($P>0.10$, F-test) between breeds.

[e]Control means were significantly different ($P<0.05$, T-test) from TrT means within each breed.

[f]Control means were not significantly different ($P>0.10$, T-test) from Trt means within each breed.

[g]% Survival = Number repleting/30 (total) x % hatch.

Hereford and Braford breeds for the % hatch of eggs laid by female lone star ticks.

There were significant differences ($P<0.01$, F-test) between breed Trt groups for the number of females repleting. Averaging the 3 challenge periods, 93.4% of all females placed in the cloth stockinette cells repleted on the Trt Hereford compared to 43.4% repleting on the Trt Braford. There were some differences between Trt and Cnt animals within the same breed (see Table 9).

The average repletion wt and egg mass wt of females recovered from the 2 breed Trt groups were also significantly different ($P<0.01$, F-test). Females repleting on the Trt Hereford averaged 423.4 mg and laid an average 284.7 mg and laid an average of 150.2 mg of eggs. In contrast, Strother et al. (1974) were unable to show significant differences for replete female wt or egg mass wt between Braford and Hereford breeds while studying resistance in various breeds of cattle to lone star ticks. Differences within the Braford breed between the Trt and Cnt animals for the egg mass wt, strongly suggest that individual cattle respond differently to repeated infestations with lone star ticks.

The % survival of females placed in the stockinette cells averaged over the 3 challenge periods was 16.7 and 7.4% for the Hereford and Braford Trt groups, respectively. It is evident from the data presented in this chapter and Chapter III that the yield of lone star ticks from Brahman crossbred cattle is significantly less than that from Hereford when the animals were compared under similar conditions. It is also evident that the biotic potential and thus the survival of ticks that had fed on Brahman crossbred animals was less than the biotic potential of ticks that had fed on Hereford. Thus, a program of crossbreeding

Brahman and Hereford animals would be an effective method of managing future populations of ticks and should provide an alternative approach to chemical control of the lone star tick.

CHAPTER V

SEROLOGICAL OBSERVATIONS ON THE HOST RESPONSE TO FEEDING BY *AMBLYOMMA AMERICANUM* (L.)

Resistance to tick attack in cattle is primarily acquired but some innate resistance exist in Brahman (Zebu) and Brahman crossbred cattle (Nelson et al. 1977, Norval 1975, O'Kelly and Spiers 1976). A greater loss of ticks from animals previously exposed than from animals previously unexposed is evidence that resistance has developed (Boese 1974, Koudstaal et al. 1978, Norval 1975, Stacey et al. 1978, Utech et al. 1978b, Williams et al. 1977, 1978). The resistance expressed by different breeds of cattle to tick attack is protective and has both humoral and cellular components (Roberts and Kerr 1976).

Few studies have demonstrated serologically both components of resistance in cattle to ticks. Riek (1962) was unable to show by various serological tests the presence of IgG or IgM immunoglobulins to tick antigens in sera of cattle infested with *Boophilus microplus* (Canestrini). However, he was able to show that resistance was associated with an immediate hypersensitivity reaction to salivary secretions of the tick. Recently, Willadsen et al. (1978) when studying resistance to *B. microplus* in English and Brahman x English crossbred cattle, demonstrated serologically an IgG or IgM immunoglobulin to a specific antigen found in the tick's salivary secretion. These workers also

correlated an increase in antibody titre with higher levels of resistance. In the immediate hypersensitivity reaction, increases in the concentration of histamine as a result of mast cell degranulation near the site of tick attachment caused ticks to detach (Kemp 1978). Thus a strong relationship was shown between resistance and the immediate hypersensitivity reaction (Willadsen et al. 1978, Schleger et al. 1976).

