https://doi.org/10.1007/s00436-020-06767-4

Gastrointestinal nematode infections in goats: differences between strongyle fecal egg counts and specific antibody responses to *Teladorsagia circumcincta* in Nera di Verzasca and Alpine goats.

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Abstract

Strongylida are gastrointestinal nematodes (GIN) of greatest importance in small ruminants throughout the world. Differences in resistance and resilience to GIN among goat breeds were reported. This study aims to investigate the mechanism underlying the breed-associated differences using a cosmopolitan (Alpine, AB) and an autochthonous (Nera di Verzasca, NV) goat breed. At first, fifteen goats from the same herd, (NV=7, AB=8), at day 0 were infected with infective larvae (L3) of mixed GIN. From the 15th day post-infection (DPI) individual parasite egg excretion (fecal egg counts, FEC) was performed on all goats, once per week, until the 63rd DPI. Afterwards, in goats under field conditions (30 AB and 30 NV reared on the same farm) individual faecal and blood samples were collected; FEC, specific antibody and PCV levels were explored. In goats with experimental GIN infection, mean eggs per gram of faeces (EPG) values were consistently lower in NV goats. In goats with natural GIN infection, EPG and prevalence values showed high variability in both breeds; among individual variables, breed had a significant influence on EPG. Further, PCV and Anti-*T. circumcincta* IgA levels were influenced by the breed. Lower PCV values were also associated with higher strongyle EPG in AB goats and anti-*T. circumcincta* IgA levels were influenced by both strongyle EPG and breed, with IgA levels being higher in AB vs. NV goats and positively associated with EPG. Neither EPG nor breed had any influence on IgE levels. Both studies on experimental and natural infection confirmed that goats of NV are more resistant to infection with gastrointestinal nematodes.

Keywords

parasites; gastro-intestinal nematodes; *Teladorsagia*; goat; resistance; resilience

1. Introduction

Gastrointestinal nematodes (GINs) infect small ruminants throughout the world and are an important cause of disease and production loss (van Houtert and Sykes, 1996; Waller 1997). The GIN of greatest importance in small ruminants are members of the order Strongylida, and among these, *Teladorsagia circumcincta* is considered one of the most important causative agents of parasitism of the gastrointestinal tract (Zajac 2006). *T. circumcincta* has been identified as the predominant GIN in small ruminant farms in cool temperate areas in Europe or Australia/New Zealand (Chartier and Reche 1992; Chartier et al. 2000; Gallidis et al. 2009; Molina et al. 1997; Vlassoff and McKenna 1994). In addition, *T. circumcincta* showed the highest abundance and prevalence values in goat herd from northern Italy (Manfredi et al. 2010; Zanzani et al. 2014).

As regards the gastrointestinal parasitism, goats can be significantly more heavily infected than sheep, and both the acquisition and expression of immune responses against GINs are less efficient in goats than sheep (Hoste et al. 2010). In goats as well as in other animal species, the distribution of worm populations in the host is aggregated (Hoste et al. 2002). Moreover, faecal egg counts (FEC) can be influenced by the season and the month of the year, as well as by the interaction between these factors and the area of study (Papadopoulos et al. 2003; Di Cerbo et al. 2010; Manfredi et al. 2010). Differences in resistance and resilience to GIN among goat breeds were also reported. Thai native goats were found to be more resistant to *Haemonchus contortus* compared to their Anglo-Nubian crosses, showing lower FEC, lower worm counts and a lower reduction in blood values (packed cell volume (PCV), haemoglobin, total protein, and albumin) (Pralomkarn et al. 1997). Another breed, the Small East African goat, showed more resistant to GIN infections than animals in Galla breed having significantly lower FEC and higher PCV at all sampling times over the reproductive cycle and particularly marked differences over the lactation period (Baker et al., 1998). Again, Nigerian West African Dwarf goats have been shown to be trypanotolerant and to resist infections with *H. contortus* very effectively: that capacity seemed to be immunologically based and genetically endowed (Chiejina and Behnke 2011).

In a previous study, Alpine breed (AB) goats, a cosmopolitan breed, and Nera di Verzasca (NV) goats, an autochthonous breed, reared in a mountain ecosystem of Lombardy, northern Italy, were studied to determine the effects of differing levels of naturally acquired GIN infections on both milk yield and quality. While GIN infections resulted in a reduction in milk yield, protein and fat contents in both breeds, the effects were more pronounced in the AB compared to the more resilient NV (Alberti et al. 2014). To further investigate the mechanism underlying these breed-associated differences, this study focuses on controlled nematode challenge studies, avoiding the influence of differences in feeding behavior, as well as exploring further explanatory variables associated with parasite egg excretion expressed by strongyle FEC under field conditions. Furthermore, differences in parasite-specific antibody and PCV levels were also explored.

2. Materials and methods

2.1.Experimental GIN challenge

2.1.1. Larvae preparation

Third-stage infective larvae of mixed gastrointestinal nematodes were cultured from a pool of fresh faeces of highly infected goats (3000-9000 eggs per gram of faeces (EPG)); faeces were obtained from 5 NV and 5 AB goats reared in the farm described below ("Natural GIN challenge" section). The faeces were collected directly from the animals' rectum and subsequently incubated for ten days at 25°C in a large container to ensure good levels of oxygen and humidity (50-80%). At the end of the coproculture, larvae were recovered by a modified Baermann method, concentrated by a sucrose-based solution and finally washed several times in water at room temperature and before being stored in water 4°C (MAFF 1986). A sub-sample of this suspension was fixed with 2% formalin, and 200 larvae were identified to genus level as described by van Wyk et al. (2004): the most prevalent genus was *Teladorsagia/Trichostrongylus* spp. (85%) followed by *Haemonchus* spp. (15%). Two days before challenge infection, larvae were kept at room temperature to allow their reactivation.

