A Laboratory Manual on *Rhipicephalus microplus*

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Raquel Cossio-Bayugar,
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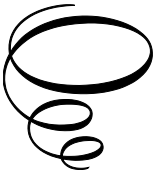

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TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

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Ticks represent one of the most important parasitic arthropods in biomedical research, second only to mosquitoes for their impact on public health and livestock production (de la Fuente et al., 2008), and play an important role in the transmission of a variety of lethal pathogens to many animal and human hosts (Dantas-Torres et al., 2012; Yu et al., 2015). Their combined action as pathogen vectors and blood sucking capacity inflicts billions of dollars in worldwide losses to the cattle, milk, beef and leather industries and a larger amount of money in medical investment to prevent and treat tick-borne diseases in livestock, domestic animals, and humans (Bulman, 2012; Narladkar, 2018; Narladkar, 2018).

Among the 900 species of ticks reported to date, some have adapted to conditions generated by humans to infest domestic animals and livestock. Those classified in the *Ixodidae* family and *Rhipicephalus* sp. genus are obligate hematophagous ectoparasites that may be found around the world, producing massive infestations on strayed dogs, wild cervids and freely grazing cattle (Tan et al., 2021). The *Rhipicephalus (Boophilus) microplus* tick has selected cattle as its preferred host, hence earning the name of the common cattle tick. It is a single-host tick, which means that it develops its entire parasitic phase on a single animal, which gives it greater chances

of survival; therefore, it is feasible to develop four to five tick generations over the course of a year. The economic losses caused by *R microplus* infestation with its associated diseases and control costs have been calculated at USD $13.9–18.7 billion per year worldwide (Jaime Betancur Hurtado and Giraldo-Ríos, 2019). In some countries, such as Mexico, economic losses due to *Rhipicephalus* sp. ticks on livestock depend on conditions such as herd size, breed, age, and tick species, since the environmental conditions of livestock exploitation are different in each country, with estimated average annual losses attributed to *R. microplus* infestations of 3.24 billion USD in Brazil (Grisi et al., 2014) and 570 million USD in Mexico (Rodríguez-Vivas et al., 2017). Other nonenvironmental factors that contribute to livestock economic losses may be attributed to tick infestation levels, from moderate to high infestations inflicting losses due to blood ingestion by *R. microplus* ticks (Abbas et al., 2014; Rajput et al., 2006), adversely impacting cattle weight gain in different parts of the world. These types of losses attributed to cattle-tick infestations in nations such as Australia, Argentina, and Mexico range from 40 to 50 kg per head per year tick-loss assessment in the livestock industry (Abbas et al., 2014; Rajput et al., 2006).

Tick control may be achieved in several ways, but in the last 100 years, chemical control has been the most commonly used procedure for that particular purpose (George et al., 2004). However, chemical control of ticks has severe drawbacks, such as gradual loss of acaricide efficacy resulting in an ever-increasing economic cost, in addition to a higher impact on public exposure to pesticides and environmental pollution because these toxic chemicals are a source of soil and water contamination (De Meneghi et al., 2016). Furthermore, ixodicide residues in meat and milk from treated animals are commonly found tainting the food supply (Alvarado Vega et al., 2022; Camoni et al., 1990). Perhaps the most dreaded consequence of frequent use of pesticides is the selection of acaricide-resistant populations of ticks diminishing the efficacy of commonly used acaricide formulations (George et al., 2004; Cossío-Bayúgar et al., 2021), which prevents us from continuing to use the same acaricide formulations indefinitely, forcing us to change formulations over time and selecting new types of acaricide-resistant tick populations (Thullner et al., 2007). Alternative procedures to pesticide treatment are necessary and require systematic study and in-depth knowledge of the parasite's morphology, biological physiology and molecular characterization of their biochemical phenotypes and tick strain properties against man-made acaricides.

The book "*Rhipicephalus microplus*: a laboratory manual" is a guide to study different biological aspects of the cattle tick *R. microplus*. It is our goal to detail procedures related to tick isolation, taxonomical classification, morphological identification and life cycle (Chapter 2), tick-rearing conditions and maintenance of reference strains on confined and restrained bovine hosts, as well as laboratory maintenance of different developmental stages of *R. microplus* strains, destined to provide a sustained supply of high-quality ticks for scientific studies and toxicological assays at any moment under rigorous laboratory conditions (Chapter 3). Detailed procedures for FAO-approved acaricide bioassays for acaricide resistance detection on field-isolated ticks are compared against reference acaricide-resistant strains of *R. microplus* (Chapter 4). These types of toxicological bioassays are designed to study the effect of acaricides on ticks; however, they may be useful for scientific assessment of plant extracts or the effect of tick pathogen microorganisms on ticks under rigorous experimental conditions.

It is also our purpose to elaborate a comprehensive guide to specific dissection procedures of *R. microplus* internal organs and describe specific bioassays that are useful to assess the physiological effects of new acaricide-like molecules on dissected tick organs (Chapters 5). Internal tick fluids are a crucial medium for tick-borne diseases and a steady supply of specialized tick cells. It is our goal to detail optimized protocols to extract *R. microplus* hemolymph (Chapter 6) and, saliva in addition to a comprehensive description of how to extract organs or tissue samples for further studies such as tick biology, physiology, in vitro cell culture, proteomic, transcriptomic or genomic studies (Chapter 5) (Xavier et al., 2018; Schwarz et al., 2014; Esteves et al., 2017; Cossío-Bayúgar et al., 2015; Mosqueda et al., 2008).

In vitro tick cell culture, growth and maintenance of *R. microplus* embryonic cell lines and primary and permanent tick cell cultures may be useful for *in vitro* testing of new generation acaricides and readily available at any time to study different aspects of tick biology, such as the relationship between ticks and tick-borne diseases, which will help to identify new strategies for tick control (Al-Rofaai and Bell-Sakyi, 2020; Escobar-Chavarría et al., 2021; Cobaxin-Cárdenas et al., 2019) (Chapter 7).

Chapter 8 describes a procedure designed to analyze the physiological response of the *R. microplus* smooth muscle as a predictor of acaricides and related molecules; these types of procedures are useful for the assessment of the effect of experimental molecules on ticks as potential muscle contraction disrupters, analogous to currently used acaricide

effects on cholinesterase and irreversibly impeded nervous impulse (Chapter 8). Alternatively, these procedures may be used to discover physiological evidence that the presence of mutations in acaricide-target molecules correlates with resistance in maintaining contractibility in muscle, despite the presence of pesticides as a new type of acaricide-resistance bioassay (Cossío-Bayúgar et al., 2015; Cossío-Bayúgar et al., 2020).

