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Immune and haematological parameters of Blackbelly ewes infected with gastrointestinal nematodes¤

Parámetros inmunológicos y hematológicos en ovejas Blackbelly infectadas con nematodos gastrointestinales

Parâmetros imunes e hematológicos de ovelhas Blackbelly infectadas por nematoides gastrintestinais

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Abstract

Background: It is necessary to identify phenotypic traits related to natural resistance against gastrointestinal nematodes (GIN) in order to know the host immunity status in productive ewes. **Objetive:** To determine haematological and immunological parameters (IgA and IgG) during pregnancy and lactation in Blackbelly ewes naturally infected with GIN. **Methods:** The number of eggs per gram (EPG), packed cell volume (% PCV), plasmatic protein (PP), and peripheral eosinophils were determined during eight months. In addition, sera and saliva samples were collected to establish IgG and IgA kinetics by indirect enzyme-linked immunosorbent assay (ELISA). **Results:** The results showed 2,592 ± 2,403 EPG and 22.2 ± 4.0% PCV during lactation and 595 ± 901 EPG and 25.1 ± 2.5% PCV during pregnancy. A higher percentage of *Trichostrongylus colubriformis* larvae were observed in pregnancy (84 to100%) than in lactation (36 to 44%). The IgA activity in serum samples showed a marked reduction (from 80 to 10%) during lambing for both *Haemonchus contortus* and *T. colubriformis* antigens. In saliva samples, IgA activity with regard to the standard decreased from 56% at 60

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days to 30% at 45 days before lambing and remained low for 45 days during lactation (23 to 32% activity). The eosinophils numbers were 2.0 x 10^9 cells L^{-1} in pregnancy and remained low at 0.7 x 10^9 cells L^{-1} in lactation. **Conclusion:** The studied variables reflect the breakdown of immunity against GIN in Blackbelly ewes before and after lambing.

Keywords: *antigens, Haemonchus contortus, immunity*, *parasites, sheep, Trichostrongylus colubriformis.*

Resumen

Antecedentes: La identificación del fenotipo relacionado con la resistencia contra nematodos gastrointestinales (GIN) es necesaria para conocer la inmunidad del huésped en ovejas en producción. **Objetivo:** Determinar los parámetros hematológicos e inmunológicos (IgA e IgG) en gestación y lactancia en ovejas Blackbelly infectadas naturalmente con GIN. **Métodos:** Se determinó el número de huevos por gramo de heces (EPG), se identificaron las larvas y se registró el porcentaje del volumen celular aglomerado (% PCV), proteína plasmática (PP) y eosinófilos periféricos durante ocho meses. Además, se colectó suero y saliva para determinar la cinética de IgG e IgA por medio de un ensayo inmuno-enzimático (ELISA) indirecto. **Resultados:** Los resultados mostraron 2.592 ± 2.403 EPG y 22,2 ± 4,0% PCV durante la lactancia y 595 ± 901 EPG y 25,1 ± 2,5% PCV durante la gestación. Se observó un mayor porcentaje de larvas de *Trichostrongylus colubriformis* en gestación (84 a 100%) que en lactancia (36 a 44%). La actividad de la IgA en las muestras de suero mostró una marcada reducción después del parto para ambos antígenos de *Haemonchus contortus* y *T. colubriformis* (80 a 10%). En saliva, la actividad de la IgA disminuyó de 56 a 30% del día 60 al 45 antes del parto y se mantuvo baja en los primeros 45 días de la lactancia (actividad de 23 a 32%). El número de eosinófilos fue de 2,0 x 10^9 células L^{-1} durante la gestación, y se redujo a 0,7 x 10^9 células L^{-1} en la lactancia. **Conclusión:** Las variables estudiadas reflejan la ruptura de la inmunidad contra GIN en ovejas Blackbelly antes y después del parto.

Palabras clave: *antígenos, Haemonchus contortus, inmunidad, ovinos, parásitos, Trichostrongylus colubriformis.*