2.1.2. Animals and experiment design

Fifteen goats from the same herd, matched for age and production stage (2-3 years old, non-lactating and non-pregnant) were used in this study. Animals were divided into two groups according to the breed: NV breed (n=7) and AB (n=8). For the duration of the study the two breeds were housed separately in two different pens without access to pasture, and no contact was possible between the two breeds. Both groups were fed with grass hay supplemented with pelleted concentrate (dry matter: 87%; crude protein: 17.2%; crude fiber: 6%; crude fat: 4%; sodium: 0.7%). At the arrival, 14 days prior to challenge, the goats were treated with netobimin (Hapadex, MSD Animal Health S.r.l, Segrate, Italy) at the dose of 15 mg/kg to remove pre-existing nematodes. Three days later the goats were moved into two new pens with clean straw. On the day of challenge, individual strongyle FEC were performed to confirm the efficacy of the anthelmintic treatment: all fifteen goats scored negative to strongyle eggs. After that, at day 0, all the goats were infected *per os* with ~6000 infective larvae (L3) of mixed GIN. From the 15th day post-infection (DPI) individual FEC was performed on all goats, once per week, until the 63rd DPI. All faecal egg counts were performed using the FLOTAC double technique with NaCl flotation solution (s.g. 1.200) (Cringoli et al., 2010).

2.1.3. Statistical analysis

A general linear model (GLM) for repeated measures with normal distribution was run to verify differences between EPG values (logarithmically transformed) at different DPI registered in NV and AB. Breed (binomial categorical variable: NV, AB) was considered as the independent variable. Five repeated measurements of EPG were considered: 15 DPI, 36 DPI, 43 DPI, 50 DPI, and 60 DPI. If present, any influential values would be removed from the analysis. The sphericity assumption and the homoscedasticity were verified through the Mauchly's test (X^2 = 14.580, *p*-value = 0.111) and Levene's test (*p*-value>0.05 in all groups). Statistical analysis was performed with SPSS (version 20.0; SPSS, Chicago, IL).

2.2. Natural GIN challenge

2.2.1. Study population and sample collection

The study was carried out on 60 adult female goats of two different dairy breeds: 30 goats of AB and 30 goats of NV breed reared on the same farm, located at 980 m of altitude (Province of Varese, northern Italy). Goats were reared in a semi-extensive way as follows: they were all kept in the fold (a paddock near the farm) from December to March, fed with hay *ad libitum* and an increasing concentrate supplementation. During March-November they were free to graze and browse in a large mountain area (~200 ha) from 900 to 1550 m a.s.l.; they grazed during the day in cooler months (March-June and September-November) and during the night in hotter months (July-August). Kidding occurred from January to March.

Individual faecal samples taken directly from the rectum were collected on a monthly basis from January to December 2013. Blood samples were collected at the same time (except in June) by jugular venepuncture using a plain and an EDTA-containing vacutainers (BD, Franklin Lakes, NJ, USA) for serum samples and haematology, respectively. Serum samples were obtained by centrifugation of clotted samples at 2120 × *g* for 15 min and serum stored at −20◦C before use. EDTA-blood was stored at 4°C prior to packed cell volume (PCV) analysis which was performed within 8 hours of collection. An anthelmintic treatment (netobimin 15 mg/kg b.w.) was administered to the whole herd in November 2012.

2.2.2. Coprological analysis

Strongyle FEC were performed using FLOTAC double technique with NaCl flotation solution (s.g. 1.200) (Cringoli et al. 2010) and expressed as EPG. Fecal samples collected in April from both breeds were pooled and cultured to evaluate the composition of the gastrointestinal helmintofauna in the studied flock: faeces were incubated for ten days at 25°C in a large vessel, ensuring adequate moisture (50- 80%); third stage larvae (L3) were isolated by Baermann technique. The first 100 randomly selected L3 were identified to the generic level according to van Wyk et al. (2004).

2.2.3. Packed cell volume (*PCV) analysis*

PCV was determined on all EDTA-blood samples using an Abbott Cell-DYN 3500 Hematology Analyzer (Abbott Laboratories, Abbott Park, IL, USA) and expressed as % total blood volume.

2.2.4. Quantification of Teladorsagia circumcincta-specific IgA and IgE in serum

Serum levels of *Teladorsagia circumcincta*-specific immunoglobulin (Ig) A and IgE were determined on blood samples collected during the months (April to December) where the goats are more infested with GIN. An indirect ELISA was performed as follows: ELISA plates (Immulon 2HB, thermoelectron 3455) were incubated overnight at 4°C with *T. circumcincta* somatic L3 antigen (2µg/ml) in 0.1M carbonate buffer pH 9·6. After washing five times in PBS containing 0.05% Tween 20 (PBS/T20), blocking buffer (PBS + 3% fish gelatine, Sigma-Aldrich G7765) was added to each well and plates incubated for 1 hr at 37°C. Plates were subsequently incubated with serum samples diluted in PBS containing 0.5% Tween 80 and 0.5 M NaCl (PBS/T80) for 1 hr at 37°C. After washing in PBS/T20, plates were incubated for 1 hr at 37°C with either mouse anti-ovine/bovine IgA (clone K84 2F9, AbDSerotec) diluted 1:1000 in PBS/T80 or anti-sheep/goat IgE (clone 2F1, culture supernatant, Moredun Research Institute) diluted 1:100 in PBS/T80. After further washing plates were incubated for 1 hr at 37°C with rabbit anti-mouse Ig-HRP (Dako P0260) diluted 1:1000 in PBS/T80. After a final wash in PBS/T20, colour reactions were developed with ortho-phenylenediamine (OPD) substrate (SigmaFast OPD, P9187) room temperature for 5-10 minutes. Reactions were stopped by the addition of 2.5M H_2SO_4 and the optical density at 492nm (OD@492) measured using a Sunrise™ microplate reader (Tecan, Männedorf, CH, Switzerland). The optimum dilution of serum samples was determined following serial two-fold dilutions from 1:5 to 1:2560 in order to ensure OD@492 values were within the linear part of the reaction curve: optimal dilutions were found to be 1:20 and 1:10 for IgA and IgE ELISAs, respectively. For each plate, a known positive control sample was analysed to normalise ODs between plates.

2.2.5. Statistical analysis

Prevalence, abundance, and intensity of strongyle infection were calculated within considered categories (breed, the month of sampling) according to Bush et al. (1997).

The effect of individual characteristics on strongyle EPG was evaluated through a generalized linear mixed model (GLMM): breed (binomial categorical variable: NV, AB), number of kidding (binomial categorical variable: primiparous, multiparous), days of lactation (continuous variable), month of delivery (categorical variable: January, February, March) were considered as independent variables. Since strongyle EPG showed an aggregated distribution, statistical model was performed with a negative binomial error distribution and log link-function.

