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COMPARATIVE RESPONSE OF IgA AND IgG ACTIVITY AND HEMATOLOGICAL PARAMETERS AMONG FOUR MAIN BEEF-CATTLE BREEDS INFECTED WITH GASTROINTESTINAL NEMATODES IN THE WARM HUMID TROPIC OF MEXICO*

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Abstract

This study determined the immunoglobulin (A and G) activity against gastrointestinal nematodes (GIN) and also the hematological parameters in four beef-calf breeds naturally infected in a tropical region of Mexico. Thirty-six infected calves were used to determine the fecal nematode egg counts (FEC), the IgA and IgG activity in serum and saliva, the packed cell volume (PCV), the plasmatic protein (PP) and the differential leukocyte counts. These parameters were measured for a five-month period. ELISA assay was performed using adult worm crude antigen (AWCA) from *Haemonchus contortus*, *Trichostrongylus colubriformis*, *Mecistocirrus digitatus* and *Cooperia punctata*. The variables were analyzed using a fixed model according to the breed group. The FEC records (means ± standard deviation) for the different breeds were: Brangus (122±115), Charolais (391±507), Guzerat (294±326) and Brown Swiss (413±395). No statistical differences (P>0.05) were found among breeds. Cooperia and *Haemonchus* were the main identified genera in the coprocultures. Differences in hematological parameters were observed among breeds (P<0.01); Guzerat showed the highest PCV (42.6±4.7%). The lowest leukocyte counts were observed in the Charolais breed (9.2±2.4 × 10^9 cells L^{-1}). Differences between Guzerat and Charolais were only detected in

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eosinophils, neutrophils and lymphocytes. Increased activity of IgA was observed against *M. digitatus* (21% OD) compared with the response against *C. punctata* in serum samples (13% OD). The lowest IgA activity in serum and saliva was found in Guzerat calves. Charolais and Brown Swiss showed the highest IgA activity in serum and the highest value in saliva was found in Brangus.

Key words: *Cooperia punctata*, *Mecistocirrus digitatus*, eosinophils, immunoglobulins, peripheral immunity, saliva

In warm and humid climates of tropical regions of the world, gastrointestinal parasitic nematodes (GIN) are considered one of the main health problems affecting cattle, particularly under grazing systems (Cardoso et al., 2013). Parasitic diseases affect the productive potential of cattle, increasing maintenance costs (Rinaldi and Geldhof, 2012; Oliveira et al., 2013), especially when anthelmintic resistance has been reported due to the intensive use of anthelmintic drugs in GIN control (Gasbarre, 2014). The widespread usage of macrocyclic lactones has resulted in the selection of drug resistant helminth parasites (Stromberg et al., 2012) and the integrated parasite control system has been proposed as a crucial strategy for preserving animal health and maximizing their productivity (Sutherland and Leathwick, 2011). The combined use of different parasite control measures can lead to reduce the use of chemical anthelmintic drugs in cattle. This system allows preserving the animal welfare, minimizing a possible environmental damage and eventually increasing the farmer's income (Pisseri et al., 2013). In cattle production farms under a regular grazing system, animals can develop natural genetic resistance against GIN infections. Over time, if susceptible animals with higher fecal nematode egg counts (FECs) are culled from herds, pasture contamination could decrease and as a consequence overall GIN infection should also decrease (Pisseri et al., 2013). Genotype selection of animals with natural resistance represents an option to counteract the effects of gastrointestinal nematodes (Hou et al., 2012).

Several indicator traits should be considered to identify the presence of genetic resistance against GIN in cattle. For instance, peripheral eosinophils, IgA, IgG and IgM antibodies are useful parameters to assess animal immune response against GIN (Bishop, 2012) and these values can be correlated with the fecal nematode egg counts. IgA activity can be detected in fluids like plasma and saliva, which is a potentially valuable tool in diagnosis and control (Prada-Jiménez et al., 2014). However, in purebreds maintained under warm weather conditions, parasitism levels and immune response against gastrointestinal nematodes are currently unknown. The cross between European and Zebu genotype has shown to improve production parameters in cattle in the tropics; although, little information is known about immune response against gastrointestinal nematodes in cross breeds (Oliveira et al., 2009). In the present study we hypothesize that the Zebu breeds show some degree of resistance against GIN expressed in some immunological and hematological parameters. The objective of the present study was to identify differences in immunological, hematological and parasitic parameters in infected and re-infected calves by gastrointestinal nematodes in four cattle breeds in a warm and humid climate.

