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Phenotypic evaluation of genetic resistance to the tick *Rhipicephalus (Boophilus) microplus* in Argentine Creole cattle

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ABSTRACT

The objective of this work was to characterize the Argentine Creole cattle breed through the identification of individual phenotypic variations in the levels of infestation with *Rhipicephalus (Boophilus) microplus*. We evaluated 179 heifers exposed to successive artificial infestations from 2015 to 2018, achieving a total of 663 observations. Tick counts were assessed with the linear mixed model, considering year of evaluation, time of infestation, dam's age and nutritional status during the evaluated period as fixed effects. The average tick count value obtained allowed to classify the breed as highly resistant to the tick charge (99.3%). Although the previous nutritional condition of the animals did not affect the individual charge response, weight gain during the trial showed a significantly negative correlation. We conclude that the Argentine Creole breed is an attractive genetic alternative for cattle breeding in endemic regions, either as a pure breed or a cross-breed.

1. Introduction

The high levels of parasitism produced by *Rhipicephalus (Boophilus) microplus*, and the pathogens it transmits (*Babesia bovis, Babesia bigemina* and *Anaplasma marginale*), are an important limitation for livestock production. This is because they decrease cattle weight gain and milk production, damage leather quality, increase mortality and morbidity rates and lead to higher tick control costs (Späth et al., 1994). In addition, these effects may be increased by the association between tick burden and myiasis (Reck et al., 2014).

The genetic basis of host response variation to tick infestation within and between breeds has been recognized for many years (Wilkinson, 1955; Francis, 1966; Cardoso et al., 2014). *Bos indicus* cattle are generally considered to be more resistant to ticks than *Bos taurus* breeds of European origin (Utech et al., 1978; Madalena et al., 1990; Frisch and O'Neill, 1998; Wambura et al., 1998; Bianchin et al., 2007; da Silva et al., 2007). This is probably due to the presence of naturally selected genes along the evolutionary process of Zebuine breeds (Ibelli et al., 2012; Ayres et al., 2013). A similar condition is observed in indigenous African cattle breeds (Afrikander, Nguni and N'Dama), which are characterized by a high tick resistance (Scholtz et al., 1991; Fivaz et al., 1992; Mattioli et al., 1993; Mattioli and Dempfle, 1995, 2000). Likewise, among the American Creole breeds, the Domestic Animal Diversity Information System (DAD-IS) indicates the resistance or tolerance of the Colombian breed Romosinuano to tick charge (FAO, 2010). In addition, the Brazilian Creole Lageano breed has manifested resistance to ectoparasites, with less severe infestations by larvae of *Dermatobia hominis* and *R. (B.) microplus*, this being associated with thinner coat (Cardoso

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et al., 2014). Another local Brazilian breed (Caracú) has shown intermediate resistance to ticks (Maiorano et al., 2019). Regarding Argentine Creole cattle (ACr), there are antecedents of tick load evaluation using natural infestations, positioning ACr cattle as having higher resistance in relation to their non-Iberian *B. taurus* pairs (Guglielmone et al., 1990). It should be noted that these results were not explored in depth because it was a biotype comparison study, and the information was limited to that generated in the 1990s.

The ACr breed originated from cattle introduced to America by the Spanish conquerors. At present, it represents a population that has suffered a process of natural selection for more than 500 years, resulting in a rustic breed capable of developing in very unfavorable environments (Rabasa et al., 2005). These conditions also determine that the ACr breed can be considered as a genetic reservoir of a species on which a strong selection pressure has been exerted by man (Giovambattista et al., 2001). In this sense, the characterization of animal genetic resources is central to respond to current and/or future productive challenges.

The evaluation of adaptive traits is of great importance for livestock production systems since it allows the identification of individuals that are more tolerant and/or resistant to biotic and abiotic stress conditions. In addition, this information can later contribute as selection criteria in animal improvement programs.

The control of *R.* (*B.*) *microplus* is based almost exclusively on the application of synthetic chemical acaricides, but this method has relevant drawbacks, such as the emergence of acaricide resistance and the accumulation of chemical residues in meat or milk. One of the feasible alternatives to reduce dependence on chemical acaricides is the incorporation of tick resistant cattle. Therefore, the objective of this work was to characterize the ACr breed through the identification of individual phenotypic variations in the levels of infestation with *R. (B.) microplus* in cattle belonging to the herd of the Instituto de Investigación Animal del Chaco Semiárido (IIACS, for its Spanish acronym). This animal population is representative of the breed at national level, since it influences the genetic conformation of many of the herds in current production.

