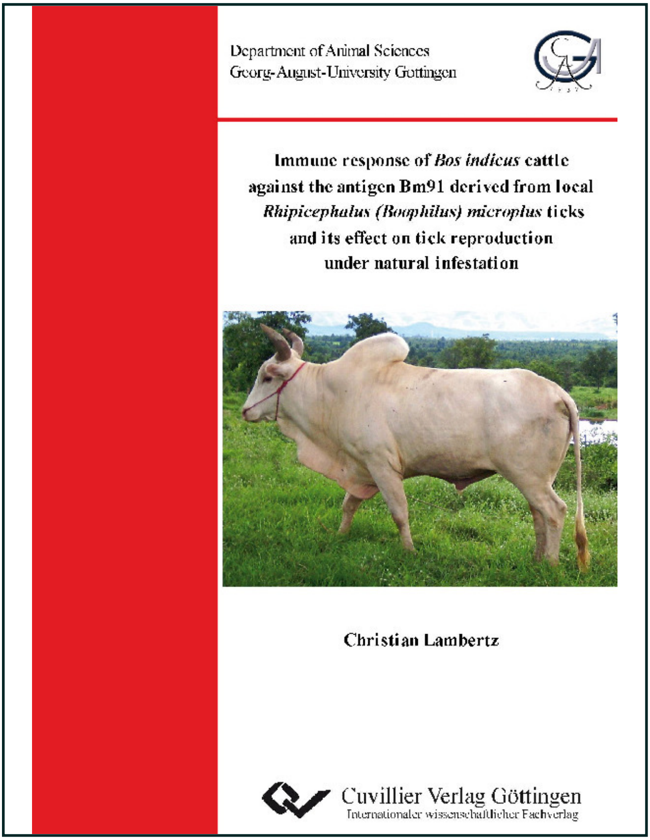
Christian Lambertz (Autor)

Immune response of Bos indicus cattle against the antigen Bm91 derived from local Rhipicephalus (Boophilus) microplus ticks and its effect on tick reproduction under natural infestation



https://cuvillier.de/de/shop/publications/357

Copyright:
Cuvillier Verlag, Inhaberin Annette Jentzsch-Cuvillier, Nonnenstieg 8, 37075 Göttingen, Germany
Telefon: +49 (0)551 54724-0, E-Mail: info@cuvillier.de, Website: https://cuvillier.de

3 INTRODUCTION

Agriculture is an integral part of Thailand's emerging economy, the 2nd largest in South East Asia. According to the 2003 Agricultural Census around 40 % of the labour force is employed in agriculture (NSO 2003). During the last years the agricultural sector contributed about 10 % to the gross domestic product of the country with 67 million inhabitants. While undergoing a substantial transformation, agriculture has been shifting towards high valued products. The proportion of livestock to the agricultural gross domestic product increased to more than 20 % (FAO 2005). In 2008 the Department of Livestock Development (DLD) amounted the cattle population in Thailand to 9.1 million head, whereof less than 500,000 head were dairy cattle (DLD 2009b). About 70 % of the cattle population are Thai indigenous cattle (DLD 2009a).

Serious problems to Thailand's livestock sector are caused by ectoparasite infestations. Like in tropical and subtropical countries generally, ticks are the most important cattle ectoparasites. The dominating species is the cattle tick *Rhipicephalus (Boophilus) microplus* which has a global importance due to its vast geographical distribution, its high reproductive capacity and its efficient transmission of tick borne pathogens. As the demand for meat and other animal products continues to increase with the growing economy, the importance of ticks and tick borne diseases (TBD) in Thailand will rise (Ahantarig et al. 2008; Chansiri 1997). One of the factors favouring tick infestations is the import of exotic cattle breeds especially of *Bos taurus* origin. These exotic breeds generally lack a natural immunity to tick infestations. Without this host resistance the cattle necessitate a more intensive use of acaricidal drugs for their survival when compared to *B. indicus* breeds (George et al. 2004). Beyond this, climate change will favour the expansion of tick-infested regions (Estrada-Peña 2001), and thus, the spread of TBD in Asia (Olwoch et al. 2007).

