

Serum antibody responses in Creole kids experimentally infected with *Haemonchus contortus*

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Abstract

The objective of this study was to evaluate the relationship of parasite-specific serum antibodies with the resistance status of Creole kids. The average breeding values on egg output predicted in a context of natural infection at 11 months of age were distant of 1.07 genetic standard deviation between resistant and susceptible animals. After drenching the animals were maintained worm-free during 1 month until experimental infection with 10,000 *Haemonchus contortus* infective larvae (L3). Enzyme-linked immunosorbent assay was carried out in serum samples to determine the level of IgG, IgA and IgE anti-*H. contortus* L3 crude extracts and adult excretion/secretion products (ESP). Parasitological and blood immunological parameters were measured on the 2 extreme groups. Despite the absence of any typical signs of haemonchosis, susceptible kids had more than 11 times higher faecal egg counts (FEC) at 35 days post-infection (d.p.i.) than resistant kids had. Levels of immunoglobulin against *H. contortus* L3 and ESP increased significantly after infection in both groups. However, no difference in the host immune response mediated by immunoglobulin against *H. contortus* was evidenced between groups. This finding suggests that, in goats previously infected by *H. contortus*, a degree of protection occurred and the phenotypic and genetic segregation in resistant and susceptible animals were not related to the humoral immune response. The correlation coefficients between FEC and IgE anti-ESP ($r = 0.593$; $P < 0.05$) was significant in both resistant and susceptible animals. Such correlation suggesting a hypersensitivity reaction dependent on worm prolificacy has never been described. This result needs further studies to understand the mechanisms underlying this observation.

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1. Introduction

The gastrointestinal nematode *Haemonchus contortus* is one of the major pathogens of sheep and goats

throughout the temperate and tropical region of the world and is thus considered as an important constraint on efficient production. The emergence of nematode populations with resistance to anthelmintics and the concern about chemical residues in animal products threaten the sustainability of this approach (Waller, 2006). Alternative strategies for the control of gastrointestinal nematode infections, include selection of genetically resistant breeds and the development of effective vaccine (Gray, 1997; Newton and Munn,

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1999). In sheep, the ability to acquire immunity and express resistance to gastrointestinal nematodes varies among and within breeds and is under genetic control (Stear et al., 1999). The knowledge of the immune mechanisms underlying such genetic variation is critical for the identification of genetically resistant livestock and immunization (Gill et al., 2000).

The immune response against gastrointestinal nematodes is well documented in sheep (Miller and Horohov, 2006). Thus genetic resistance is immunologically mediated by proliferation of mucosal mast cells, globule leukocytes and eosinophils (Meeusen et al., 2005). The response against gastrointestinal nematodes also involves production of parasite-specific immunoglobulin A (IgA), IgG1 and IgE (Pfeffer et al., 1996; Kooyman et al., 1997; Shaw et al., 1998).

Although goats are markedly susceptible to infection with gastrointestinal nematodes, some breeds are genetically resistant to infection (Pralomkarn et al., 1997; Behnke et al., 2006). Recently, we demonstrated the feasibility of breeding for improved resistance to nematode within the Creole goats breed (Mandonnet et al., 2001, 2006). Few studies have investigated the goat immune response to *Teladorsagia circumcincta*, *Trichostrongylus colubriformis* and *H. contortus* infections (Huntley et al., 1995; Fakae et al., 1999; Perez et al., 2001, 2003). However, the humoral response in relation with the resistance/susceptible status to gastrointestinal nematode infection together with parasitological measures has not been investigated in goats. This study was carried out to evaluate the relationship of parasite-specific serum IgG, IgA and IgE and parasitological parameters with the resistance and susceptibility to *H. contortus* within the Creole goats breed.

2. Materials and methods

2.1. Animals and experimental design

Twenty five 10-month old female Creole goats reared on pasture were chosen from the flock of INRA-Gardel, according to their breeding value for resistance (Mandonnet et al., 2001). In this flock, the pedigree of each animal was available from the foundation generation of 1979. In addition, faecal samples have been regularly collected (weeks 6 and 7 after drenching) at 7 and 11 months of age for genetic evaluation on the average of 2 faecal egg counts (FEC) measures, since 1995. The resistant and susceptible average predicted breeding values on egg output in a context of natural infection at 11 months of age were distant of 1.07 genetic standard deviation in this experiment. The

animals were drenched with moxidectine (Cydectine[®], Fort Dodge Veterinaria S.A., Tours, France, 300 µg/kg) and housed indoors under worm-free conditions in a single pen 1 month before the start of the experiment. During this period, nematode faecal egg counts remained at zero. Limited infection with coccidia occurred despite diclazuril treatments (Vecoxan, Janssen-Cilag, 1.5 mg/kg) before the start of the experiment, but without any clinical signs of coccidiosis. All animals received granulated feed adapted to their age, as well as forage and tap water *ad libitum*. Animals were reared following European Union recommendations for animal welfare in accordance with the regulations of the Animal Care Committee of INRA.