Resumo

Antecedentes: A identificação de traços fenotípicos relacionados à resistência natural contra nematóides gastrintestinais (GIN) é necessária para saber a imunidade do hospedeiro em ovelhas produtivas. **Objetivo:** Determinar parâmetros hematológicos e imunológicos (IgA e IgG) em períodos de gestação e lactação em ovelhas Blackbelly naturalmente infectados com GIN. **Métodos:** O número de ovos por grama (EPG), volume empacotado de células (% PCV), proteína plasmática (PP) e eosinófilos periféricos foram determinados durante oito meses. Além disso, as amostras de soro e saliva foram recolhidas para determinar a cinética de IgG e IgA por ELISA indireto. **Resultados:** Os resultados mostraram 2.592 ± 2.403 EPG e 22,2 ± 4,0% PCV durante a lactação e 595 ± 901 EPG e 25,1 ± 2,5% PCV durante a gravidez. A percentagem mais elevada de larvas de *Trichostrongylus colubriformis* foi observada na gravidez (84 a 100%) do que na lactação (36 a 44%). A atividade de IgA em amostras de soro mostrou uma redução acentuada (80 a 10%) durante o parto nos antígenos de *Haemonchus contortus* e *T. colubriformis*. Em amostras de saliva, a atividade de IgA diminuiu de 56 a 30% do dia 60 ao 45 antes do parto e permaneceu baixa por 45 dias durante a lactação (atividade de 23 a 32%). O número de eosinófilos foi de 2,0 x 10^9 células L^{-1} na gravidez e manteve-se baixo, com 0,7 x 10^9 células L^{-1} na lactação. **Conclusaõ:** As variáveis estudadas refletem a quebra da imunidade contra GIN em ovelhas Blackbelly antes e depois do parto.

Palavras chave: *antigénios, Haemonchus contortus, imunidade, ovinos, parasitas, Trichostrongylus colubriformis.*

Introduction

Tropical climate conditions have a strong influence on the high prevalence of gastro-intestinal nematodes (GIN), which are highly pathogenic parasites in grazing sheep. Some nematode species such as *Haemonchus contortus* and *Trichostrongylus colubriformis* are the main problem in endemic areas because they present anthelmintic resistance (Cruz-Rojo *et al.,* 2012; Macarthur *et al.,* 2013). Selective breeding of resistant sheep able to regulate nematode

infection could be a sustainable method for GIN control (Shakya *et al.,* 2011; Shaw *et al.,* 2013).

It becomes necessary to identify the phenotypic traits related to natural resistance against GIN in order to obtain information related to host immunity in productive ewes. In sheep, low immune responses against GIN are frequently observed around parturition, and identifying resistant ewes is particularly important in animal selection (Kahn *et al.,* 2003; Rocha *et al.,* 2004). Number of eggs per gram of faeces (EPG) has been the main phenotypic trait used to determine GIN resistance and is a reference to establish the relationship between nematode burden and immune system response. Protective immunity to GIN dependens on innate and acquired immune factors such as eosinophilia and immunoglobulin (Ig) A and G, which could help confirm the host resistance against GIN (Alba-Hurtado and Muñoz-Guzmán, 2012; Bishop, 2012). For instance, the high association between IgA response and *T. circumcincta* infection observed by Strain *et al.* (2002) suggests the role of this antibody (Ab) as a possible biomarker for resistance to GIN infections. Sheep challenged with GIN were able to increase IgA levels against carbohydrate larval surface antigen (CarLA; Harrison *et al.,* 2003a; 2003b). The widespread presence of CarLA in various nematode species has been suggested as a biomarker that offers practical, rapid, and easy diagnostic possibilities for identifying GIN resistance in sheep (Shaw *et al.,* 2012).

In order to conduct selection of resistant ewes, physiological aspects such as lactation and gestation shoul be evaluated because they can affect the development of the host immune system against GIN (Beasley *et al.,* 2010). The aim of the present study was to determine the influence of physiological stage in the variability of parasitological, haematological and immunological parameters of Blackbelly ewes naturally infected with gastrointestinal nematodes in tropical conditions.

Material and methods

Ethical considerations

This project was approved by the Research Institute of Animal Science at Universidad Autónoma Chapingo (approval number 145503001, from March 2014).

The study was conducted in Salto de Agua (Chiapas, Mexico), classified as a tropical wet climate (Aw) and located at 17°34′ N and 92°29′ W at 85 m.a.s.l.. The mean temperature in 2013 and 2014 was 26.6 °C, with 3,298 mm of rainfall (Kottek *et al.,* 2006).

Experimental design

Twenty-five Blackbelly ewes (2.5 years old) in their second gestation were selected from a flock. Ewes grazed through the study period; therefore, they were naturally infected with GIN. Faecal samples, blood, saliva, and live weight were taken every 2 weeks through the study (December 2014 to July 2015).

The ewes were maintained in rotational grazing in paddocks of star grass (*Cynodon plectostachyus*) and humidicola grass (*Brachiaria humidicola*) at a stocking density of fifteen ewes per hectare. Lactating ewes were supplemented with 250 g of a mixed feed (sorghum, corn, soy, and ground sugar cane). A model of accelerated lambing was implemented in the flock (González *et al.,* 2010). The model considered three lambing seasons (April, August, and December 2013-2014).