Material and methods

Location of experimental area

This research was conducted at the "San Fernando" farm located in the Candelaria municipality, Campeche State, Mexico at 17° 49' 00" N and 90° 14' 00 W. The climate of the region is classified as Aw on the Köeppen scale (Kottek et al., 2006), and defined as an equatorial savannah with dry winter, with 26.6°C average temperature and with 1360 mm annual rainfall (CONAGUA, 2014).

Animals

Thirty-six three-month-old calves were randomly distributed into four groups of nine animals each as follows: Guzerat (*Bos indicus*, n=9), Charolais (*Bos taurus,* n=9), Brown Swiss (*Bos taurus,* n=9), and also Brangus (crossbred originated from Brahman and Angus; *B. taurus* × *B. indicus,* n=9).

Animals of each breed were kept in different paddocks under a continuous grazing system on *Brachiaria brizanta* and *B. humidicola* grass over a five-month period (December 2013 – March 2014).

A prevention and control program against *Clostridium* spp. through vaccination was implemented in 3-month-old calves.

The experiment was divided into two stages. In the first stage, blood and feces samples were taken once a month during December, January and February in order to determine the immunological and copro-parasitological parameters among the animals prior to an anthelmintic treatment. The second stage comprised samplings at 15 and 45 days post treatment (dpt). This stage was established to determine the antibody changes after re-infection in each breed.

Anthelmintic efficacy assessment

The efficacy of the administered drugs was determined with the fecal egg count reduction test (FECRT) using the following formula (1 – mean treated group/mean control group *100). Two groups of ten calves each were formed: Brangus and Brown Swiss calves (Group 1) and Charolais and Guzerat calves (Group 2). Additionally, 16 calves (mixed breeds) were considered as a control group (Group 3) without any anthelmintic treatment. The anthelmintic efficacy was evaluated according to the WAVVP recommendations (Coles et al., 1992). Group 1 was treated with Albendazole (BZ, Desparazole, Wellco Corporation, 5.8 mg kg^{-1}) by oral route and Group 2 was subcutaneously treated with IVM (Endectin, Novartis, 0.2 mg kg^{-1}) in the neck next to the left shoulder.

Blood samples collection and analysis

The experiment was conducted to determine the antibody level changes before and after treatment to know antigenic response in the different breeds. Nine calves were used per breed (n=9). Jugular blood samples were taken once a month and collected into 5 mL EDTA-vacutainers to perform hematological assays. Additional samples were taken to recover serum for later antibody analysis. The hematological parameters analyzed were: the packed cell volume (% PCV) by the micro-hematocrit

method; plasmatic protein concentration (PP, g dL^{-1}) using a refractometer (Atago, China), and leukocytes counts (cells/L of blood) using a hemocytometer (Improved Neubauer Bright Line, USA). In addition, thin blood smears were carried out and stained with a commercial staining kit (Hemocolorante rápido, Hycel SA de CV, Mexico). The first 100 leukocytes were counted and classified morphologically as neutrophils, eosinophils, basophils, monocytes and lymphocytes and the percentage of each one was calculated.

Saliva samples collection

Saliva samples were collected using cotton swabs (Protec, DeGasa, Mexico) according to Shaw et al. (2012). The swab was clasped in long-nose surgical forceps, close to the teeth for 10 s. The collected swab was then placed into 15-ml eppendorf tubes and centrifuged for 5 min at 400 × *g*. The extracted saliva was diluted at 1/10 in sample dilution buffer (10 mM phosphate buffer and 0.25 M NaCl, pH 7.2 containing 1.0% Tween 20 and 0.02% sodium azide), and then stored at –20°C until use.