Infective larvae (L3) of *H. contortus* were obtained 42 days before challenge from cultures of faeces taken from monospecifically infected Creole goats with isolates previously obtained from Creole goats reared on pasture in different farms in Guadeloupe (Aumont and Cabaret, 1999). Kids were orally infected with a single dose of 10,000 *H. contortus* third-stage larvae (L3) at Day 0. Here we analyzed the response of 8 resistant and 8 susceptible animals according to their FEC during experimental infection.

2.2. Parasitological techniques, blood and serum samples

Faecal samples were collected to determine FEC using a modified McMaster method for rapid determination (Aumont, 1997). Blood samples from each animal were recovered once a week and centrifuged for 5 min at 5000 rpm. Serum was then frozen at –20 °C until analysis. Blood samples were collected in EDTA coated tubes (Becton Dickinson, Plymouth, UK) to measure the number of circulating eosinophils according to the method of Dawkins et al. (1989). Eosinophils were counted with a Malassez cell counter. The packed cell volume was measured using the capillary microhaematocrit method.

2.3. Antibodies detection in serum by indirect ELISA

2.3.1. Worm antigen preparation

Prior to experimentation five donor goats infected with 10,000 L3 of *H. contortus* were sacrificed at 42 d.p.i. and adult worms were harvested from the abomasum. These worms were thoroughly washed in PBS (pH 7.4) containing penicillin (100 IU/ml) and streptomycin (1 mg/ml). Fifty adult worms per millilitre of the same buffer were maintained in a 5% CO₂

atmosphere at 37 °C overnight. Next, the supernatant containing excretory/secretory products (ESP) was collected, filtered (0.2 µm) and stored at −70 °C until further use. A crude extract of *H. contortus* L3 was prepared after three cycles of freezing and thawing (−70 °C, +25 °C), homogenization at 4 °C and centrifugation at 30,000 *g* for 30 min at 4 °C. The supernatant was used as the crude extract of the L3 antigen. The protein concentration of both antigenic preparations was determined with the method of Bradford (1976).

2.3.2. Serum specific IgA and IgE

Briefly, crude extract of *H. contortus* L3 and ESP were diluted at 2 µg/ml in carbonate buffer (pH 9.6), distributed in 96-well plates (Nunc surface, Nunc, Denmark), incubated overnight at 4 °C. The wells were washed three times with PBST (0.01 M phosphate, 0.15 M sodium chloride, pH 7.2 and 0.1% Tween 20). Non-specific binding sites were blocked by 3 h incubation with PBS-1% bovine serum albumin (BSA, Sigma, St. Louis, USA), 5% sucrose. Duplicate serum samples diluted 1:2 (IgA), and 1:320 (IgE) in PBST were incubated for 2 h at room temperature (RT). The plates were washed three times with PBST before addition of a horseradish peroxidase-conjugated Rabbit anti-goat IgA (Alpha Diagnostic) diluted 1:1000 in carbonate buffer (60 min of incubation at RT). For IgE ELISA, plates were sequentially incubated with mouse anti-ovine IgE monoclonal IE7 (Kooyman et al., 1997) diluted 1:1000 (kindly provided by Dr. F.N.J. Kooyman, Utrecht University, The Netherlands) and rabbit anti-mouse HRP conjugate (Serotec, Oxford, England). Three final washes with PBST were carried out before addition and incubation at RT of 100 µl per well of the chromogen (2,2'-azino-bis, 3-ethylbenzthiazoline-6-sulfonic acid or 3,3',5,5'-tetramethylbenzidine for IgA and IgE respectively). After 20 min, the optical densities were determined with a spectrophotometer by measuring the absorbance at 405 nm for IgA. For IgE detection the colour reaction was stopped by the addition of 50 µl per well of 2N H₂SO₄ and the optical density was measured at 450 nm (OD₄₅₀). In order to compare results between assays, a positive control consisting of a pool of sera containing IgA and IgE antibody was included on each plate, and the OD₄₀₅ or OD₄₅₀ of unknown samples were altered in proportion with changes of this standard.

