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IMMUNOLOGY AND HOST-PARASITE INTERACTIONS - ORIGINAL PAPER

Variation in phenotypic resistance to gastrointestinal nematodes in hair sheep in the humid tropics of Mexico

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Abstract

The objective of the study was to evaluate phenotypic resistance against gastrointestinal nematodes in Blackbelly, Pelibuey and Katahdin ewes before pregnancy in the humid tropics of Mexico. Individual faecal and blood samples were taken in 59 Pelibuey, 69 Blackbelly and 73 Katahdin ewes. The egg count per gram of faeces (EPG) of gastrointestinal nematodes (GINs) was determined. The percentage of packed cell volume (PCV) and body condition score (BCS) of each animal were also recorded. The ewes were segregated as susceptible, intermediate or resistant based on the EPG using the quartile method. The data were analysed using the general linear method, and the means between breeds were compared by Tukey's test. The relationships between the EPG, PCV and BCS were evaluated by Spearman correlation. The Katahdin ewes showed the highest EPG counts (3613.6 ± 5649) compared to the Blackbelly and Pelibuey ewes (576.1 ± 1009 and 56.8 ± 187, respectively, $P < 0.01$). The PCV values between breeds were similar ($P > 0.05$). The susceptible ewes had the highest EPG counts and the lowest PCV percentage (5069 ± 6404 and 22.8% ± 8.1% respectively) compared to the resistant ewes ($P < 0.01$). A higher percentage of Katahdin ewes were susceptible compared to the other breeds ($P < 0.05$). The main GIN species were *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Cooperia curticei*. In conclusion, Katahdin ewes showed susceptibility to GIN compared to Blackbelly and Pelibuey ewes before the pregnancy period in the humid tropics of Mexico.

Keywords Resistance · Susceptibility · EPG · Pelibuey · Blackbelly · Katahdin

Introduction

Infection by gastrointestinal nematodes (GIN) is one of the main limiting factors for sheep production in grazing systems in tropical regions (Torres-Acosta et al. 2012). GINs affect the health, welfare and productive and reproductive behaviour of animals (Keane et al. 2006; Knox et al. 2006). Parasitic infections in grazing sheep are generally mixed (Asmare et al. 2016), although *Haemonchus contortus* has received special attention because of its high pathogenicity and blood-sucking habit, which can cause severe health problems in sheep (Bishop 2012; Besier et al. 2016). Other species such as *Trichostrongylus colubriformis*, *Cooperia curticei* and *Oesophagostomum columbianum* also are present and cause pathogenic effects in sheep (Roeber et al. 2013).

Currently, GIN control through anthelmintics is inefficient due to the development of anthelmintic resistance (AR) in many GIN species (Herrera-Manzanilla et al. 2017). It is necessary to generate alternative control strategies, such as the selection of sheep resistant to GIN infection (Palomo-Couoh et al. 2016). In the present context, resistance is the immunological capacity of sheep to control GIN infection (Alba-

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Hurtado and Muñoz-Guzmán 2013). The selection of animals resistant to GIN has been previously predicted based on phenotypic markers (Castillo et al. 2011), such as egg count per gram of faeces (EPG), packed cell volume (PCV), blood eosinophils (BE), FAMACHA© and body condition score (BCS) (Saddiqi et al. 2012). Native breeds were found to be more resistant to GINs than introduced and improved breeds (Notter et al. 2017). In particular, the hair sheep breeds Blackbelly (Yazwinski et al. 1979; Vanimisetti et al. 2004) and Pelibuey (Ojeda-Robertos et al. 2017) and the composite breed Katahdin (Notter et al. 2017) have shown greater resistance to GIN under artificial infection with *Haemonchus contortus* compared to wool breeds. However, there is a lack of information on the resistance of these sheep to GIN under grazing conditions.