Currently, tick control relies largely on the use of acaricides. The high tick prevalence in most parts of Thailand requires intensive treatments with these drugs. However, a number of serious drawbacks are associated with this tick control method. These include the development of acaricide-resistant tick populations, environmental contamination, contamination of animal products, high costs for farmers as well as expenses to develop new drugs. Therefore, new approaches to control *R. (B.) microplus* more sustainably are necessitated. Among the alternative tick control methods including host resistance to ticks, management strategies and biological control agents, anti-tick vaccines showed the most encouraging results. Two different vaccines based on the tick gut antigen Bm86 were developed in the 1990s in Australia and Cuba (Rodríguez et al. 1995a; Willadsen et al. 1995). An anti-tick vaccine targeting Thai *R. (B.) microplus* strains would constitute a major advance for Thailand's livestock sector. Nevertheless, the efficacy of these vaccines is still the reason for the limited application in practice. Beside the commercialised antigen other potential antigens have been isolated. One of these candidates is Bm91. The protein was isolated by Riding et al. (1994) and its efficacy against the cattle tick was shown when it was used combination with Bm86 (Willadsen et al. 1996). The results observed warrant the evaluation of Bm91 as a possible tool to control cattle ticks in Thailand in a more sustainable way. Therefore, Bm91 derived from a local *R. (B.) microplus* strain was tested in two immunisation trials under field conditions of natural tick infestation.

The specific objectives were

1. to evaluate the humoral immune response against the Bm91 antigen in *B. indicus* cattle,
2. to assess the efficacy of the Bm91 immunisation on the reproductive performance of *R. (B.) microplus* ticks under field conditions of natural infestation.

4 LITERATURE REVIEW

4.1 Taxonomy and life cycle of ticks

Ticks belong to the phylum Arthropoda and are related to crustaceans, insects, spiders, scorpions, and mites. As invertebrates they have an exoskeleton protecting the organs. They are further classified as members of the Arachnida class which can be distinguished from insects by the number of legs. While Arachnida have four pairs of legs, insects have three. Together with mites ticks share the sub-class Acari. The sub-order Ixodida (ticks) can be divided into the two main families Argasidae (soft ticks) and Ixodidae (hard ticks). The hard sclerotised shield, the scutum, which is found on the anterior dorsal surface of hard ticks, is absent in soft ticks. In soft ticks the mouthparts are located ventrally and in hard ticks anterior. The third family Nuttalliellidae is of minor importance and contains only one single species. Out of the 899 listed tick species by Barker & Murrell (2004) around 80 % belong to the Ixodidae and are divided into 12 genera.

After ticks crawl onto the host animal, they attach to the skin with their mouthparts. These consist of the chelicerae, the hypostome, and the palps. The chelicerae and the hypostome form a tube which penetrates the host's skin. Often cement is secreted with the tick saliva. This glues the palps to the outer epidermis and the rough chelicerae sheath and the toothed hypostome to the dermis. The chelicerae consist of moveable rods with sharp claws at the end. These cut a hole into the dermis and break the capillary blood vessels close to the surface of the skin. This forms the feeding lesion. The ticks feed on the blood and the lymph which is released into this lesion.

Ixodid ticks feed slow because the body wall has to grow before it can expand to take a very large blood meal. Larvae take typically 3 to 5 days to fully engorge with blood, nymphs 4 to 8 days, and females 5 to 20 days. After full engorgement with blood the ticks detach from the host's skin and drop to the ground. Males of most species feed enough for their reproductive organs to mature but do not expand like the females. Ticks find their hosts in several ways. Most commonly they live in open environments and crawl onto vegetation to wait for their host passing by. This behaviour is called questing. Differentiated by the place where moulting takes place three different types of life cycle can be distinguished in ixodid ticks. Ticks moult either on the host or after detaching from the host on the ground. Each of the life cycles consists of the four stages egg, larva, nymph, and adult. Most common is the three-host life cycle, in which the larvae develop in the eggs and hatch usually within several weeks. After feeding once on the host, the larvae detach from the host and moult to nymphs on the ground. When the nymphs completed feeding on the host, they detach and moulting takes place on the ground. Females feed on a new host, detach when they are fully engorged, and lay one batch of eggs on the ground. The depleted female dies after egg laying is completed. The three-host life cycle is slow and takes from 6 months up to several years. Only the larvae and the nymphs feed on the same host, whereas the adults engorge on another host in the two-host life cycle. The one-host life cycle is less common but occurs in the most important sub-genus *Boophilus*. The larvae hatch after several weeks of development on the soil and crawl onto vegetation to quest for a host. After the larvae finished feeding, they stay on the host for moulting to nymphs, before they continue to feed on this host. Moulting to adults takes place on the same host. When the adults are partially fed, they mate, and thereafter fully engorge. The fully engorged females detach and lay a single batch of 2,000 to 20,000 eggs on the ground. This life cycle is usually rapid and takes around three weeks (Hitchcock 1955). Feedings at all stages of their live cycle are parasitic.

