UNIVERSITI PUTRA MALAYSIA

EFFECTS OF *BOOPHILUS MICROPLUS* LARVAL INFESTATION ON KEDAH-KELANTAN CATTLE AND THEIR *BOS TAURUS* CROSSES

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FPV 1997 9

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By

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Thesis submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Veterinary Medicine and Animal Science Universiti Putra Malaysia

April 1997

ACKNOWLEDGEMENTS

All praise to Almighty Allah, the Merciful and the Benevolent. Had it not been due to His will and favour, the completion of this study would not have been possible.

I would like to express my sincere gratitude and appreciation to my supervisor, Associate Professor Dr. Shaik Mohd Amin-Babjee, for his invaluable advice, guidance, supervision, support and encouragement throughout the duration of this study.

I wish to express my sincere gratitude to Professor Dr Sheikh Omar Abdul Rahman and Dr. M.R. Jainudeen for their invaluable advice and guidance towards the completion and editing of my theses. I would also like to express my gratitude to Associate Professor Dr. Dahlan Ismail for assistance in statistical analyses; and to Associate Professor Dr. M.K Vidyadaran for his assistance in electron microscopy.

I have also been very fortunate in receiving assistance from a number of staff whom I would like to thank, in particular : Associate Professor Dr. Rasedee Abdullah and members of Clinical Pathology Laboratory, for their excellent technical assistance in haematology

I would like to thank the staff members of the Parasitology Laboratory, En. Bohari, Yaccob, Nuzulazuan, Ishak, Lee Chu Chong and Mohd Nazeri and all other staff members of the Faculty of Veterinary Medicine and Animal Science, University Pertanian Malaysia for their invaluable assistance.

I wish to thank Dr Fauziah Othman, for permission to use the facilities of the Electron Microscope Unit and to Mr Ho Oi Kuan and Puan Aminah Jusoh for their kind assistance.

Last but not least, I wish to express my appreciation to my wife, Fadiah Jammal who has not only given me encouragement, moral and loving support throughout the course of my studies but also has been very patient in taking care of our children whenever I needed to be alone.

My special thanks also goes to my son, Mohd Zead and daughters, Zeenah, Shrean and Marrowa for their patience and understanding and sacrifice during the course of my study.

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April 1997

Chairman: Associate Professor Dr. S.M. Amin-Babjee Ph. D

Faculty: Veterinary Medicine and Animal Science

Tick resistance and the effects of dexamethasone and anti-histamine were investigated in four Kedah-Kelantan (KKKK), four F1 Kedah-Kelantan X Friesian (KKFF), and four 25% Kedah-Kelantan X 75% Frieisian (KFFF) using experimental tick infestations. Experimental animals were infested (20,000 larvae) on four occasions with an interval of two months between infestations to determine tick responses and haematological and cellular responses of the host. Subsequently, the effects of dexamethasone and anti-histamine were investigated.

Number of ticks, their weight, weight of egg masses and number of hatched larvae were significantly ($P<0.05$) lower in KKKK than in their crosses. At tick attachment sites, infiltration and degranulation of eosinophils and basophils were the striking changes. KKKK expressed intense eosinophil and basophil response than their crosses.

Mast cells were completely ablated at 24 h post infestation indicative of their degranulation.

Animals treated with dexamethasone were susceptible to *B. microplus* larvae as shown by the production of a high number of engorged female ticks, high mean weight of replete ticks, mean weight of egg masses and number of larvae. There was a reduction or complete ablation of cellular infiltration particularly of eosinophils at tick feeding sites in all genotypes.

There was little or no effect of anti-histamine treatment on the acquisition of resistance. On the contrary, animals treated with anti-histamine and dexamethasone became more susceptible to *B. microplus* larvae. Eosinophil number was higher in anti-histamine treated than those receiving both anti-histamine and dexamethasone in all genotypes.