The most common sheep breeds in the Mexican tropics are Pelibuey, Blackbelly, Katahdin, Dorper and their crosses (Muñoz-Osorio et al. 2016). In the sub-humid tropics of Mexico, Palomo-Couoh et al. (2016, 2017) reported that the Pelibuey breed showed greater resistance to GIN compared to the Katahdin breed based on the EPG and BE count. Currently, in Tabasco, the Blackbelly, Pelibuey and Katahdin breeds are raised in sheep production systems. Productive ewes are continuously grazed because this form of production is the cheapest and only receive grain supplements during the lactation period. However, the humid tropical climate of the region enables a high rate of parasitic infection throughout the year, compromising sheep health and production. It is important to evaluate the resistance of these hair sheep to GIN given the higher nematode infection gradient during grazing before breeding as a result of low BCS and the increased animal parasite burden and pasture contamination during this period (Soto-Barrientos et al. 2018). The evaluation of ewe resistance against GIN during this period will enable better anthelmintic management considering breed differences. Hence, the objective of the present study was to evaluate phenotypic resistance against gastrointestinal nematodes in hair sheep ewes (Blackbelly, Pelibuey and Katahdin) before pregnancy in the humid tropics of Mexico.

Materials and methods

Location

The study was carried out from February to May during the period of lower precipitation (274 mm) in three sheep farms located in Tabasco, Mexico (17° 59′ 13″ N and 92° 55′ 10″ W), at an altitude of 9 masl. The climate in the region is hot humid, with an average temperature of 27 °C, relative humidity greater than 90% and rainfall of 1677.4 mm per year (CONAGUA 2017).

Animals and management

The manager of each participating farm was previously informed about the study. The animal management and sample protocols were performed under the consent and supervision of the managers.

Adult ewes were selected from three flocks containing solely Blackbelly, Pelibuey or Katahdin sheep. Only ewes that were non-lactating, non-pregnant and non-dewormed for at least 60 days before the sampling date were considered. In total, 69 Blackbelly, 59 Pelibuey and 73 Katahdin sheep ewes were selected, with an average age of 3.5, 3 and 3, respectively, and an average BCS of 2.2, 3.1 and 2.6, respectively.

Feed management

The Pelibuey sheep were located at the Centre for Training and Reproduction of Minor Species (CECAREM). At this location, sheep grazed 8 h/day in paddocks of Bermuda grass (*Cynodon dactylon*; 7.6% crude protein [CP]) and were provided with a commercial feed supplement (14.0% CP). The Blackbelly sheep were located at a second farm and grazed 10 h/day in paddocks of African star grass (*Cynodon plectostachyus*; 7.0% CP). The Katahdin sheep were located at a third farm and grazed 10 h/day in paddocks of Koronivia grass (*Brachiaria humidicola*; 7.0% CP). The Blackbelly and Katahdin sheep breeds did not receive supplements during this period.

Measurements

Samples were taken by Veterinarians that were experts in sheep medicine and followed the guidelines for sheep welfare and avoiding pain and suffering according to the Bioethics Committee of the Campus of Biological and Agricultural Sciences of the Autonomous University of Yucatan, Mexico. Briefly, a helper held the sheep safely to prevent lesions during sampling and a Veterinarian took the samples of the animals.

Egg count per gram of faeces

Faecal and blood sampling was performed once when each farm was visited. Faecal samples were taken directly from the rectum of each sheep and placed in polyethylene bags. The faecal samples were processed by the modified McMaster technique to determine the EPG of GINs (Rodríguez-Vivas and Cob-Galera 2005). The number of EPG was adjusted by a correction factor of 50.

Nematode genera

Coprocultures were performed for each breed according to Corticelli and Lai (1963). The infective larval genera were

determined according to Van Wyk and Mayhew (2013). The GIN species were determined by PCR following the procedure described by Encalada-Mena et al. (2014).

Packed cell volume

Blood samples were taken from each ewe by venepuncture of the jugular vein. The samples were processed to determine the percentage of PCV using a blood analyser (Medonic CA 620/530 Vet, Brand Boule Medical AB, Stockholm, Sweden).

Body condition score

The BCS was recorded according to the scale of Russel (1984).

Animal segregation in resistant and susceptible to GIN

The cutoff point for categorising animals as resistant or susceptible was determined using the quartile method described by Palomo-Couoh et al. (2016). Animals were ordered according to EPG count from lowest to highest, and the number of animals in Q1 and Q3 was calculated to classify resistant and susceptible sheep, respectively.