The biological potential of lone star ticks that have successfully fed on various breeds of cattle have been shown to be significantly different (see Chapter III and IV). These differences may arise as a result of an immunological response by the host to the feeding of the tick. The mechanism of this host-tick system may involve humoral antibodies such as the IgG, IgM or IgE immunoglobulins. Attempts were made in this study by using the enzyme-linked immunospecific assay (ELISA) and the indirect haemagglutination tests to demonstrate circulating IgG or IgM immunoglobulins to antigens in salivary gland secretions of the lone star tick. In addition, serum from animals previously exposed to ticks was passively transferred to a calf with no prior tick experience in an attempt to demonstrate IgE immunoglobulins by the Prausnitz-Kustner (PK) test.

Materials and Methods

Serum Samples

Test sera were collected from the cattle used in the studies discussed in Chapters III and IV. Blood was drawn into 20 ml silicone coated vacutainer tubes from the jugular vein of each animal and allowed to clot. The clotted blood was centrifuged at 2000 rpm for 5 min and

the serum drawn off and stored at -20°C until needed. Serum samples were taken at the beginning of each study before exposure to ticks and at 2 week intervals thereafter.

Preparation of Crude Antigens

Tick Oral Secretions. Laboratory reared female ticks were allowed to feed on sheep for 5-7 days. The partially engorged females were removed and stimulated to salivate by injection via the anus of 1 to 2 µl of 10^{-2}M epinephrine in phosphate buffered saline (PBS) at pH 7.4 (PBS formula = NaCl, 8 g; KH_2PO_4, 0.2 g; $Na_2HPO_4 \cdot 12\ H_2O$, 2.9 g; KCl, 0.2 g; NaN_3, 0.3 g; bring to 1 liter with distilled H_2O). Oral secretions were collected by capillary action into a finely drawn glass tube. Of the ticks stimulated, only 60-70% were able to secrete. Salivary secretions from 60-100 female ticks were pooled and stored at -20°C until needed.

Homogenization of Tick Salivary Glands. Female lone star ticks were allowed to feed on sheep for 5-7 days, harvested, and the salivary glands removed. Salivary glands from 40-60 females were ground in a pyrex tissue homogenizer (Matheson Scientific Co., Houston, Texas) for 5 min in 2 ml of carbonate buffered saline, pH 9.6. The homogenate was centrifuged at 2000 rpm for 5 min and the supernatant collected and stored at -4°C until needed.

Protein Concentrations. Protein concentrations for both crude antigen preparations were determined by the Lowry protein test. Preparations of both crude antigens were used in all serological test and the results compared.

Serological Tests

Enzyme-Linked Immunospecific Assay. Experimental procedures for this test are essentially the same as described by Bullock and Walls (1977). Briefly, microtitre plates (Flow Laboratories, Rockville, Maryland) were sensitized by placing 50 µl of crude antigen preparation (100 µg protein/ml in carbonate buffer - formula = Na_2CO_3, 1.59 g; $NaHCO_3$, 2.93 g; NaN_3, 0.2 g; dilute to 1 liter with distilled H_2O) in each well and incubating overnight at 37°C. Each tray was washed 3-5 times with PBS containing 0.05% Tween 20 (Sigma Chemical Co., St. Louis, Missouri). Two fold serial dilution of the test sera in PBS-Tween 20 were added to each plate, incubated for 1 hr at 37°C, decanted, and washed 3-5 times with PBS-Tween 20. A 1:100 dilution of peroxidase conjugated rabbit anti-bovine gamma globulin, light and heavy chains (Cappel Laboratories, Inc., Cockranville, Pennsylvania) was added to each well, incubated for 1 hr, decanted, and washed as before. A solution of 5-aminosalicylic acid (Aldrich Cehmical Co., Milwaukee, Wisconsin) was prepared by dissolving 80 mg of 5-aminosalicylic acid in 100 ml distilled H_2O at 70°C, pH 6.0, and 9 ml of this solution was added to 1 ml of 0.05% H_2O_2. The aminosalicylic acid plus H_2O_2 solution was then added to each well, allowed to react for 30 min, and read visually. A brown-purple color was considered as a positive reaction.