2.4. Statistical analysis

All variables were log transformed (except PCV and weights) in order to normalize the variances. Kinetics of

each variable was modeled using mixed procedure of SAS software release 3.1 (SAS institute Inc., 1999). The peaks of the different variables were localized using also mixed procedure of SAS software, and including resistance status and time as discrete variation factors. The same procedure was used to compare animals of the 2 extreme groups. The results are presented after back-transformation. The association between data was determined using Pearson's rank correlation (MINITAB software, release 12.2).

3. Results

3.1. Parasitological and zootechnical measures

The FEC remained at zero until 21 days post-infection (d.p.i.) in both groups. Susceptible kids showed a peak in egg output between 28 and 42 d.p.i. whereas resistant kids' egg output began to increase significantly at 42 d.p.i. (Fig. 1a and Table 1). From 21 d.p.i., kinetics of egg output was significantly higher in susceptible group ($P < 0.01$). At 35 d.p.i. FEC in susceptible group was more than 11 times higher than in resistant one.

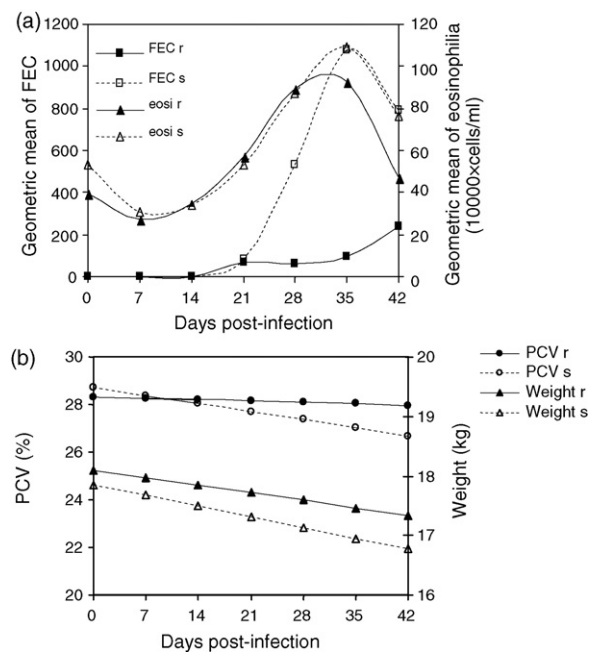


Fig. 1. (a) Geometric mean of faecal egg counts (FEC, ■ resistant; □ susceptible) and number of blood eosinophils/ml (eosi, ▲ resistant; △ susceptible) comparing resistant (r) and susceptible (s) Creole kids. (b) Mean of packed cell volume (PCV, ● resistant; ○ susceptible) and weight (▲ resistant; △ susceptible) comparing resistant (r) and susceptible (s) Creole kids.

Table 1

Significance (*P* value) of circulating eosinophils and egg output levels in reference to levels at 0 and 21 days post-infection (d.p.i.) respectively.

Status ^a	d.p.i. ^b	7	14	21	28	35	42
Resistant	Eosinophil counts	0.072	0.005	0.007	<0.0001	0.073	n.s.
	Egg output				n.s.	n.s.	0.098
Susceptible	Eosinophil counts	n.s.	0.002	n.s.	0.014	0.092	0.028
	Egg output				<0.001	0.002	0.0035

n.s., non-significant (*P* > 0.10).^a Status, genetic predisposition against gastrointestinal nematode infection.^b Days post-infection, number of days after the animals were orally infected with a single dose of 10,000 of *H. contortus* third-stage larvae (L3).

Eosinophils counts in blood significantly increased after infection (*P* < 0.001). A slight peak was shown between 28 and 35 d.p.i. in resistant kids (Fig. 1a and Table 1). In susceptible kids eosinophilia was high for days 28, 35 and 42 post-infection and a peak was shown at 35 d.p.i. (Fig. 1a and Table 1). No significant difference was shown in the kinetics of the resistant and susceptible groups.

PCV values did not change during the experiment (Fig. 1b). The average values were not significantly different between the 2 groups: 28.1% for resistant kids and 27.7% for the susceptible one.