Embryology is an important tool in cattle-tick control. Most acaricides are designed to affect egg development and parameters such as egg mass weight, hatching rate and egg mortality, which are complemented by comprehensive egg staining using embryology-specializing procedures; DAPI egg staining for the description and study of tick embryology is used to assess acaricide effects on egg development as described in Chapter 9 (Iturbe-Requena et al., 2020).

We are in the molecular biology age, and DNA and omics technologies involve molecular tools for handling of nucleic acids and proteins specific for *R. microplus* dedicated studies. Chapter 10 provides a thorough description of obtaining the nucleic acids and proteins used in studies of specialized proteomics transcriptomics and genomics of the cattle tick. Chapter 11 describes the current use of bioinformatics sequence data mining for the discovery of critical genes and their protein expression products (Xavier et al., 2018; Schwarz et al., 2014; Xavier et al., 2019; Esteves et al., 2017).

Acaricide resistance has dominated scientific research on cattle ticks for the last 40 years, and an abundance of molecular markers have been proposed as indicators according to the type of acaricide resistance in each country where it has been detected (Gupta et al., 2016; Fular et al., 2020; Miranda-Miranda et al., 2009; Cossío-Bayúgar et al., 2018). Zymography procedures as well as biochemical analysis and PCR acaricide resistance identification by laboratory assays are covered in Chapter 12, complemented by the identification and analysis of *R. microplus* xenobiotic metabolizing enzymes by zymography and transcriptome-based bioinformatics that may be applied to taxonomical classification, tick acaricide resistance diagnosis and acaricide resistance gene discovery by bioinformatics. On the other hand, Chapter 13 contains traditional bioassay laboratory methods designed to characterize acaricide resistance from the well-proven methods of the larval packet test, adult immersion test, and larval immersion test compared to novel emerging biochemical and molecular bioassays.

Tick-control and tick-acaricide resistance are the major challenges to the cattle industry since the first reports of acaricide-resistant strains of the cattle tick were published and made evident that the complete control of

R. microplus by massive use of acaricides was inevitably going to fail, and it was just a matter of time. These simple facts made a priority in the search for alternatives to acaricides destined for tick control, such as entomopathogenic microorganisms (Castro-Saines et al., 2021; Miranda-Miranda et al., 2010; (Fernández-Ruvalcaba et al., 2010; Miranda-Miranda et al., 2012; Fernández-Ruvalcaba et al., 2010). Other studies have focused on tick depredatory arthropods (Miranda-Miranda et al., 2011) and the acaricidal properties of plant extracts (Ghosh et al., 2013; Kumar et al., 2016; Kumar et al., 2021). Biocontrol is a major scientific trend and an important research topic for cattle-tick control, both of which are thoroughly described in Chapter 14, complemented with a complete guide for the identification of naturally occurring *R. microplus* entomopathogenic microorganisms; additionally, some useful methods for the extraction of natural products against the tick *R. microplus* as alternatives to acaricide tick control are described in Chapter 15.

Tick-borne diseases are probably the costliest factor affecting the bovine herd affected by cattle-tick infestations, and the loss in cattle, meat, milk, and leather production must add to medical intervention and drug-treatment costs. A complete description of the diagnosis of *Anaplasma* sp and *Babesia* sp procedures as well as scientific efforts to survey tick-borne diseases are necessary before pharmaceutical herd treatment; most tick-borne pathogen diagnostics are made by drawing blood from cattle and making lab procedures that increase in precision, cost and complexity (Shimada et al., 2004; Torioni de Echaide et al., 1998; Mosqueda et al., 2012; Bock et al., 2004; Kocan et al., 1980; Kocan et al., 2002). Identification of tick parasites and microorganisms by traditional and recently developed techniques is covered in Chapter 16, and diagnosis of *Babesia* sp. and *Anaplasma* sp. in fluids obtained from cattle ticks is the subject of Chapter 17, where the most used diagnostic procedures as well as the most recent and technologically advanced procedures are analyzed and detailed.

It is the objective of this book to provide in a single source the laboratory methods necessary to study the biology and control of the cattle tick, for which there already exists excellent specialized information from different sources (Cossío-Bayúgar et al., 2015; Seixas et al., 2012; Granda-Garcia et al., 2014; Mosqueda et al., 2008; Miranda-Miranda et al., 2009; Miranda-Miranda et al., 2010; Miranda-Miranda et al., 2012; Tidwell et al., 2021; Gupta et al., 2016; Aguilar-Díaz et al., 2018; Fular et al., 2020; Miranda et al., 2005; Martinez Ibañez et al., 2021; Aguilar-Díaz et al., 2022). Therefore, the purpose of this book is to compile in a single volume a guide for most of the subjects related to the systematic study of cattle

ticks by laboratory and bioinformatic procedures. We hope that the Manual will be useful for academics, students, and researchers in the daily activities they develop in the laboratory: if we can approach this goal, we will have fulfilled the task we have set for ourselves.

References

Abbas, R.Z., Zaman, M.A., Colwell, D.D., Gilleard, J., Iqbal, Z., 2014. Acaricide resistance in cattle ticks and approaches to its management: the state of play. Vet. Parasitol. 203, 6–20. https://doi.org/10.1016/j.vetpar.2014.03.006.

Aguilar-Díaz, H., Esquivel-Velázquez, M., Quiroz-Castañeda, R.E., Miranda-Miranda, E., Conde-Baeye, R.J.P., Cobaxín-Cárdenas, M., Ostoa-Saloma, P., Cossío-Bayúgar, R., 2018. Comparative hemolymph proteomic and enzymatic analyses of two strains of *Rhipicephalus* (*Boophilus*) microplus ticks resistant and susceptible to ixodicides. BioMed Res. Int. 2018, 1–16. https://doi.org/10.1155/2018/9451547.

Aguilar-Díaz, H., Quiroz-Castañeda, R.E., Salazar-Morales, K., Miranda-Miranda, E., 2022. A newly optimized protocol to extract high-quality hemolymph from the cattle tick *Rhipicephalus microplus*: Improving the old conditions. *CRPVBD* 2, 100066. https://doi.org/10.1016/j.crpvbd.2021.100066.

Al-Rofaai, A., Bell-Sakyi, L., 2020. Tick Cell Lines in Research on Tick Control. Front Physiol 11, 152. https://doi.org/10.3389/fphys.2020.00152.