4.1.1 The cattle tick *Rhipicephalus (Boophilus) microplus*

Of all tick species *R. (B.) microplus* (Canestrini, 1887), also known as the cattle tick or the pan tropical blue tick, is the most important species for livestock production in the world. During the latter half of the 19^{th} century it spread from its origin in South-East Asia throughout the tropics and subtropics including Australia, East and Southern Africa, and South and Central America (Jongejan & Uilenberg 2004; Labruna et al. 2009). Different strains with marked morphological differences resulted from evolutionary processes associated with habitat adaptation following biogeographical separation (García-García et al. 1999). Recent molecular and morphological studies revealed that the five species of *Boophilus* make the genus *Rhipicephalus* paraphylic meaning that some species of the Rhipicephalinae are more closely related to *Boophilus* species than to other *Rhipicephalus* species (Murrell & Barker 2003). Therefore, *Boophilus* was synonymised with the Rhipicephalinae. This one-host tick completes its parasitic part of the life cycle in about three weeks and egg laying can be completed in about four weeks (Roberts 1968a).

In tropical climates *R. (B.) microplus* infestations occur throughout the whole year with peak infestations during the rainy season (Gomes et al. 1989; Lima et al. 2000; Turner & Short 1972). The cattle tick *R. (B.) microplus* is known to be highly reproductive and an efficient vector of the most important tick-borne parasites. Two characters which make this tick species a threat to livestock production in tropical and subtropical regions. As predicted with a simulation model for African tick species, climate change will favour the expansion of tick-infested regions, and thus, the spread of TBD (Olwoch et al., 2007). This is supported by observations made in different African countries where *R. (B.) microplus* is spreading fast and displaces other Rhipicephalinae species (Lynen et al. 2008; Madder et al. 2010). The higher reproductive performance together with the shorter generation interval compared to other tick species have been the most important factors favouring the efficient spread of the cattle tick.

4.1.2 Other important tick species

On the African continent *Amblyomma variegatum* and *A. hebraeum* are the most widely distributed and most important tick species for domestic livestock. The former is known as the tropical bont tick and is widespread throughout tropical Sub-Saharan Africa while the latter is known as the South African bont tick and inhabits the south-eastern part of the African continent. Another widespread species is *R. (B.) annulatus* which is present in the Mediterranean region, Southern Russia, the Near and Middle East, Western and Southern Africa, and Mexico. Confined to Africa the blue tick, *R. (B.) decoloratus*, is the most common one-host tick species in Africa. Spread in East and South Africa, *R. appendiculatus,* the brown ear tick, is the vector of East Coast Fever that is caused by an infection with *Theileria parva*, whereas *H. anatolicum anatolicum* is the main vector of the disease Tropical Theileriosis, a *T. annulata* infection in Southern Europe, and the Middle and Far East (Jongejan & Uilenberg 2004; 1994; Walker et al. 2003).

4.2 Impact of ticks on livestock

Ticks are among the most important ectoparasites of cattle in the tropics and subtropics and approximately 80 % of the world's cattle population is at risk from ticks and TBD (FAO 1984). Heavy tick infestations have adverse physiological effects on the host and result in decreased live weight gain (Jonsson 2006). Engorging ixodid ticks increase their live weight by 100 to 200 times (Kemp et al. 1982). The actual amount of blood ingested by the tick is even greater because the blood is concentrated and the remaining fluid is excreted in the saliva. Anaemia is a symptom of heavy infestations. It is characterised by a decrease of the packed cell volume (PCV) of the blood, of its haemoglobin content (Hb), and of the red blood cell count (RBC) (Riek 1957). Global losses of tick infestations and TBD have been estimated to be more than US$ 18 billion (de Castro 1997). In Brazil alone yearly losses caused by ticks and TBD have been estimated at US$ 2 billion (Grisi et al. 2002). Depending on location and farming system, costs for the chemical control of ticks have been estimated between US$ 2.50 and US$ 25.00 per animal per year (Pegram 2001). Prevalence studies undertaken in Thailand emphasised the importance of this ectoparasite for cattle, although a very limited number of cattle was observed (Changbunjong et al. 2009; Sarataphan et al.