Statistical analysis

Means, standard errors and quartiles were obtained using the proc. univariate procedure in the SAS statistical software (SAS Institute 2004). The data were analysed using the general linear modelling procedure in the same software (SAS Institute 2004). The EPGs were transformed to logarithm [Log (EPG +1)] to homogenise the variance and approximate the model to a normal distribution. The following statistical model was generated:

$$Y_{ijk} = \mu + \gamma_i + \delta_j + \gamma \times \delta_{ij} + \zeta_k + \gamma \times \zeta_{ik} + \varepsilon_{ijkl}$$

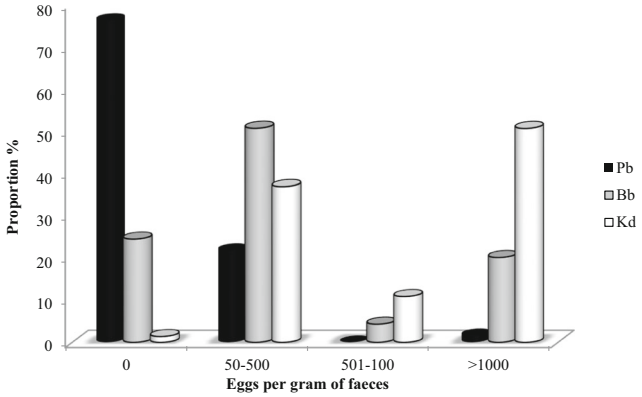

Fig. 1 Proportion of Pelibuey (Pb), Blackbelly (Bb) and Katahdin (Kd) ewes shedding eggs per gram of faeces of gastrointestinal nematode infected under grazing condition in the hot humid tropics of Mexico

where Y_{ijk} = the response variable (EPG, PCV), μ = the general mean, γi = the fixed effect of the flock (i = Blackbelly, Katahdin, Pelibuey), δ_j = the effect of segregation (susceptible, intermediate and resistant), $\gamma \times \delta_{ij}$ = the joint effect of the flock and segregation, ζ_k = the effect of the BCS, $\gamma \times \zeta_{ik}$ = the joint effect of the flock and BCS and ε_{ijkl} = the random error ~ IIDN (0, σ^2). The means were compared using Tukey's test. The relationships between EPG, PCV and BCS between breeds and segregation categories (susceptible, intermediate and resistant) were evaluated using Spearman correlations.

Results

Katahdin ewes showed the highest EPG and lowest PCV values compared to Blackbelly and Pelibuey ewes ($P < 0.01$). Pelibuey ewes showed a higher PCV ($P < 0.05$) than Blackbelly but had a similar EPG (Table 1).

Around half (50.7%) of the Katahdin ewes excreted more than 1000 EPG, whereas 76% of the Blackbelly ewes excreted less than 501 EPG (Fig. 1). Notably, 76.3% of the Pelibuey ewes excreted 0 EPG.

Table 1 Mean ± standard deviation, confidence interval 95%, median and rank of eggs per gram of faeces of GIN and percentage of package cell volume in Pelibuey, Blackbelly and Katahdin ewes infected during grazing in the hot humid tropics of Mexico

Variables	*N*	Mean ± SD		Confidence interval 95%	Median	Rank	
						Minimun	Maximun
EPG							
Pelibuey	59	56.8 ± 187	b	8–106	0	0	1300
Blackbelly	69	576.1 ± 1009	b	334–818	150	0	4950
Katahdin	73	3613.6 ± 5649	a	2226–4862	1050	0	24,100
PCV							
Pelibuey	59	30.6 ± 4.7	a	29.4–31.8	30.5	21.9	43.1
Blackbelly	69	23.5 ± 5.1	b	22.2–24.7	23.2	12.8	39.5
Katahdin	68	21.7 ± 5.53	c	20.4–23.0	21.3	12.6	39.6

Different letter in same column means statistically differences $P < 0.05$

Table 2 Pearson correlation between EPG and PCV in Pelibuey, Blackbelly and Katahdin ewes segregated as resistant, intermediate and susceptible to GIN infections grazing in hot humid tropics of Mexico