2. Materials and methods

2.1. Environmental conditions

The IIACS is located in the depressed plain region, dry-subhumid saline subregion (Zuccardi and Fada, 1985) of the province of Tucumán, Argentina, located at 27° 11″ south latitude and 65° 14″ west longitude (Fig. 1). This region has a warm, dry and sub-humid mesoclimate (Torres Bruchman, 1977) that corresponds to Cwa in the Köppen-Geiger climate classification system (Beck et al., 2018). Frosts occur between May and September, with a frequency of 16 frosts per year. The average annual temperature is 19.5 °C (25 °C in January and 12 to 12.5 °C in July). In the period 2010–2020, the average annual precipitation was 877.8 mm (12.9% coefficient of variation [CV] between years) and the highest percentage of millimeters fell between October and April (more than 90%). Potential evapotranspiration is 1.090 mm per year.

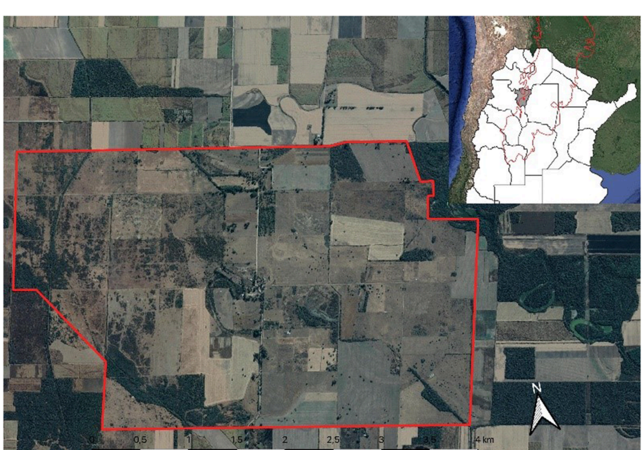

Fig. 1. Location of the Instituto de Investigación Animal del Chaco Semiárido (IIACS - INTA); Leales, Tucumán, Argentina.

2.2. Experimental population and phenotypic data

The ACr cattle herd was formed in 1959. It was the first experimental herd of IIACS, initially composed of 35 cows and two bulls. It later expanded by the introduction of animals acquired in the Argentine provinces of Salta, Jujuy, Chaco, Santiago del Estero and Tucumán. In spite of the geographical isolation within the vast national territory, which determines certain genetic differences, this herd is representative of the breed at national level, since many of the herds currently in production in Argentina were conformed from this population.

At present, the IIACS population has a total of 115–125 females, with an annual mating season. Three to four bulls are used per year, assigned to lots of approximately 30 females and taking into account minimum relationship between parents. Mating is natural in the field and stabled in December, January and February. Therefore, births occur between September and December of each year. The forage base consists of megathermic grasses (*Chloris gayana, Megathyrsus maximus, Brachiaria brizantha* and *Cynodon dactilon*). Annually, female calves are weaned and rebred, while males are mostly sold for fattening.

A total of 179 ACr heifers were exposed to successive artificial infestations with approximately 10,000 *R. (B.) microplus* larvae every 35 days from 2015 to 2018 (Fig. 2). From the total number of individuals evaluated, 126 animals had four consecutive tick counts and 53 had three ($n = 663$ total observations). Infestation dates were 07/16, 08/20, 09/24 and 10/29 in 2015; 06/09, 07/14, 08/18 and 09/22 in 2016; 07/13, 08/17, 09/21 and 10/26 in 2017; and 06/08, 07/13, 08/17 and 09/21 in 2018. The corresponding counts were made for five consecutive days, beginning on 08/03, 09/07, 10/12 and 11/16 in 2015; 06/27, 08/01, 09/05 and 10/10 in 2016; 07/31, 09/04, 10/09 and 11/13 in 2017; and 06/26, 07/31, 09/04 and 10/09 in 2018. Each contemporary group of heifers consisted of approximately 45 animals, ranging in age from 9 to 15 months and with an average initial weight of 144.1 kg.