1998). With the rising demand for meat and other animal products the importance of ticks and TBD in Thailand will further increase (Ahantarig et al. 2008; Chansiri 1997).

4.2.1 Direct effects of ticks

Direct effects of ticks are caused by the blood loss, tick burdens as well as toxicoses. Injurious tick bites can cause severe hide damage including abcessation and can be routes for secondary infections. Furthermore, crumbled ear pinnae, sloughed tits, missing tail tips, lameness, and foot rot can result from tick infestations (Holdsworth et al. 2006).

In Australia high-yielding Holstein-Friesian dairy cows with a low previous tick exposure were artificially infested weekly with an increasing number of tick larvae over a period of 12 weeks. By the end of the trial infested cows showed a 2.86 l/d reduced milk yield and a 10.6 kg reduced live weight when compared to non-infested cows (Jonsson et al. 1998).

In subsequent studies, Jonsson (2006) estimated that on average each engorging tick is responsible for the loss of more than 1 g of body weight. Differences between purebred *B. taurus* and crossbred *B. taurus* x *B. indicus* cattle were insignificant. In a previous study, Sutherst et al. (1983) amounted the loss of live weight gain to 0.6 to 1.5 g per engorging *R. (B.) microplus* tick. The magnitude of losses caused by ticks varies with the cattle genotype (Scholtz et al. 1991), the tick species (Norval et al. 1997a; Norval et al. 1997b) and the level of infestation (Sutherst et al. 1983).

4.2.2 Indirect effects of ticks

Beside these direct effects, ticks indirectly affect their hosts by transmitting a greater variety of pathogenic micro-organisms than any other arthropod vector group while taking their blood meal (Jongejan & Uilenberg 2004). Among the pathogens of economic importance for livestock are babesias, anaplasmas, theilerias, arboviruses, rickettsias, and tularaemia. These pathogens cause serious diseases responsible for high economical losses of livestock producers. *R. (B.) microplus* is the major vector of TBD in the tropics and subtropics and, among others, transmits the pathogens *Babesia bovis*, *B. bigemina, Anaplasma marginale,* and *Theileria parva* and *T. annulata* (McCosker 1979).

4.2.2.1 Babesiosis

Babesiosis, commonly known as cattle or tick fever, is caused by the two protozoa *B. bigemina* and *B. bovis*. Babesiae, in general, are characterised by their asexual multiplication in erythrocytes of vertebrates, their sexual reproduction in the tick, and the production of sporozoites in the salivary gland of the tick. These two protozoa of major importance for livestock can be divided by the size of the merozoites[1] into large (*B. bigemina*) and small babesiae (*B. bovis*). The former is more widespread whereas the latter is more pathogenic (Homer et al. 2000). Clinical symptoms of *B. bovis* include fever followed by inappetence, depression, increased respiratory rate, weakness, and a reluctance to move. Due to the fact that haemoglobinuria is often present, the disease is also known as redwater (Bock et al. 2004). *B. bigemina* infections are characterised by erythrocyte destruction and haemoglobinuria is present earlier, whereas fever is less pronounced than in *B. bovis* infections (Bock et al. 2004; Riek 1964). In acute *B. bovis* and *B. bigemina* infections the percentage of infected erythrocytes in the circulating blood does not exceed 1 % (Levy & Ristic 1980). Compared to taurine breeds, zebus show milder clinical symptoms to primary infections. This is assumed to be the result of the evolutionary relationship between *B. indicus* cattle, ticks, and *Babesia* spp. (Bock et al. 1997). Live attenuated vaccines against both pathogens were developed (Callow 1977) and showed at least 90 % protection in vaccinated animals after challenge with field isolates in recent studies (Alvarez et al. 2004).