Fecal egg count

The fecal samples were directly collected from the rectum of each animal every 28–31 days, always in the morning. The EPG were determined by the McMaster technique (Thientpont et al., 1986) with sensitivity of 50 eggs per gram. Cultures were prepared with the feces of each breed and sampling date, according to the technique of Curticelli (Thientpont et al., 1986), for determining the prevalent nematode genera (Van Wyk and Mayhew, 2013).

Antigen preparation

Adult worm crude antigen (AWCA) was prepared from *M. digitatus* and *C. punctata* worms that were collected from the abomasum and intestine of calves, respectively. *Haemonchus contortus* and *T. colubriformis* from sheep were also used as antigens (Ag). All these species have been recorded as very common in the region (von Son-de Fernex et al., 2014; González-Garduño et al., 2013).

Adult nematodes were washed twice in PBS pH 7.2/0.01% sodium azide and extracted by grinding using a mortar with 10% 1 mM phenylmethylsulphonyl fluoride (PMSF, Sigma-Aldrich, St Louis Missouri, USA) and kept on ice. The pellet was homogenized overnight with buffer and 2% (w/v) reduced Triton X-1060 (Aldrich). Samples were collected by centrifugation at 20,000 x *g* /20 min at 4°C. The supernatant, containing AWCA was collected and stored at –20°C (Bowdridge et al., 2013). The protein concentration was estimated by the Bradford method (Bradford, 1976) and confirmed by polyacrylamide gel electrophoresis (SDS-PAGE) (data not shown).

Immuno-enzymatic assay

Before the anthelmintic treatments, the ELISA assay was performed using saliva and sera samples with the four nematode antigens previously described, while in the second stage tests were conducted only for nematode antigens from cattle (*M. digitatus* and *C. punctata*) according to previously-obtained results.

Antigens were diluted at 2.5 μg mL^{-1} in buffer carbonate (pH 9.6) distributed in 96-well plates (NUNC MaxiSorb, Denmark) incubated overnight at 4°C. The wells were washed three times with PBST (pH 7.2 and 0.05% Tween 20). Non-specific binding sites were blocked by incubation at 37°C with 0.5% skimmed milk in PBST for 1 h. Next duplicate serum samples were diluted 1:100 and saliva at 1:20 and added to wells to determine the IgA and IgG activity. The samples were incubated at 37°C for 1 hour. After washing a horseradish peroxidase-conjugated rabbit anti-bovine IgA or IgG (Bethyl Laboratories, Montgomery, AL, USA) diluted at 1:5,000 in PBST incubated for 45 min at 37°C, was used. The tetramethyl benzidine (TMB, Sigma Aldrich, St Louis Missouri, USA) substrate was used as chromogen solution for 15 min of incubation at room temperature (RT) and stopped with 1M H_2SO_4. The optical densities (OD) were determined with a microplate absorbance reader (Imark, Bio-Rad) by measuring the absorbance at 450 nm for IgA or IgG. In order to compare results between assays, a negative control containing IgA and IgG antibodies against nematodes was included in each plate. Subsequently, blank absorbance was subtracted from all samples to correct for non-specific binding (Ramírez-Restrepo et al., 2010).

A positive control serum for the nematode species was obtained from calves with the highest immunoglobulin activity. Results are expressed as the percentage of the OD of the positive control serum and employing the following formula:% OD = [(OD mean of the tested serum – OD negative control serum) / (OD mean of the positive control serum – OD mean negative control serum)] × 100 (Cardoso et al., 2013).

Statistical analysis

Data corresponding to the EPG were log10 (EPG + 1) transformed to reduce the variance (Williams et al., 2010). The variables PCV, peripheral leukocytes percentages, and ELISA activity were separately analyzed for each stage. After anthelmintic treatment a factorial design was made to maximize the degrees of freedom for each main effect according to the following model:

$$Y_{ijk} = \mu + \tau_i + \zeta_j + \tau^*\zeta_{ij} + \varepsilon_{ijk}$$

Y_{ijk} = is the response variable,
μ = overall mean,
τ_i = effect of the treatment (anthelmintic treated and non-treated),
ζ_j = effect of the breed (Charolais, Brangus, Brown Swiss, Guzerat),
$\tau^*\zeta_{ij}$ = interaction of treatment and breed,
ε_{ijk} = residual error.