Alvarado Vega, C., Briceño Guevara, S., Matarrita-Rodríguez, J., Masís-Mora, M., Pérez-Rojas, G., WingChing-Jones, R., 2022. Residuos de acaricidas en leche entera bovina de Costa Rica. URJ 14, 3787. https://doi.org/10.22458/urj.v14i1.3787.

Bock, R., Jackson, L., de Vos, A., Jorgensen, W., 2004. Babesiosis of cattle. Parasitology 129 Suppl, S247-269. https://doi.org/10.1017/s0031182004005190.

Bulman, M.G., 2012. Pérdidas económicas directas e indirectas por parásitos internos y externos de los animales domésticos en Argentina. Anales de la Acad. Nac. Agron. Vet. LXVI, 76–176.

Camoni, I., Ghirotti, M., De Meneghi, D., Diverio, S., Generali, T., Izzo, P., Mwaka, B., 1990. Chlorfenvinphos residues in milk from traditionally managed cows in Southern Province, Zambia. Vet. Res. Commun. 14, 503–506. https://doi.org/10.1007/BF00367062.

Castro-Saines, E., Hernandez-Ortiz, R., Lagunes-Quintanilla, R., Peña-Chora, G., 2021. Characterization of a strain of *Serratia* sp. with

ixodicide activity against the cattle tick *Rhipicephalus microplus*. Exp. Appl. Acarol. 85, 101–111. https://doi.org/10.1007/s10493-021-00640-4.

Cobaxin-Cardenas, M.E., Aguilar Díaz, H., Olivares Avelino, P., Salinas-Estrella, E., Preciado-de la Torre, J.F., Quiroz-Castañeda, R.E., Amaro-Estrada, I., Cossío-Bayúgar, R., Rodríguez-Camarillo, S., 2019. Primer abordaje para la propagación de *Anaplasma marginale* (MEX-31-096) en células de garrapata Rm-sus. RECEIN 13, 67–80. https://doi.org/10.26457/recein.v13i51.2200.

Cossío-Bayúgar, R., Martínez-Ibañez, F., Aguilar-Díaz, H., Miranda-Miranda, E., 2018. Pyrethroid Acaricide Resistance Is Proportional to P-450 Cytochrome Oxidase Expression in the Cattle Tick *Rhipicephalus microplus*. Biomed Res. Int. 2018, 8292465. https://doi.org/10.1155/2018/8292465.

Cossío-Bayúgar, R., Miranda-Miranda, E., Aguilar Díaz, H., Reynaud, E., 2021. Type of Resistance, in: The Entomological Guide to *Rhipicephalus*, Insects and Other Terrestrial Arthropods: Biology, Chemistry and Behavior. Nova Scientific Pulishers, Inc, New York, NY, pp. 147–176.

Cossío-Bayúgar, R., Miranda-Miranda, E., Fernández-Rubalcaba, M., Narváez Padilla, V., Reynaud, E., 2015. Adrenergic ligands that block oviposition in the cattle tick *Rhipicephalus microplus* affect ovary contraction. Sci Rep 5, 15109. https://doi.org/10.1038/srep15109.

Cossío-Bayúgar, R., Miranda-Miranda, E., Martínez-Ibañez, F., Narváez-Padilla, V., Reynaud, E., 2020. Physiological evidence that three known mutations in the para-sodium channel gene confer cypermethrin knockdown resistance in *Rhipicephalus microplus*. Parasit. Vectors 13, 370. https://doi.org/10.1186/s13071-020-04227-7.

Dantas-Torres, F., Chomel, B.B., Otranto, D., 2012. Ticks and tick-borne diseases: a One Health perspective. Trends Parasitol. 28, 437–446. https://doi.org/10.1016/j.pt.2012.07.003.

de la Fuente, J., Estrada-Pena, A., Venzal, J.M., Kocan, K.M., Sonenshine, D.E., 2008. Overview: Ticks as vectors of pathogens that cause disease in humans and animals. Front. Biosci. 13, 6938–6946. https://doi.org/10.2741/3200.

De Meneghi, D., Stachurski, F., Adakal, H., 2016. Experiences in tick control by acaricide in the rraditional cattle sector in Zambia and Burkina Faso: possible environmental and public health implications. Front. Public Health 4. https://doi.org/10.3389/fpubh.2016.00239.

Escobar-Chavarría, O., Cossío-Bayúgar, R., Ramírez-Noguera, P., Prado-Ochoa, M.G., Velázquez-Sánchez, A.M., Muñoz-Guzmán, M.A.,

Angeles, E., Alba-Hurtado, F., 2021. *In vivo* and *in vitro* apoptosis induced by new acaricidal ethyl-carbamates in *Rhipicephalus microplus*. Ticks Tick Borne Dis. 12, 101603. https://doi.org/10.1016/j.ttbdis.2020.101603.

Esteves, E., Maruyama, S.R., Kawahara, R., Fujita, A., Martins, L.A., Righi, A.A., Costa, F.B., Palmisano, G., Labruna, M.B., Sá-Nunes, A., Ribeiro, J.M.C., Fogaça, A.C., 2017. Analysis of the Salivary Gland Transcriptome of Unfed and Partially Fed *Amblyomma sculptum* Ticks and Descriptive Proteome of the Saliva. Front Cell Infect. Microbiol. 7, 476. https://doi.org/10.3389/fcimb.2017.00476.

Fernández-Ruvalcaba, M., Peña-Chora, G., Romo-Martínez, A., Hernández-Velázquez, V., de la Parra, A.B., De La Rosa, D.P., 2010. Evaluation of *Bacillus thuringiensis* pathogenicity for a strain of the tick, *Rhipicephalus microplus*, resistant to chemical pesticides. J. Insect. Sci. 10, 186. https://doi.org/10.1673/031.010.14146.

Fular, A., Gupta, S., Sharma, A.K., Kumar, S., Upadhaya, D., Shakya, M., Nagar, G., Ghosh, S., 2020. Standardization of tick specific biochemical tools for estimation of esterases, monooxygenases and glutathione S-transferase for characterization of acaricide resistance. Pestic. Biochem. Physiol. 164, 130–139. https://doi.org/10.1016/j.pestbp.2020.01.008.

George, J.E., Pound, J.M., Davey, R.B., 2004. Chemical control of ticks on cattle and the resistance of these parasites to acaricides. Parasitology 129, S353–S366. https://doi.org/10.1017/S0031182003004682.