The high tick resistance of Kedah-Kelantan (KKKK) may be attributed to reduction of ticks numbers, presence of degranulated eosinophils and basophils, the absence of immunosuppression, and release of histamine at tick attachment sites from degranulated eosinophils and basophils. Histamine initiates an immediate hypersensitivity reaction leading to self-grooming. As a result, the number of engorged ticks were reduced or absent in Kedah-Kelantan than in their crosses.

Abtrak thesis yang dikemukakan kepada Senat Universiti Putra Malaysia
bagi memenuhi syarat-syarat untuk Ijazah Doktor Falsafah

KESAN SERANGAN LARVA *BOOPHILUS MICROPLUS* TERHADAP LEMBU KEDAH-KELANTAN DAN KACUKAN *BOS TAURUSNYA*

Oleh

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April 1997

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Ketahanan sengkenit dan kesan deksametason dan anti-histamina telah diselidik dalam empat ekor lembu Kedah-Kelantan (KKKK), empat ekor F1 Kedah-Kelantan X Friesian (KKFF), dan empat ekor 25% KK X 75% Friesian (KFFF) melalui serangan sengkenit ujikaji. Haiwan ujikaji telah diserangkan (20,000 larva) empat kali, berselang dua bulan di antara serangan, untuk menentukan gerak balas sengkenit dan hematologi dan sel pada perumah. Berikutannya, kesan deksametasone dan anti-histamina pula diselidik.

Bilangan sengkenit dan beratnya, berat jisim telur dan bilangan larva menetas adalah tererti ($P<0.05$) lebih rendah dalam KKKK daripada kacukannya. Pada tapak lekatan sengkenit, penyusupan dan penyahgranulan eosinofil dan basofil merupa perubahan yang menonjol. KKKK menunjukkan gerak balas eosinofil dan

basofil lebih tinggi daripada kacukannya. Sel masta didapati terhapus lansung pada 24 j pascaserangan, menunjukkan ia telah mengalami penyahgranulan.

Haiwan terperlakukan deksametason didapati rentan terhadap larva *B. microplus* seperti ternyata daripada penghasilan bilangan sengkenit betina sebak, min berat sengkenit kenyang, min berat jisim telur dan bilangan larva yang tinggi. Pada tapak makan sengkenit ini terdapat pengurangan atau pengablatan sepenuhnya penyusupan sel terutama sekali eosinofil.

Kesan perlakuan dengan anti-histamina terhadap perolehan ketahanan ini adalah paling sedikit atau tiada langsung. Disebaliknya, haiwan terperlaku dengan anti-histamina dan deksametason menjadi lebih rentan terhadap larva *B. microplus*. Dalam kesemua genotip, bilangan eosinofil lebih tinggi tererti pada yang terperlaku anti-histamina daripada yang menerima kedua-duanya sekali, anti-histamina dan deksametason.

Ketahanan sengkenit tinggi pada lembu Kedah-Kelantan (KKKK) ini boleh disabitkan kepada pengurangan dalam bilangan sengkenit, kewujudan eosinofil dan basofil ternyahgranul, ketiadaan pengimunotindasan, dan pembebasan histamina daripada eosinofil dan basofil ternyahgranul pada tapak lekatan sengkenit. Histamina mencetus tindak balas kehiperpekaan serta merta yang membawa kepada kelakuan membersih diri lembu tersebut. Akibatnya, bilangan sengkenit sebak telah menjadi kurang atau tiada langsung dalam lembu Kedah-Kelantan dan kacukannya.

CHAPTER I

INTRODUCTION

The most important indigenous Zebu breed of cattle in Malaysia is the Kedah -Kelantan (KK). The other zebu breed in Malaysia is the Local Indian Dairy (LID). The population of KK is about 400,000 head and constitutes about 80 per cent of the cattle population in Malaysia (Mahyuddin, 1993). KK possesses a well-developed hump, brown in colour with a height of about 100 cm at the withers. They are hardy, well-adapted to the tropical environment and widely distributed in Peninsular Malaysia, particularly among the northern states of Kelantan and Kedah.