Blood samples were collected fortnightly to measure haematological traits. The % PCV was determined by the micro-haematocrit method and plasmatic protein was expressed in g dL^{-1}. In addition, leukocytes were counted and classified morphologically as neutrophils, eosinophils, basophils, monocytes, and lymphocytes. Saliva samples were collected fortnightly according to the technique cited by Shaw *et al.* (2012). Faecal samples were collected at 15-day intervals for 8 months. The number of EPG was determined by the McMaster technique. Identification of the prevalent nematode species was carried out in adults and larvae were recovered in faecal cultures (Thientpont *et al.,* 1986) and identified by morphometric characterization (Van Wyk and Mayhew, 2013).

Crude worm antigen (CWA) was extracted from adult species of *H. contortus* (n = 50) and *T. colubriformis* (n = 200). Nematodes were collected from the abomasum and intestine of infected donors. Tissues were extracted by grinding nematodes in a mortar with 10% 1 mM phenylmethylsulphonyl fluoride (PMSF, Sigma-Aldrich, St Louis Missouri,

USA). Samples were collected by centrifugation at 20,000 x *g* for 20 min at 4 °C. The supernatant, containing CWA, was collected and stored at -20 °C. In addition, hot water extraction of L3 (HWEL) was performed for surface antigens from exsheathed larvae of *H. contortus* and *T. colubriformis* (Harrison *et al.,* 2008). Protein concentration of both antigenic preparations was determined with the method by Bradford (1976) and confirmed by SDS-PAGE.

Sera and saliva samples were analysed using indirect ELISA against CWA and HWEL antigens from *H. contortus* and *T. colubriformis,* respectively. Briefly, antigenic products were diluted to 2.5 μg mL^{-1} in carbonated buffer (pH 9.6), distributed into 96-well plates (NUNC MaxiSorb, Denmark) and incubated overnight at 4 °C. The wells were washed three times with PBST (0.1 M phosphate, 0.14 M sodium chloride, 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. Duplicate serum samples diluted 1:100 (IgA and IgG) in PBST and saliva samples diluted at 1:20 (IgA) were incubated for 1 h at 37 °C. The plates were washed with PBST before the addition of a horseradish peroxidase-conjugated rabbit anti-sheep IgA and IgG (Bethyl Laboratories, Montgomery, AL, USA) diluted at 1:5,000 in PBST and incubated for 45 min at 37 °C. Then, 50 μL of tetramethylbenzidine (TMB, Sigma Aldrich, St Louis Missouri, USA) substrate solution was added and allowed to incubate for 15 min at RT. The reaction was stopped with 50 μL 1 M H_2SO_4 and optical densities (OD) were determined with a microplate absorbance reader (Imark, Bio-Rad, Mexico, DF) at 450 nm for IgA and IgG. A pool of serum of ewes with high level of IgA against *H. contortus* and *T. colubriformis* served as positive control to standardize values between plates. A negative control and three wells for each antigen without serum (blank) were included on each plate. Therefore, the blank absorbance was subtracted from the samples to correct for non-specific binding (Ramírez-Restrepo *et al.,* 2010). The activity of IgG was expressed as the percentage of the positive standard serum using the formula by Cardoso *et al.* (2013).

Statistical analyses

The data obtained per ewe around parturition were re-arranged from the calendar date to the day relative to each sheep parturition date. After this, dates were grouped in fifteen days to analyse the effect of time. All data were analysed using SAS software release 9.2. (SAS, 2004). In addition, all variables were log-transformed (except PCV) to approximate a normal distribution. Repeated measures were used to test EPG, packed cell volume (% PCV), peripheral eosinophils, and Ig activity (Williams *et al.,* 2010). The model used was:

$$Y_{ijk} = m + \rho_i + \alpha_{j(i)} + \tau_k + \rho\tau_{ik} + \varepsilon_{ijk}$$

Where

Y_{ijk} = is the FEC, PCV, or DWG.

μ = is the mean.

ρ_i = is the fixed effect of physiological stage (gestation and lactation).

$\alpha_{j(i)}$ = is the random effect of j-esim animal in the i-esim physiological stage $\alpha_{j(i)} \sim N(0, \sigma^2_a)$.

$\tau_{k(i)}$ = is the fixed effect of the time (-150, -120, -105, -90, -75, -60, -45, -30, -15, 15, 30, 45, 60, 75, 90).

$\rho\tau_{ik}$ = is the combined effect of physiological stage and time.

$\varepsilon_{ijk} \sim N(0, \sigma^2_e)$ = is the residual error.

A Pearson correlation analysis was used to determine the relationship between indicators of immunity and the other parameters.

Results

Ewes had higher EPG (2,592 ± 2,403) during lactation compared with the gestation period (595 ± 901; Figure 1).