The effect of strongyle EPG on selected haematological and biochemical data was subsequently considered. Separate GLMMs (normal distribution, log function) were run using PCV, IgA, and IgE as dependent variables, and strongyle EPG (continuous variable), breed and their interaction as independent variables.

Goat ID and month of sampling were entered in all GLMMs as nested random effects. Final models were developed with backward elimination considering the goodness of fit with the Akaike information criterion corrected (AICC). Statistical analysis was performed with SPSS (version 20.0; SPSS, Chicago, IL).

3. Results

3.1.Part 1: experimental GIN infection

EPG data is shown in Fig. 1. Starting from the 15 DPI, egg excretion increased in both breeds at a relatively constant rate, reaching a peak at the end of the study at 63 DPI. Mean EPG values were consistently lower in NV goats throughout the experiment (Fig. 1).

GLM for repeated measures showed significant differences with DPI (df=4, MS=40.846, F=78.552, *p*-value<0.0001) and among the interaction DPI×breed (df=4, MS=1.506, F=2.896, *p*-value=0.035), reflecting the increase in EPG over time and a difference in the EPG dynamic between the two breeds. This was further explored using contrast analysis of each DPI level which indicated that EPG at each DPI was statistically different to the previous time-point (p=0.001) with the exception of the first and second sampling time-points (15 DPI vs. 36 DPI, p=0.216), reflecting a significant

increase in EPG over time. Furthermore, a significant DPI×breed interaction effect was only seen with the 36 DPI vs. 43 DPI contrast (*p*-value=0.02) which reflected an earlier rise in EPG in the NV breed at 36 DPI (Fig 1).

3.2.Part 2. Natural GIN infection

Mean EPG data during natural GIN infection is shown in Fig. 2. A first peak in EPG values was reached in April in both breeds; subsequently, EPG values fell in the NV goats while they remained high in the AB goats until August, when in both breeds EPG values decreased to remain low until the end of the study period (Fig. 2).

At the beginning of the study, larval culture showed that *Teladorsagia circumcinca/Trichostrongylus* spp. were the most common GINs in the studied flock, representing 85% of baermannized L3; the remaining 15% was *Haemonchus contortus*.

EPG and prevalence values showed high variability in both breeds; in AB goats the mean EPG values over the course of the study were more than twice as high as in NV goats, being 818.9 (SD=1,415.97) and 334.40 (SD=663.65) respectively. Further, AB goats showed a higher maximum EPG value (10452) compared to NV goats (4300). During the year of sampling, minimum and maximum prevalence values were 68.75-100% in AB and 51.52-100% in NV goats.

The influence of individual variables on strongyle EPG was evaluated. In the first GLMM, all the investigated individual variables (breed, parity, days of lactation, and month of kidding) were associated with strongyle EPG (Table 1). Breed had a significant effect on EPG, with significantly higher EPG in AB compared to NV goats. EPG was also significantly higher in primiparous compared to multiparous goats, increased within increasing days of lactation and was higher in goats that kidded in March compared to goats that kidded in January and February (Table 1).

Subsequently, PCV levels, and *T. circumcincta* serum antibodies (IgA and IgE) were considered in the two breeds within the study period (Fig 3, Fig 4). PCV and IgG levels were higher in NV than AB goats, while IgA mean values were higher in AB than NV goats. In the following GLMMs, the

effects of strongyle infections on PCV, IgA, IgE were evaluated. PCV values were influenced by the interaction of strongyle EPG with breed, with lower PCV values associated with higher strongyle EPG in AB but not NV goats. Anti-*T. circumcincta* IgA levels were influenced by both strongyle EPG and breed, with IgA levels being higher in AB vs. NV goats and positively associated with EPG. Neither EPG nor breed had any influence on IgE levels. Table 2 resumed the results obtained in the GLMMs, while in Fig 5 scatter plots of strongyle EPG against PCV, IgA and IgE in AB and NV goats were represented.

4. Discussion

Parasitic infections affect small ruminants worldwide and, mainly in grazing flocks, several of them are probably not eradicable. In northern Italy, the threat of parasitism in grazing ruminants involves both public health and animal production (Manfredi et al. 2011). Parasitic infections can be influenced by several risk factors, such as management, geospatial determinants and individual features (Di Cerbo et al. 2010; Manfredi et al. 2010). Particularly, in dairy goats from northern Italy, a daily milk yield strongly related to GINs burden was observed, with significant differences between cosmopolitan breeds and the autochthonous breed NV (Alberti et al. 2012; 2014).

Both studies on experimental and natural infection confirmed that goats of NV are more resistant to infection with gastrointestinal nematodes. Furthermore, it was demonstrated that these differences in resistance were in part due to inherent differences between the two breeds and not purely related to differences in grazing behaviour or parasite exposure, as the results were replicated in a controlled GIN challenge study in which all the goats were on the same diet and received the same GIN challenge.

PCV percentage is generally used to estimate the levels of anaemia as a result of parasitism by hematophagous strongyles such as *H. contortus*, although more subtle effects have been observed in experimental infection in kids by *T. circumcincta* (Richard and Cabaret 1993).

Indeed, PCV has been used to evaluate the resistance/resilience of small ruminants to GIN (Saddiqui et al. 2012). In the current study PCV, was negatively correlated with FEC under natural infections. PCV was also influenced by breed, being higher in NV than AB goats. Helminth infections in surveyed goats caused a significant reduction of PCV levels during the peaks of strongyle egg output which occurred in two periods (summer and autumn) when a higher number of infective larvae are available at pastures. The PCV decrease during GIN infection was likely due to the presence of *H. contortus* as confirmed by coprocultures of pooled faeces of the goat herd collected from both breeds at the start of the blood sampling period, with *H. contortus* representing 15% of the GIN species within the pool.

As regards the humoral immune response against *T. circumcincta* in the two goat breeds with natural infection, variations were found in antibody levels over time that may be related to the seasonality of parasite life cycle in the study area, where several generations of *T. circumcincta* can develop during a grazing season. Consequently, the increasing and decreasing of antibody levels could reflect the variation in the quantities of ingested infective larvae. Particularly, in AB, IgA was greatest in August and September when the EPG is lowest; this could potentially indicate that IgA is positively associated with protection in this breed. In contrast IgA levels were fairly consistent over the study period in NV (Fig 4).