Ghosh, S., Tiwari, S.S., Srivastava, S., Sharma, A.K., Kumar, S., Ray, D.D., Rawat, A.K.S., 2013. Acaricidal properties of *Ricinus communis* leaf extracts against organophosphate and pyrethroids resistant *Rhipicephalus* (*Boophilus*) *microplus*. Vet. Parasitol. 192, 259–267. https://doi.org/10.1016/j.vetpar.2012.09.031.

Granda-García, I., Miranda-Miranda, E., Reynaud, E., Fernández-Ruvalcaba, M., Cossío-Bayúgar, R., 2014. Descripción morfológica de la embriogénesis de la garrapata del ganado *Rhipichephalus* (*Boophilus*) *microplus* (Acari:Ixodidae). Entomol. Mex. 13, 43–48.

Grisi, L., Leite, R.C., Martins, J.R. de S., Barros, A.T.M. de, Andreotti, R., Cançado, P.H.D., León, A.A.P. de, Pereira, J.B., Villela, H.S., 2014. Reassessment of the potential economic impact of cattle parasites in Brazil. Rev. Bras. Parasitol. Vet. 23, 150–156. https://doi.org/10.1590/s1984-29612014042.

Gupta, S., Ajith Kumar, K.G., Sharma, A.K., Nagar, G., Kumar, S., Saravanan, B.C., Ravikumar, G., Ghosh, S., 2016. Esterase mediated

resistance in deltamethrin resistant reference tick colony of *Rhipicephalus* (*Boophilus*) *microplus*. Exp. Appl. Acarol. 69, 239–248. https://doi.org/10.1007/s10493-016-0032-7.

Iturbe-Requena, S.L., Prado-Ochoa, M.G., Velázquez-Sánchez, A.M., García-Hernández, F., Cossío-Bayúgar, R., Muñoz-Guzmán, M.A., Ángeles, E., Alba-Hurtado, F., 2020. Oogenesis and embryogenesis inhibition induced by two new ethyl-carbamates in the cattle tick *Rhipicephalus microplus*. Ticks Tick Borne Dis. 11, 101326. https://doi.org/10.1016/j.ttbdis.2019.101326

Jaime Betancur Hurtado, O., Giraldo-Ríos, C., 2019. Economic and Health Impact of the Ticks in Production Animals, in: Abubakar, M., K. Perera, P. (Eds.), Ticks and Tick-Borne Pathogens. IntechOpen. https://doi.org/10.5772/intechopen.81167.

Kocan, K.M., De La Fuente, J., Blouin, E.F., Garcia-Garcia, J.C., 2002. Adaptations of the tick-borne pathogen, *Anaplasma marginale*, for survival in cattle and ticks. Exp. Appl. Acarol. 28, 9–25. https://doi.org/10.1023/a:1025329728269.

Kocan, K.M., Teel, K.D., Hair, J.A., 1980. Demonstration of *Anaplasma marginale* Theiler in ticks by tick transmission, animal inoculation, and fluorescent antibody studies. Am. J. Vet. Res. 41, 183–186.

Kumar, K.G.A., Tayade, A.B., Kumar, R., Gupta, S., Sharma, A.K., Nagar, G., Tewari, S.S., Kumar, B., Rawat, A.K.S., Srivastava, S., Kumar, S., Ghosh, S., 2016. Chemo-profiling and bioassay of phytoextracts from Ageratum conyzoides for acaricidal properties against *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) infesting cattle and buffaloes in India. Ticks Tick Borne Dis. 7, 342–349. https://doi.org/10.1016/j.ttbdis.2015.12.005.

Kumar S, Sharma AK, Kumar B, Shakya M, Patel JA, Kumar B, Bisht N, Chigure GM, Singh K, Kumar R, et al. 2021. Characterization of deltamethrin, cypermethrin, coumaphos and ivermectin resistance in populations of *Rhipicephalus microplus* in India and efficacy of an antitick natural formulation prepared from Ageratum conyzoides. Ticks Tick Borne Dis. 12,101818.

Martínez Ibáñez, F., Cossío-Bayúgar, R., Miranda-Miranda, E., Jasso-Villazul, C.E., 2021. Reference tick strains as an important biological material for resistance characterization, in: The Entomological Guide to *Rhipicephalu*s. Nova Scientific Pulishers, Inc, New York, NY, pp. 177–200.

Miranda, E.M., Osorio, J.M., Cossío-Bayúgar, R.C., 2005. Detección de resistencia a los acaricidas en la garrapata del ganado *Boophilus*

microplus mediante análisis de zimogramas. REDVET. Revista Electrónica de Veterinaria VI, 1–17.

Miranda-Miranda, E., Cossío-Bayúgar, R., Quezada-De, M.D.R., F.O.-V., Neri-Orant, S., 2009. Age-induced carboxylesterase expression in acaricide-resistant *Rhipicephalus microplus*. Res. J. Parasitol. 4, 70–78. https://doi.org/10.3923/jp.2009.70.78.

Miranda-Miranda, E., Cossio-Bayugar, R., Martinez-Ibañez, F., Bautista-Garfias, C.R., 2011. *Megaselia scalaris* reared on *Rhipicephalus* (*Boophilus*) *microplus* laboratory cultures. Med Vet Entomol 25, 344–347. https://doi.org/10.1111/j.1365-2915.2010.00930.x.

Miranda-Miranda, E., Cossío-Bayúgar, R., Martínez-Ibáñez, F., Casasanero-Orduña, R., Folch-Mallol, J., 2012. Natural occurrence of lethal aspergillosis in the cattle tick Rhipicephalus (*Boophilus*) *microplus* (Acari:Ixodidae). Parasitology 139, 259–263. https://doi.org/10.1017/S0031182011001843.

Miranda-Miranda, E., Cossío-Bayúgar, R., Quezada-Delgado, Ma.D.R., Sachman-Ruiz, B., Reynaud, E., 2010. Staphylococcus saprophyticus is a pathogen of the cattle tick *Rhipicephalu*s (*Boophilus*) *microplus*. Biocontrol Science and Technology 20, 1055–1067. https://doi.org/10.1080/09583157.2010.505325.

Mosqueda, J., Cossío-Bayúgar, R., Rodríguez, E., Falcón, A., Ramos, A., Figueroa, J.V., Alvarez, A., 2008. Primary midgut, salivary gland, and ovary cultures from *Boophilus microplus*. Ann. N.Y. Acad. Sci. 1149, 49–52. https://doi.org/10.1196/annals.1428.050.