Animals slightly lost weight (*P* < 0.0001) across the experiment (Fig. 1b). Resistant kids lost less weight than susceptible ones but the differences were not statistically significant. The loss was around 0.6 kg in resistant group and around 1 kg in susceptible one.

3.2. Serum antibody responses

The OD's levels of sera from parasite-free kids used as negative control were not significantly different from the background (data not shown). Following infection with *H. contortus* the levels of IgA anti-L3 response

increased in both groups to peak at 14 d.p.i., and then decreased rapidly to reach a baseline from 28 d.p.i. to the end of the infection (Fig. 2a and Table 2). Susceptible animals had an IgA anti-L3 response more pronounced than resistant without statistical differences (Fig. 2a). The IgE anti-L3 response increased significantly after infection to peak at 21 and 28 d.p.i., in susceptible and resistant groups (Table 2). The level of IgE anti-L3 was higher in susceptible group but without significant differences (Fig. 2a).

The levels of IgA specific antibody response to *H. contortus* adult excretion/secretion products (IgA anti-ESP) increased rapidly in both groups to peak between 14 and 21 d.p.i., then decreased and reach the baseline at 35 d.p.i. (Fig. 2b) but no difference was observed between groups. The IgA anti-ESP response was more pronounced than the IgA response against *H. contortus* L3 crude extract (Fig. 2a and b; *P* < 0.005). IgE anti-ESP increased significantly from 14 d.p.i. in resistant kids and from 7 d.p.i. in susceptible ones. The level of IgE anti-ESP increase continuously in both groups throughout the infection (Fig. 2b). The levels of IgG anti-L3 and IgG anti-ESP were weak and no difference was evidenced between groups (data not shown).

Table 2

Significance (*P* value) of immunoglobulin (Ig) secretion levels in relation to secretion at 0 days post-infection (d.p.i.), in resistant and susceptible kids.

Antigen ^a		L3 ^b							ESP ^c					
Status ^d	d.p.i. ^e	7	14	21	28	35	42		7	14	21	28	35	42
Resistant	IgE	n.s.	n.s.	<0.001	<0.001	0.081	0.053	n.s.	n.s.	0.052	<0.001	<0.001	<0.001	<0.001
	IgA	n.s.	<0.001	0.006	n.s.	n.s.	n.s.	n.s.	n.s.	<0.001	<0.001	0.015	0.020	n.s.
Susceptible	IgE	n.s.	0.051	<0.001	<0.001	n.s.	n.s.	0.007	0.019	0.002	<0.001	<0.001	<0.001	<0.001
	IgA	0.006	<0.001	<0.001	0.171	0.122	0.025	n.s.	<0.001	<0.001	<0.001	<0.001	0.010	0.102

^a Antigen, soluble protein extract of *Haemonchus contortus*.^b L3, third-stage larvae (L3) crude extracts.^c ESP, adult nematode excretory/secretory products.^d Status, genetic predisposition against gastrointestinal nematode infection.^e Days post-infection, number of days after the animals were orally infected with a single dose of 10,000 *H. contortus* third-stage larvae (L3).

Table 3

Overall correlation between immunological and parasitological variables measured during the experimental infection.

	Circulating eosinophils	IgA anti-L3	IgA anti-ESP	IgE anti-L3	IgE anti-ESP
IgA anti-L3	−0.018				
IgA anti-ESP	0.239	0.767***			
IgE anti-L3	0.583**	0.458*	0.684**		
IgE anti-ESP	0.588**	0.169	0.286	0.649**	
FEC	0.254	−0.147	0.084	0.444	0.593**

* $P < 0.10$.** $P < 0.05$.*** $P < 0.001$.

3.3. Correlation coefficients between parasitological and immunological variables

Since the serum antibody response showed similar profiles in susceptible and resistant animals with no statistical differences the analysis of correlation coefficients was realized between variables of all animals. Values of circulating eosinophils, IgE anti-L3 and IgE anti-ESP were positively correlated ($r = 0.583$ and $r = 0.588$, $P < 0.05$, Table 3). Values

of FEC were only correlated with IgE anti-ESP ($r = 0.593$, $P < 0.05$). IgA and IgE response against *H. contortus* L3 crude extract or adult ESP were highly positively correlated ($r = 0.767$, $P < 0.001$ and $r = 0.649$, $P < 0.05$, respectively).