In sheep higher levels of IgA and IgE are usually associated with higher resistance to GIN and subsequent lower FEC. For example, in Caribbean hair sheep, the infection with *H. contortus* was characterised by higher IgA and IgE levels and lower FEC than in conventional wool lambs (MacKinnon et al. 2010). Henderson and Stear (2006) have shown that in sheep variation in parasite-specific IgA and eosinophilia responses are strongly negatively correlated with *T. circumcincta* adult worm length. In the other hand, an association between high plasma IgE activity against a major surface allergen of *T. circumcincta* L3 and low FEC was demonstrated in naturally infected crossbred lambs (Huntley et al. 2001). IgA and eosinophils are also associated with regulation of worm size and fecundity (Stear et al. 1995; Stear et al. 2002).

Scarce data on this topic are available for goats. De la Chevrotière et al. (2012) showed that in Creole goats IgA response against L3 or excretory/secretory products of adults *H. contortus* was positively correlated to FEC, in contrast with the negative correlation between IgE against L3 of *H. contortus* and FEC, that seemed to be one key point of the immune response for development of resistance to gastrointestinal nematode infections in Creole goats. In the study on natural infection, in the Alpine goats, higher IgA levels were found throughout the sampling period, probably related to the greater parasite burden expressed by FEC. The drop in FEC in the months of August and September seemed to be associated with increased IgA and IgE circulating levels. After that period, EPG rose again, suggesting that a turnover of the nematode population took place, despite the increased antibody levels. The natural decrease in FEC in August and September seems suggestive of a self-cure phenomenon caused by the high intake of GIN L3 in the previous months. Indeed, in August the egg excretion was reduced by 83.3% in AB and by 90.6% in NV goats when compared with July EPG. Similarly, Uriarte et al. (2003) in GIN infected ewes from NE Spain hypothesized that this phenomenon appeared to occur by the end of August when egg excretion was reduced by 70%. However, a few authors affirmed that the reduced larval establishment and expulsion of adult worms are rarely observed in goats (Hoste et al. 2010). We could therefore assume that plants containing natural anthelmintics were selectively ingested by goats and control of parasite infections was so achieved leading a sharp drop of EPG (Villalba et al. 2014).

The NV goats gave evidence of a more effective response to nematodes than the other breed, as visible from the lower FEC almost throughout the sampling period. Moreover, this response was more persistent. In fact, after the earlier decrease in the EPG level, no second rising in FEC was observed. Therefore, the higher resistance to trichostrongyle nematode infection seen in the NV breed does not appear to be related to circulating levels of *T. circumcincta*-specific IgA or IgE against L3 stage (Fig 4). Similar results, but on *H. contortus*, were found in the resistant breed of sheep Santa Ines that showed lower FEC and lower IgA in abomasal mucus than Suffolk and Ile de France sheep (Amarante et al. 2005).

Thus, there are still some hypothesis to verify in order to understand better the mechanisms involved in the immune response against *T. circumcincta* in goats and NV breed. As no significant role either of IgA or IgE directed against *T. circumcincta* L3 crude antigen was found in the more resistant breed, it is possible that different parasite antigens are targeted between the resistant and the susceptible breed. Another hypothesis is that different results could be achieved if antibody responses against different stages of the parasite (L_4, L_5 (immature adult stage) or adult parasites) were analysed. In fact, it is demonstrated that "each parasite stage expresses a unique antigen profile and could, therefore, be considered as a distinct antigenic organism in the context of the host's immune response" (Meeusen et al. 2005).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

The collection of biological samples from live animals were performed in the respect of animal welfare according to current legislation. The study was conducted with the approval of the Institutional Animal Care and Use Committee of Università degli Studi di Milano (Permission OPBA_34_2013).

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Alberti EG, Zanzani SA, Ferrari N, Bruni G, Manfredi MT (2012) Effects of gastrointestinal nematodes on milk productivity in three dairy goat breeds. Small Rum Res 106:S12-S17. https://doi.org/10.1016/j.smallrumres.2012.04.027

Alberti EG, Zanzani SA, Gazzonis AL, Zanatta G, Bruni G, Villa M, Rizzi R, Manfredi MT (2014) Effects of gastrointestinal infections caused by nematodes on milk production in goats in a mountain ecosystem: comparison between a cosmopolite and a local breed. Small Rum Res 120:155–163. https://doi.org/10.1016/j.smallrumres.2014.04.017

Amarante AFT, Bricarello PA, Huntley JF, Mazzolin LP, Gomes JC (2005) Relationship of abomasal histology and parasite-specific immunoglobulin A with the resistance to *Haemonchus contortus* infection in three breeds of sheep. Vet Parasitol 128:99–107. https://doi.org/10.1016/j.vetpar.2004.11.021

Baker RL, Mwamachi DM, Audho JO, Aduda EO, Thorpe W (1998) Resistance of Galla and Small East African goats in the sub-humid tropics to gastrointestinal nematode infections and the peri-parturient rise in faecal egg counts. Vet Parasitol 79:53–64. https://doi.org/10.1016/S0304-4017(98)00151-4

Bush AO, Lafferty KD, Lotz JM, Shostak AW (1997) Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83:575-83.