Mosqueda, J., Olvera-Ramírez, A., Aguilar-Tipacamu, G., Canto, G.J., 2012. Current advances in detection and treatment of babesiosis. Curr. Med. Chem. 19, 1504–1518. https://doi.org/10.2174/092986712799828355

Narladkar, B.W., 2018. Projected economic losses due to vector and vector-borne parasitic diseases in livestock of India and its significance in implementing the concept of integrated practices for vector management. Vet. World 11, 151–160. https://doi.org/10.14202/vetworld.2018.151-160.

Rajput, Z.I., Hu, S., Chen, W., Arijo, A.G., Xiao, C., 2006. Importance of ticks and their chemical and immunological control in livestock. J. Zhejiang Univ. - Sci. B 7, 912–921. https://doi.org/10.1631/jzus.2006.B0912.

Rodríguez-Vivas, R.I., Grisi, L., Pérez de León, A.A., Silva Villela, H., Torres-Acosta, J.F. de J., Fragoso Sánchez, H., Romero Salas, D., Rosario Cruz, R., Saldierna, F., García Carrasco, D., 2017. Potential

economic impact assessment for cattle parasites in Mexico. Review. Rev. Mex. Cienc. Pecu. 8, 61. https://doi.org/10.22319/rmcp.v8i1.4305.

Schwarz, A., Tenzer, S., Hackenberg, M., Erhart, J., Gerhold-Ay, A., Mazur, J., Kuharev, J., Ribeiro, J.M.C., Kotsyfakis, M., 2014. A systems level analysis reveals transcriptomic and proteomic complexity in *Ixodes ricinus* midgut and salivary glands during early attachment and feeding. Mol. Cell Proteomics 13, 2725–2735. https://doi.org/10.1074/mcp.M114.039289.

Seixas, A., Oliveira, P., Termignoni, C., Logullo, C., Masuda, A., da Silva Vaz, I., 2012. *Rhipicephalus* (*Boophilus*) *microplus* embryo proteins as target for tick vaccine. Vet. Immunol. Immunopathol. 148, 149–156. https://doi.org/10.1016/j.vetimm.2011.05.011.

Shimada, M.K., Yamamura, M.H., Kawasaki, P.M., Tamekuni, K., Igarashi, M., Vidotto, O., Vidotto, M.C., 2004. Detection of *Anaplasma mar*ginale DNA in larvae of *Boophilus microplus* ticks by polymerase chain reaction. Ann. NY. Acad. Sci. 1026, 95–102. https://doi.org/10.1196/annals.1307.012.

Tan, L.P., Hamdan, R.H., Hassan, B.N.H., Reduan, M.F.H., Okene, I.A.-A., Loong, S.K., Khoo, J.J., Samsuddin, A.S., Lee, S.H., 2021. *Rhipicephalus* tick: a contextual review for Southeast Asia. Pathogens 10, 821. https://doi.org/10.3390/pathogens10070821

Thullner, F., Willadsen, P., Kemp, D., 2007. Acaricide rotation strategy for managing resistance in the tick *Rhipicephalus* (*Boophilus*) *microplus* (Acarina: Ixodidae): laboratory experiment with a field strain from Costa Rica. J. Med. Entomol. 44, 817–821. https://doi.org/10.1603/0022-2585(2007)44 [817: arsfmr]2.0.co;2

Tidwell, J.P., Treviño, D.E., Thomas, D.B., Mitchell, R.D., Heerman, M.C., Pérez de León, A., Lohmeyer, K.H., 2021. Pictorial dissection guide and internal anatomy of the cattle tick, *Rhipicephalus* (*Boophilus*) *microplus* (Canestrini). Ticks Tick Borne Dis. 12, 101685. https://doi.org/10.1016/j.ttbdis.2021.101685

Torioni de Echaide, S., Knowles, D.P., McGuire, T.C., Palmer, G.H., Suarez, C.E., McElwain, T.F., 1998. Detection of cattle naturally infected with *Anaplasma marginale* in a region of endemicity by nested PCR and a competitive enzyme-linked immunosorbent assay using recombinant major surface protein 5. J. Clin. Microbiol. 36, 777–782. https://doi.org/10.1128/JCM.36.3.777-782.1998

Xavier, M.A., Tirloni, L., Pinto, A.F.M., Diedrich, J.K., Yates, J.R., Gonzales, S., Farber, M., da Silva Vaz, I., Termignoni, C., 2019. Tick Gené's organ engagement in lipid metabolism revealed by a combined

transcriptomic and proteomic approach. Ticks Tick Borne Dis. 10, 787–797. https://doi.org/10.1016/j.ttbdis.2019.03.013

Xavier, M.A., Tirloni, L., Pinto, A.F.M., Diedrich, J.K., Yates, J.R., Mulenga, A., Logullo, C., da Silva Vaz, I., Seixas, A., Termignoni, C., 2018. A proteomic insight into vitellogenesis during tick ovary maturation. Sci. Rep. 8, 4698. https://doi.org/10.1038/s41598-018-23090-2

Yu, Z., Wang, H., Wang, T., Sun, W., Yang, X., Liu, J., 2015. Tick-borne pathogens and the vector potential of ticks in China. Parasit. Vectors 8, 24. https://doi.org/10.1186/s13071-014-0628-x

CHAPTER 2

RHIPICEPHALUS MICROPLUS: TICK CLASSIFICATION, MORPHOLOGICAL IDENTIFICATION AND LIFE CYCLE

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Abstract

Rhipicephalus (Boophilus) microplus, the tropical cattle tick, mainly infests cattle, deer and buffalo, but it can also be found on horses, goats, sheep, donkeys, dogs, pigs and some wild mammals. *R. microplus* is found worldwide in subtropical and tropical regions. This tick is endemic in the Indian region, much of tropical and subtropical Asia, northeastern Australia, Madagascar, southeastern Africa, the Caribbean, and many countries in South and Central America and Mexico. *R. microplus* is a vector of protozoans (*Babesia bovis* and *B. bigemina*) and the rickettsia *Anaplasma marginale,* which have a severe impact on the cattle industry. Previously, this tick species taxonomically was classified as *Boophilus microplus,* but

it was reclassified based on its morphological characteristics. This is important to differentiate it from other tick species such as *Rhipicephalus annulatus*.

In this chapter, we examine the most important features of the classification and morphology of *R. microplus*, including relevant taxonomic aspects, using original figures and drawings, as well as the identification of proper features of this tick species.