4. Discussion

While there is evidence that some breeds of goats differ from others in their susceptibility to nematode infection (Cabaret and Gruner, 1988; Pralomkarn et al., 1997), there is as yet little information available about within-breed variation in nematode resistance on which to base a breeding programme. Previous work has shown that resistance to naturally acquired strongyles infections in Creoles goats seems to be under genetic control (Mandonnet et al., 2001). In the present study we evaluated the phenotypic and genetic resistant/susceptible status of these animals during experimental infection with *H. contortus* and showed a good genetic and phenotypic segregation between resistant and susceptible kids. At 35 d.p.i. FEC in resistant animals was 11 times lower than in susceptible. Thus, in resistant animals worm establishment rate seems to be lower and female prolificacy and/or worm maturation delayed. In accordance with this hypothesis resistance of sheep to *H. contortus* has been associated, with the lengthening of prepatent periods, lower parasite faecal egg output, lower establishment rate and, consequently, lower adult worm burdens as well as an increase in larval inhibition in the abomasal mucosa (Adams, 1993; Stear et al., 1995a).

Despite a good segregation in FEC between animal groups, the typical signs of *H. contortus* infection, such as anorexia, prostration, apathy and anaemia were not observed throughout the experiment, thus characterizing the infection as subclinical. The number of infective larvae used in this experiment resulted in mild infection, rather than the more accentuated clinical response obtained with a similar infective dose employed in

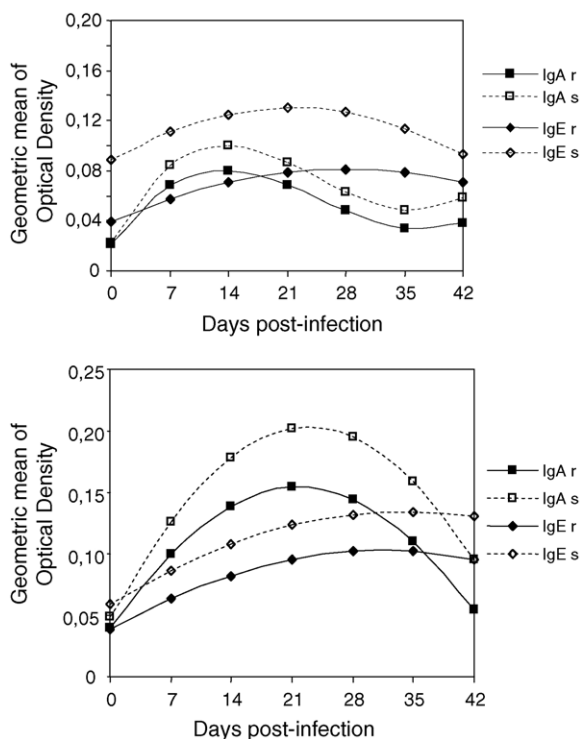


Fig. 2. (a) Systemic IgA (■ resistant; □ susceptible) and IgE (◆ resistant; ◇ susceptible) response against *H. contortus* crude extract of L3 (anti-L3) antigens in resistant (r) and susceptible (s) Creole kids. (b) Systemic IgA (■ resistant; □ susceptible) and IgE (◆ resistant; ◇ susceptible) response against adults *H. contortus* excretion products (anti-ESP) in resistant (r) and susceptible (s) Creole kids.

previous studies with *H. contortus* within different goats and sheep breeds (Perez et al., 2001, 2003; Lacroux et al., 2006).

In this experiment the animals were fed with a high amount of protein (40 g crude protein/day) what could be, at least in part, responsible for the absence of signs. Indeed, several studies have showed that protein or urea supplementation in the diet of sheep was able to increase resilience and resistance to infection by endoparasites (Houtert et al., 1995; Wallace et al., 1998; Knox and Steel, 1999).

It is generally assumed that blood eosinophilia and the infiltration of target tissues by eosinophils are characteristic outcomes of helminth infection in mammals. Many studies suggested that this cell population play a role in resistance to helminth infection since significant correlations between resistance/susceptibility to endoparasites infection and the magnitude of the peripheral eosinophil response has been shown (Meeusen et al., 2005). More recently it has been shown that eosinophils could interact with and damage gastrointestinal nematode larvae *in vivo* (Balic et al., 2006). Herein eosinophilia increased significantly after infection, but no correlations between peripheral blood eosinophilia and protection/resistance were found. One reason for this might be that eosinophilia should have been measured locally rather than in peripheral blood. Nonetheless, it has been shown that in parasitized sheep a high protein diet could increase eosinophils counts and attenuate physiopathological effects of haemonchosis (Datta et al., 1998). The subclinical haemonchosis observed in our experiment where resistant and susceptible animals showed no differences in eosinophilia, is in keeping with this previous study, but this hypothesis needs further experiments to be validated.

