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Variation of Feeding Performance in Females of *Haemaphysalis longicornis* Neumann (Acarina: Ixodidae) with Reference to Acquired Immunity

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Introduction

For many years *Haemaphysalis longicornis* Neumann, 1901, has been incorrectly referred to as *H. bispinosa* by many workers in some literature. It is morphologically very close to *H. bispinosa* which is a tropical species occurring in Southeast Asia. As a matter of fact, the tick *H. longicornis* belongs to the subgenus *Kaiseriana*, and one of *H. (K.) bispinosa* group of Asian haemaphysalids (Hoogstraal et al 1968[16], Hoogstraal et al 1970[15], Hoogstraal and Yamaguti 1970[18] and Saito and Hoogstraal 1972)[36]. Meanwhile, the name of *H. neumanni* has also been used by Nikol'sky and Meshcheryakova (1964)[28], Belikova and Somov (1967)[5] and Hoogstraal and Trapido (1966)[17]. Later, the taxon *H. longicornis* has been resurrected by Hoogstraal et al (1968)[16] and it has been distinguished from *H. bispinosa*, then the taxon *H. neumanni* has been regarded as a synonym of *H. longicornis*.

H. longicornis has long been shown to be the vectors of *Coxiella burneti*, the agent of Q fever (Smith 1942[37], as *Rickettsia burneti* and *H. bispinosa*) and *Theileria mutans* (probably *T. sergenti*, see Uilenberg 1976[42], Uilenberg et al 1978[43] and Brocklesby 1978)[8] the agent of Australian bovine theileriosis (Riek 1966[34], as *H. bispinosa*) in Australia. The species has also been recognized as the proven vectors of *Theileria sergenti* the agent of bovine theileriosis in Russia, Japan, Australia, Korea and elsewhere it occurs. Hoogstraal (1966[14], as *H. neumanni*) has reported that the species is the vector of the Russian spring-summer encephalitis virus and L'vov (1978)[25] has mentioned that the species is the vector of the Powassan virus and the Khasan virus belonging to the families Togaviridae and Bunyaviridae, respectively. Recently, Minami and Ishihara (1980)[26] have suggested that the species would be the vector of *Babesia ovata* sp. n. the new specific name for the protozoan agent causing so-called large-type piroplasmosis in Japan and probably in Korea, too.

Some noticeable references on the cytological significance of this species, such as Bremner (1959[7], as *H. bispinosa*), Oliver and Bremner (1968)[30], Khalil(1972)[24], Oliver et al (1973[31] & 1974)[32], Oliver(1977)[29], and Dicker and Sutherst (1981)[9] are available. Kang (1980)[19,20,21] has reviewed extensively the classification, identification, geographical distribution, epidemiological and epizootiological vector role, biological and genetical significance of the species and produced an intensive work on the ecological and physiological properties of the tick with reference to colonization for mass production and water balance mechaism.

Rabbits are usually used as the experimental hosts in the colonization for all instars of the tick, and it was repeatedly experienced that the first batch of the ticks always showed a significantly sufficient feeding performance, while the following batches showed remarkably reduced feeding performance in the repeated feeding trials. Such phenomena suggest that rabbits acquired immunity against the ticks after one infestation with them.

A series of experiments was therefore designed and carried out in order to assess the acquired immunity to the tick in the experimental host rabbit. the ecological findings on the variation of feeding performance of the adult females in the primary and repeated feeding trials are reported here and the histopathological studies on the lesion produced by the tick bites are also reported separately (Kang 1981).[22]

Materials and Methods

Rabbits as Host: The rabbits inexperienced to any tick feeding experiments were used as experimental host in the laboratory colonization of the tick. They were all males aged three to seven months at the beginning of the first trial and were of the conventional breed, New Zealand White, supplied by the centre for Laboratory Animals, Bush Estate. They were reared in the separate standard cages and fed on a standard diet, SG1 Oxoid.