Rhipicephalus microplus is a member of the family Ixodidae (hard ticks). Hard ticks have a dorsal shield (scutum), and their mouthparts (capitulum or gnatosome) protrude forward when they are seen from above. *Rhipicephalus* ticks have a hexagonal basis capitulum. The spiracular plate is rounded or oval, and the palps are very short, compressed, and ridged dorsally and laterally. Males have adanal plates and accessory plates. The anal groove is absent or indistinct in females and faint in males. There are no festoons or ornamentation. *R. microplus* adults have a short, straight capitulum. The legs are pale cream, and there is a wide space between the first pair of legs and the snout. The body is oval to rectangular, and the shield is oval and wider at the front. The snout is short and straight. The nymphs of this species have an orange–brown scutum. The body is oval and wider at front. The body color is brown to blue–gray, with white at the front and sides. *R. microplus* larvae have a short, straight capitulum and a brown to cream body. Larvae have six legs instead of eight.

Keywords: taxonomy, tick biology, larvae, nymph, engorged female, growth.

1. Introduction

Ticks (Acari: Ixodida) are blood-feeding ectoparasites second only in importance to mosquitoes as vectors of human diseases, and their importance as vectors of animal diseases is widely recognized (Strickland, 1976; Balashov et al, 1983; Bautista, 2016). Ticks are distributed worldwide as parasites of wild and domestic vertebrates except fishes. These parasites belong to the phylum Arthropoda, class Arachnida, subclass Acari, order Parasitiformes and suborder Ixodida. Ticks share the order Parasitiformes with the suborders Holothyrida, Mesostigmata (commonly known as mites) and Opilioacarida. Ixodida contains three families: Argasidae (soft ticks that have a dorsum without chitin), Ixodidae (hard ticks that have a dorsum totally or partially covered with chitin) and Nuttalliellidae (an ill-known monotypic family represented by *Nuttalliella namaqua*). In turn, according to morphological characters, the family Ixodidae is subdivided into the

Prostriata group (genus Ixodes) and Metastriata group (all other genera in Ixodidae).

Ticks parasitizing animals have existed for at least 99 million years (Peñalver *et al*., 2017). Its external structures are very similar to those of present ticks (Figure 1). Ticks probably transmitted pathogenic microorganisms to vertebrates millions of years ago. In this context, Poinar (2015) found rickettsial-like cells in the 15-million-year-old tick *Cornupalpatum burmanicum*.

2. General external morphology of ticks

Hereafter, figures show the process to identify Ixodidae members and how to differentiate in a fast and simple form, *R. microplus* ticks from other genres and species that may be found infesting cattle. The differences between hard (Ixodidae) and soft (Argasidae) ticks are shown in Figure 1 and 2 (dorsal view).

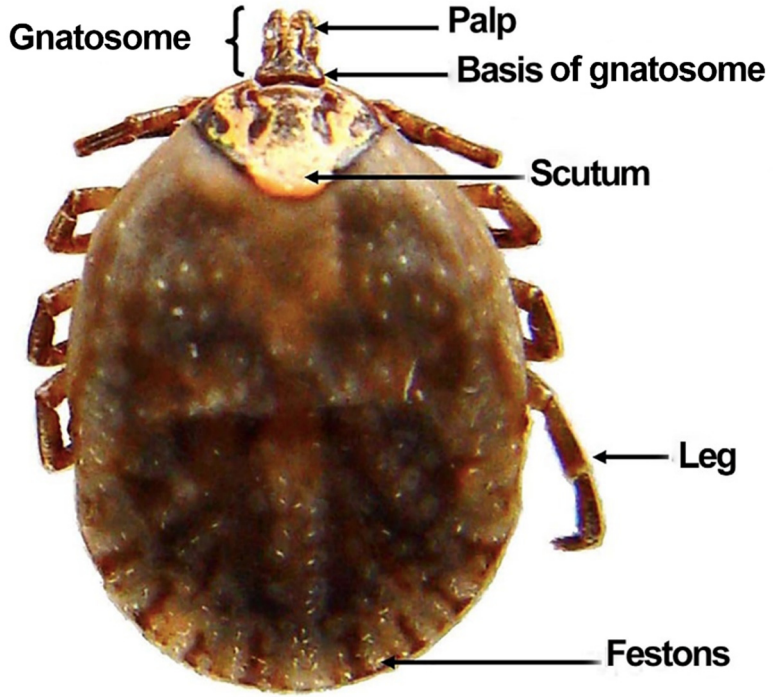

Figure 1. Ixodid tick external morphology.

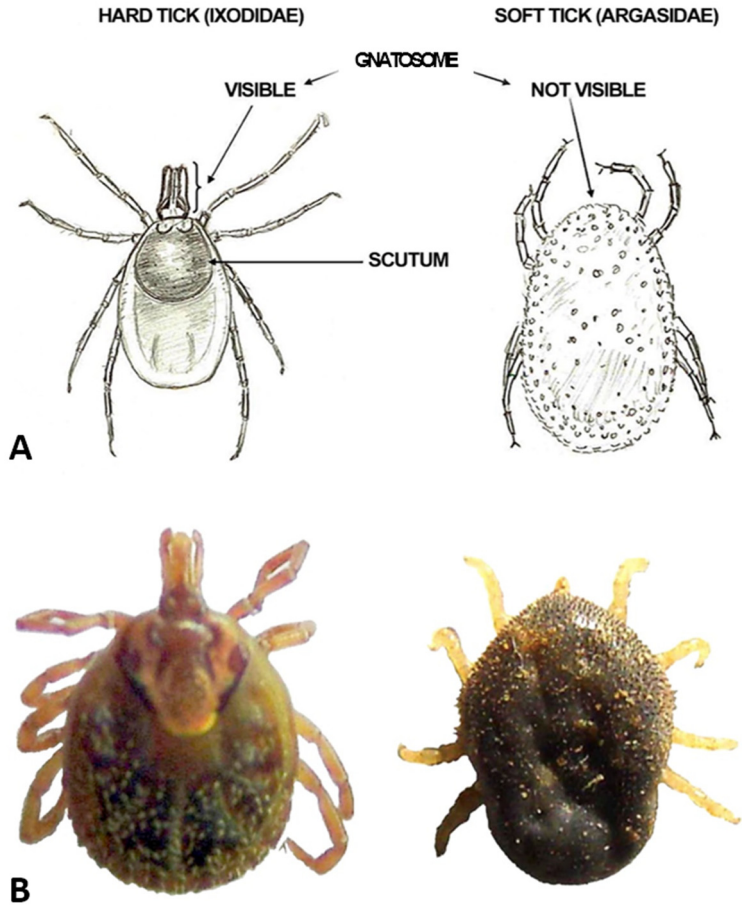

Figure 2. Differences between hard and soft ticks. Panel A) Morphological differences between a hard tick, *Ixodes scapularis*, (Ixodidae) and a soft tick, *Argas persicus* (Argasidae). Panel B) Left, *Amblyomma mixtum*, a hard tick (Ixodidae); right, *Otobius megnini*, a soft tick (Argasidae).