In the first stage the sample size was nine calves per breed without analyzing the effect of treatments. The analyses were carried out using the SAS statistical package version 9.2., with the GLM procedure (SAS, 2008). Pearson correlation analysis was used to ascertain the relationship between indicators of immunity. The differences among treatments were detected using the Tukey test.

Results

Parasitological parameters and fecal egg reduction test (FECRT)

During the first stage in the initial infection of calves, the FECs were similar among the four beef-cattle breeds sampled ($P>0.05$). The general means and standard deviation were: in Brangus, 122±115; Charolais, 391±507; Guzerat, 294±326; and Brown Swiss calves, 413±395. *Cooperia* and *Haemonchus* were the predominant genera found in coprocultures with 51.6% and 32.6%; respectively, followed by *Oesophagostomum* (13.9%) and *Strongyloides* (1.9%).

In the FECRT the general means and standard deviation before treatment (0 day) and 15 days post-treatment, respectively, were: in Group 1 Albendazole, 175±300 and 0±0 EPG; Group 2 IVM, 206±309 and 174±336 EPG; Group 3 control, 216±237 and 268±342 EPG. With these results Albendazole showed 100% efficacy and IVM reduced the EPG count only in 35% with regards to the control group. At 45 dpt the FECs (126±174 EPG) were similar in all breeds due to the natural re-infection rate. Nematode infective larvae in coprocultures revealed the presence of *Cooperia* after anthelmintic treatment.

Hematological parameters

Differences in PCV were only observed in the Charolais breed pre- (32%) and post-treatment (28%). The highest PCV was found in the Guzerat calves, before and after the anthelmintic treatment (42.6±4.7% and 43.3±3.9%, respectively). A lower number of leukocytes was observed in the Charolais breed (9.2±2.4 and 8.1±1.6 × 10^9 cells L^{-1}, respectively) with significant differences from Brangus and Guzerat breeds. High variation in eosinophils, neutrophils and lymphocytes was observed; for such reason differences only between Guzerat and Charolais were detected (Table 1). The hematological values did not change between breeds by the anthelmintic effect (second stage) and are globally presented in Table 1.

Immunoglobulin A (IgA)

In the evaluation of IgA in the first stage (four antigens), the highest IgA activity (% OD) corresponded to *M. digitatus* in serum and *M. digitatus* and *C. punctata* in saliva (Table 2). No response in IgA activity against *T. colubriformis* antigen in serum and saliva was observed in any breed.

The Brangus and Brown Swiss calves had higher IgA activity in serum and saliva than Guzerat and Charolais (Table 2). The Guzerat breed showed the lowest values in serum against *C. punctata* and *M. digitatus* (4.4 and 13.8% OD, respectively), while the major response was recorded against *H. contortus* antigen (24.9% OD). Brangus showed significantly higher activity of salivary IgA against *C. punctata* and *M. digitatus* when compared with other breeds (41.2 and 39.3% OD, respectively).

In the second stage regarding antigens from cattle, the Charolais calves showed high serum immune response (IgA) against both the *M. digitatus* (57% OD) and *C. punctata* antigens (68% OD). In saliva, the greatest response occurred in the Brangus breed against *M. digitatus* (30.5% OD). Once again, the response of the Guzerat calves was the lowest of all breeds (Figure 1).

Table 1. Hematological values in naturally infected calves of four breeds in Campeche, Mexico