Ticks as Parasite: The strain of the tick *H. longicornis* used in these experiments was the progeny of the bisexual diploid females originally collected from cattle in Weongseon District, Gangweon-do Province located in the eastern central part of the Korean Peninsula and colonized in the laboratory, Zoology Department, the University of Edinburgh. The identification of the species was mainly based on the morphological characteristics of the unfed adults and on the views of Hoogstraal et al (1968)[16], Roberts (1970)[35], and Yamaguti et al (1971).[48]

Colonization and Mass Production: For the colonization and mass production, numbers of the identified unfed ticks were applied to rabbits' ears in the technique divised by the author (Kang 1980).[19] For the sake of reference and uniformity it was desirable to maintain the colony within glass screw-capped jars (100mm×100mm×180mm) of known relative humidity 85.0 % R.H., controlled by saturated potassium chloride solution at 25°C constant temperature provided by an electric thermostatic incubator and the complete darkness throughout the course of the colonization and experiments as possible.

Collection and Handling of Ticks: Female ticks were dropped off naturally when fully engorged, whereas, males tended to stay on the host for a further period, so that the margination of feeding periods of the males was not clearly defined. Collection of the engorged females was done nine to eleven o'clock every morning and they were counted and weighed in the afternoons as a rule. Weighing was done individually on an electric substitution balance (Oertling, Release-0-matic, Model HO3 5908) accurate to the nearest 10^{-2}mg. After that, they were confined in clean glass tubes(18mm×50mm) plugged with non-absorbent coloured-cotton-wool. All unfed adult ticks were handled with the aid of very small hair-brushes or a pair of fine opthalmic forceps, while, the engorged females were manipulated by means of ordinary water-colour painting brushes.

Definitions: Feeding performance was investigated according to the reliable sources such as the feeding rates, feeding periods and velocities, engorged weights and feeding efficiencies. The feeding rate was defined here as the percentage of the number of females fed successfully (i.e., engorged up to 100mg body weight or heavier, and dropped naturally), out of the number of females applied initially. The feeding period was defined as the interval in days unit, which elapses between the application of the tick to the host and the natural dropping of the engorged tick, while, the feeding velocity (V) was defined as the reciprocal value of the actual feeding period of the tick. The engorged weight was defined as the immediate body weight when the tick engorged on and dropped from the host and