Chartier C, Reche B (1992) Gastrointestinal helminthes and lungworms of French dairy goats: prevalence and geographical distribution in Poitou-Charentes**.** Vet Res Commun 16:327-355

Chartier C, Etter E, Hoste H, Pors I, Mallereau M-P, Broqua C, Mallet S, Koch C, Massé A (2000) Effects of the initial level of milk production and of the dietary protein intake on the course of natural nematode infection in dairy goats. Vet Parasitol 92: 1-13

Chiejina SN, Behnke JM (2011) The unique resistance and resilience of the Nigerian West African Dwarf goat to gastrointestinal nematode infections. Parasit Vectors 4 :12. https://doi.org/10.1186/1756-3305-4-12

Cringoli G, Rinaldi L, Maurelli MP, Utzinger J (2010) FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc 5 :503–515. https://doi.org/10.1038/nprot.2009.235

de la Chevrotière C, Bambou J-C, Arquet R, Jacquiet P, Mandonnet N (2012) Genetic analysis of the potential role of IgA and IgE responses against *Haemonchus contortus* in parasite resistance of Creole goats. Vet Parasitol 186:337–343. https://doi.org/10.1016/j.vetpar.2011.11.071

Di Cerbo AR, Manfredi MT, Zanzani S, Stradiotto K (2010) Gastrointestinal infection in goat farms in Lombardy (Northern Italy): analysis on community and spatial distribution of parasites. Small Rum Res 88:102–112. https://doi.org/10.1016/j.smallrumres.2009.12.017

Fraquelli C, Zanzani SA, Gazzonis AL, Rizzi R, Manfredi MT (2015) Effects of condensed tannin on natural coccidian infection in goat kids. Small Rum Res 126:19-24. https://doi.org/10.1016/j.smallrumres.2015.01.019

Gallidis E, Papadopoulos E, Ptochos S, Arsenos G (2009) The use of targeted selective treatments against gastrointestinal nematodes in milking sheep and goats in Greece based on parasitological and performance criteria. Vet Parasitol 164:53–58. https://doi.org/10.1016/j.vetpar.2009.04.011

Gazzonis AL, Veronesi F, Di Cerbo AR, Zanzani SA, Molineri G, Moretta I, Moretti A, Piergili Fioretti D, Invernizzi A, Manfredi MT (2015) *Toxoplasma gondii* in small ruminants in Northern Italy – prevalence and risk factors. Ann Agric Environ Med 22:62–68. https://doi.org/10.5604/12321966.1141370

Gazzonis, AL, Alvarez Garcia, G, Zanzani SA, Ortega Mora L, Invernizzi A, Manfredi MT (2016) *Neospora caninum* infection in sheep and goats from north-eastern Italy and associated risk factors. Small Rum Res 140:7-12. https://doi.org/10.1016/j.smallrumres.2016.05.010

Henderson NG, Stear MJ (2006) Eosinophil and IgA responses in sheep infected with *Teladorsagia circumcincta*. Vet Immunol Immunopathol 112:62–66. https://doi.org/10.1016/j.vetimm.2006.03.012

Hoste H, Le Frileux Y, Goudeau C, Chartier C, Pors I, Broqua C, Bergeaud JP (2002) Distribution and repeatability of nematode faecal egg counts in dairy goats: a farm survey and implications for worm control. Res Vet Sci 72:211–215. https://doi.org/10.1053/rvsc.2002.0546

Hoste H, Sotiraki S, Landau SY, Jackson F, Beveridge I (2010) Goat–Nematode interactions: think differently. Trends Parasitol 26: 376-381. https://doi.org/10.1016/j.pt.2010.04.007

Huntley JF, Redmond J, Welfare W, Brennan G, Jackson F, Kooyman F, Vervelde L (2001) Studies on the immunoglobulin E responses to *Teladorsagia circumcincta* in sheep: purification of a major high molecular weight allergen. Parasite Immunol 23:227–235.

MacKinnon KM, Zajac AM, Kooyman FNJ, Notter DR (2010) Differences in immune parameters are associated with resistance to *Haemonchus contortus* in Caribbean hair sheep. Parasite Immunol 32:484–493. https://doi.org/10.1111/j.1365-3024.2010.01211.x

Ministry of Agriculture, Fisheries and Food (MAFF) (1986) Manual of Veterinary Parasitological Laboratory Techniques. 3 rd ed. Her Majesty's Stationary Office (HMSO), London, 160 pp.

Manfredi MT, Di Cerbo AR, Zanzani S, Stradiotto K (2010) Breeding management in goat farms of Lombardy, northern Italy: risk factors connected to gastrointestinal parasites. Small Rum Res 88:113–118. 10.1016/j.smallrumres.2009.12.018

Manfredi MT, Di Cerbo AR, Zanzani S, Meriggia A, Fattori D, Siboni A Bonazza V, Filice C, Brunetti E (2011) Prevalence of echinococcosis in humans, livestock and dogs in northern Italy. Helmintologia 48:59-66. https://doi.org/10.2478/s11687-011-0011-9

Meeusen ENT, Balic A, Bowles V (2005) Cells, cytokines and other molecules associated with rejection of gastrointestinal nematode parasites. Vet Immunol Immunopathol 108:121–125. https://doi.org/10.1016/j.vetimm.2005.07.002

Molina JM, Gutierrez AC, RodriguezPonce E, Viera JA, Hernandez S (1997) Abomasal nematodes in goats from the subtropical island of Grand Canary (Spain). Vet Res 28:259–270.

Papadopoulos E, Arsenos G, Sotiraki S, Deligiannis C, Lainas T, Zygoyiannis D (2003) The epizootiology of gastrointestinal nematode parasites in Greek dairy breeds of sheep and goats. Small Rum Res 47:193–202. https://doi.org/10.1016/S0921-4488(02)00258-4

Pralomkarn W, Pandey VS, Ngampongsai W, Choldumrongkul S, Saithanoo S, Rattaanachon L, Verhulst A (1997) Genetic resistance of three genotypes of goats to experimental infection with *Haemonchus contortus*. Vet Parasitol 68:79–90. https://doi.org/10.1016/S0304-4017(96)01073-4

Richard S, Cabaret J (1993) Primary infection of kids with *Teladorsagia circumcincta* - susceptibility and blood-constituents. Vet Parasitol 47: 279-287.

Saddiqi HA, Sarwar M, Iqbal Z, Nisa M, Shahzad MA (2012). Markers/parameters for the evaluation of natural resistance status of small ruminants against gastrointestinal nematodes. Animal 6: 994–1004.