Item	Breed							
	Brangus		Charolais		Guzerat		Brown Swiss	
	Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD	
Before anthelmintic treatment (First stage)								
PCV (%)	34.1 b	7.1	32.0 b	4.8	42.6 a	4.7	33.3 b	4.9
Plasmatic protein (g dl^{-1})	6.49 b	0.44	6.91a	0.31	6.53 b	0.36	6.64 ab	0.38
Leukocytes (× 10^9 L^{-1})	12.2 ab	2.6	9.2 c	2.4	13.5 a	4.3	11.0 bc	2.7
Basophils (%)	1.2 a	1.4	0.8 a	1.6	1.1 a	1.1	1.6 a	1.9
Neutrophils (%)	18.5 a	15.3	11.0 b	7.7	15.9 a	5.5	12.5 ab	13.1
Eosinophils (%)	3.9 a	4.2	1.6 b	2.0	3.8 a	6.9	1.8 ab	1.7
Monocytes (%)	0.8 a	0.8	1.0 a	1.0	1.1 a	1.3	1.0 a	0.9
Lymphocytes (%)	75.6 ab	20.1	85.7 b	25.2	77.9 a	26.4	83.0 ab	21.0
After anthelmintic treatment (Second stage)								
PCV (%)	35.6 b	7.5	28.6 c	4.1	43.3 a	3.9	32.8 b	3.9
Plasmatic protein (g dl^{-1})	6.9 a	0.4	6.2 b	0.5	6.4 b	0.3	6.7 a	0.7
Leukocytes (× 10^9 L^{-1})	11.6 a	2.1	8.1 b	1.6	10.6 a	2.9	11.3 a	3.0
Basophils (%)	2.3 a	1.4	3.8 a	2.5	3.2 a	1.8	2.4 a	1.9
Neutrophils (%)	16.6 a	11.2	12.8 b	9.3	18.0 a	6.9	16.6 a	13.6
Eosinophils (%)	2.0 ab	1.7	2.3 b	2.4	4.7 a	4.9	3.5 ab	3.6
Monocytes (%)	1.1 ab	1.0	0.9 b	0.9	1.1 ab	1.0	1.3 a	1.1
Lymphocytes (%)	77.9 a	17.8	80.2 b	17.0	73.1 a	23.0	76.2 a	17.7

PCV – Packed cell volume. Different letters by stage in each row are significant (P<0.01).

Table 2. Percentage of IgA optical density in serum and saliva against adult gastrointestinal nematodes crude antigens in four naturally infected breeds grazing in warm climate

Antigen	Guzerat		Brangus		Charolais		Brown Swiss		Mean
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Serum									
M. digitatus	13.8	3.2	21.3	8.0	23.7	4.1	26.1	6.3	21.3 a
C. punctata	4.4	1.9	11.4	2.8	15.6	2.9	19.1	4.8	12.6 b
H. contortus	24.9	7.0	17.2	6.0	7.5	2.0	22.1	4.0	17.9 c
T. colubriformis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0 d
Saliva									
M. digitatus	14.6	5.0	39.3	9.0	17.9	6.0	32.6	7.0	26.1 a
C. punctata	12.9	3.0	41.2	8.0	15.1	4.0	27.2	5.0	24.1 a
H. contortus	11.4	3.0	0.0	3.0	0.0	0.0	0.0	0.0	2.9 b
T. colubriformis	0.8	0.5	0.8	0.5	0.0	0.0	1.8	1.0	0.8 b

IgA activity (% OD) in respect to a positive control. Different letters in the last column are statistically different (P<0.01).

Immunoglobulin G (IgG)

In the Guzerat calves similar IgG activity in serum to both antigens was observed and little differences were observed when comparing all breeds (Figure 2). The IgG activity in saliva was too low in all breeds and not significant differences were detected (P>0.05); for such reason, those values were not presented.

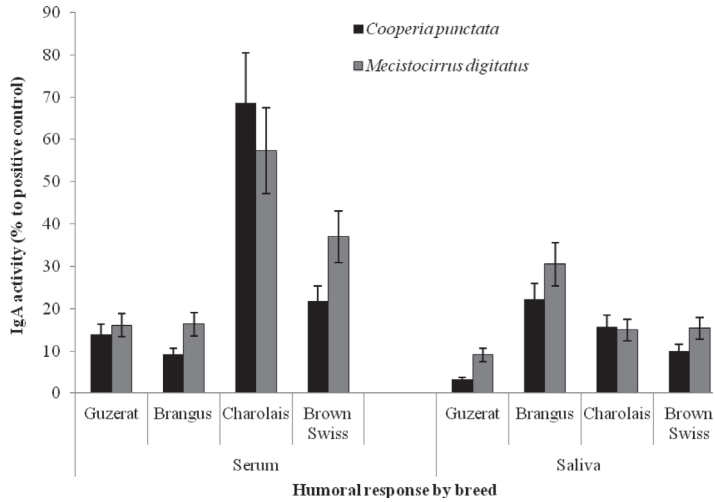

Figure 1. Mean of serum and saliva immunoglobulin A activity against adult worm crude antigen of *Cooperia punctata* or *Mecistocirrus digitatus* in four calf breeds naturally infected by gastrointestinal nematodes, shown as optical density (% OD) with regards to positive control. Means ± standard error