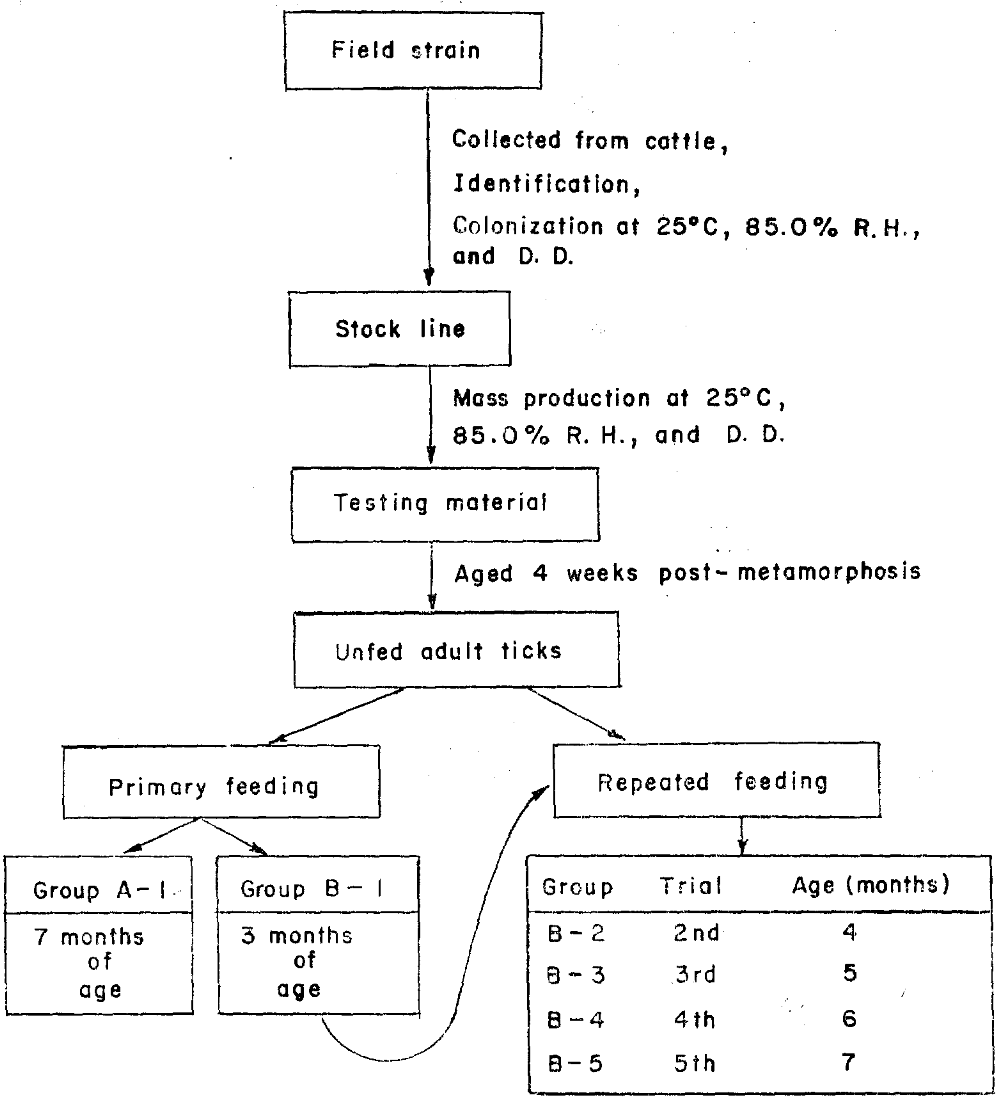


Fig. 1. Experimental feeding schedule.

in the other hand, the feeding efficiency was defined as the percentage rate (F.E. %) of net increase in the body weight of the tick.

Statistical Analysis: The sample mean ($\bar{X}$) was defined here as the simple average value of the sample population observed, so that in this report all means were written with the standard deviations (SD, s) and the estimated standard errors (SE, s/Vn). For the comparison of the means of two small samples, the modified form of "Student's t-test" was used. For the analysis of variance, the F ratio between treatment-mean-squares and error-mean-squares was calculated and compared with the one-tailed value of the F-statistics at appropriate probability level. For the significance of individual differences between treatment means, least sign-ificant difference (LSD) was used in certain statistical analysis. Probability was best defined here in relation to the frequency distribution of the ideal population.

Feeding Schedule: To assess the variation of the feeding performance of the adult females, an experimental colonization and feeding schedule was designed as shown in Fig. 1., and the unfed ticks were applied to the rabbit ear in equal numbers of twelve individuals of both sexes.

Results

Feeding Performance of the Ticks in the Primary Feeding Trials: The summarized data for the feeding performance of the females in a total of sixteen trials of the primary feeding experiment were shown in Table 1.

Table 1. Summarized Data for Feeding Performance of *H. longicornis* Femsles in Primary Feeding Trials

	Experimental Group		Pooled
	A-1	B-1	
Age (months) of Rabbits	7	3	—
No. of Trials (Rabbit Ears)	4(8)	4(8)	8(16)
Age(weeks) of Ticks	4	4	—
Pairs of Ticks Applied per Ear	12	12	—
Total No. of Ticks Collected	81	79	160
Successful Feeding Rate (%)	84.33	82.29	83.33
Mean Feeding Period (days)	9.10	9.34	9.22
Mean Feeding Velocity	0.1099	0.1070	0.1085
Mean Unfed Weight of Ticks (mg)	1.838	1.832	1.835
Mean Fed Weight of Ticks (mg)	254.06	253.39	253.73
Mean Feeding Efficiency (%)	13723	13731	13727

An overall mean successful feeding rate of 84.38% (totally 81 engorged out of 96 attached, Mean=10.1, SD=1.642, SE=0.581, n=8) was obtained in the experimental Group A-1 (rabbits, seven months of age), whereas, 82.2% (totally 79 engorged out of 96 attached, Mean=9.9, SD=1.808, SE=0.639, n=8) in the experimental Group B-1 (rabbits, three months of age). The statistical analysis showed that there was no significant difference (t=0.232, 14 degrees of freedom, p>0.05) between the experimental groups.

In Group A-1, an overall mean feeding period of 9.10 days (Min.=7, Max.=13, SD=1.454, SE=0.162, n=8) was observed, while in Group B-1, mean 9.34 days (Min.=7, Max.=13, SD=1.568, SE=0.176 n=8). There was no significant difference (t=1.017, 158 degrees of freedom, p>0.05) between the groups. The velocity defined as the reciprocal value of the actual feeding period was 0.1099 (Mean) in Group A-1, and 0.1070 (Mean) in Group B-1.