From birth to weaning, the animals were exposed to natural infestations with common bovine ticks on megathermic pastures. Before starting the artificial infestations, population immunity for *Anaplasma* spp., *B. bovis* and *B. bigemina* was evaluated by serological techniques. Sera were processed by competitive enzyme-linked immunosorbent assay (cELISA) for *Anaplasma* spp. (Sarli et al., 2020) and indirect ELISA (iELISA) for *Babesia* spp. (OIE, 2012) at the Laboratorio de Inmunología y Parasitología, Estación Experimental Agropecuaria, INTA Rafaela (Rafaela, Argentina). In addition, prior to the first infestation of each contemporary group, antiparasitic drugs were applied for the control of internal (15% injectable ricobendazole) and external (immersion bath with amitraz 12.5%) parasites, taking into account the residual times of the inputs used. From then on, no partial quantification controls for the presence of other parasites were carried out. Anticlostridial and respiratory viral vaccines were also applied. After each infestation, the animals were kept in tick-free pastures, achieved through the sowing of an annual grass (*Avena strigosa*) to avoid errors in subsequent counts as a result of the incorporation of larvae in the pastures. The forage-based feeding of the animals was supplemented with ration composed of ground corn grain (1% live weight) and sunflower expeller (0.5% live weight) to achieve adequate daily weight gains for rearing and subsequent mating season.

The tick larvae used for infestations were obtained after incubation of engorged female ticks (27 ± 1 °C and at least 85–86% humidity) collected from naturally infested cattle in the field (IIACS native tick

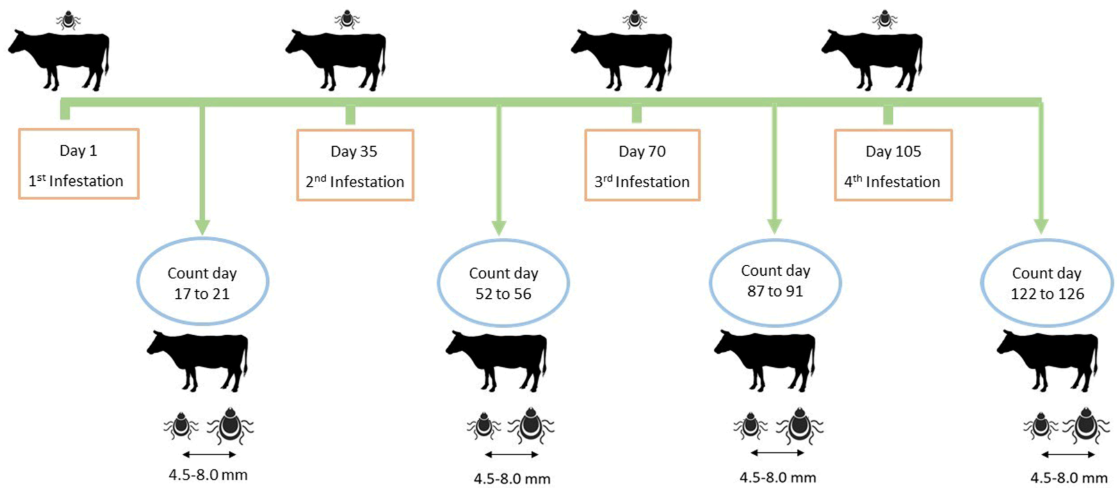

Fig. 2. Experimental scheme of the artificial infestations with *R. (B.) microplus* carried out on Argentine Creole heifers.

population). After 12 days of incubation, 0.5 g aliquots of eggs (corresponding to ≅ 10,000 larvae) were placed in individual jars and returned to the incubation chamber under the same conditions until hatching. All larvae used in infestations were 15 to 20 days old. Within each contemporary group, animals were artificially infested at separate periods every 35 days by emptying the contents of a tube into the dorsal area of each animal.

Between 17 and 21 days after each infestation, the amount of partially engorged females (4.5–8.0 mm ticks) was evaluated following the criteria established by Wharton et al. (1970). In addition, animal weights were obtained at weaning and at the beginning of tick counts in each infestation using a cattle scale. Individual blood samples were obtained by puncture of the jugular vein and placed in tubes with anticoagulant ethylenediaminetetraacetic acid (EDTA) for subsequent genotyping.