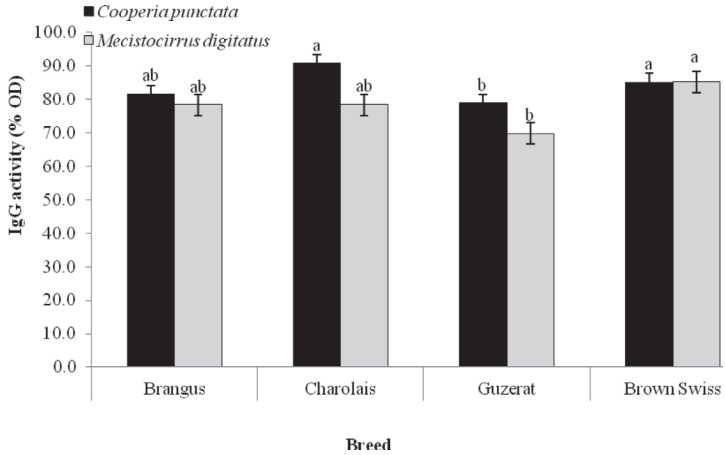

Figure 2. Mean of serum immunoglobulin G activity against adult worm crude antigen of *Cooperia punctata* or *Mecistocirrus digitatus* in four calf breeds naturally infected by gastrointestinal nematodes, shown as optical density (% OD) with regards to positive control. Means ± standard error

Correlations

Pearson correlations among the most important studied variables (PCV, PP, eosinophils and Ig´s) are described in Table 3. Many of the variable correlations were not significant due to the high variability among individuals. Correlations between eosinophils and IgA in serum and saliva were not significant except in Charolais and Guzerat breeds in serum (Table 3). A strong positive correlation between antigens (*C. punctata* and *M. digitatus*) was significant, but there was no relationship between IgA in serum and saliva.

Table 3. Correlation coefficients between immune parameters in four breeds

	General	Brown Swiss	Charolais	Brangus	Guzerat
Eosinophils with					
C. punctata IgA saliva	0.08ns	–0.24ns	–0.04ns	0.52ns	–0.16ns
M. digitatus IgA saliva	0.05ns	–0.19ns	0.04ns	0.55ns	–0.26ns
C. punctata IgA serum	0.13ns	0.39*	0.26ns	0.36ns	0.52ns
M. digitatus IgA serum	0.18ns	0.23ns	0.35*	0.41ns	0.87**
C. punctata IgA saliva with					
C. punctata IgA serum	0.14ns	0.28ns	0.01ns	–0.41ns	0.61ns
M. digitatus IgA serum	0.22ns	0.45*	0.12ns	–0.38ns	0.25ns
M. digitatus IgA saliva	0.93**	0.96**	0.95**	0.75**	0.98**
M. digitatus IgA saliva with					
C. punctata IgA serum	0.20ns	0.29ns	0.10ns	–0.48ns	0.53ns
M. digitatus IgA serum	0.27*	0.45*	0.20ns	–0.39ns	0.14ns
C. punctata IgA serum with					
M. digitatus IgA serum	0.89**	0.89**	0.93**	0.98**	0.87**
Packed cell vollume with					
Plasma protein	0.347*	0.591*	0.696**	0.678**	0.593**
Eosinophils	0.356ns	0.302ns	0.447*	0.581*	0.183ns
C. punctata IgA saliva	0.204ns	0.124ns	0.001ns	0.792*	0.213ns
M. digitatus IgA saliva	0.123ns	0.181ns	–0.065ns	0.438*	0.141ns

* Significant (P<0.05), ** Highly significant (P<0.01), ns – not significant (P>0.05).

