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Short communication

Impact of the post-weaning parasitism history on an experimental *Haemonchus contortus* infection in Creole goat kids



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ABSTRACT

Gastrointestinal nematode (GIN) infections have an important negative impact on small ruminant production. The selection of genotypes resistant to these parasitic infections is a promising alternative control strategy. Thus, resistance against GIN is an important component of small ruminant breeding schemes, based on phenotypic measurements of resistance in immune mature infected animals. In this study we evaluated both the impact of the post-weaning parasitism history on the response to an experimental *Haemonchus contortus* infection of resistant and susceptible Creole kids chosen on the basis of their estimated breeding value, and the interaction with the kid's genetic status. During the post-weaning period (from 3 months until 7 months of age) Creole kids were reared at pasture according to four different levels of a mixed rotational stocking system with Creole cattle: 100% (control), 75% (GG75), 50% (GG50), and 25% (GG25) of the total stocking rate of the pasture. The level of infection of the kids decreased significantly at 50% and 25% of the total stocking rate. After the post-weaning period at pasture, at 11 months of age kids were experimentally infected with *H. contortus*. The faecal egg counts (FEC) were significantly lower in the groups showing the highest FEC at pasture. This result suggests that a degree of protection against an experimental *H. contortus* infection occurred during the post-weaning period and was dependant on the level of parasitism. Interestingly, no interaction was observed between this level of protection and the genetic status. In conclusion, the level of post-weaning natural parasitism history at pasture would not influence the genetic status evaluation. More generally our results suggest that it would be better to expose kids to a high level of gastrointestinal parasitism during the post-weaning period in order to increase the basal level of resistance thereafter.

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1. Introduction

In the Tropics, kids and lambs are reared mainly at pasture for meat production. Under such conditions,

gastrointestinal nematode (GIN) infections are a major cause of their morbidity and mortality. Chemotherapy alone is no longer an efficient solution because of the spread of anthelmintic resistance in GIN and the increasing public demand of chemical-free animal products. The philosophy of the different research programs is to develop more sustainable alternative control strategies in order to reach a favourable equilibrium for animal production between host and parasites.

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The genetic selection of resistant genotype is a promising alternative control strategy of GIN in the long term (Bishop and Morris, 2007). Thus, resistance against GIN is an important component of small ruminant breeding schemes which include this trait as an objective. These breeding schemes are often based on phenotypic measurements of resistance in immune mature (i.e. around 8–9 months for kids and 5–6 months for lambs) infected animals. The faecal egg count (FEC) is always measured and according to the schemes other relevant traits related to the pathophysiology of GIN infections (e.g. anaemia, blood eosinophil counts) could be measured (Hunt et al., 2013).

It has been shown that partial protective immunity against GIN in lambs can be observed after vaccination with either live nematodes or specific nematode antigens (Emery et al., 1999, 2000; McClure et al., 1998; Vervelde et al., 2001). These results suggest that in kids and lambs the level of maturation of the immune system is sufficient to generate a partial protective response against GIN infections. Thus, the post-weaning parasitism history of kids and lambs could thereafter influence their response to an experimental or a natural GIN infection for phenotypic measurements of resistance.

Naturally infected tropical pastures allow genetic evaluation of Creole goat resistance to GIN (Mandonnet et al., 2006). The estimated breeding values (EBV) for FEC under natural mixed infection conditions, of the Creole goat flock at INRA-Gardel are routinely calculated. This study was conducted to evaluate both the impact of the post-weaning parasitism history on the response to an experimental *Haemonchus contortus* infection of resistant and susceptible Creole kids, and the interaction with the kid's genetic status.

2. Materials and methods

All animal care, handling techniques, procedures as well as license for experimental infection and blood sampling were approved by INRA, according to the certificate number A-971-18-02 of authorization to experiment on living animals issued by the French Ministry of Agriculture, before the initiation of the experiment.

2.1. Animals, management and experimental design

All kids were born and reared on pasture at INRA-Domaine de Gardel until 7 months of age. The pedigree of each animal was available from the foundation generation of 1979. Here, the EBV for FEC of each kid was calculated on the basis of the phenotypic measurement of its ascendants. The flock grazed all year on irrigated *Digitaria decumbens* pastures contaminated with *H. contortus* (55–75%), *Trichostrongylus colubriformis* (20–40%) and *Oesophagostomum columbianum* (0–10%) (unpublished data from coprocultures regularly performed on “sentinel” animals). Three trials were involved in this study with a total of 169 male kids chosen from 3 successive cohorts of the Creole flock of INRA-Domaine de Gardel. The experimental design at pasture has been previously described (Mahieu, 2013). Briefly, kids were reared at pasture from 3 months (weaning) until 7 months of age in a mixed