Stear MJ, Bishop SC, Doligalska M, Duncan JL, Holmes PH, Irvine J, McCririe L, McKellar QA, Sinski E, Murray M (1995). Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. Parasit Immunol 17: 643-652

Stear MJ, Henderson NG, Kerr A, McKellar QA, Mitchell S, Seeley C, Bishop SC (2002). Eosinophilia as a marker of resistance to *Teladorsagia circumcincta* in Scottish Blackface lambs. PARASITOLOGY 124:553-560

Uriarte J, Llorente MM, Valderrábano J (2003) Seasonal changes of gastrointestinal nematode burden in sheep under an intensive grazing system. Vet Parasitol 118: 79–92

van Wyk JA, Cabaret J, Michael LM (2004) Morphological identification of nematode larvae of small ruminants and cattle simplified. Vet Parasitol 119:277-306

Van Houtert MF, Sykes AR (1996) Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. Int J Parasitol 26:1151-1167

Villalba JJ, Miller J, Ungar ED, Landau SY, Glendinning J (2014) Ruminant self-medication against gastrointestinal nematodes: evidence, mechanism, and origins. Parasite 21: 31

Vlassoff A, McKenna PB (1994) Nematode parasites of economic importance in sheep in New Zealand. New Zeal J Zool 21:1-8. https://doi.org/10.1080/03014223.1994.9517971

Waller PJ (1997) Sustainable helminth control of ruminants in developing countries. Vet Parasitol 71:195–207

Zajac AM (2006) Gastrointestinal nematodes of small ruminants: life cycle, anthelmintics, and diagnosis. Vet Clin North Am Food Anim Pract 22:529–541. https://doi.org/10.1016/j.cvfa.2006.07.006

Zanzani SA, Gazzonis AL, Di Cerbo A, Varady M, Manfredi MT (2014). Gastrointestinal nematodes of dairy goats, anthelmintic resistance and practices of parasite control in Northern Italy. BMC Vet Res 10:114. https://doi.org/10.1186/1746-6148-10-114

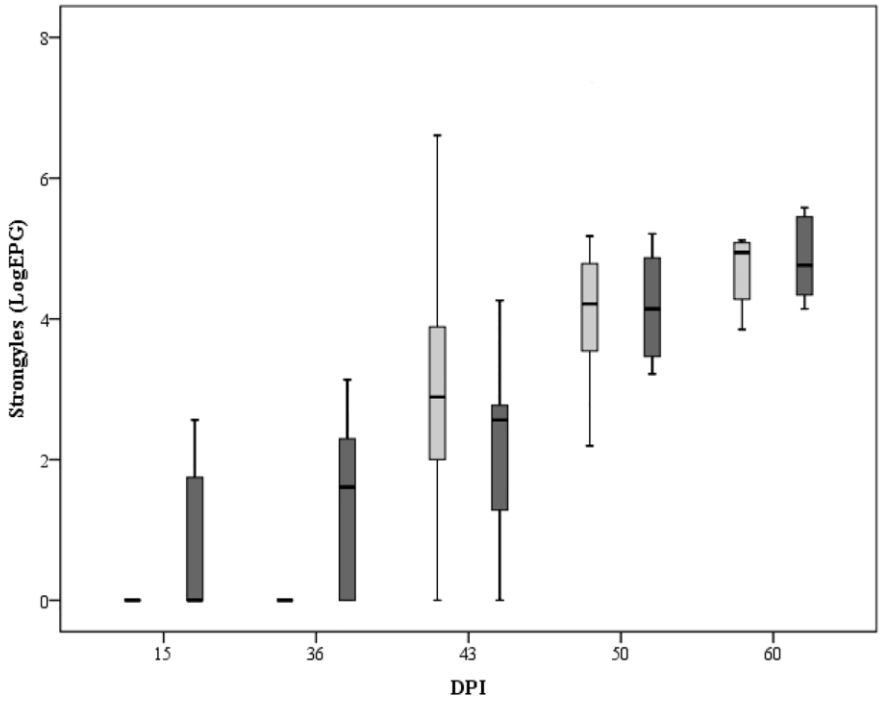

Fig 1. Distribution of gastrointestinal nematode EPG expressed by log of two goat breeds throughout a 63-days study period. Box-plot with logEPG mean and whiskers to minimum and maximum of the of the EPG values; outliers (°) and extreme values (*) are reported. In grey Alpine EPG and in black Nera di Verzasca EPG.

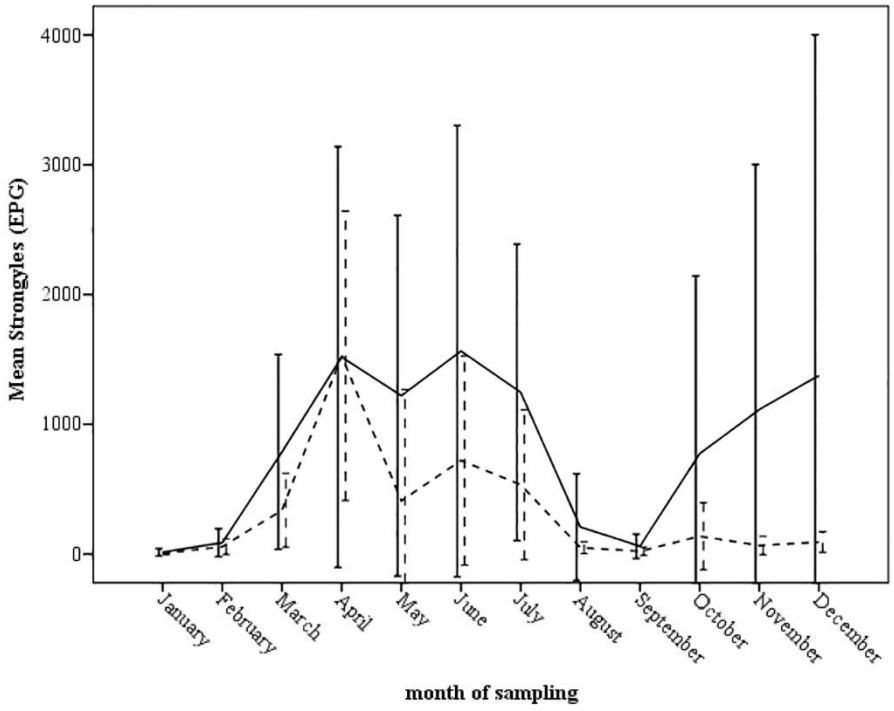

Fig 2. Distribution of EPG mean values according to sampling in two goat breeds (Alpine: solid line, Nera di Verzasca: dashed line) naturally infected by gastrointestinal nematodes. Whiskers represent the standard deviation of the mean.