The PCV was approximately 25.1 ± 2.5% during pregnancy and this parameter was markedly reduced to 20 ± 1.3 and 19.2 ± 3.3% at 30 and 45 days after lambing. In addition, plasmatic protein was 6.5 ± 0.5 g dL^{-1} during pregnancy, and decreased to 5.7 ± 0.4 g dL^{-1} 30 days post-partum. Body weight increased 17% (from 30.1 to 36.2 Kg) in the last two months of pregnancy, and lambing reduced this value in the same proportion (Figure 2).

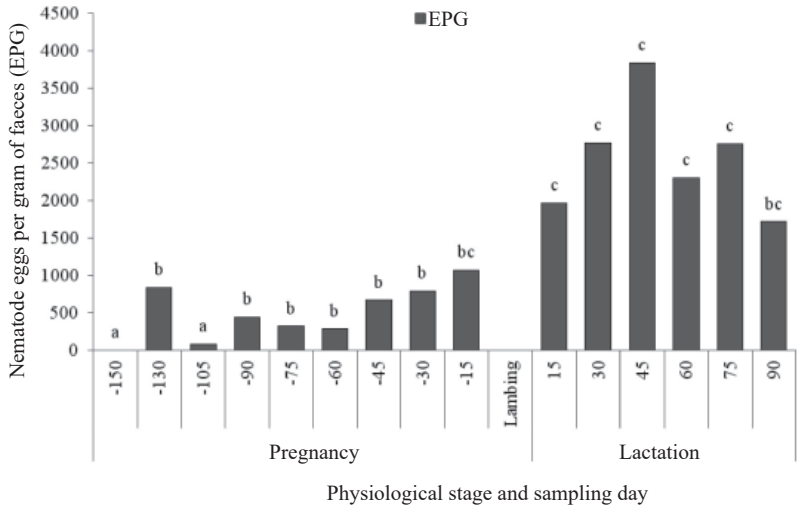

Figure 1. Faecal egg count in Blackbelly ewes naturally infected during pregnancy and lactation.

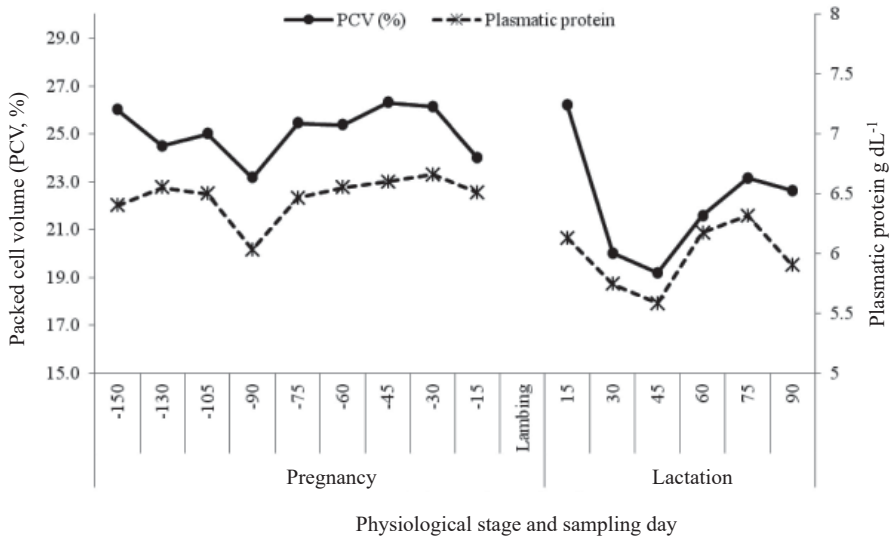

Figure 2. Percentage of packed cell volume (PCV, %) and plasmatic protein (PP) of Blackbelly ewes during pregnancy and lactation.

Three main species of GIN were identified as *H. contortus*, *T. colubriformis*, and *Cooperia curticei.* The *T. colubriformis* population was the major species identified during pregnancy, followed by *H. contortus*. During lactation, *H. contortus* was the dominant nematode species. Other nematodes, such as *Strongyloides papillosus* and *Oesophagostomum columbianum,* were also identified (Table 1).

Kinetics of IgA decreased during pregnancy until the lambing period and reached the lowest levels between 5.71 ± 1.53 to 7.2 ± 1.62% compared with the standard, for *H. contortus* CWA, and 10.29 ± 2.72 to 12.60 ± 2.79% compared with standard for *T. colubriformis* CWA (Figure 3a). The IgA kinetics changed 30 days after parturition when the levels began to increase, as shown in Figure 3a. The IgG anti-CWA levels were erratic, and no differences were observed between pregnancy and lactation ($p > 0.05$; Figure 3b).