rotational stocking system with Creole cattle. Kids were allocated to 4 grazing groups according to the stocking rate based on the metabolic live weight ($LW^{0.75}$): 25% (kids 150 kg $LW^{0.75}$ and cattle 450 kg $LW^{0.75}$), 50% (kids 300 kg $LW^{0.75}$ and cattle 300 kg $LW^{0.75}$), 75% (kids 450 kg $LW^{0.75}$ and cattle 150 kg $LW^{0.75}$) and 100% (kids 600 kg $LW^{0.75}$). For a given plot, the kids grazed for 7 days. The 8th day when the kids left this first plot, the grass available for cattle grazing on each square meter of the pasture is on average 3 times higher on the plot with the 25% kid partial stocking rate compared with the 75% kid partial stocking rate. The cattle balanced out the kid stocking rate by spending more time and grazing more on each square meter of the pasture with the 25% kid partial stocking rate than on the pasture with the 75% goat partial stocking rate. At 7 months of age, FEC of all kids were determined twice at one-week interval. Thereafter, all kids were drenched with levamisole (Polystrongle, Coophavet, Ancenis, France, 8 mg/kg) and placed indoors. During this period, limited nematode infection occurred but without any clinical signs. At 10 months (one month before experimental infection), kids were drenched with ivermectin (Oramec, Merial, Lyon, France, 0.3 mg/kg) and they received *ad libitum* parasite-free *Dichanthium* spp. hay. At 11 months of age, all kids were infected with a single dose of 10,000 *H. contortus* L₃. The FEC, packed cell volume (PCV) and blood eosinophil counts were recorded at 0, 28 and 35 days post-infection (d.p.i.).

2.2. Faecal egg counts (FEC) and blood sampling

To determine FEC, faecal samples of approximately 10 g were collected weekly during experimental infection directly from the rectum of each kid. The faeces were kept in plastic tubes to avoid contamination and immediately transported to the laboratory in refrigerated vials. All samples were individually analyzed using a modified McMaster method for rapid determination and FEC was expressed as the number of eggs/g faeces (Aumont et al., 1997). Blood samples were individually collected once a week by jugular venipuncture from each Creole kid by using disposable syringes and 20-Ga needles. A 2.5-mL portion of each blood sample was placed in commercial anticoagulant tubes (ethylenediamine tetraacetic acid K₃, EDTA tubes; Becton Dickinson, Plymouth, UK). Blood samples previously placed in EDTA coated tubes were used to measure the number of circulating eosinophils according to the method of Dawkins et al. (1989) and counted with a Malassez cell (Fisher Scientific, Illkirch, France). The packed cell volume was measured using the microhaematocrit method (NCCLS, 1993).

2.3. Statistical analysis

Data (FEC, PCV, blood eosinophil counts) were analyzed using PROC MIXED of SAS (v. 9.1, SAS Inst. Inc., Cary, NC, 2003) considering the genetic status, the grazing group, the cohort, the days post-infection and their interactions as fixed effects when significant. The FEC and blood eosinophil counts were log transformed in order to normalize the variances. For all traits, the kid was considered as the

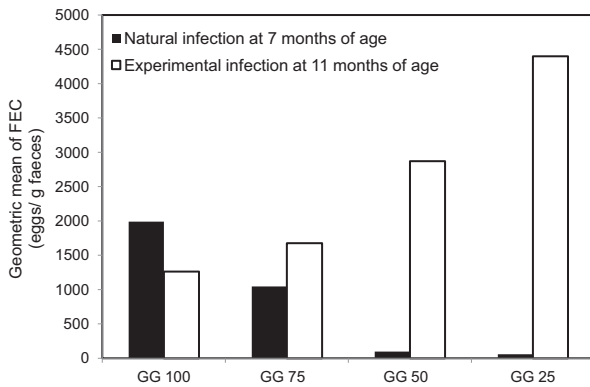


Fig. 1. Geometric means of faecal egg counts (FEC) of kids naturally infected at 7 months of age at pasture by gastrointestinal nematodes then experimentally infected with *Haemonchus contortus* at 11 months of age. Significantly different means are indicated by different superscripted letters ($P < 0.05$). ¹ Kids reared at pasture from 3 months (weaning) until 7 months of age into mixed grazing system with Creole cattle, were allocated in 4 grazing groups according to the stocking rate based on the metabolic live weight ($LW^{0.75}$): 25% (GG25, kids 150 kg $LW^{0.75}$ and cattle 450 kg $LW^{0.75}$), 50% (GG50, kids 300 kg $LW^{0.75}$ and cattle 300 kg $LW^{0.75}$), 75% (GG75, kids 450 kg $LW^{0.75}$ and cattle 150 kg $LW^{0.75}$) and 100% (GG100, kids 600 kg $LW^{0.75}$).

experimental unit and was included in the model as a random effect. Significance was declared at $\leq 5\%$ of probability; comparisons between means were tested by the least squares means procedure with adjustment for multiple comparisons (Tukey–Kramer).