The level of resistance was determined according to Utech (1978), who used parasite infestations with approximately 20,000 larvae and calculated resistance as the average percentage of tick larvae that failed to mature into adult females, assuming a 1:1 sex ratio of larvae placed on the animals. This meant that cattle with 100 and 1000 engorged females (of the 20,000 larvae applied) would have 99 and 90% resistance, respectively. Thus, cattle tick resistance was classified as follows: $>$ 98%, highly resistant; 95 to 98%, moderately resistant; 90 to 95%, lowly resistant; and $<$ 90%, very lowly resistant (Jonsson et al., 2014).

2.3. Statistical analysis

A linear mixed model (REML) was used to describe tick counts (non-negative discrete quantitative variable). Due to the overdispersion of the data (variance $>$ mean), we obtained a negative binomial distribution, but we proceeded with the logarithmic transformation of the data to facilitate the calculation. The experimental unit was the left flank of the animal. It was assumed that tick infestation could be affected by fixed factors, namely, time of count, age of the mother, nutritional status and year of evaluation. The time of counting refers to the successive infestations carried out on the animals (1, 2, 3 and 4). Since pre-weaning weight gain of ACr calves is influenced by the age of the mother at calving (Holgado et al., 2021), the following categories were considered for the evaluation of females: heifer (mother's age up to 3 years), young cow (mother's age between 4 and 9 years), and adult cow (mother's age over 10 years). In addition, because all physiological processes within the body are affected by the availability of nutrients, including the immune system, the weight gain of the animals during the trial was evaluated, taking into account the categories weight loss (negative values between weighing), adequate gain (weight gain between 0 and 200 g/day), and high gain (more than 200 g/day). The mathematical model used was as follows:

$$yij = \beta_1 x1_{ij} + \beta_1 x_{1ij}^2 + ... + \beta_4 x_{4ij}\beta_4 x_{4ij}^2 + b_{i1}z_{1ij} + b_{i2}z_{2ij} + b_{i3}z_{3ij} + b_{i4}z_{4ij} + \varepsilon_{ij}$$

ere y_{ij} corresponds to the log average tick count response variable for the particular case ij, β_1 to β_4 are the fixed effect coefficients, x_{1ij} to x_{4ij} are the fixed effect variables (predictors) for observation j in group i, b_{i1} to b_{i4} are the random effect coefficients assumed to be multivariate normally distributed, z_{1ij} to z_{4ij} are the random effect variables (predictors), and ε_{ij} is the error for case j in group i where the error for each group is assumed to be multivariate normally distributed. The quadratic value was included to incorporate flexibility into the model and improve its behavior.

The data were analyzed using the R statistical program RStudio version 1.4.1103. The package used was *lme4*, which provides reliable and easy-to-interpret output for mixed-effects models (Bates and Maechler, 2010). The *Mass* package (Venables and Ripley, 2002) was used to obtain the negative binomial hypothetical distribution parameters (size and mu).

2.4. Ethics statement

All procedures performed on the animals were reviewed and approved by the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) of the School of Veterinary Sciences of the National University of La Plata (Protocol n° 41.2.14T).

3. Results and discussion

Resistance response to *R. (B.) microplus* is generally considered as the average value of the partially engorged female count (4.5–8.0 mm body length of ticks) during days 17 to 21 post-infestation. Fig. 3 exhibits the distribution of the average tick counts. An excessive number of values

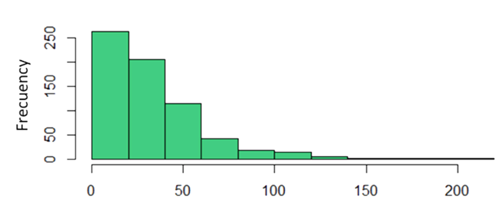

Fig. 3. Distribution of average tick counts, *R. (B.) microplus,* on Argentine Creole females.

close to 0 can be observed, showing an overdispersion of the data (variance = 831.53; mean = 33.46) and suggesting a hypothetical negative binomial distribution, which was confirmed by obtaining the corresponding parameters (size = 1.52; mu = 33. 48). This distribution was used by Maiorano et al. (2019) for a crossbreed comparative study of tick resistance using generalized linear mixed models, and the authors indicated that models with negative binomial distribution were not very frequently used in that type of studies.