Discussion

In spite of the numerical differences observed in the FECs among the four studied breeds, they were not statistically significant (P>0.05); this fact is explained by the high variation of intra-breed individual response in EPG, which was greater than the variation between breeds in a similar way to the results reported by Cardoso et al. (2013). This high variability in EPG has been indicated in other studies about genetic resistance (Morris, 2007) in which considerable animal-to-animal variation in the degree of susceptibility to GIN is reported, and also in the period of time over which infection persists in the animals. The high variation in the FECs also can be due to the abundance and oviposition rate produced by the nematode burden in the host. In cattle, *Haemonchus* and *Mecistocirrus* are the main GIN listed (50–60%) (Canul-Ku et al., 2012; Von Son-de Fernex et al., 2014) and also the most prolific genera that parasitize ruminants; in those cases counts tend to be very high. However, *Cooperia* has also been indicated as a very common genus (Bartley et al., 2012), which has a lower oviposition rate. Other less frequent genera but which also contribute to FECs are: *Trichostrongylus*, *Bunostomum* and *Oesophagostomum* (Idika et al., 2012). The abundance of each GIN species, and therefore the FEC, depends on the seasons, which clearly modulate the population dynamics (Peña et al., 2000; Quijada et al., 2006).

With respect to the hematological variables, the PCV in the Guzerat breed is remarkably high (42.6±4.7%), being higher than the other breeds in the present study, and even higher than the PCV indicated for this breed in another (Alves-Júnior et al., 2009), but the value was comparable to that reported in Nelore (41.5±0.65%) in Brazil (Oliveira et al., 2013). The opposite case occurred with the lowest values observed in Charolais, but the results were consistent with those obtained in purebreds of the same breed (Umpapol et al., 2014), and also with the % PCV of crosses of this breed and Limousine in Ireland (Lejeune et al., 2010). The % PCV obtained in Brangus and Brown Swiss are in the ranges indicated in several breeds in Africa (Van Wyk et al., 2013). The lower PCV obtained in the Charolais breed in the second stage cannot be indicated as a result of GIN infection, because the FECs were similar among breeds, but this reduction of PCV was most likely associated with the presence of ticks, which were noted only in this breed, but they were not accurately evaluated.

Hematological values have been used to explain differences in adaptation between the Zebu and European breeds to warm environment (Macêdo et al., 2013), which affect the cattle physiology and productivity. In the tropics it has been reported that the Zebu breeds have a higher hematocrit value than the European genotypes suggesting that the Guzerat breed has a high hematocrit because it is a breed adapted to the tropical environmental conditions (Rosse et al., 2017). It is possible that the high hematocrit value could be responsible for the better productive performance of the Guzerat breed compared to the Creole breed in the Mexican tropics (Martínez-Velázquez et al., 2006).

The hematocrit reflects the concentration of erythrocytes but not the total mass of these (Barrios et al., 2011). Therefore, additional studies are required to evaluate other parameters that consider the hemoglobin concentration and its relationship with the hematocrit.

Studies on peripheral immunity show very little effect of the production system (indoor or outdoor) and greater immunological differences originated between seasons of the year, especially related to nutritional aspects (Lejeune et al., 2010), as well as by age (Brun-Hansen et al., 2006) and the physiological state (Sattar and Mirza, 2009). However, few studies have been done to know the hematological and immunological levels among breeds in a warm weather in Mexico, so the observed results are important because in our study we found hematological differences among breeds in % PCV, leukocytes, neutrophils, eosinophils and lymphocytes. The mean count of eosinophils and neutrophils was higher in the Guzerat and Brangus breeds, which are considered as breeds adapted to the tropics and with some degree of resistance to parasites, as indicated in Brazil (Bricarello et al., 2007). Possibly these breeds have the best innate immune response, which is mediated primarily by neutrophils and eosinophils and is the first line of defense, reducing the initial number of pathogens and improving the acquired immune response. Production of eosinophils is observed in response to primary infections by helminths, possibly providing protection for the host (Macêdo et al., 2013).

In the humoral immune response, the low IgA activity against *T. colubriformis* Antigen (Ag) in both serum and saliva is an indicator that infections with *T. colu-*

briformis do not seem to be important in the area of study, and animals have not developed immunity against this species, which coincided with the low frequency of this species (4%) found in the copro-cultures of cattle in tropical areas from México (Canul et al., 2011) and also in Brazil where the prevalence was lower than 1% (Lima, 1998).