Table 1. Variables affecting EPG (eggs per gram) of gastrointestinal nematodes in naturally infected Nera di Verzasca (NV) and Alpine (AB) goats.

Variable	Nr of goats	Prevalence: positive/examined	EPG Abundance (SD)	EPG Intensity (SD)	Min-max	F	DF	β±SE	Exp(β)(95 % CI)	*p*-value
Intercept								1.961±0.31		0.0001
Breed						20.929	1			0.0001
AB	37	289/330 (87.6%)	818.59 (1415.81)	934.72 (1476.805)	0-10452			0.825±0.18	2.283 (1.602-3.253)	0.0001
NV (reference)	34	309/352 (87.8%)	334.28 (663.66)	380.8 (695.823)	0-4300			0	1	
Number of kidding						63.394	1			0.0001
Primiparous	16	111/139 (79.9%)	1008.76 (1709.71)	1263.23 (1828.335)	0-10452			2.262±0.284	9.598 (5.494-16.765)	0.0001
Multiparous (reference)	55	481/537 (89.6%)	459.63 (880.93)	513.14 (916)	0-7884			0	1	
Days of lactation (continuous variable)						139.257	1	0.01±0.001	1.01 (1.008-1.012)	0.0001
Month of delivery						3.411	2			0.034
January	40	348/389 (89.5%)	489.6 (936.39)	547.29 (974.054)	0-7884			-0.64±0.25	0.527 (0.323-0.862)	0.011
February	11	105/125 (84%)	403.62 (696.47)	480.5 (735.564)	0-4044			-0.624±0.283	0.536 (0.308-934)	0.028
March (reference)	11	99/105 (94.3%)	1124.3 (1778.23)	1192.44 (1809.387)	0-10452			0	1	

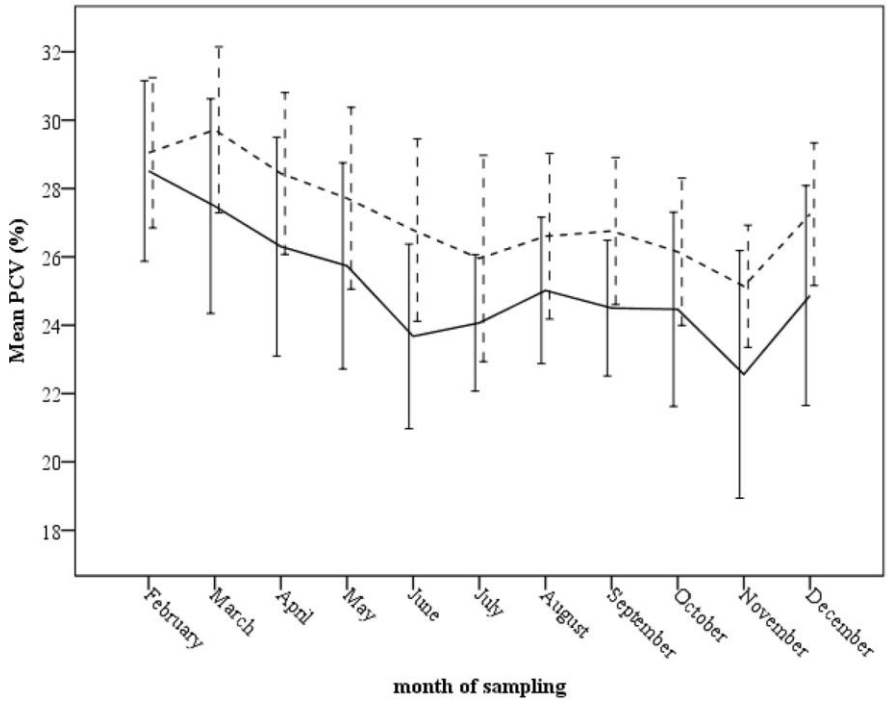

Fig 3. Distribution of PCV mean values according to sampling in two goat breeds (Alpine, AB: solid line, Nera di Verzasca, NV: dashed line) naturally infected by gastrointestinal nematodes. Whiskers represent the standard deviation of the mean.