Tick counts on the animals evaluated ranged from 0 to 134 (89% CV). The mean and median values of the average tick count in the ACr breed were 18 and 14, respectively. In the data set, the first quartile was 6, while the third quartile was 25. The mean value obtained for ACr heifers classified them as having high resistance to tick load. This implied a general 99.3% resistance value (high resistance according to Jonsson et al., 2014).

Natural resistance to ectoparasites in certain American Creole breeds has been reported in previous works (Guglielmone et al., 1990; Cardoso et al., 2014; FAO, 2010; Maiorano et al., 2019). The coexistence of Creole cattle with *R. (B.) microplus* in American territory for many years allowed natural selection to act on individuals, preserving those genotypes favorable for ectoparasite resistance over numerous generations. At the same time, natural selection favored ticks with a certain capacity to evade host defenses, so that the parasite/host interface based on an equilibrium relationship. In this way, and considering the evolution of the ACr cattle breed, it could adapt to the presence of ticks, the same as ectoparasites did to its immune system. In this sense, it has been postulated that resistance to trypanosomiasis and tick infestation in the African taurine breed N'Dama has developed through continuous and prolonged contact between the host and the parasite during several thousand years (Mattioli and Wilson, 1996; Mattioli et al., 2000). Similar hypotheses were formulated by Bennet and Wharton (1968), Seifert (1971) and Barré et al. (1988) regarding resistance to *R. (B.) microplus, Rhipicephalus (Boophilus) decoloratus* and *Amblyomma variegatum* in the Australian Brahman, Africander and local Creole Zebu breeds of Guadeloupe Island.

In this study, differences in tick count distributions were observed in the evaluations conducted between 2015 and 2018. The estimation of the fixed effect corresponding to the year of evaluation resulted in significant differences (Table 1). The intercept corresponded to the year 2015, and for each change with respect to the base level, the coefficient for 2016 represented a significant (0.50) tick load reduction (65% fewer ticks). In 2018, although the coefficient was conditioned by a 0.16 increase (17% more ticks), it was not significant. The year 2017 evidenced a non-significant 0.16 reduction with respect to the intercept (17% fewer ticks). These results arose from the interpretation of the coefficient when the variable was transformed according to the formula (EXP (0.28291)−1) *100 (Table 1).

Table 1
Effects of the sequence of infestation (linear and quadratic terms), mother's age (MA), type of average daily gain (ADG) and year on the tick counts of *R. (B.) microplus* in ACr heifers. Parameter estimates (coefficients) are the change in the response associated with predictors regarding the baseline given by the next reference levels: Year 2015, MA Adult cow and adequate ADG.

	Estimate value	Standard error	t-value
(Intercept)	−241.42	81.98	−2.945
Linear term coefficient	−22.398	0.98320	−2.278
Quadratic coefficient term	35.218	101.522	3.469
MA young cow	0.1527	0.11264	1.356
MA heifer	0.10212	0.14377	0.710
ADG high gain	−0.28291	0.09937	−2.847
ADG weight loss	0.07243	0.10475	0.691
Year 2016	−0.50293	0.11857	−4.241
Year 2017	−0.15950	0.12366	−1.290
Year 2018	0.16081	0.11815	1.361

MA, mother's age; ADG, (average daily gain. Base levels: Year 2015, MA adult cow and adequate ADG. t-value, two-tailed Student's *t*-test.

It is worth mentioning that from 2011 to 2017, the sexual precocity of females in the ACr cattle herd of the IIACS was evaluated as part of the productive characterization of the breed. For instance, 15-month-old heifers were first mated, obtaining low birth weight calves, low pre-weaning weight gain due to a limited dairy aptitude of the mother and, consequently, low weaning weight (Holgado et al., 2017). The offspring of adult ACr breed cows, 7 to 10 years old at calving, achieved the highest values of pre-weaning weight gain with respect to cows of other ages (Holgado et al., 2021). Since longevity is a quality of the breed, the age at calving of ACr cows belonging to the IIACS herd can be up to approximately 17 years. This pre-existing condition in the evaluated animals could alter their health status at the time of evaluation (Galyean et al., 1999), taking into account that nutrition plays a fundamental role in the immune response and nutrients can affect several, if not all, aspects of the immune response (Ingvartsen and Moyes, 2013). Results of the fixed effect of dam age on the maximum tick count response variable showed that the previous nutritional condition of the heifers had no significant effect (Table 1). Visually, each year of evaluation presents a different pattern, independently of the age of the dams of the heifers in each contemporary group (Fig. 4).