The mean engorged weight of the females in Group A-1 was 254.06mg (SD=34.263, SE=3.807, n=81), whereas, mean 253.29mg (SD=32.168, SE=3.619, n=79) in Group B-1. There was also no significant difference (t=0.810, 158 degrees of freedom, p>0.05) between the groups.

It was therefore concluded that the feeding performance of *H. longicornis* females in the laboratory colonization was not influenced by the age of the rabbits inexperienced to any tick infestation prior to the experiment.

Feeding Performance of the Ticks in the Repeated Feeding Trials: There was significant difference of the feeding performance of the females in the repeated feeding trials on the same host rabbit at one-month intervals. The summarized data were shown in Table 2.

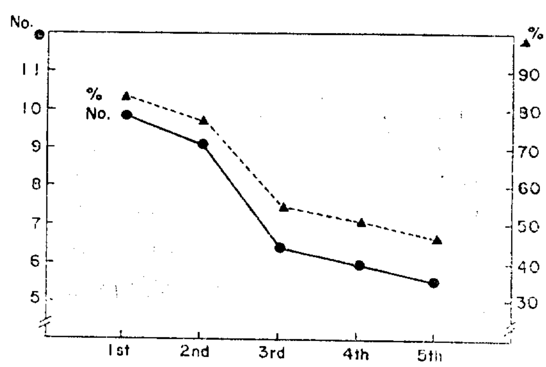
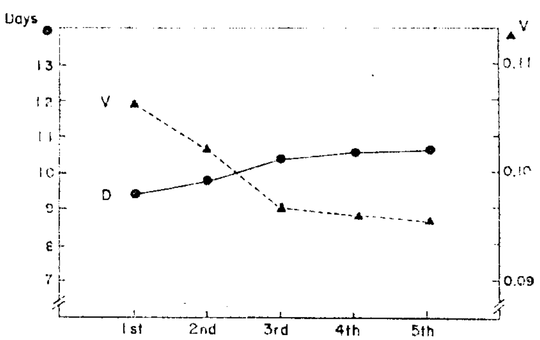


Fig. 2. Number and percentage rate of *H. longicornis* females engorged successfully in repeated feeding trials.

Table 2. Summarized Data for Feeding Performance *H. longicornis* in Repeated Feeding Trials

	Feeding Trials				
	1st	2nd	3rd	4th	5th
Age (months) of Rabbits	3	4	5	6	7
No. of Trials (Rabbit Ears)	8·········→	8·········→	8·········→	8·········→	8
Age(weeks) of Ticks	4	4	4	4	4
Pairs of Ticks Applied per Ear	12	12	12	12	12
Total No. of Ticks Collected	79	73	51	48	45
Mean No. of Ticks per Ear	9.875	9.125	6.375	6.000	5.625
Mean Successful Feeding Rate (%)	82.29	76.04	53.13	50.00	46.87
Mean Feeding Period (days)	9.34	9.74	10.31	10.42	10.47
Mean Feeding Velocity	0.1070	0.1027	0.0970	0.0960	0.0955
Mean Unfed Weight of Ticks (mg)	1.832	1.829	1.837	1.824	1.830
Mean Fed Weight of Ticks (mg)	253.39	242.58	190.73	187.85	184.18
Mean Feeding Efficiency (%)	13731	13163	10283	10199	9964



Feeding trials

Fig. 3. Feeding periods and velocities of *H. longicornis* females in repeated feeding trials.

As mentioned ealier, a mean number of 9.875 females engorged successfully per rabbit ear (Mean feeding rate=82.29%, SD=15.065, SE=5.326, n=8) was recorded in the experimental Group B-1, the primary feeding trials. However, the feeding performance of the females in the repeated feeding trials was varied according to the repeating trials, such as, mean 9.125 females (Mean feeding rate=76.04%, SD=16.925, SE=5.984, n=8) in Group B-2, the second trials; mean 6.375 (53.13%, SD=15.390, SE=5.441, n=8) in Group B-3, the third trials; mean 6 (50%, SD=22.712, SE=8.030, n=8) in Group B-4, the forth trials; mean 5.625 (46.87%, SD=15.356, SE=5.429, n=8) in Group B-5, the fifth trials.

As shown in Fig. 2., the number of females engorged successfully per rabbit ear was reduced as the number of repeating trials increased and the statistical analysis of variance (Table 3.) showed that there was a very highly significant difference (F=7.147, with 4/35 degrees of freedom, $p<0.001$) within the experimental treatment groups.