In this study the humoral immune response observed was not a primary response since the animals were previously exposed to nematode infection on pasture. But in contrast to studies in Texel and Castellana sheep showing a stronger IgG and IgA response in animals previously exposed to infection (Schallig et al., 1995; Gomez-Munoz et al., 1999), in Creole kids naturally parasitized on pasture before the experimental infection, a weak response was observed except for IgA anti-ESP which showed amounts similar to primo-infected sheep. Previous studies in sheep suggest that IgA may be the major mechanism controlling fecundity of *H. contortus* and worm length and fecundity of *Teladorsagia circumcincta* (Stear et al., 1995b; Strain et al., 2002). In

agreement, Amarante et al. showed in three breeds of sheep (Tropical Santa Ines, European Suffolk and Ile de France) an inverse relationship between FEC and IgA anti-L3 and IgA anti-L5 in the mucus (Amarante et al., 2005). A significant IgA response was observed in both groups against *H. contortus*, but no relationship was found between IgA anti-L3 or IgA anti-ESP and FEC.

In mammals, elevated IgE response following parasitic nematodes infection is well documented for a long time. Studies on IgE response in ruminant were as yet restricted to sheep and cattle. Thus, in sheep increased total and nematode-specific serum IgE levels after gastrointestinal nematode infection have been reported in many studies (Huntley et al., 1998; Pernthaner et al., 2005). Furthermore, a negative correlation between worm counts and total serum IgE levels was found in sheep parasitized with *H. contortus* (Kooyman et al., 1997). Our results showed a significant rise in L3 crude extract and ESP specific serum IgE in both animal groups after *H. contortus* infection. But in contrast to previous studies in sheep, no statistical difference was evidenced between resistant and susceptible animals. A significant positive correlation was found between FEC and IgE anti-ESP ($r = 0.593$; $P < 0.05$), suggesting that hypersensitivity response in Creole kids infected with *H. contortus* might be associated with worm burden and/or female prolificacy. Altogether these data emphasise the intriguing complexity of the immune response to nematode infection.

In conclusion, in accordance to our previous results (Mandonnet et al., 2001, 2006) this study showed a within-breed variation in resistance to experimental haemonchosis. This genetic and phenotypic variation was not related to the host humoral response level, probably in part due to a degree of immunization of the animals on pasture in natural infection and a balanced diet during the course of the experiment. In further research, we aim to investigate the primo immune response locally in the abomasal mucosa and mucus together with the humoral response of Creole kids infected with *H. contortus*.