Race	Correlation coefficient	Category	Correlation coefficient
Pelibuey	0.01^{ns}	Susceptible	− 0.61**
Blackbelly	− 0.48**	Intermediate	− 0.49**
Katahdin	− 0.57**	Resistant	− 0.36*

*$P < 0.05$, **$P < 0.01$

The relationship between the EPG and PCV for the different breeds and resistance categories is shown in Table 2. The EPG negatively affected the PCV, resulting in a 48% reduction in the Blackbelly breed and a 57% reduction in the Katahdin breed. No relationship was found between EPG and PCV in the Pelibuey breed. In relation to the resistance categories, a negative correlation was found between the EPG and the level of resistance.

The EPGs of the different breeds per resistance category are shown in Fig. 2. Katahdin ewes presented the highest EPGs in all resistance categories compared to Pelibuey and Blackbelly ewes ($P < 0.05$). In relation to the PCV, Pelibuey ewes maintained high values independently of the resistance category. In contrast, Blackbelly and Katahdin sheep showed decreasing values associated with susceptibility (Fig. 3).

A negative relationship was found between BCS and EPG in Katahdin sheep in relation to the resistance category.

The coprocultures showed a high prevalence of *Haemonchus contortus* larvae (68.9%), followed by *Trichostrongylus colubriformis* (30.9%) and *Cooperia curticei* (0.6%) larvae. The species were determined by genotyping using PCR and by morphological traits (Fig. 4).

Discussion

The present study is the first in the humid tropics of Mexico comparing the phenotypic resistance of two hair sheep breeds (Pelibuey and Blackbelly) and a composite breed (Katahdin) to GIN infection under grazing conditions.

Pelibuey and Blackbelly ewes showed greater resistance to GINs compared to Katahdin sheep, as evidenced by differences in EPG ($P < 0.05$) between breeds. Blackbelly ewes showed a similar EPG as Pelibuey ewes and a lower EPG than Katahdin ewes. Previously, Blackbelly sheep showed greater resistance to GIN than wool sheep breeds (Yazwinski et al. 1979) and composite breeds (Vanimisetti et al. 2004) under artificial infection with *H. contortus*. Similar results were reported with respect to other French sheep breeds such as INRA 401 (Gruner et al. 2003). For many years, Blackbelly sheep were exploited because of their prolific potential. However, the increasing demand for sheep meat has shifted attention to more efficient sheep breeds (Muñoz-Osorio et al. 2016). The results of the present study suggest that the inclusion of Blackbelly sheep in the breeding schemes of sheep production systems under grazing conditions should be reconsidered because of their resistance to GIN infection.

Notably, Katahdin ewes showed lower resistance to GIN infection in terms of EPG than Blackbelly and Pelibuey ewes despite their intermediate BCS. Compared to the wool breeds of Hampshire (Burke and Miller 2002) and Dorset (Vanimisetti et al. 2004), Katahdin sheep are considered to have intermediate resistance to GIN infection, but, are still less resistant than hair sheep breeds (Ngere et al. 2018). Another study found similar results to those of the present study wherein Katahdin ewes showed higher EPG than Pelibuey ewes or lambs (Palomo-Couoh et al. 2016, 2017). However, the EPG has an inheritance index of only 0.26 to 0.38 (Bishop et al. 2004), indicating that environmental