3. Identification of *Rhipicephalus microplus* based on external morphological features

The next figures show a process for the easy identification of *R. microplus*, starting with an unknown tick (Figure 3) and following the red arrows, based on the appearance of the capitulum and the presence of scutum.

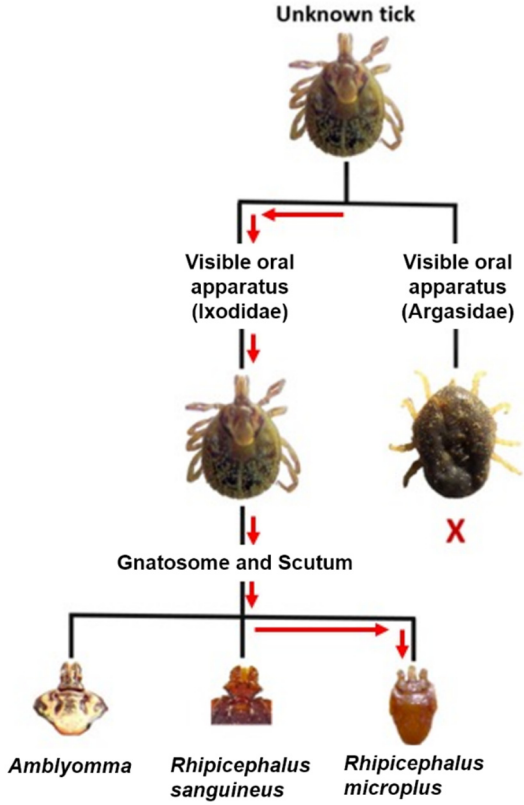

Figure. 3. First step of identification of *Rhipicephalus spp.* ticks. Identification of *Rhipicephalus spp.* based on the gnatosome and scutum. To differentiate *R. (B) microplus* from *R. annulatus;* other characteristics are observed and pointed out later.

After identification as a hard tick based on the capitulum and scutum, the differentiation of males and females of *R. microplus* and *R. annulatus* is carried out following the red arrows (Figure 4, panel A). Based on the presence of a caudal appendage or caudal process on male ticks of *R. (B) microplus* (Figure 4 panel B) and spurs in the second and third coxae on female ticks (Figure 4, panel C).

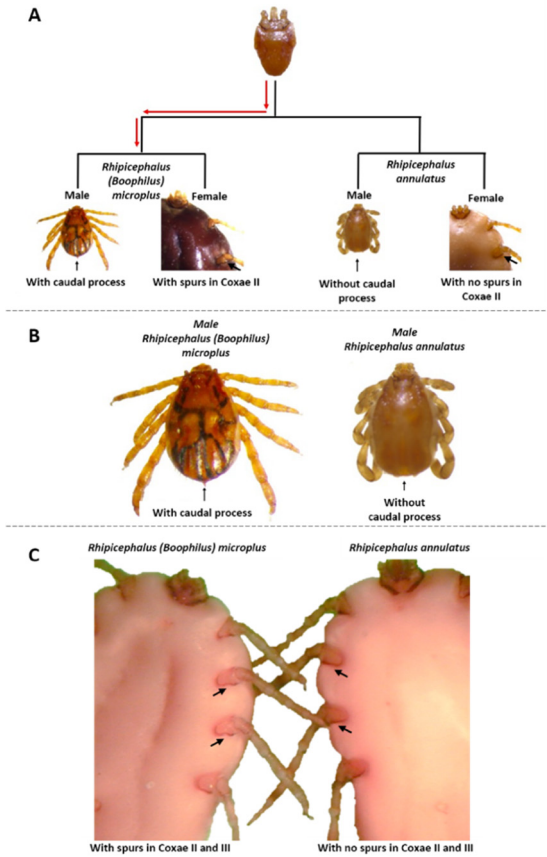

Figure 4. Second step of identification of ticks. Panel A) Identification of *Rhipicephalus (Boophilus) microplus* and *R. annulatus* males and females. Panel B) Main difference between *R. (B) microplus* and *R. annulatus* males (dorsal view). Panel C) Main difference between *R. (B) microplus* and *R. annulatus* females (ventral view).

A schematic guide in colors for the identification of the most important parts of the anatomy of *R. microplus* adult female (Figure 5) and male ticks (Figure 6) is presented in the following figures to help distinguish each segment. The key parts for taxonomic identification are marked with an asterisk (*).

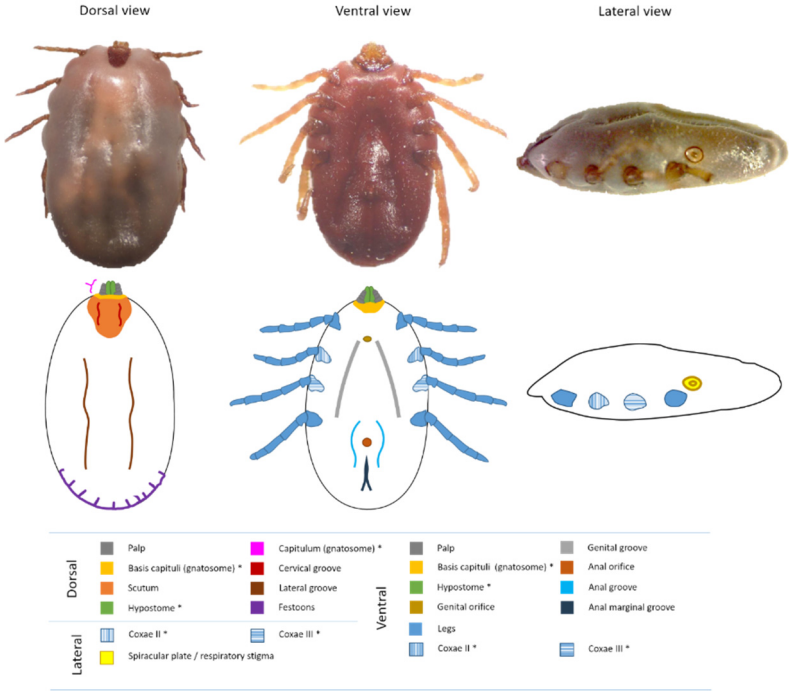

Figure 5. Adult female *R. microplus*, key external characteristics for identification. The capitulum (capituli or gnatosome) has a slightly more protuberant hypostome than the palps. The basis of the gnatosome has a hexagonal shape (dorsal view). Second and third coxae have an inverted heart shape (ventral view). Spiracular plate (respiratory stigma) is located a few millimeters caudal to the fourth coxae. Festoons are barely distinguished in adults and not identifiable in completely engorged females.