As mentioned above, host immunity requires adequate metabolic energy to maintain optimal functioning and it fluctuates depending on the demands of other physiological systems. These demands, in turn, will be variable depending on environmental conditions, such as nutrient availability (Demas et al., 2011). The heifers evaluated in this work had different weight gains throughout the period of artificial infestation. A weight gain above 200 g/d allowed to significantly differentiate animals carrying a lower number of ticks (32% less tick abundance in absolute number). Although the study design was not specifically developed to evaluate the relationship between tick infestation and weight gain, the effect of tick load on weight gain in cattle is widely recognized (O' Kelly et al., 1971; Seebeck et al., 1971; Springell et al., 1971; Gee et al., 1971; Holroyd and Dunster, 1978; O' Rourke, 1982; Mellor et al., 1983; Sutherst et al., 1983; Sholtz et al., 1991; Jonsson, 2006; Rossner et al., 2022). This fact highlights the benefit of incorporating tick resistant cattle into a herd not only from an animal health perspective but also from a productive standpoint.

If we consider the time at which each infestation was carried out, tick counts decreased as the number of challenges progressed and the second infestation presented a greater data dispersion, allowing a better separation of genotypes with higher and lower resistance. Between the first and third infestations, a significant decreasing linear effect was observed in the number of ticks on the animals (Table 1). However, at the fourth infestation, a positive quadratic effect correlated with a non-significant increase in the number of ticks. The combination of both effects describes a parabolic behavior in the average tick counts. It may be suspected that after the second infestation, the host developed a stronger defense mechanism. Both domestic and laboratory animals have been reported to acquire resistance to repeated tick infestations (Trager, 1939; Dipeolu and Harunah, 1984; Jongejan et al., al.,1989; Dipeolu, 1989; Rechav and Kostrzewski, 1991; Dipeolu et al., 1992). In addition, the nutritional status of the animals after the second infestation may have contributed to the immune response to tick infestation. The non-significant load increase observed in the last infestation could be related to the physiological state of the females, which were close to puberty.

Finally, it is important to indicate that the parasitic phase of the common bovine tick develops entirely on the host and its duration ranges from 20 to 41 days, being normally 23 days (modal day) (Nuñez et al., 1982). When applying artificial infestations, tick counts between days 17 to 21 after infestation are adequate for many purposes. The development dynamics of the tick cycle in ACr animals showed that female tick counts (between 4.5 and 8.0 mm) on days 17 and 18 post infestation were not high, while they were higher from day 19, reaching maximum values on days 20 and 21. Recommendations in the Food and Agriculture Organization (FAO) manuals indicate that tick counts

