Phenotype Characterization of Pelibuey Native Lambs Resistant to *Haemonchus contortus*

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Specific phenotypic traits of the blood-feeder nematode *Haemonchus contortus* were characterized on Pelibuey native, cross-breed sheep in a tropical region of Mexico with background of anthelmintic problems. Data were collected on 4-month-old lambs ($n = 63$) and analyzed weekly in three stages: stage 1 (S1, not infected (0–3 weeks)); S2, first infection with 350 *H. contortus* infective larvae (L_3) per kg of body weight by oral route (3–10 weeks); and S3, second infection with 175 *H. contortus* L_3 per kg of body weight by oral route (10–18 weeks). Once infection took place, sheep grazed in a free-nematode experimental paddock, and hemoncosis-related traits, such as the number of eggs per gram of feces (*epg*), packed cell volume (%*pvc*), and eosinophils (%*eo*), were measured each week. Results were analyzed on specific traits and stages using SAS statistics. Statistical differences of the main phenotypic traits ($P < 0.05$) were observed throughout S2 on Pelibuey high (*hr*) and low (*lr*) responders, respectively: *epg* mean, 475 ± 340.5 and 1439 ± 243.6; %*pvc* mean, 31 ± 0.3 and 27 ± 0.2; and %*eo* mean, 2.5 ± 0.3 and 1.99 ± 0.2. In addition, *lr* and *hr* sheep grazed for 3 months on an area infected with *Haemonchus*, *Cooperia,* and *Strongyloides*, and the *hr* sheep showed a low number of *epg* after being exposed to multiple infections, so that no further treatment was required. These results showed that the selection of animals resistant to nematodes can be an alternative method of control against anthelmintic resistance problems in order to improve animal health and avoid a negative environmental impact.

Key words: **phenotype; Pelibuey breed; *Haemonchus contortus***

Introduction

Tropical regions provide a suitable habitat for increased parasitic nematode populations affecting grazing sheep. The most pathogenic gastrointestinal nematode in the tropics is *Haemonchus contortus,* which affects the sheep abomasum tissues where it lacerates superficial blood vessels and sucks blood into its oral cavity.[1] As a result, sheep raisers strategically treat all animals with chemical anthelmintic compounds at extremely frequent intervals (i.e., every 28 days). Thus, there is a concern about multiple anthelmintic resistance developed by *H. contortus*.[2] Selection of responder sheep might improve the natural resistance against *H. contortus* as an alternative control on tropical farms where native Pelibuey crossbreed sheep are threaten by haemonchosis. Thus, the aim of this study was to characterize

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specific haemonchosis traits that could have an important implication in the selection of animals resistant to sheep haemonchosis.

Materials and Methods

This study was carried out in "Las Margaritas" Experimental Station (INIFAP), situated in a tropical region of Mexico, Hueytamalco, State of Puebla, Mexico. Sixty-three, parasitic-free, 4-month-old lambs were kept in an experimental paddock. This research was divided into three experimental stages. The first one, S1 (from week 0 to 3) was for animal adaptation to the experimental paddock. In the second stage, S2, 350 *H. contortus* infective larvae (L_3) per kg of body weight (BW) were orally administrated at week 3 in every animal. In the last stage, S3, a re-infection of the animals was performed on week 10 using a dose of 175 *H. contortus* L_3 per kg BW by the same route. Lambs were fed commercial growing food and water *ad libitum*. Fecal samples were obtained from the rectum, and the number of eggs per gram of feces (*epg*) was estimated using the McMaster technique and recorded for 18 weeks. Blood samples were taken from the jugular vein to determine the percentage of packed cell volume (%*pvc*) and eosinophils (%*eo*), which were counted in blood smears. Statistical analysis was carried out with raw data for all fixed phenotypic traits throughout the three stages using PROC MIXED (Version 8, 1999; SAS Institute Inc., Cary, NC). Repeated measures were analyzed using least square means (LSM, PROC MIXED), which included fixed effects (i.e., sex, BW). The LSM obtained from the parasitological parameters per animal was compared using a multiple *t*-test. Differences were considered statistically significant when $P < 0.05$.

Results

The *epg*, %*pvc*, and %*eo* data analyses showed differences among stages, associated to low response (*lr*) and high response (*hr*) against *H. contortus*. The LMS for lambs remained normal at S1, and no statistical differences ($P > 0.05$) were observed for *epg* (*lr,* 123.3 ± 114.4; *hr,* 319.3 ± 79.5*)* and for %*pvc* (*lr,* 32 ± 0.2; *hr,* 32 ± 0.3). In contrast, remarkable differences between *lr* and *hr* ($P < 0.05$) throughout S2 were observed. For instance, the mean *epg* was 1439 ± 240.5 for *lr* and 475.3 ± 340.5 for *hr* and the mean %*pvc* was 27 ± 0.2 for *lr* and 31.3 ± 0.3 for *hr*. In contrast, S3 showed that most of the experimental lambs were recovering *epg* and *pvc* normal parameters; the mean *epg* was 37.6 ± 45.15 for *lr* and 148 ± 31.83 for *hr* and %*pvc* was 29 ± 0.02 for *lr* and 32 ± 0.3 for *hr*. All experimental sheep were then exposed for 3 months to *Haemonchus*, *Cooperia, and Strongyloides* genera in a naturally infected paddock, and *epg* acutely increased in 13 animals. In contrast, 50 sheep showed a mild response; thus, anthelmintic treatment was applied 12 weeks after infection for almost all the flock. The fact that those animals did not need an anthelmintic treatment before such a wider period between stages could be considered as a beneficial achievement in terms of reducing the number of anthelmintic treatments. On the other hand, the %*eo* indicated differences ($P < 0.05$) between stages and the means were as follows: S1 = 0.8 ± 0.1 for *lr* and 1.45 ± 0.2 for *hr*; S2 = 1.99 ± 0.2 for *lr* and 2.5 ± 0.3 for *hr*; S3 = 0.2 ± 0.16 for *lr* and 2.7 ± 0.2 for *hr*. Moreover, live weight gain showed statistical differences between *lr* and *hr* ($P < 0.05$); however, there were not statistical differences in the live weight gain between females and males ($P > 0.05$).

Discussion

This study analyzed some haemonchosis traits in Pelibuey lambs and allowed us to identify the relationship between phenotype and environmental pressure on a tropic farm. *Haemonchosis* is the most commonly found parasitic disease in less-developed farming systems,

causing severe damage to the sheep flock.[2] Although, there are effective commercial anthelmintic drugs, a number of problems derived from the use of such compounds are increasing, such as anthelmintic resistance (which triggers a high incidence of parasitic nematodes) and the possible negative impact of some drugs on beneficial organisms in nature.[2] It is important to explore different control measures that could be helpful for farmers, such as phenotype selection of native resistance breeds. For instance, New Zealand and Australia have applied animal selection as an integrated control of parasites.[3] Other countries, such as the United Kingdom and United States, have also demonstrated the importance of associating nematode infection with genotypes.[3,4] This study might contribute to the selection of animals resistant to nematode parasites and encourage animal health management. Further studies should be considered to explore the influence of specific genes related to *H. contortus*-resistant sheep in tropical regions.

Conflicts of Interest

The authors declare no conflicts of interest.

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