The KK has a high fertility rate, but it provides less than 50 per cent of the meat supply in Malaysia. Since the 1950's, many exotic dairy and beef breeds, have been introduced to Malaysia. However only in 1970's was a serious attempt undertaken to improve the productivity of KK and LID in Malaysia (Dahlan et al., 1982). The purpose of introducing these breeds into the country was to boost milk and beef production. The imported breeds were mainly, Jersey for milk and Aberdeen Angus and the Hereford for beef.

These cattle from temperate regions encountered several problems under the harsh tropical environment. There was a decline in their reproductive performance, high mortality due to babesiosis *(Babesia bovis)* transmitted by *Boophilus microplus* tick, poor adaptability to high environmental temperatures and nutritional deficiencies (Lingam et al., 1977).

With the failure of *Bos taurus* cattle breeds to survive in Malaysia, steps were taken to import Zebu-*Bos taurus* crossbred strains suitable for our environment. The imported beef breeds were Droughtmaster and Santa Gertrudis and the dairy breeds were the Australian Milking Zebu and Sahiwal X Friesian (Dahlan et al., 1982).

Crossbreeding provides an opportunity to increase production by combining the desirable characteristics of two or more breeds and taking advantage of heterosis. Reproductive efficiency and maternal performance of the cow and increased growth rate of the calf are traits that have shown the most gain from heterosis (Dahlan et al., 1982).

The high fertility and adaptive tolerance to high ambient temperatures and humidity of these cattle (KK) can be combined with the growth efficiency and desirable carcass characteristics of improved *Bos taurus* (Hereford, Friesian) and *Bos indicus* breed (Brahman), to produce high performing dairy and beef crossbreeds (Dahlan et al., 1982).

In any crossbreeding programme, Kedah-Kelantan should constitute the base herd just as much as the Brahman cattle in Australia. One of the most valuable traits of Zebu cattle is resistance to ticks (Utech et al., 1978).

Ticks cause severe economic reduction in livestock production, and without tick control it would be virtually impossible to raise livestock economically in many areas of the word. The tick *B. microplus,* is an important parasite of cattle in Australia, Asia, South America and Africa (Drummond, 1970).

Field observations indicate that Kedah-Kelantan cattle are highly tick resistant compared with their crosses. But no detailed study has been conducted in Malaysia to determine the mechanism by which KK reject ticks. Thus a series of studies was undertaken to determine the effects of experimental infested of KKKK and their crosses with the tick *B. microplus.*

The thesis is divided into several chapters. Introduction (Chapter 1), after a review of the literature (Chapter II), the materials and methods used in all the experiments represented (Chapter III), before proceeding to determine the resistance of Kedah-Kelantan cattle and their crosses to experimental infestation with *Boophilus microplus* larvae (Chapter IV) and the hosts response to infestation (Chapter V). Finally the mechanism of immunosuppression (Chapter VI) and anti-histamine (Chapter VII) in tick resistance was investigated.

CHAPTER II

REVIEW OF LITERATURE

TICKS

Ticks (superfamily Ixodoidae) are obligate temporary ectoparasites of terrestrial vertebrates.

Taxonomic classification of Ticks

Ticks, together with the mites, belong to the order Acari or Acarina, class Arachnida, phylum Arthropoda. Ticks are placed in the superfamily Ixodoidea of suborder Ixodides.(Roberts, 1970).

Three families are recognised, Nuttalliellidae, Ixodidae, Argasidae.

Nuttalliellidae: This family contains a single species, *Nuttalliella mammaqua* (Bedford, 1932) Which occurs in south-west Africa and is considered to posses characters intermediate between the Argasidae and Ixodidae (Roberts, 1970).

Ixodidae: These are the 'hard' ticks with a dorsal scutum in all stages, which covers practically thr entire dorsum in the male but only the anterior portion in the female, nymph and larvae. The capitulum is terminal in all stages, and in the female the basis is furnished with porose areas (Roberts, 1970).