The IgA response using HWEL Ag decreased significantly 45 days before the lambing period

Table 1. Percentage of nematode larvae obtained from copro-cultures of naturally infected Blackbelly ewes during pregnancy and lactation under tropical conditions.

Physiological status	Sampling period		Species			
			Haemonchus contortus	*Cooperia curticei*	*Trichostrongylus colubriformis*	*Oesophagostomum columbianum*
Pregnancy	-120	-90	0.0	0.0	100.0	0.0
	-90	-60	0.5	0.0	84.8	14.8
	-60	-30	5.1	18.7	66.1	10.1
	-30	-1	4.1	24.3	69.5	2.1
Lactation	1	30	42.7	12.7	43.7	0.9
	30	60	57.0	4.4	36.1	2.5
	60	90	51.5	0.0	48.5	0.0

**Strongyloides papillosus* appeared in copro-cultures of all samples.

(30.6 ± 5.1% respect to standard) and remained low during the 45 days postpartum (23 to 37%). Subsequently, IgA increased at 60 to 75 days (64 to 70% respect to standard). The response of IgA against *H. contortus* and *T. colubriformis* HWEL was similar, as shown in Figure 4.

The number of eosinophils remained high (2.0 x 10^9 cell L^{-1}) from the beginning of the pregnancy period and decreased significantly 45 days before lambing ($p<0.01$) respect to the lowest levels, which occurred virtually throughout the lactation period (0.7 x 10^9 cell L^{-1}). The number of total leukocytes remained high during pregnancy (15.7 x 10^9 cell L^{-1}) and a decrease was observed from early lactation to 60 days post-lambing to a low level (12.2 x 10^9 cell L^{-1}; Figure 5). Differences were found between -45 days before lambing and 60 days post-partum ($p<0.01$). The other PMN cells did not show important changes.

A negative correlation occurred between EPG and PCV (Table 2). While EPG increased, PCV decreased by 48% during lactation. In pregnancy, the correlation was also negative but was only 38% ($p<0.01$). The same behaviour was observed between EPG and PP during lactation. An important and positive correlation was determined between plasmatic protein concentration and PCV in the lactation period ($r = 0.53$). A slight negative correlation was observed between number of eosinophils and the EPG only in gestation ($r = -0.28$).

In addition, in the lactation period, positive correlations were observed in IgA between the *H. contortus* HWEL Ag and the CWA from *H. contortus* in serum samples ($r = 0.45$). For the HWEL, the correlation between *H. contortus* and *T. colubriformis* was high ($r = 0.95$ to 0.98) in both pregnancy and lactation. Furthermore, high values of IgA were found in the *H. contortus* and *T. colubriformis* antigens ($r = 0.83$ to 0.88; Table 3).

Discussion

The haematological and immune responses of Blackbelly ewes showed differences between the pregnancy and lactation periods. The nematode infection caused by *H. contortus* and *T. colubriformis* was observed three weeks prior to parturition, when there was an acute increase in the number of excreted EPG. At approximately the peri-parturient time, the increased number of EPG observed in late pregnancy and beginning of lactation periods agrees with PCV reduction, lower eosinophils count, and decrease of IgA. The peri-parturient rise (PPR) is a well-known phenomenon, and has been observed in several wool breeds relating to the effect of female sex hormones (Kahn *et al.,* 2003; Williams *et al.,* 2010).

At the same time, when EPG counts increased the % PCV was reduced, especially during lactation, as a result of the increased susceptibility of females to GIN –particularly blood-sucking. The PCV reduction from