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References

- Adams, D.B., 1993. Systemic responses to challenge infection with *Haemonchus contortus* in immune Merino sheep. *Veterinary Research Communications* 17.
- Amarante, A.F.T., Bricarello, P.A., Huntley, U., Mazzolin, L.P., Gomes, J.C., 2005. Relationship of abomasal histology and parasite-specific immunoglobulin A with the resistance to *Haemonchus contortus* infection in three breeds of sheep. *Veterinary Parasitology* 128, 99–107.
- Aumont, G., Cabaret, J., 1999. Etude comparée du succès reproductif du nématode parasite *Haemonchus contortus*, en situation de co-évolution chez des hôtes petits ruminants sensibles et résistants en zone tropicale. Appel d'offres recherche du Bureau des Ressources Génétiques: Recherches méthodologiques pour l'amélioration des processus de gestion et de conservation des ressources génétiques animales, végétales et microbiennes Rapport d'étape 1997–1998.
- Aumont, G. R. P. a. N. M. 1. 1997. Le dénombrement des éléments parasitaires: Un outil pour l'étude de la résistance génétique aux endo-parasites chez les petits ruminants. Workshop final de l'ATP CIRAD-MIPA 72/94, Guadeloupe, France.
- Balic, A., Cunningham, C.P., Meeusen, E.N.T., 2006. Eosinophil interactions with *Haemonchus contortus* larvae in the ovine gastrointestinal tract. *Parasite Immunology* 28.
- Behnke, J.M., Chiejina, S.N., Musongong, G.A., Fakae, B.B., Ezeokonkwo, R.C., Nnadi, P.A., Ngongeh, L.A., Jean, E.N., Wakelin, D., 2006. Naturally occurring variability in some phenotypic markers and correlates of haemonchotolerance in West African Dwarf goats in a subhumid zone of Nigeria. *Veterinary Parasitology* 141, 107–121.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72.
- Cabaret, J., Gruner, L., 1988. Genetic variability of resistance to parasites. In: *Proceedings, 3rd World Congress on Sheep and Beef Cattle Breeding*, Volume 1, 19–23 June 1988, Paris.
- Datta, F.U., Nolan, J.V., Rowe, J.B., Gray, G.D., 1998. Protein supplementation improves the performance of parasitised sheep fed a straw-based diet. *International Journal for Parasitology* 28.
- Dawkins, H.J.S., Windon, R.G., Eagleson, G.K., 1989. Eosinophil responses in sheep selected for high and low responsiveness to *Trichostrongylus colubriformis*. *International Journal for Parasitology* 19.
- Fakae, B.B., Chiejina, S.N., Behnke, J.M., Ezeokonkwo, R.C., Nnadi, P.A., Onyenwe, W.I., Gilbert, F.S., Wakelin, D., 1999. The response of Nigerian West African Dwarf goats to experimental infections with *Haemonchus contortus*. *Research in Veterinary Science* 66, 147–158.
- Gill, H.S., Altmann, K., Cross, M.L., Husband, A.J., 2000. Induction of T helper 1-and T helper 2-type immune responses during *Haemonchus contortus* infection in sheep. *Immunology* 99, 458–463.
- Gomez-Munoz, M.T., Cuquerella, M., Gomez-Iglesias, L.A., Mendez, S., Fernandez-Perez, F.J., Fuente, C.d.I., Alunda, J.M., 1999. Serum antibody response of Castellana sheep to *Haemonchus contortus* infection and challenge: relationship to abomasal worm burdens. *Veterinary Parasitology* 81.
- Gray, G.D., 1997. The use of genetically resistant sheep to control nematode parasitism. *Veterinary Parasitology* 72, 345–366.
- Houtert, M.F.J., Barger, I.A., Steel, J.W., 1995. Dietary protein for young grazing sheep: interactions with gastrointestinal parasitism. *Veterinary Parasitology* 60.
- Huntley, J.F., Patterson, M., MacKellar, A., Jackson, F., Stevenson, L.M., Coop, R.L., 1995. A comparison of the mast-cell and eosinophil responses of sheep and goats to gastrointestinal nematode infections. *Research in Veterinary Science* 58, 5–10.
- Huntley, J.F., Schallig, H.D.F.H., Kooyman, F.N.J., MacKellar, A., Millership, J., Smith, W.D., 1998. IgE responses in the serum and gastric lymph of sheep infected with *Teladorsagia circumcincta*. *Parasite Immunology* 20.
- Knox, M.R., Steel, J.W., 1999. The effects of urea supplementation on production and parasitological responses of sheep infected with *Haemonchus contortus* and *Trichostrongylus colubriformis*. *Veterinary Parasitology* 83.
- Kooyman, F.N.J., VanKooten, P.J.S., Huntley, J.F., MacKellar, A., Cornelissen, A.W.C.A., Schallig, H.D.F.H., 1997. Production of a monoclonal antibody specific for ovine immunoglobulin E and its application to monitor serum IgE responses to *Haemonchus contortus* infection. *Parasitology* 114, 395–406.
- Lacroux, C., Nguyen, T.H.C., Andreoletti, O., Prevot, F., Grisez, C., Bergeaud, J.P., Gruner, L., Brunel, J.C., Francoise, D., Dorchie, P., Jacquet, P., 2006. *Haemonchus contortus* (Nematoda: Trichostrongylidae) infection in lambs elicits an unequivocal Th-2 immune response. *Veterinary Research* 37, 607–622.
- Mandonnet, N., Aumont, G., Fleury, J., Arquet, R., Varo, H., Gruner, L., Bouix, J., Khang, J.V.T., 2001. Assessment of genetic variability of resistance to gastrointestinal nematode parasites in Creole goats in the humid tropics. *Journal of Animal Science* 79, 1706–1712.
- Mandonnet, N., Menendez-Buxadera, A., Arquet, R., Mahieu, M., Bachand, M., Aumont, G., 2006. Genetic variability in resistance to gastro-intestinal strongyles during early lactation in Creole goats. *Animal Science* 82, 283–287.
- Meeusen, E.N.T., Balic, A., Bowles, V., 2005. Cells, cytokines and other molecules associated with rejection of gastrointestinal nematode parasites. *Veterinary Immunology and Immunopathology* 108, 121–125.
- Miller, J.E., Horohov, D.W., 2006. Immunological aspects of nematode parasite control in sheep. *Journal of Animal Science* 84 (Suppl.), E124–E132.
- Newton, S.E., Munn, E.A., 1999. The development of vaccines against gastrointestinal nematode parasites, particularly *Haemonchus contortus*. *Parasitology Today* 15, 116–122.
- Perez, J., Garcia, P.M., Hernandez, S., Martinez-Moreno, A., las Mulas, J.M., Camara, S., 2001. Pathological and immunohistochemical study of the abomasum and abomasal lymph nodes in goats experimentally infected with *Haemonchus contortus*. *Veterinary Research* 32, 463–473.
- Perez, J., Garcia, P.M., Hernandez, S., Mozos, E., Camara, S., Martinez-Moreno, A., 2003. Experimental haemonchosis in goats: effects of single and multiple infections in the host response. *Veterinary Parasitology* 111, 333–342.
- Pernthaner, A., Shaw, R.J., McNeill, M.M., Morrison, L., Hein, W.R., 2005. Total and nematode-specific IgE responses in intestinal lymph of genetically resistant and susceptible sheep during infection with *Trichostrongylus colubriformis*. *Veterinary Immunology and Immunopathology* 104, 69–80.
- Pfeffer, A., Douch, P.G.C., Shaw, R.J., Gatehouse, T.K., Rabel, B., Green, R.S., Shirer, C.L., Jonas, W.E., Bisset, S., 1996. Sequential cellular and humoral responses in the abomasal mucosa and blood