Table 3. Analysis of Variance for Number of *H. longicornis* Females Engorged Successfully in Repeated Feeding Trials

Sources of Variance	Sums-of-Squares	Degrees of Freedom	Mean Squares	F
Within Treatments	122.1	4	30.525	7.147***
Error	149.5	35	4.271	
Total	271.6	39	—	

On the other hand, having computed the least significant difference (LSD=2.09, t=2.023, 35 degrees of freedom, $p<0.05$), there were significant difference between the pairs of means in

Groups B-1 and B-2, and Groups B-3, B-4 and B-5.

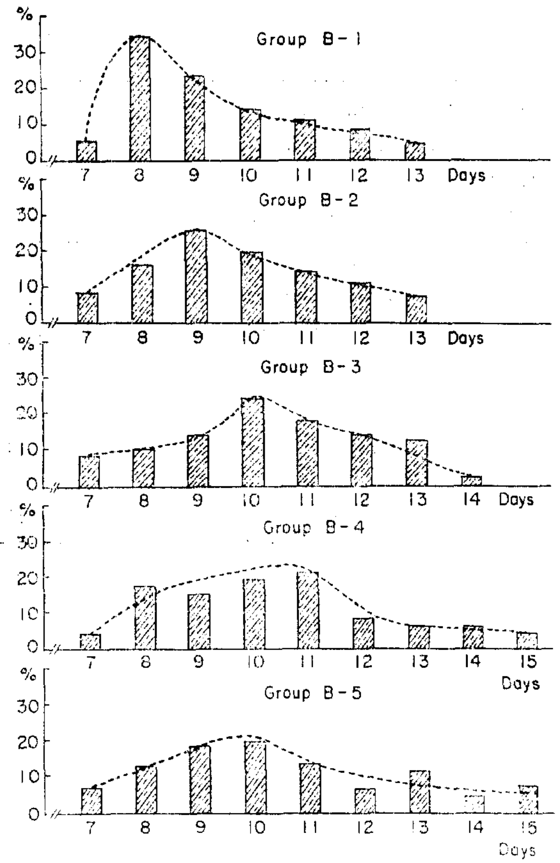
The mean feeding periods and velocities of the females in the experimental repeated feeding trials were shown as in Fig. 3. In the primary feeding trials (B-1), the females showed the mean 9.34 days of feeding period (SD=1.568, SE=0.176, n=79) and then it was delayed progressively according to the increased number of repeating trials, such as, mean 9.74 days (SD=1.675, SE=0.196, n=73), mean 10.31 days (SD=1.816, SE=0.254, n=51), mean 10.42 days (SD=2.061, SE=0.297, n=48) and mean 10.47 days (SD=2.232, SE=0.333, n=45) in Groups B-2, B-3, B-4 and B-5, respectively. The feeding velocity was also retarded from 0.1070 (mean) in Group B-1, to the means of 0.1027, 0.0970, 0.0960 and 0.0955 in Groups B-2, B-3, B-4 and B-5, respectively. The statistical analysis of variance (Table 4.) showed that there was a highly significant difference ($F=4.588$, with $4/\infty$ degrees of freedom, $p<0.001$) within the treatments.

Table 4. Analysis of Variance for Feeding Periods in Days of *H. longicornis* Females in Repeated Feeding Trials

Sources of Variance	Sums-of Squares	Degrees of Freedom	Mean Squares	F
Within Treatments	61.663	4	15.416	4.588**
Error	977.675	291	3.360	
Total	1039.338	295	—	

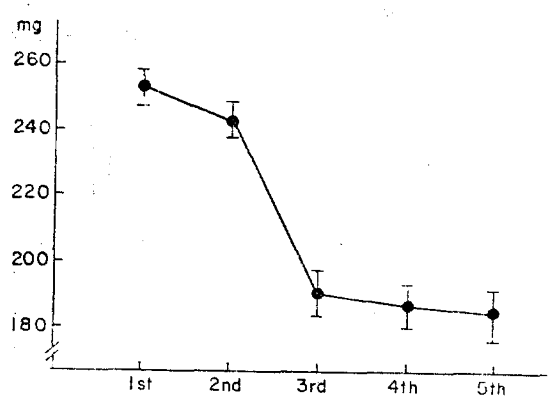
On the other hand, the percentage frequency distribution of the feeding periods of *H. longicornis* females in the repeated feeding trials was shown in Fig. 4.