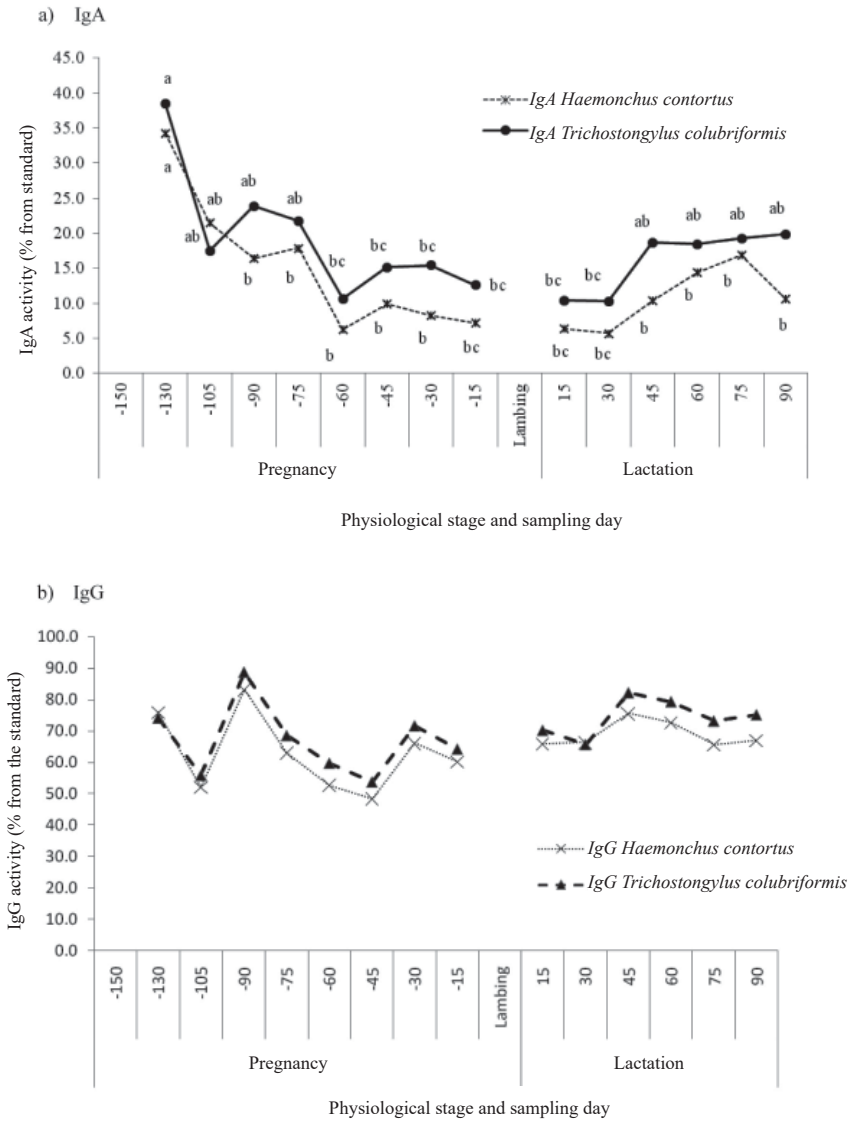

Figure 3. Dynamics of IgA and IgG activity against *Haemonchus contortus* and *Trichostrongylus colubriformis* in serum during pregnancy and lactation of Blackbelly ewes under tropical conditions. Different letters on each variable indicate statistical differences ($p<0.05$).

27% to less than 20% is considered an important health parameter in susceptible sheep because the infection can cause death if an animal is not treated in a timely manner (Macarthur *et al.,* 2013; González-Garduño *et al.,* 2014). In addition, the low % PCV during lactation was associated with an increase in the number of *H. contortus* larvae in cultures, as shown in Table 3. During lactation, the percentage of *H. contortus* larvae increased from 4 to 40% in the coprocultures. Differences observed between larvae percentage during pregnancy and lactation might be attributed to the host response against nematode species. For instance, resistant ewes better resisted infection with *T. colubriformis* than infection with *T. circumcincta* (Williams *et al.,* 2010).

In normal physiological processes the weight of Blackbelly ewes change during pregnancy with a positive gain of 5 Kg in the last two months before lambing. Similar results were found in non-supplemented and supplemented Merino ewes in the same physiological stages (Kahn *et al.,* 2003). Because

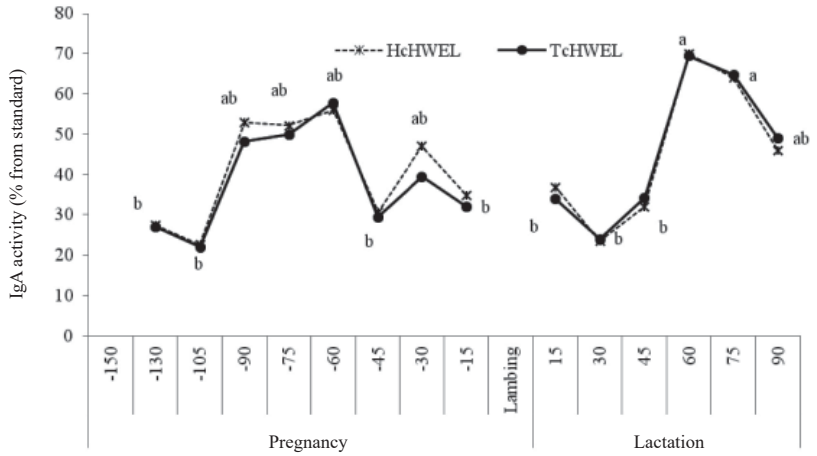

Figure 4. IgA activity in saliva with HWEL antigen of *Trichostrongylus colubriformis* (TcHWEL) and *Haemonchus contortus* (HcHWEL) during the pregnancy and lactation periods of Blackbelly ewes under tropical conditions. HWEL: Hot water extract larval antigen. Different letters on each variable indicate statistical differences ($p<0.05$).