The IgA low activity in saliva against *H. contortus* could be due to the fact that the antigen for this nematode species was obtained from sheep, the same situation occurred in the response of IgG, which did not show activity against AWCA of sheep (*H. contortus* and *T. colubriformis*) since the activity was similar between controls and naturally infected calves.

Cross-reactivity between *M. digitatus* and *Haemonchus* AWCA was observed in the Guzerat calves because in the study, only *Haemonchus* was determined in coprocultures, and the immune reaction was higher for *M. digitatus*. As indicated in sheep, cross-antigenicity among trichostrongyloidea could be found (Cuquerella et al., 1994). With IgG differences were detected between the Guzerat and Brown Swiss against *M. digitatus* Ag. The IgG results in this study behaved similarly to the results of another study, in which serum IgG activity was relatively high in the breeds by the end of the trial (Cardoso et al., 2013).

Largest differences were found in the IgA activity between breeds in saliva. The Guzerat breed had the lowest values, while the Brangus and Brown Swiss breeds showed the highest values against both *M. digitatus* and *C. punctata* in contrast with another study in which there were no differences in the mean activity of immunoglobulin (G and A) against *C. punctata* and *H. placei* antigens in cattle (Cardoso et al., 2013). The results obtained in this study are important because the evaluation of IgA activity against AWCA in serum and saliva by indirect ELISA can determine immunological differences intra- and between breeds due to the presence of antigens in parasitic nematode species of cattle (*C. punctata* and *M. digitatus*); however, the levels of nematode egg excretion were similar in all breeds.

The low activity of IgA in serum in the Guzerat calves, could be explained by the observation made by other authors that little IgA appears in the plasma during infection because mucosal IgA will bind to nematodes and excess IgA will move into the lymph and subsequently appear in the plasma (Prada-Jiménez et al., 2014; Stear et al., 2003). Low plasma IgA values occur in animals with a low intensity infection or a heavily infected animal in which most of the mucosal IgA is bound to parasite antigens. High plasma IgA means that the response to infection is strong (Prada-Jiménez et al., 2014).

Although the Guzerat breed had lowest levels of IgA, the cellular response had opposite effect, since the number of eosinophils was higher in Guzerat than in the other breeds, so that the humoral immune response could be complementary to the cellular immune response and therefore the expulsion of nematode eggs found was similar to all other breeds. But to determine the differences in immunoglobulin production, more accurate studies are required in which monospecific infections would be considered, because under natural infections is not possible to distinguish whether the immune response is due to one or two species of GIN and the possible interactions between them with the humoral and cellular response.

In sheep a negative correlation between the Ab and FEC was indicated (r=−0.570) as well as a positive association between Ab and EOS (r=0.506) because of GIN infection (Figueroa-Castillo et al., 2011). However, the correlations observed between eosinophils and FECs in the calves of the four breeds were not significant, but the correlation with IgA in serum coincides with that observed in sheep. In cattle apparently the effects of gastrointestinal nematodes are not as strong and only serum IgA activity is moderately correlated with IgA responses in the abomasal mucus (Sinski et al., 1995).

The correlation of the PCV was high only with the PP, but there was a positive correlation with the IgA in the Brangus breed. This response was possibly due to the small sample size and the few changes occurred in the PCV during the study period. With those samples it was not possible to determine any relationship with IgA and IgG by effect of nematode infection. No differences in faecal excretion of gastrointestinal nematodes eggs occurred in the calves of the four beef-cattle breeds, but less IgA activity against both *M. digitatus* and *C. punctata* antigens was observed in Guzerat with regards to the other breeds; however, the highest activity with respect to the *H. contortus* antigen was found in the Guzerat calves. In addition, the neutrophils and eosinophils counts were higher in Guzerat compared with the Charolais calves. The highest packed cell volume occurred in Guzerat breed.

High correlations between *M. digitatus* and *C. punctata* crude antigens were found; this indicates that crude antigens detected by ELISA are highly conserved and for such reason in the diagnostic test could be used for general nematode diagnosis.

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