The mean engorged weight of the females in the primary feeding trials (B-1) was 253.39mg (SD=32.168, SE=3.619, n=79), but it was reduced as shown in Fig. 5. as mean 242.58mg (SD=34.251, SE=4,009 n=73) in Group B-2, mean 190.73mg (SD=41.451, SE=5.804, n=51) in Group B-3, mean 187.85mg (SD=40.025, SE=5.967, n=48) in Group B-4 and mean 184.15mg (SD=40.025, SE=5,967, n=45) in Group B-5. The statistical



Feeding periods

Fig. 4. Percentage frequency distribution of feeding periods of *H. longicornis* females in repeated feeding trials.



Feeding trials

Fig. 5. Engorged weights of *H. longicornis* females in repeated feeding trials

analysis of variance as shown in Table 5., showed that there was a very highly significant difference ($F=49.21$, with 4 degrees of freedom, $<p\ 0.001$) witin treatment groups.

Table 5. Analysis of Variance for Engorged Weights of *H. longicornis* Females in Repeated Feeding Trials

Sources of Variance	Sums-of Squares	Degrees of Freedom	Mean Squares	F
Within Treatments	0.2757	4	0.0689	49.21***
Error	0.4067	291	0.0014	
Total	0.6826	295	—	

Discussion

Since Trager (1939)[40,41] showed that one infestation of guinea pigs with larvae of the tick *Dermacentor variabilis* induced an acquired immunity which is solid enough to prevent larvae from engorging and to reduce the amount of blood taken by nymphs and adults, there have been several interesting reports (Hitchcock 1955[13], Riek 1962[33], Feldman-Muhsam 1964[10], Gregson 1966[12], Allen and Wikel 1978[2], Francis and Little 1978[11], Kemp 1978[23] and Wikel and Allen 1978).[47] However, the immunological approach of resistance and host-tick relation has not been studied sufficiently when compared with the progress which has been made in understanding the mechanism of immunity to microorganisms and helminth parasites. But there is some evidence that the acquired immunity to ticks and the cellular reactions of immune animals to the ticks provide several interesting similarities to the reactions in conventional immunology for microorganisms an helminth parasites(Moorhouse 1969[27], Tatchell and Moorhouse 1968[38] & 1969[39], Arthur 1970[3], Allen 1973[1], Wikel and Allen 1976[46], Bagnall 1978[4] and Whitwell 1978).[44] It has long been recognized that the secretions from salivary glands or stings of blood-sucking insects or hazardous insects act as antigens. The first bite or sting produces a sensitization and wheal, resulting from the bite or sting of certain insects on susceptible animals, following subsequent bites or stings is in the nature of an immunological allergic reaction.

Feldman-Muhsam (1964)[10] alluded to the relation of tick feeding and immune response; during multiple feedings on a host, the first infestation sensitized the host, enabling the second infestation of ticks to feed better, and subsequently the host became immunized. Gregson (1966)[12] has recommended that considering the possibilities of an immunity build up, it is better to aim at a large and healthy initial infestation than to reinfest and encounter resistance. As another immunological problem, Gregson (1966)[12] has also indicated that attention must be paid to the formation of immunity in individuals of the tick stock-line from repeated infestations on the same species of host, therefore, continued use of the same species of host is not recommended for the feeding of certain species of ticks. However, the author (Kang 1980)[19,20] has not been experienced yet such phenomenon in the colonization of *H. longicornis*.

Branagan (1969)[6] observed that an increase in the number of brown ear tick *Rhipicephalus appendiculatus* nymphs applied resulted in an overall reduction in the length of the feeding period, and suggested two plausible conjectures to explain the association of shorter nymphal engorgement periods with heavier infestations: the first of those was 'clustering behavior', the tendency for applied nymphs to congregate to each other before attaching; and the second, 'rejection mechanism', a manifestation of the acquisition by the host of resistance to tick infestation. However, Kang (1980)[19] has reported the very reverse of the host-parasite relation between feeding and population of the tick *Haemaphysalis longicornis* in his work. There was some difficulty in accepting Branagan's suggestion; firstly, the clustering habit of ticks was not able to be reckoned as a pre-requisite behavior for attachment but it could be regarded as an attempt to get out of the tangled tick-pile and to find a host to feed on; secondly, with regard to the hosts do not carry any innate immunity to the tick infestation or do not have any acquired immunity with the tick intestation at the beginning of the feeding experiment, the conjecture of the 'rejection mechanism' was hardly understandable (Kang 1980).[19] It should be noted that after first meeting the antigen which is a kind of protein produced by the tick, there is an interval of about two weeks before the acquired antibody can be detected. Therefore, in the present experiments the author has tried to assess the difference of feeding performance between the experimental tick groups of primary and repeated feeding trials.