Argasidae: These are the 'soft' ticks with the body integument leathery and roughened and without a dorsal shield or scutum. The capitulum in ventral in adults and nymphs, rarely visible from above, terminal or subterminal in the larvae (Roberts, 1970).

Family Ixodidae

This family consists of the Prostriata and Metastriata. The Prostriata includes a single subfamily, Ixodinae, with a single genus, *Ixodes,* in which the anal grooves embrance the anus anteriorly and usually unite in a arch or point. In the Metastriata, the anal groove embraces the anus posteriorly or is obsolete. There are two subfamilies in Metastriata, the Amblyomminae with the genera *Amblyomma*, *Aponoma*, *Dermacentor*, *Anocentor*, and *Haemaphysalis*, and theRhipicephalinae with the genera *Rhipicephalus*, *Boophilus*, *Margaropus*, *Cosmiomma*, *Hyalomma*, *Nosomma* and *Rhipicentor* (Roberts, 1970),

Effects of Infestation

Feeding Habits: During feeding, salivary secretion is injected into the host and the salivary glands remove excess fluid from the blood meal. Some animals develop a local allergic reaction to the salivary gland secretion and this local reaction may limit or completely prevent tick infestation. This phenomenon appears to be better developed in *Bos indicus* (Moorhouse, 1969) and in general these animals carry substantially lower burdens especially of one-host ticks (Moorhouse and Tatchell, 1966)

Local tissue irritation: Infestation generally results in local irritation, resulting in wounds that are susceptible to bacterial infection and screw-worm infestation (Balashov, 1972).

Econrmic Losses: Ticks cause severe economic reductions in livestock production, and without tick control it would be virtually impossible to raise livestock economically in many areas of the world (Drummond, 1970). The tick, *B. microplus* is an important parasite of cattle in Australia, Asia, Central and South America and Africa. Heavy tick burdens also result in loss of production of meat, milk, wool and eggs and decreased value of hides. Severe infestation may cause anaemia and even death (Drummond, 1970).

The United States Department of Agriculture (1965) estimated that ticks-caused losses of US$60 million annually in cattle production and US$4.7 million annually in sheep production in the United States. Since the outbreak of the disease in Texas in August 1972 (Cattleman Staff, 1974), about US$4 million has been spent on control.

The estimated annual loss to the cattle industry of US$8 billion worldwide results from direct parasitisation (tick feeding), transmission of pathogenic microorganisms, and/or the development of secondary bacterial infections at the attachment sites (Steelman, 1976).

Studies in Australia indicate that total annual loss caused by cattle tick amounts to about US$5 per head of cattle, or 4% of the gross value of cattle slaughtered in 1972/1973 (Bram, 1975). Significant economic losses occur as a direct result of tick infestation and tick-borne pathogens (Steelman, 1976), and ixodid resistance to acaricides is a serious cause for concern, since tick control is almost totally dependent on these chemicals (Wharton, 1976). This has stimulated interest in integrated pest management strategies employing non-chemical methods of tick control involving innate (natural) and acquired (immune) host resistance to ticks (Utech et al., 1978). Alternative measures using biological control such as "pasture spelling" and tick resistant cattle (Wharton and Norris, 1980) can reduce tick burdens.

Disease Transmission: Tick saliva plays a role in transmission of viruses, rickettsia (including *Anaplasma*) and bacteria (including *spirochetes*) (Hoogstraal, 1973). Little appears to be known of the location and development of these organisms in the tick salivary gland, which in many cases, merely act as an organ of transmission rather than being involved in a cycle of development of the microorganism. A more complete relationship exists between ticks and the piroplasma, babesia (Mahoney,1977) and theileria (Schein and Friedhoff, 1978).