Indirect Haemagglutination Test. The tanned cell indirect haemagglutination technique described by Herbert (1967) and Willadsen et al. (1978) was used also to determine antibody titres. Sheep red blood cells were tanned with 1/10000 (w/v) tannic acid in 10 ml PBS. The tanned cells were incubated in a water bath at 37°C for 15 min with crude antigen

containing 300 μg of protein. Cells were washed 3 times in PBS containing 0.5% bovine serum albumin and titrated against 2-fold serial dilutions of the test serum. All sera to be tested were heat-inactivated at 56°C for 30 min then absorbed with uncoated tanned cells. The highest dilution agglutinating was taken as the endpoint titre.

Prausnitz-Kustner Reactions. Skin on a 4 month old calf raised free of ticks was shaved with an electric clipper and marked off into squares with a felt tip pen. Each site (square) was injected with a 0.1 ml volume of undiluted serum or serum diluted 1:5, 1:25, or 1:125 in PBS. Control sites were injected with 0.1 ml PBS. Four days later the calf was injected intravenously with 25 ml of 5% Evans Blue dye (Warner Chilcott Laboratories, Morris Plains, New Jersey), and each injection site injected a second time with 0.1 ml of the antigen preparations, 5 μg protein. Control sites were injected with 0.1 ml volumes of PBS, the antigen preparations or histamine, 550 μg/ml. Measurements at right angles were taken on the swollen areas 30 min after injection of crude antigen. An average measurement larger than the controls was considered a positive reaction.

Results and Discussion

Serological Tests

No circulating antibodies were detected by either the ELISA or indirect haemagglutination test in any of the serum samples tested. However, these results are not conclusive since it was not determined which protein (or proteins) in the crude preparations could be antigenic or if the concentration of that protein (or proteins) was sufficient to

elicite a response. It may be possible after isolation and purification of the specific antigenic proteins in the saliva of female lone star ticks to detect a circulating antibody by either ELISA or indirect haemagglutination tests.

One serum, from a Brahman x Hereford crossbreed, gave a positive reaction in the P-K test (Table 10). This animal was also observed to be highly irritated by the presence of ticks on the skin and to produce considerable serous exudate at the site of tick feeding. Also, fewer ticks were able to complete engorgement on this animal. Production of serous exudate has been associated with an immediate hypersensitivity reaction in cattle to feeding by B. microplus (Riek 1962, Koudstaal et al. 1978, Willadsen et al. 1978, Schleger et al. 1976). The mechanisms involved in the cattle-lone star tick host response system may be similar to those observed for the cattle-B. microplus host response system. However since only a crude antigen preparation was used in these tests, further research toward purification and isolation of specific antigens found in the saliva of lone star ticks is needed before this system can be serologically elucidated.

Table 10.--Immunological tests on serum from previously exposed and unexposed cattle to infestation with Amblyomma americanum (L.).

Breed and Animal Number	Serological Tests and Results[a]					
	ELISA		IH		P-K	
	before infestation	after infestation	before infestation	after infestation	before infestation	after infestation
Hereford 1	-	-	-	-	-	-
Hereford 2	-	-	-	-	-	-
Hereford 3		-		-		-
Hereford 4	-	-	-	-	-	-
Hereford 5	-	-	-	-	-	-
Braford[b] 6						
Braford 7	-	-	-	-	-	-
Braford 9	-	-	-	-	-	+[c]
Braford 11						
Braford 16						
Hereford[d] 18	-	-	-	-	-	-
Hereford 19	-	-	-	-	-	-
Braford[d] 30	-	-	-	-	-	-
Braford 20	-	-	-	-	-	-
Braford 21	-	-	-	-	-	-
Controls						
Saline	-	-	-	-	+	+
Histamine (550 µg/ml)	-	-	-	-	+	+

[a]Serological tests were enzyme-linked immunospecific assay (ELISA), indirect haemagglutination (IH), and Prausnitz-Kustner reactions (P-K).

[b]Brahman x Hereford crossbreed.

[c]Titre 1:5. Control swollen areas averaged 6.5 mm and postive reaction was 9 mm in diameter.

[d]Serum was from animals on pasture (see Chapter III).

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