- of Romney sheep dosed with *Trichostrongylus axei*. *International Journal for Parasitology* 26, 765–773.
- Pralomkarn, W., Pandey, V.S., Ngampongsai, W., Choldumrongkul, S., Saithanoo, S., Rattanaachon, L., Verhulst, A., 1997. Genetic resistance of three genotypes of goats to experimental infection with *Haemonchus contortus*. *Veterinary Parasitology* 68, 79–90.
- Schallig, H.D.F.H., VanLeeuwen, M.A.W., Hendrikx, W.M.L., 1995. Isotype-specific serum antibody-responses of sheep to *Haemonchus contortus* antigens. *Veterinary Parasitology* 56, 149–162.
- Shaw, R.J., Gatehouse, T.K., McNeill, M.M., 1998. Serum IgE responses during primary and challenge infections of sheep with *Trichostrongylus colubriformis*. *International Journal for Parasitology* 28, 293–302.
- Stear, M.J., Bairden, K., Duncan, J.L., Murray, M., 1995a. A comparison of the responses to repeated experimental infections with *Haemonchus contortus* among Scottish Blackface lambs. *Veterinary Parasitology* 60.
- Stear, M.J., Bishop, S.C., Doligalska, M., Duncan, J.L., Holmes, P.H., Irvine, J., McCririe, L., McKellar, Q.A., SINSKI, E., Murray, M., 1995b. Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. *Parasite Immunology* 17, 643–652.
- Stear, M.J., Strain, S., Bishop, S.C., 1999. Mechanisms underlying resistance to nematode infection. *International Journal for Parasitology* 29, 51–56.
- Strain, S.A.J., Bishop, S.C., Henderson, N.G., Kerr, A., McKellar, Q.A., Mitchell, S., Stear, M.J., 2002. The genetic control of IgA activity against *Teladorsagia circumcincta* and its association with parasite resistance in naturally infected sheep. *Parasitology* 124, 545–552.
- Wallace, D.S., Bairden, K., Duncan, J.L., Eckersall, P.D., Fishwick, G., Gill, M., Holmes, P.H., McKellar, Q.A., Murray, M., Parkins, J.J., Stear, M.J., 1998. The influence of dietary supplementation with urea on resilience and resistance to infection with *Haemonchus contortus*. *Parasitology* 116.
- Waller, P.J., 2006. From discovery to development: current industry perspectives for the development of novel methods of helminth control in livestock. *Veterinary Parasitology* 139, 1–14.