Summary and Conclusions

A series of experiments was designed and carried out to assess the variation of feeding performance of the adult female ticks of *Haemaphysalis longicornis* in the primary and repeated feeding trials on the same experimental host rabbit under the laboratory conditions and the results obtained were summarized and concluded as follows:

1. The feeding performance of *H. longicornis* females was not influenced by the age of the rabbits inexperienced to any tick infestation prior to the experiments.

2. The mean successful feeding rate (%) oer rabbit ear was 82.29% in the primary feeding trials but it was progressively reduced as the number of repeating trials increased on the same host.

3. The females showed the mean of 9.34 days of feeding period in the primary feeding trials and then the following batches showed delayed feeding periods in the repeated feeding trials. In other words, the feeding velocity was retarded in the repeated feeding trials.

4. The mean engorged weight of the females in the primary feeding trials was 253.39mg, however, it was remarkably reduced in the repeated feeding trials.

5. It was concluded that an acquired immunity was indeed developed, that the feeding performance was reduced by repeated feeding of the ticks on the same host, ant that it was not true economy to use a rabbit more than twice for the colonization of the tick *H. longicornis*.

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Haemaphysalis longicornis 진드기에 있어서의 獲得免疫에 依한 雌蟲 吸血成就度의 變化에 관한 研究

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抄 錄

진드기는 生物學的 여러分野의 研究對象으로 또는 진드기 媒介傳染病의 疫學과 防除를 爲한 研究材料로 供試되는데, 이를 爲하여는 實驗作群에 依한 集團生產이 要求된다. 그러나 이러한 實驗作群에 있어서 同一한 家兎를 實驗用 宿主로 反復하여 使用했을 境遇, 진드기의 吸血成就度가 低下되는 現象을 經驗하게 된다. 이러한 現象에 興味를 가지고, 이를 實驗用 家兎에 對한 *Haemaphysalis longicornis* 未飽血 雌虫의 反復吸血에 있어서의 吸血成就度 變化를 調査해 보고자 몇가지 實驗이 設計 遂行된 바, 結果는 다음과 같이 要約된다.

1. 最初(一次) 吸血試圖에 있어서 供試된 진드기의 吸血成就度는 實驗用 宿主인 家兎의 月齡別 比較群間에 有意性 있는 差異가 認定되지 아니 하였다.

2. 成功的 吸血率은 最初(一次) 吸血試圖에서 82.29%를 보였으나, 反復吸血試圖 次數가 增加함에 따라, 76.04%(二次), 53.13%(三次), 50%(四次), 46.87%(五次)로 漸次的으로 低下되는 成績을 보였다.

3. 平均吸血期間은 最初(一次)吸血試圖에서 9.34日을 보였으나, 反復吸血試圖 次數가 增加함에 따라, 9.74日(二次), 10.31日(三次), 10.42日(四次), 10.47日(五次)로 漸次 遲延 되었으며, 平均吸血速度는 0.1070에서 0.1027, 0.0970, 0.0960, 0.0955로 漸次 減速化 되었다.

4. 平均 吸血體重에 있어서는 最初(一次) 吸血試圖에서 253.39mg의 成績을 보였으나, 吸血試圖가 反復됨에 따라, 242.58mg(二次), 190.73mg(三次), 187.85mg(四次), 184.18mg(五次)으로 漸次 輕量化되는 傾向을 나타냈다.

5. 結論的으로 實驗用 家兎에 對한 *Haemaphysalis longicornis* 진드기의 吸血成就度는, 反復吸血試圖 次數가 增加함에 따라, 宿主體內에 形成 累積되는 진드기에 對한 獲得免疫의 影響을 받아 漸次 低下되며, 따라서 *H. longicornis* 진드기의 集團生產을 爲한 實驗作群이나 血統保存을 爲한 繼代吸血에 있어서 同一 家兎를 2回以上 反復使用하는 것은 바람직한 일이 아닌 것으로 思料된다.