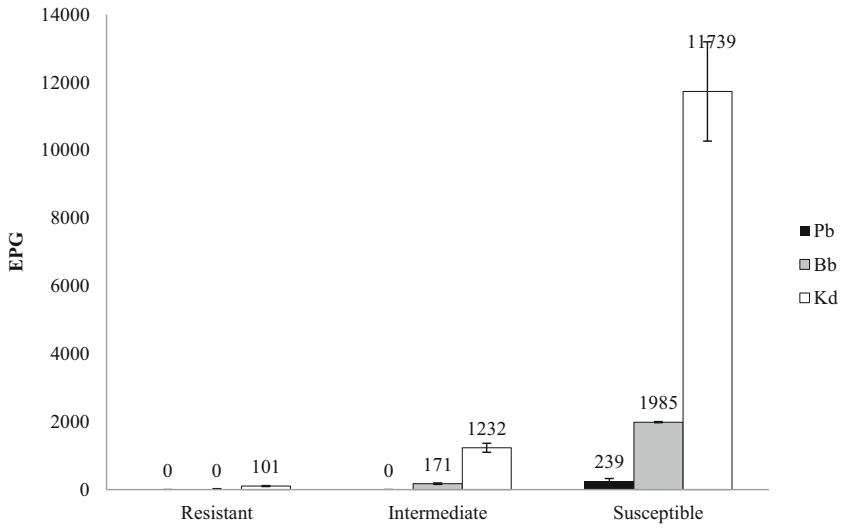

Fig. 2 Egg count per gram of faeces (EPG) of GIN in Pelibuey (Pb), Blackbelly (Bb) and Katahdin (Kd) ewes segregated as resistant, intermediate and susceptible to gastrointestinal nematode infection under grazing condition in the hot humid tropics of Mexico

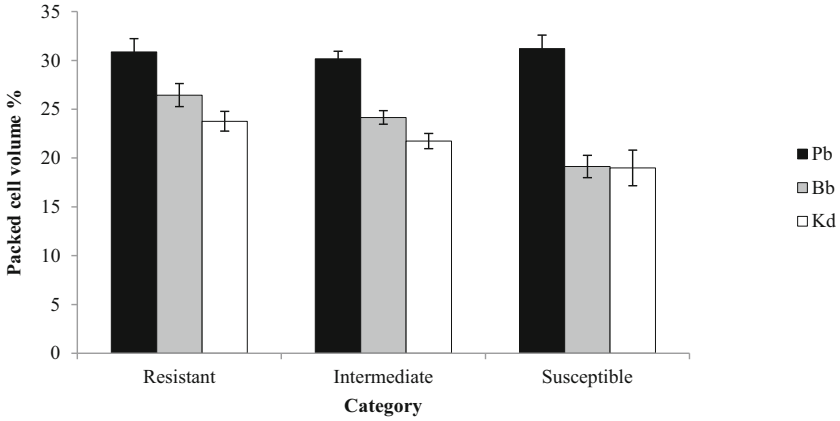

Fig. 3 Percentage of packed cell volume of Pelibuey (Pb), Blackbelly (Bb) and Katahdin (Kd) ewes segregated as resistant, intermediate and susceptible to infection with gastrointestinal nematodes under grazing condition of the hot humid tropics of Mexico

factors play an important role in determining the parasitic gradient, which can ultimately be considered a function of climatic conditions, animal management and animal breed (Morgan and Van Dijk 2012). The susceptibility of breeds to GIN infection also increases as the level of infection increases (Besier et al. 2016). For example, humidity and temperature were previously related to GIN larval abundance in the grass in the tropics and may hence influence the level of infection (Molento et al. 2016). The climatic and environmental conditions of Tabasco likely promote increased GIN infection levels in pastures grazed by ewes.

Finally, the BCS is a practical measure indicating the energy reserves of an animal but has also previously been used as an indicator of the intensity of GIN infection (Idika et al. 2012;