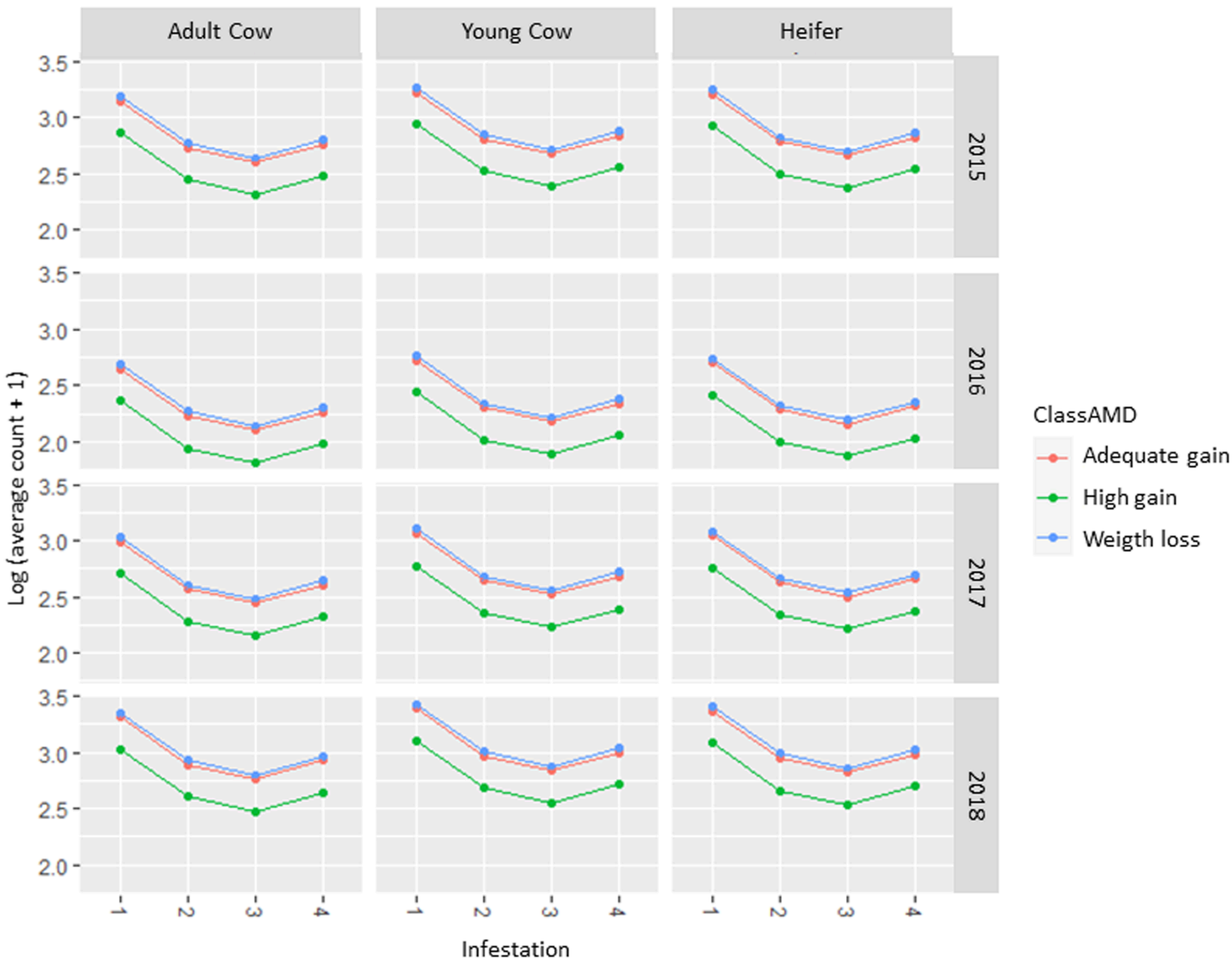

Fig. 4. Graphical representation of the transformed data of average tick counts, *R. (B.) microplus,* in ACr heifers in relation to the age of the mother (adult cow, young cow and heifer), body weight gain (classAMD) and year of evaluation.

should be done on days 20 and 21 post infestation in Zebuine cattle and on days 19 and 20 in European cattle (FAO, 1987). In the case of ACr cattle, the moment to count ticks that complete their development on the animal after artificial infestations should coincide with that of Zebuine cattle, i.e., days 20 and 21 post infestation.

4. Conclusions

The *R. (B.) microplus* count values in Argentine Creole cattle after artificial infestations showed that it could be classified as a highly resistant breed. Thus, it can be proposed as an attractive genetic alternative for cattle breeding in endemic regions for the mentioned ectoparasite, either as a pure breed or a crossbreed. The nutritional condition of cattle prior to common tick resistance evaluations did not affect the individual response to tick load. However, weight gain during the evaluation period and tick counts showed a significantly negative correlation. Probably, the host developed a stronger defense mechanism after the second infestation, and could be therefore proposed as an appropriate methodology to evaluate a larger number of animals, together with the performance of two infestations with their corresponding reading on days 20 and 21 post infestation. This could help obtain more phenotypic data for the genetic evaluation of the trait. Additionally, the assessment of hematological and physiological parameters in future studies would allow to broaden the present discussion.

CRediT author statement

María Florencia Ortega: Conceptualization, Methodology, Research, Resources, Writing- Original draft; Guillermo Giovambattista: Conceptualization, Methodology, Writing - Original draft, Supervision; Christian Cutullé: Conceptualization, Methodology, Writing - Original draft, Supervision; Daniel Dos Santos: Formal analysis; Santiago Nava: Writing - Original draft, Supervision; Martín Bonamy: Conceptualization, Methodology; Fernando Holgado: Conceptualization, Methodology, Resources.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Data availability

Data will be made available on request.

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