3. Results and discussion

In this study, we first evaluated the impact of the post-weaning parasitism history on the response to an experimental *H. contortus* infection of Creole kids. During the post-weaning period (*i.e.* from 3 to 7 months of age) Creole kids were reared at pasture according to four different levels of a mixed rotational stocking system with Creole cattle. Indeed, previous studies supported the hypothesis that cattle could be used for pasture decontamination because no significant GIN cross-infection was observed between small ruminants (goats and sheep) and cattle either after experimental infection or alternate grazing (Mahieu and Aumont, 2009; Rocha et al., 2008). In accordance with these results, our hypothesis was that the level of GIN infection exposure may vary according to the proportion of kids to cattle grazing in a rotational stocking system. At weaning, Creole kids were allocated to four grazing groups into a mixed rotational stocking system with Creole cattle according to the stocking rate: 25% (25% kids and 75% cattle, GG25), 50% (50% kids and 50% cattle, GG50), 75% (75% kids and 25% cattle, GG75) and 100% (100% kids, GG100). We showed that during the post-weaning period, FEC were significantly higher in GG100 and GG75 compared with GG50 and GG25 (Fig. 1, $P < 0.001$). This result concerning 169 kids of the 757 kids in the experimental design of Mahieu (2013), showed that the rotational stocking system of Creole kids and cattle decreased the level of infection of the kids, measured *via* the FEC, at 50% (GG50) and 25% (GG25) of the total stocking rate. Similar

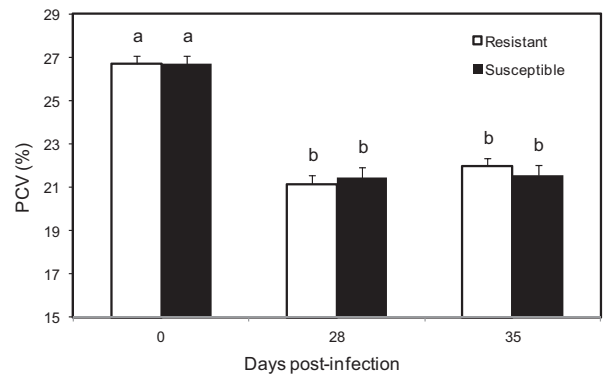


Fig. 2. Mean packed cell volume (PCV) of resistant and susceptible kids experimentally infected with *Haemonchus contortus* at 11 months of age. Significantly different means are indicated by different superscripted letters ($P < 0.05$).

results, showing reduced FEC and improved growth rates in lambs reared in a mixed grazing system with cattle and sheep, were previously found (Marley et al., 2006). After the post-weaning period at pasture, kids were experimentally infected with *H. contortus* at 11 months of age. In contrast with the results observed at pasture, the FEC were significantly lower in GG100 and GG75 compared with GG50 and GG25 (Fig. 1, $P < 0.001$). This result suggests that a degree of protection against an experimental *H. contortus* infection occurred during the post-weaning period and was dependent on the level of parasitism. This result is in keeping with previous vaccination studies showing the capacity of kids and lambs to mount a partial protective immunity (Colditz et al., 1996; Smith and Angus, 1980).

Resistance against GIN infections is measured after experimental or natural challenge of adult animals. Therefore, there is a need to evaluate the interaction between the level of natural GIN infection during the post-weaning period and the response to further natural or experimental infections. In this study, no interaction was observed between this level of protection acquired during the post-weaning period and the genetic status. Indeed, after the experimental infection, susceptible kids had more than 2.5 times higher FEC on average at 28 and 35 d.p.i. than resistant kids had ($P < 0.001$, data not shown). No interaction of the genetic status \times GG was observed ($P > 0.05$).

Blood parameters (*i.e.* PCV and blood eosinophil counts) were also monitored after the experimental infection. The average PCV values decreased significantly irrespective of the genetic status, from 26% at 0 d.p.i. to 21% at 35 d.p.i. ($P = 0.0001$, Fig. 2). No effect of the genetic status nor the GG was observed ($P > 0.05$). Blood eosinophil counts increased significantly irrespective of the genetic status ($P < 0.001$, Fig. 3). At 35 d.p.i. blood eosinophil count was significantly higher in susceptible kids ($P = 0.03$). No effect of the GG was observed. This result supports our previous hypothesis that in Creole goats, blood eosinophil count is a marker of the level of infection (Bambou et al., 2009).

In conclusion, the level of post-weaning natural parasitism history at pasture would not influence the genetic status evaluation. More generally our results suggest that it would be better to expose kids to a high level of

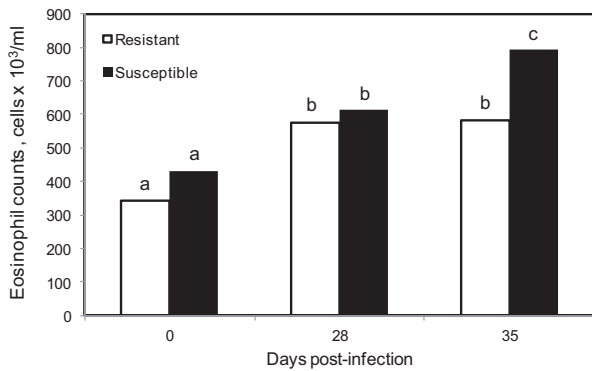


Fig. 3. Geometric mean blood eosinophil counts of resistant and susceptible kids experimentally infected with *Haemonchus contortus* at 11 months of age. Significantly different means are indicated by different super-scripted letters ($P < 0.05$).

gastrointestinal parasitism during the post-weaning period in order to increase the basal level of resistance thereafter.

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