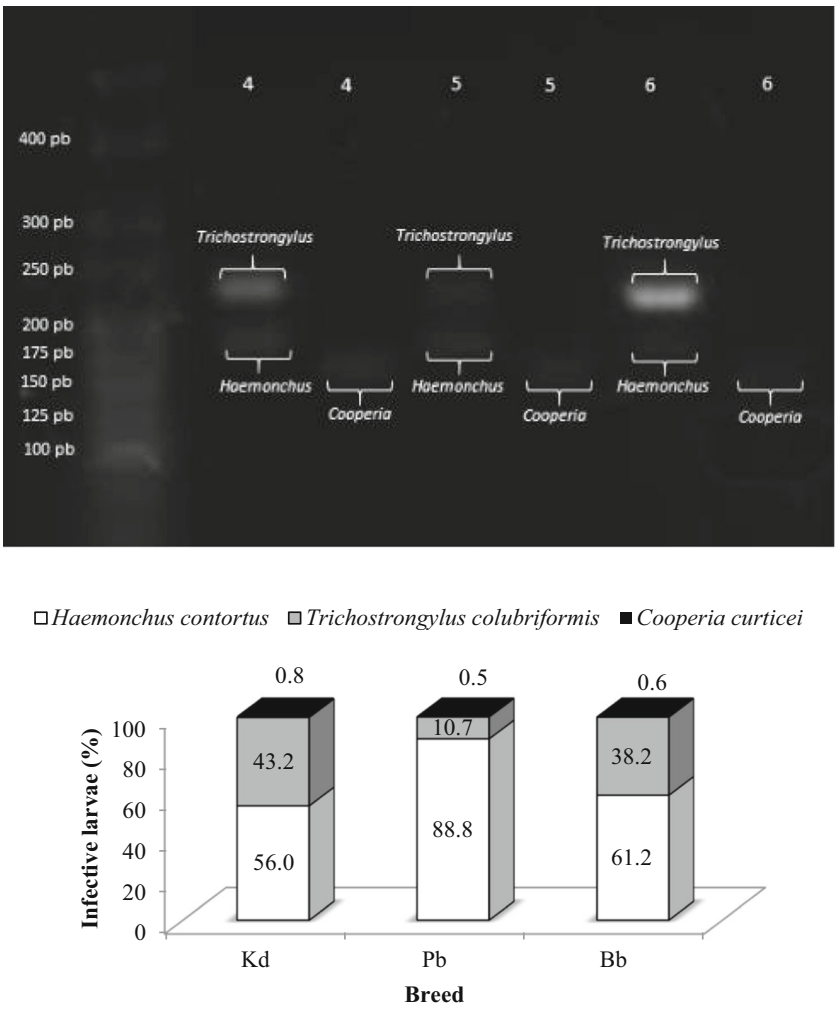

Fig. 4 Infective larvae of gastrointestinal nematodes in hair sheep (4. Katahdin (Kd), 5. Pelibuey (Pb), 6. Blackbelly (Bb)) assessed with genotyping by PCR and morphological identification in faeces of ewes grazing in the humid tropic of Mexico

Soto-Barrientos et al. 2018) and has been shown to exert a decisive influence on EPG count (Cornelius et al. 2014). Higher BCS is associated with a greater immune response against GIN (Greer and Hamie 2016). Similarly, in the present study, the Pelibuey ewes presented the best BCS and the lowest EPG counts. Meanwhile, the Katahdin ewes showed high variation in BCS; thinner animals (BCS 1) had a higher EPG compared to those with a BCS of 3 or 4. Also, the Blackbelly ewes with a BCS of 3 showed a lower EPG compared to thinner animals. It appears that feeding and flock management influenced the phenotypic expression of GIN in Pelibuey ewes. These latter ewes received supplementation and had a comparatively high BCS, which probably induced low EPG. This could explain the lack of a correlation between the PCV and EPG ($r = 0.01$) in this breed. In contrast, in the Katahdin and Blackbelly ewes, the PCV and EPG were highly and negatively related (Reynecke et al. 2011) because of the presence of the hematophagous nematode *H. contortus*, which decreased the PCV. In these latter breeds, the correlation coefficient between EPG and PCV was dependent on the resistance category. Susceptible ewes also showed a higher correlation between EPG and PCV. It is possible that the selection of resistant animals can decrease the risk of death by anaemia due to *H. contortus* (Lôbo et al. 2009).

Conclusions

Katahdin ewes showed higher susceptibility to GIN infections than Blackbelly and Pelibuey ewes. Pelibuey ewes showed the highest resistance to GIN infections under hot, humid tropical conditions. A relationship was found between EPG, BCS and PCV. Hence, PCV could be used as an indicator for alternative methods for diagnosing the impact of nematodes on animal health. Finally, the results of the present study suggest that the inclusion of Blackbelly sheep in sheep production breeding schemes under grazing conditions should be reconsidered because of their resistance to GIN infections.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Statement on the welfare of animals The Bioethics Committee of the Campus of Biological and Agricultural Sciences of the Autonomous University of Yucatan, Mexico, approved the development of the present study under authorisation number CB-CCBA-D-2017-001.

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