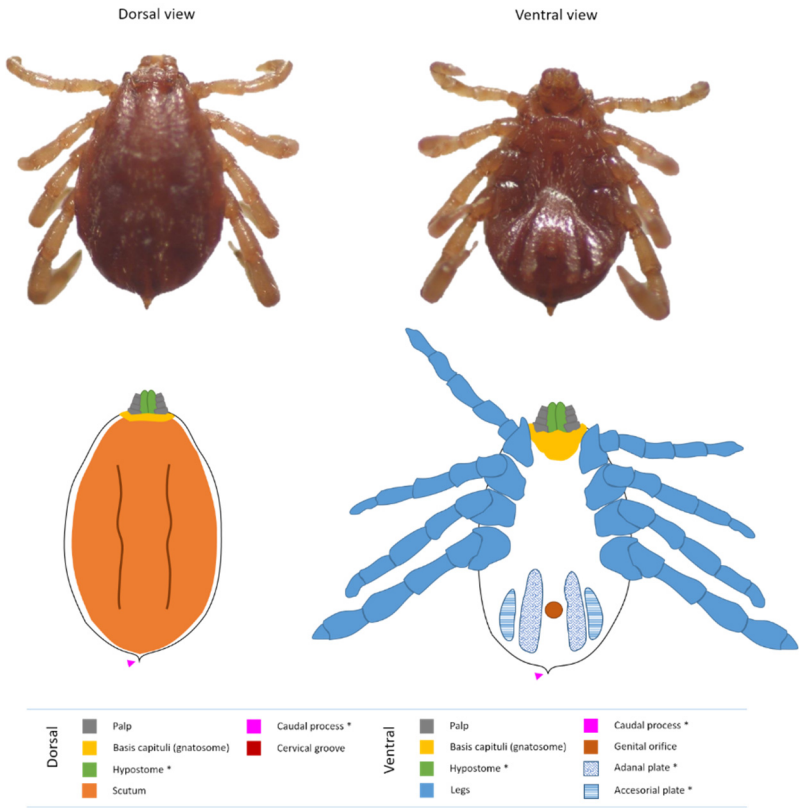

Figure 6. Adult male *R. microplus*, key external characteristics for identification**.** In addition to female ticks, the capitulum (capituli or gnatosome) has a slightly more protuberant hypostome than the palps. The basis of the gnatosome has a poorly defined shape. The second and third coxae are not key for identification. Scutum occupies the majority of the dorsal space of the body. The presence of adanal plates (or shields) and accessory plates are key for the identification of *R. microplus* male ticks, as well as the presence of the caudal process (appendage).

Finally, Figure 7 shows the comparative size among a blood-replete female, adult male and larvae (dorsal view).

Figure. 7. External morphology of *Rhipicephalus microplus*. Left, adult engorged female; center, adult male; right, larvae.

4. Life cycle of *Rhipicephalus microplus* ticks

Rhipicephalus (*Boophilus*) *microplus* is a single host tick; it spends all its life stages on a single host. Ticks in general go through four stages of development: egg, larva, nymph and adult (Figure 8). The larval stage is different from the nymph and adult stages by having only three pairs of legs, and nymphs and adults have four pairs. There are also two intermediate phases of development known as mutant phases, which are characterized by the detachment of the outer skin or cuticle (molt) of the larva in its passage to nymph and of the nymph in its passage to adult. Both events occur after taking a blood meal from the host (Anderson and Magnarelli 2008; Sonenshine et al. 2002).

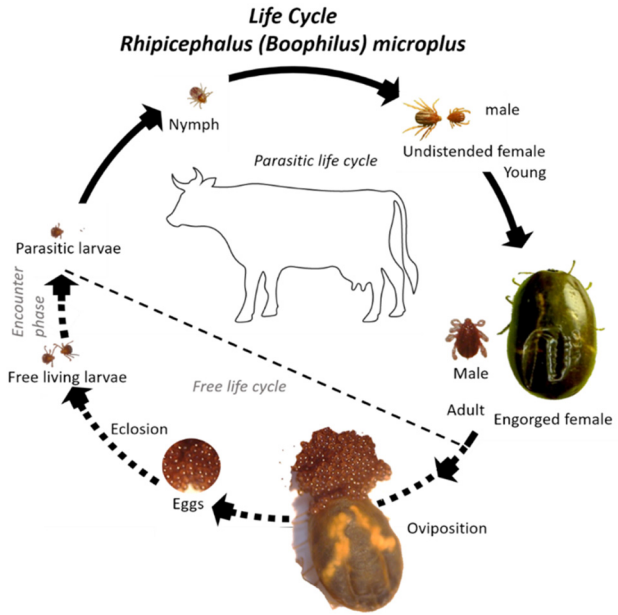

Figure 8. Life cycle of *R. microplus*. Nonparasitic life begins at the moment adult engorged ticks drop from bovines and start oviposition until larvae emerge from the eggs and start looking for a bovine to feed on; this period is known as the encounter phase (when the larvae have matured from their food reserve and are able to climb onto the host). Once on the cattle, the larvae molt into nymphs, and these molt into adults without leaving the host. Usually, the vegetation that the females look for is grasses less than 30 cm high; if the grasses are tall, the females do not oviposit.

The life cycle of *R. microplus* ticks begins with the hatching of the egg laid by the gravid female tick in a moist and protected site, from which the larva emerges. Oviposition lasts from 14 to 24 days as a general range from the moment the first to the last egg is laid. This period varies depending on climatic conditions from summer to winter (Senbill et al., 2018; Nuñez et al., 1985). The number of eggs laid also varies, between 2500 and almost 5000 eggs were counted. Eggs usually take between 15 and 60 days before larvae start to emerge. These numbers vary according to climatic conditions (Senbill et al., 2018). Of these eggs, under field conditions, usually only 80% or fewer yield live larvae (Lahille, 1917).