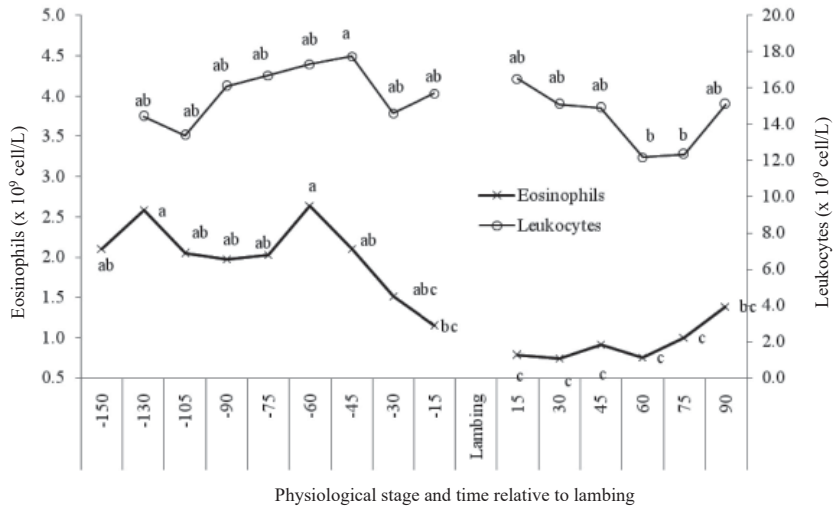

Figure 5. Circulating eosinophils and total leukocytes during the pregnancy and lactation periods of Blackbelly ewes. Different letters on each variable indicate statistical differences ($p<0.05$).

Table 2. Correlation coefficients of % PCV, EPG, plasmatic protein, and eosinophils during pregnancy and lactation periods in Blackbelly ewes.

Lactation	Pregnancy			
	EPG	**% PCV**	**Plasmatic protein**	**Eosinophils**
EPG	1	-0.38**	-0.22 NS	-0.28*
PCV	-0.48**	1	0.28*	0.09 NS
Plasmatic protein	-0.40**	0.53**	1	0.40**
Eosinophils	-0.04 NS	-0.03 NS	0.07 NS	1

Above the diagonal, coefficients correspond to pregnancy. Below the diagonal, coefficients correspond to lactation. *Significant ($p<0.05$), **Highly significant ($p<0.01$), NS: Not significant ($p>0.05$). EPG: Nematode eggs per gram of faeces. PCV: Packed cell volume (%).

Table 3. Correlation coefficients of IgA or IgG in serum and saliva during the pregnancy and lactation periods in Blackbelly ewes.

Lactation		Pregnancy					
		Haemonchus contortus **antigen**			*Trichostrongylus colubriformis* **antigen**		
		Serum		**Saliva**	**Serum**		**Saliva**
		CWA Ag		**HWEL Ag**	**CWA Ag**		**HWEL Ag**
		IgA	**IgG**	**IgA**	**IgA**	**IgG**	**IgA**
H. contortus	CWA-IgA	1	0.47**	0.19^{NS}	0.88**	0.41**	0.16^{NS}
	CWA-IgG	0.33**	1	0.21^{NS}	0.31*	0.95**	0.16^{NS}
	HWEL-IgA	0.45*	-0.12^{NS}	1	0.42*	-0.09^{NS}	0.98**
T. colubriformis	CWA-IgA	0.83**	0.52**	0.24^{NS}	1	0.48**	0.21^{NS}
	CWA-IgG	0.38**	0.96**	0.20^{NS}	0.33*	1	0.15^{NS}
	HWEL-IgA	0.42*	-0.17^{NS}	0.95**	0.39*	-0.12^{NS}	1

Above the diagonal, coefficients correspond to pregnancy; below the diagonal, coefficients correspond to lactation. *Significant ($p<0.05$), **Highly significant ($p<0.01$), NS: Not significant ($p>0.05$). HWEL: Hot water extract larval antigen for saliva. CWA: Crude worm antigen for serum.

pregnancy involves natural factors such as the foetal fluid, foetus and placenta, the pre-partum period is considered the time with the most severe nutrient pressure (Houdijk *et al.,* 2005). Consequently, nutrition is the main strategy for developing mature immunity during late pregnancy and lactation against parasitic infections (Beasley *et al.,* 2010). The weight change supports the theory that the peri-parturient relaxation in immunity is probably linked to the increasing needs of energy and protein for foetal growth and hormonal changes (Mahieu and Amount, 2007).