4.2.2.2 Anaplasmosis

On a global scale anaplasmosis is the most prevalent TBD of cattle caused by *A. marginale* (Ristic 1968). Its prevalence and incidence is highest in *R. (B.) microplus* endemic regions (Lincoln et al. 1987). The pathogen belongs to the order Rickettsiales

1 During the tick bite vertebrate hosts are infected by the injection of sporozoites with the tick saliva. These penetrate directly into the RBCs and the parasite produces two merozoites by binary fission of the sporozoite. After the lysis of the erythrocyte each merozoite invades a new erythrocyte (Chauvin et al. 2009).

and is an obligate intracellular bacterium found in membrane-bound vacuoles in the host cell cytoplasm. It develops persistent infections in mammalian and tick hosts which both serve as reservoirs for infection of susceptible hosts. Bovine erythrocytes are the only known site of replication and during acute infections up to 70 % of the erythrocytes may become infected (Richey 1981). When infected erythrocytes are taken up with the blood meal of the tick, tick gut cells become infected. Later the infection spreads to several other tick tissues including the salivary glands (Kocan 1992). The acute phase of the disease is characterised by weight loss, fever, abortion, and lowered milk production. Cattle often die after acute infections. As proposed by Kocan et al. (2004) immunising cattle with a combination of anti-tick antigens and *A. marginale*-derived proteins may provide an effective means of controlling infection and transmission. Vaccine formulations against a broad spectrum of strains has been developed, but provided only partial protection (Kocan et al. 2001).

4.2.2.3 Theileriosis

Protozoa of the genus *Theileria* are causative agents of a variety of disease symptoms in domestic and wild ruminants which are collectively responsible for economic losses amounting to hundreds of millions of dollars annually in Sub-Saharan Africa and Asia (Bishop et al. 2004). Globally the two most important species causing theileriosis in cattle are *T. parva* and *T. annulata*. Neither of the two protozoa has been found in Thailand (Ahantarig et al. 2008; Sarataphan et al. 1998). Nevertheless, benign *Theileria* which belongs to the *T. buffeli/orientalis/sergenti* group and causes mild disease, is highly prevalent in Thailand (Sarataphan et al. 1998).

4.2.2.4 Endemic stability of tick-borne diseases

Cattle breeds that are indigenous to regions where TBD are endemic often have a certain degree of natural resistance to these diseases and the consequences of infection are not as serious as when exotic, especially *B. taurus*, breeds are infected. This endemic stability is defined as 'a stable endemic situation with high prevalence of infection but no or little clinical disease in the target population caused by a high transmission rate of the parasites between vector tick and the vertebrate host' (Tatchell

1992). Passively acquired resistance from the colostrum lasts about 2 months when it is followed by an innate immunity from 3 to 9 months of age (Bock et al. 2004; Riek 1968). For *B. bovis* an inverse relationship between age and resistance with young animals being more resistant was found (Trueman & Blight 1978). Under conditions of endemic instability some animals will fail to become infected for a considerable period after birth and may therefore develop severe, life threatening symptoms when exposed to the protozoa later in life (Callow 1984). The import of exotic cattle, especially of taurine origin, from regions where ticks and TBD are non-endemic into tropical countries, as it is practised in Thailand, is accompanied by significant losses (Callow 1977). Prevalence studies have shown that up to 96.7 % of the cattle in Thailand are seropositive for *Babesia* spp. infections (Nishikawa et al. 1990). In a recent prevalence study by Iseki et al. (2010) the infection rate of cattle in the Northern regions of Thailand was around 70 % for both *B. bovis* and *B. bigemina* with more than half of the cattle being infected with both pathogens. Comparable values exist for Anaplasmataceae. In a survey of 7 provinces throughout Thailand 74 % of the calves were seropositive for *A. marginale* (Phrikanahok et al. 2000). Half of the beef cattle in Nan province, Northern Thailand, were found to be infected with benign *Theileria*, but no clinical signs of disease were observed (Kaewthamasorn & Wongsamee 2006). The specific tick species transmitting *Theileria* parasites in Thailand has not been identified yet (Ahantarig et al. 2008).

4.3 Immune response to tick infestation

The tick-host-pathogen interface is characterised by complex immunological interactions. Tick feeding stimulates the immune system of the vertebrate host and innate as well as specific acquired immune defences are involved in the responses of the host to the infestation. There is a dynamic balance between host responses and tick countermeasures against these mechanisms which is affecting the tick engorgement and the transmission of pathogens (Wikel 1996). After early studies focused mainly on the immunity to tick infestation directing at the development of anti-tick vaccines, later studies paid attention to the tick-host interaction with a particular view on how ticks can modulate host immune mechanisms and facilitate the transmission of TBD (Wikel & Bergman 1997).