Tick-induced reduction of lymphocyte responsiveness might have important implication in tick-borne pathogen relationships, even though host resistance to infestation has been shown to alter the transmission of tick-borne pathogens to the host (Wikel, 1980)

Many ticks transmit protozoa, bacteria rickettsia and viruses to the man and domestic animals. Various species act not only as vectors but also as reservoirs of a number of diseases in different parts of the world. Ticks are known vectors of agents that cause many economically important diseases in domestic livestock (Hoogstraal, 1970). Some of the most important diseases or agents are: anaplasmosis (Piercy, 1956), East Coast Fever (theileriosis) (Shaw, 1973), babesiosis (Riek, 1968), equine encephlomyelitis virus (Syverton and Berry, 1941) and Q fever virus (Stoker and Marmion, 1955)..

Life Cycle of Ticks

The whole life cycle usually last less than one year, but some ticks like *Ixodes ricinus* may take up 3 years to complete the life cycle. There are three parasitic stages in the life cycle of ixodid ticks - the larvae, nymph and adult. The larvae may spend most of its life-span away from the host. Once attached to a suitable host, it ingests blood or plasma for 3-7 days to complete engorgement. From this stage, the life cycle differs according to tick species (Arthur, 1962)

One-Host Ticks

The one-host tick has the ability to pass its whole life cycle on one host moulting to the nymph and adult and leaving the host only as a replete female as in the genus *Boophilus*.

These larvae lose the ability to move soon after the body begins to distend. Moulting takes place on the host and the succeeding nymphal instar, immediately on hatching, is pale brown in colour (Urquhart et al., 1987).

Two-Host Ticks

Rhipicephalus evertsi is one of the few hard ticks with a two-host type of life cycle with both immature stages living on the same host. The larvae attache itself to the host, feeds and then moult to the nymph while it is still on the host. When hardened the nymph feeds on the same host until it is engorged and then drops to the ground, where it undergoes moulting. As adults they seek a new host. These adults generally attach themselves to domestic or wild herbivores, but often the immature stages may parasitise insectivores, rodents and hares. The site of attachment by the adults are in the perianal region under the base of the tail, less frequently on the teats, in the axillae of the legs or on the scrotum. Both larvae and nymphs attach themselves deep in the ear, especially at the base of the auricule, and more rarely on the flank (Arthur, 1962) .

Three-Host Ticks

The vast majority of the hard ticks have all stages feeding either on the same host or on different hosts. The larvae attach to host A, drops from the host when replete, metamorphoses to nymph which then attacks another host B, either of the same or different species from host A, and again feeds to repletion before dropping off. The engorged nymph then transforms to either male or female, and the latter attaches itself to host C, feeds and drops off to lay eggs. Thus, three separate hosts of the same or different species are necessary for the life cycle to be completed (Arthur, 1962).

Boophilus **Species**

Boophilus is one of the most important genera of ticks in domestic animals throughout the world. At present five species of *Boophilus* have been recognised in the world, *B. kohlsi*, *B. geigyi* (Aeschlimann and Morel (1965), *B. annulatus*, *B. microplus* and *B. decoloratus* (Arthur (1962) all of which have small yellow males and pod-shaped but somewhat larger females. *B. annulatus* is a North American tick which presumably originally parasitised deer and buffalo, subsequently infesting cattle and transmitting *Babesia bigemina*, the causative agent of American Texas Fever, *B. microplus* occurs in Central and South America, Australia, the Orient, the southern part of Florida, South and East Africa where it appears to be extending its range. *B. decoloratus* is essentially an African species and is extensively distributed south of the Sahara in regions where the rainfall is heavy and there is adequate shrub cover (Arthur, 1962).

Boophilus microplus

The cattle tick, is an important species infesting cattle in most cattle rearing countries particularly in the tropical and sub-tropical regions of the world.

Primary Host

The primary host of *B. microplus* is domestic cattle, although records from sheep, goats and horses have been reported. From an economic point of view this species is important as a vector of *Babesia bovis, B. bigemina, B. berbera* and *Anaplasma marginale* in cattle (Piercy, 1956), *Babesia ovis* causing babesiosis of sheep (Riek, 1968), and biliary fever (*Nuttalia equi*) of horses (Arthur, 1962).