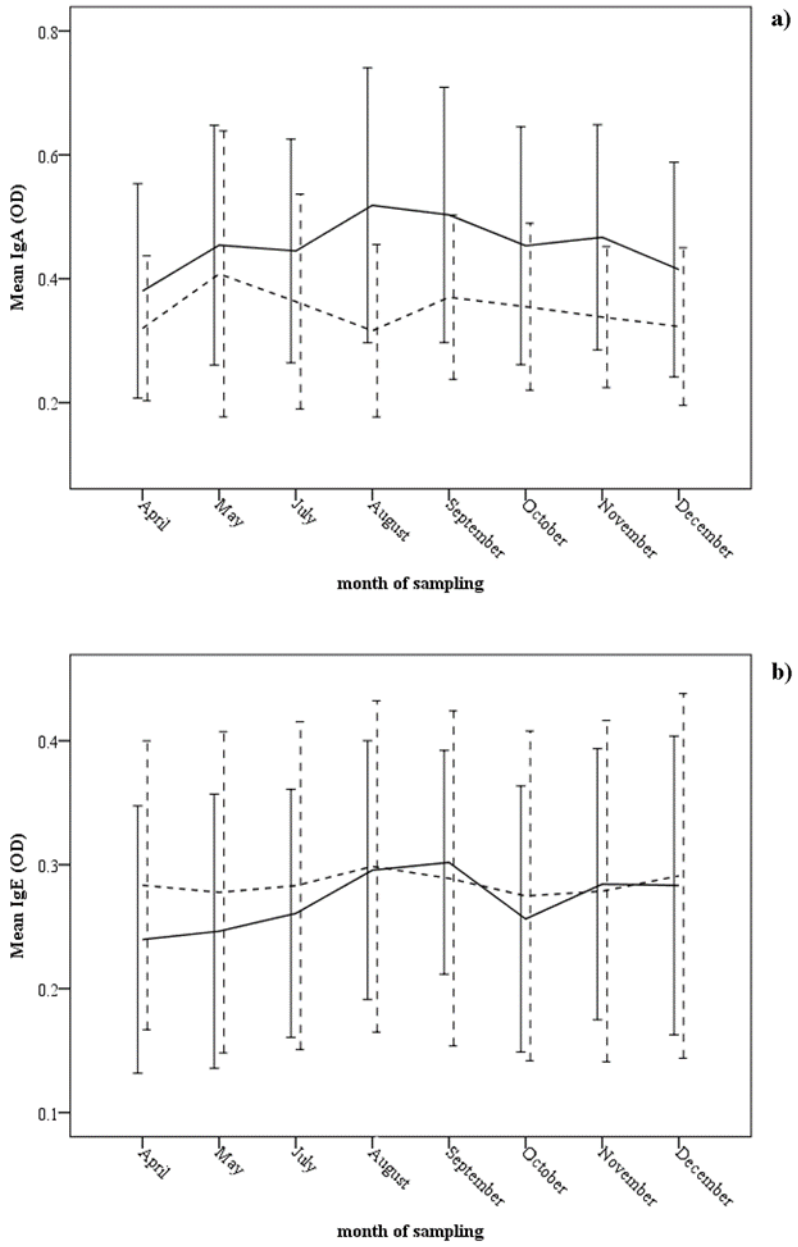

Fig 4. Distribution of IgA (a) and IgE (b) mean values according to sampling in two goat breeds (Alpine, AB: solid line, Nera di Verzasca, NV: dashed line) naturally infected by gastrointestinal nematodes. Whiskers represent the standard deviation of the mean.

Table 2. Influence of strongyle EPG values on *T. circumcincta*-specific IgA and PCV in naturally infected Alpine (AB) and Nera di Verzasca (NV) goats (Generalized Linear Mixed models, normal distribution, log function).

Dependent variable	**Independent var.**	**F**	**DF**	**β±SE**	**p-value**
IgA	**Breed**	5.688	1		
	AB			0.246±0.093	0.008
	NV			0	
	Strongyle EPG	5.144	1	-0.0001±0.0001	0.024
PCV	**Breed**	10.395	1		0.001
	AB			-0.065-0.020	0.001
	NV			0	
	Strongyle EPG	2.177	1	0.0001±0.0001	0.141
	Breed×strongyle EPG	6.412	1		0.012
	AB			-0.0001±0.0001	
	NV			0	

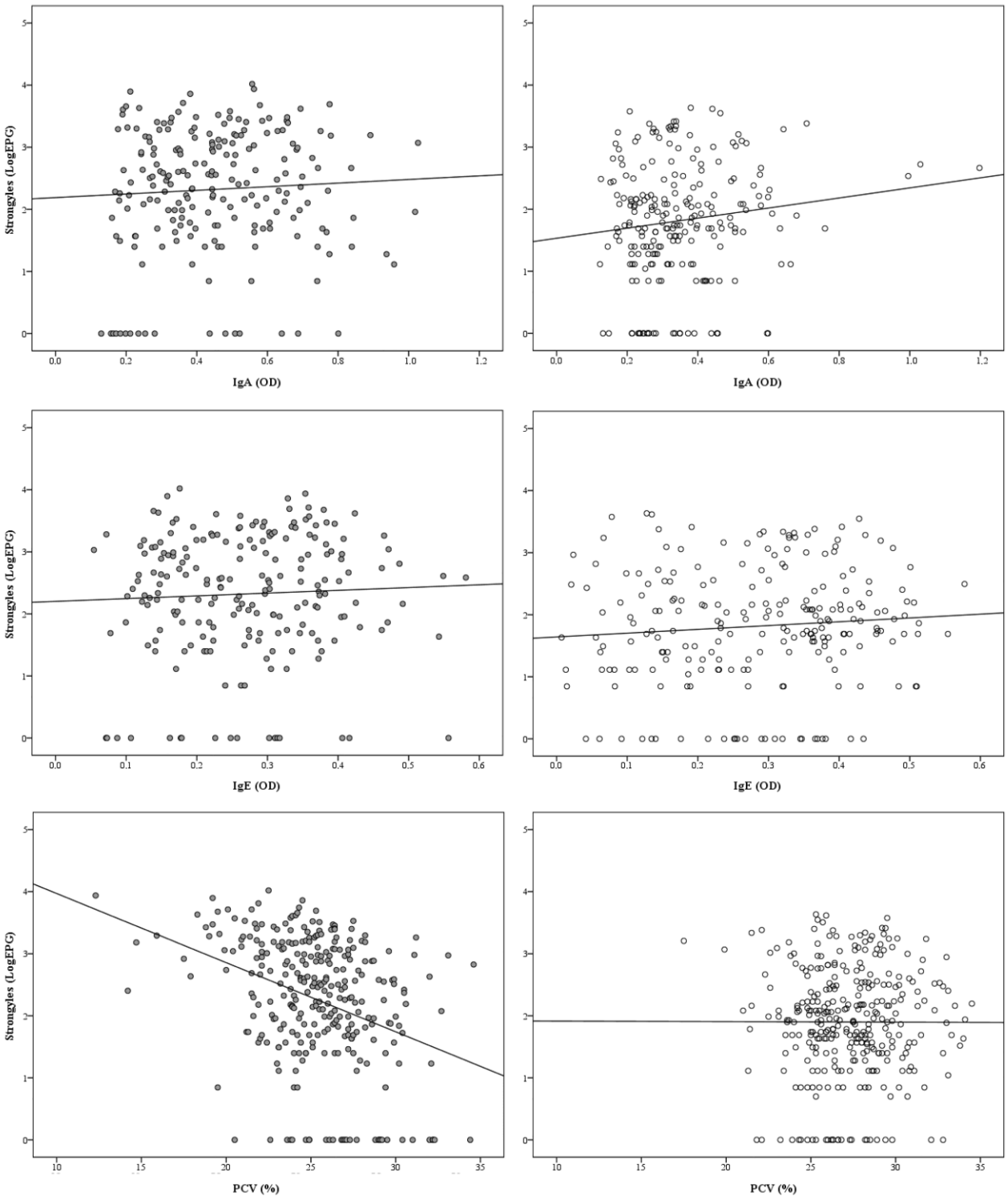

Fig 5. Scatter plots showing correlation of IgA, IgE and PCV means values with logEPG in two goat breeds naturally infected by gastrointestinal nematodes (black circle Nera di Verzasca breed, gray dot Alpine breed).