In order to obtain a blood meal successfully haematophagous arthropods generally must overcome host blood coagulation, platelet aggregation, and pain/itch responses. Therefore, the saliva contains a complex mixture of proteins with biological activity (Brossard & Wikel 2004). To counteract the host immune mechanisms, ticks have evolved numerous mechanisms (Ribeiro 1989; Ribeiro 1995). These complex interactions can be viewed as a balance between the different host defence mechanisms raised against the parasite and tick evasion strategies facilitating feeding and the transmission of pathogens. First of all, a local inflammatory response develops after the tick mouthparts penetrate the host skin. In this reaction host neutrophils as one of the first responders of the innate immune system participate (Brossard & Wikel 2004). The movement and activity of the neutrophils is controlled by the chemokine interleukin 8. Shown in *in vitro* experiments the salivary gland extract of several tick species inhibits the binding of interleukin 8 to its receptors (Hajnická et al. 2001). With this mechanism tick saliva is able to control the infiltration and activation of neutrophils at the attachment site. One of the tick salivary gland proteins that interacts with elements of the host immune system is calreticulin (Ferreira et al. 2002). This protein is expressed in all tissues and all developmental stages of ticks. It is a conserved calcium-binding protein with a diversity of biological functions. However, the specific roles of this molecule in tick-host interactions remain to be determined.

The complex of the host response to tick infestations was studied by Roberts (1968a). Eight days after a moderate *R. (B.) microplus* infestation resistance was acquired in cattle with no previous tick exposure. Between different animals a varying degree of resistance was observed. Contrary to non-exposed animals, those with previous tick exposure manifested their levels of resistance immediately after infestation. The author concluded that the degree of resistance exhibited by the cattle is the result of an immune response of the host. By transferring plasma from highly and lowly tick-resistant cattle and from tick-naive cattle to tick-unexposed calves, the involvement of a humoral component in tick resistance was demonstrated (Roberts & Kerr 1976). Plasma from highly resistant cattle conferred some degree of resistance to unexposed calves, whereas the plasma of lowly resistant cattle had no significant effect.

Further studies on the immune response to tick infestation specified the complex array of host immune responses induced by tick feeding (Wikel 1996; 1982; Wikel, Ramachandra, & Bergman 1994). Acquired resistance of cattle after repeated tick infestations resulted in a diminished engorgement weight, increased duration of feeding, decreased numbers of ova, reduced viability of ova, blocked moulting, and death of engorging ticks (Wikel 1996). In the immune response antigen presenting cells, antibodies, T and B cells[2], cytokines, complement[3], basophils, eosinophils, as well as mast cells, and a number of bioactive molecules are involved. First of all, immune responses in the skin of tick-infested cattle evoked a stronger hypersensitivity reaction with increasing tick resistance in *B. taurus* cattle (Schleger et al. 1981) involving the infiltration of the two leukocyte fractions basophils (Askenase et al. 1978) and eosinophils (Schleger et al. 1976) at the tick attachment site. Further studies of the hypersensitivity reaction associated the bioactive molecule histamin with the expression of acquired resistance to tick feeding (Wikel 1982). It was suggested that histamine-binding proteins will be found in the majority of tick species (Brossard & Wikel 2004). In *R. (B.) microplus* infested cattle the release of histamin has been found to cause skin irritations and to result in increased host grooming (Koudstaal et al. 1978). Regarding the complement system, higher serum complement levels are associated with a higher host resistance (Wambura et al. 1998).

Wikel (1996) reported that tick feeding induces the production of immunoglobulin G antibodies against tick saliva antigens. Comparing antibody levels of tick-susceptible (Holstein-Friesian) and tick-resistant (Nelore) breeds after heavy tick infestations Kashino et al. (2005) found that tick infestations suppressed the immunoglobulin G antibody response in susceptible breeds.

2 T cells and B cells belong to the lymphocytes and have immunoregulatory and effector functions in the immunity to ticks (Wikel & Bergman 1997).

3 The complement system is a biochemical cascade supporting the antibodies to clear pathogens from the host.