The IgA might be an indicator of immunity regulation due to the reduction of Ab titters when pregnancy is progressing, and the lowest level occurred across the first months of lactation; the opposite situation occurred with faecal egg counts ($r = -0.23$). These same results were observed in the study by Beasley *et al.* (2010) with under or overfed ewes, with total plasma Ab showing low titres in pregnant ewes regardless of the diet. The rapid recovery of systemic total Ab following early weaning suggests an important role in the modulation of the EPG count around parturient rise (Beasley *et al.,* 2010). After the challenge of a nematode infection, hair-ewes had numerous and rapid humoral immune responses, including the ability to reduce the number of EPG and maintain significantly high titres of circulating specific IgA (Bowdridge *et al.,* 2013).

Increased production of specific IgA after infection is also an indicator of immune responsiveness according to the response to *H. contortus* (Amarante *et al.,* 2005) and *T. circumcinta* (Martínez-Valladares *et al.,* 2005; Henderson *et al.,* 2006). Plasma IgA titres in early weaned ewes increased, becoming significantly higher to those measured in suckled ewes (Beasley *et al.,* 2010). In pregnant sheep, the high concentration of IgA in sera samples in middle lactation may be more reflective of recovery from infection (Bowdridge *et al.,* 2013), as shown in Figure 3. The decrease in EPG number may be due to IgA protection mechanisms, which include larval immobilization (Harrison *et al.,* 2003a) and suppression of the EPG number (Martínez-Valladares *et al.,* 2005; McCoy *et al.,* 2008). This together with cellular mechanisms, including eosinophils counts.

In this study, ELISA was used to confirm that HWEL, as well as CarLa, is a highly conserved carbohydrate Ag (Harrison 2003a). The antibody immune responses in saliva to larval *H. contortus* and *T. colubriformis* HWEL Ag were similar. So it could be used as a diagnostic test for infection with GIN.

However, the values in serum and saliva showed no high correlation coefficients because the response in the two fluids occurs with different intensity over time. This behaviour has been observed because IgA has local rather than peripheral action (Prada-Jiménez *et al.,* 2014).

A high correlation coefficient (r = 0.83) between CWA to both species (*H. contortus* and *T. colubriformis*) was found, but the correlation value were lower than found in saliva (r = 0.95), so CWA could also be used in general diagnostics against GIN, because trends between the two species are similar. However, with CWA in serum, it is possible to detect some individual differences in Ag in both nematode species.

Salivary IgA represents a possible means for detecting ewes with high resistance to GIN, especially during lactation. At this stage, it should be important to detect the ewes that have the ability to maintain high immunity levels despite the PPR. The IgA titres can be measured in blood, nasal secretions, and saliva because IgA is secreted in the gastrointestinal mucus and distributed to mucosal secretions through the blood (Prada-Jiménez *et al.,* 2014). Considering our results, it is possible to determine basal levels one month before gestation to 45 days post-partum, whereas the peri-partum levels of salivary IgA are reduced as part of PPR.

According to Henderson *et al.* (2006) and Macarthur *et al.* (2013), evidence exists for a general immune response of the peri-parturient ewe beginning on day -29, as measured by a low number of eosinophil cells. In contrast, antibody titres in gut tissue of infected ewes remain elevated (Houdijk *et al.,* 2005). In the same sense, the present study showed a low number of eosinophils in pregnant ewes one month before lambing, but a reduction in IgA levels occurred two months before partum in serum samples and 45 days in saliva. In addition, the recovery of the IgA levels was approximately 45 days post-partum when the IgA titres in serum and saliva occurred. However, the highest amounts of eosinophils were noted 90 days post-partum at the end of lactation period.

The increased number in EPG from day -29 to the lambing date was associated with the low number of eosinophils. Possibly, this type of response prior to parturition is a leading indicator of the general reduction of immunity associated with the peri-parturient physiology (Macarthur *et al.,* 2013). During the final stages of pregnancy and across the lambing period, systemic immunity remained depressed as shown by the lower levels of circulating eosinophils and antibody titres. In particular, blood eosinophils concentrations drop precipitously across the span of the lambing period (Beasley *et al.,* 2010). The number of eosinophils found at the beginning of lactation in the present study (0.7×10^9 L^{-1}) exhibited a tendency similar to those indicated by Beasley *et al.* (2010) in Merino ewes, whereas the counts in the Blackbelly ewes in this study were higher than other unlike so reports in sheep (Cardia *et al.,* 2011), but similar values were recorded for the same breed in another study (Tibbo *et al.,* 2005).

In conclusion, IgA in saliva and serum reflects the breakdown of immunity before and after lambing. The reduction in IgA levels occurred two months before partum in the serum and 45 days in saliva. The recovery of immunity occurred close to 45 days post-partum when IgA titres in serum and saliva occurred.

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Conflict of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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