UNIVERSITÀ DEGLI STUDI DI MILANO

SCUOLA DI DOTTORATO IN SANITÀ E PRODUZIONI ANIMALI: SCIENZA, TECNOLOGIA E BIOTECNOLOGIE

DIVET - DIPARTIMENTO DI SCIENZE VETERINARIE E SANITÀ PUBBLICA

CORSO DI DOTTORATO IN IGIENE VETERINARIA E PATOLOGIA ANIMALE – XXV CICLO

Tesi di Dottorato di Ricerca

INVESTIGATING THE HOST–PARASITE INTERACTION: INTRASPECIFIC DIFFERENCES IN A GOAT MODEL

VET/06

Dottorando: dr. Eric Giovanni Alberti

Matr. R08508

Tutor: prof. Maria Teresa Manfredi

Coordinatore: prof. Giuseppe Sironi

A.A. 2011/12

INDEX

1. INTRODUCTION

1.1. Main gastrointestinal nematodes of goats and sheep

Gastrointestinal nematodes (GIN) infect small ruminants throughout the world and are an important cause of disease and production loss. The GIN of greatest importance in small ruminants are members of the nematode order Strongylida and most of them belong to the superfamily Trichostrongyloidea. Almost the totality of grazing sheep and goats are infected with a community of these strongylid nematodes whose combined clinical effect is the condition known as parasitic gastroenteritis. Other nematodes belonging to different taxonomic orders also can be commonly found in the intestine of sheep and goats (*Capillaria* and *Strongyloides* in the small intestine, *Skrjabinema* and *Trichuris* in the large intestine), but they cause disease only seldom and are not considered important pathogens (Zajac, 2006).

One important species of the strongylid nematodes (the most important in the tropical and subtropical regions) is *Haemonchus contortus*. *H. contortus* adults are abomasal parasites of small ruminants. Female *H. contortus* reach about 3 cm in length: it is one of the largest and most prolific of the strongylid nematodes of ruminants; a female worm may produce thousands of eggs each day and larvae can rapidly accumulate on pastures. *H. contortus* develops most successfully in warm wet climates and does not tolerate cold temperatures well. The prepatent period (length of time elapsing between infection of the host and parasite maturity) of *Haemonchus* is about 3 weeks. *H. contortus* has great pathogenic potential because 5^{th}-stage larvae and adults feed on host blood, so high parasite numbers can cause fatal anaemia. Unlike many other parasites of the gastrointestinal tract, *H. contortus* is not a primary cause of diarrhoea. Consequently, its effects on a flock or herd are often insidious because routine observation of animals may not reveal the extent of infection and owners may not appreciate that disease is present until deaths occur (Taylor *et al*., 2010; Zajac, 2006).

Another relevant species of trichostrongylid nematodes (the most important in the temperate and subtropical regions) is *Teladorsagia circumcincta* (formerly *Ostertagia circumcincta*). *T. circumcincta* is a smaller parasite than *H. contortus* (females are ∼1 cm long). It is a parasite of the abomasum that does not feed primarily on blood, whose females are not so prolific as *Haemonchus*. Following infection, *T. circumcincta* larvae spend a variable period of time developing in the abomasal gastric glands. These larvae cause changes in the glands that lead to formation of visible nodules on the mucosal surface of the abomasum. In heavy infections, sheep or goats may develop diarrhoea, anaemia, or hypoproteinemia, and in severe cases death can occur. More commonly, moderate infections of *T. circumcincta* could contribute to parasitic gastroenteritis with signs including diarrhoea, decreased appetite and weight loss. The prepatent period of this parasite is again approximately 3 weeks. Adult *Teladorsagia* and *Haemonchus* can live only for a few months in the host. *Teladorsagia* (and *Trichostrongylus*) is more successful than *Haemonchus* in cooler, drier conditions; peak of transmission of these nematodes in Europe occur in two periods, from March to June and August to October (Taylor *et al*., 2010; Zajac, 2006).

Trichostrongylus is a third common genus of trichostrongylid nematodes that causes disease in heavy infections, but more often is a contributor to parasitic gastroenteritis. Several species of *Trichostrongylus* infect small ruminants, including *T. axei*, an abomasal parasite that can infect also cattle and horses, *T. colubriformis* and *T. vitrinus*, which are found in the small intestine. These small worms (<1 cm) are not readily visible in abomasal and intestinal contents. Adult *Trichostrongylus* are longer lived than *Teladorsagia* and *Haemonchus* adults and can survive over winter as adults in the host. Heavy burdens of *Trichostrongylus* can cause severe diarrhoea, weight loss, and death (Taylor *et al*., 2010).

Cooperia curticei, *Nematodirus battus*, *N. spathiger*, and *N. filicollis* are common parasites of the small intestine of sheep and goats, as is *Oesophagostomum venulosum* in the large bowel. *Bunostomum trigonocephalum* and *Chabertia ovina* are less common parasites of the small and large intestine, respectively. These species are rarely pathogenic alone, but may contribute to parasitic gastrointestinal disease (Zajac, 2006).

Although all grazing sheep and goats are infected with GIN, parasitic gastroenteritis occur most often in young, non immune animals, adult animals whose immunity has been compromised, or in sheep or goats exposed to very high levels of infection. Low worm burdens usually have little impact on animal health; as worm numbers increase, subclinical effects in the form of reduced weight gain and decreased appetite occur. With heavier worm burdens clinical signs, including weight loss, diarrhoea, anaemia, and bottle jaw, will develop.

The same life cycle generally applies to all of the economically important strongylid parasites of small ruminants. Adult female parasites in the abomasum or intestines produce eggs that are passed in the faeces. Development occurs within the faecal mass, which provides some protection from environmental conditions. After hatching, 1st-stage larvae feed on bacteria and undergo two moults to reach the infective 3rd larval stage (L_3). 3rd-stage larvae make their way out of the faecal material and onto the forage where they are ingested by sheep and goats. *Nematodirus* spp. are an exception to the life cycle described above. The L_3 develops inside the egg before hatching, that is stimulated by a period of low temperature followed by higher temperature, leading to the synchronous appearance of a great number of larvae on pastures (Zajac, 2006).

L_3 can migrate laterally and vertically in films of moisture on herbage. The ability of larvae to migrate is affected by air temperature, soil moisture, and relative humidity (Krecek *et al*., 1995). The length of time that infective larvae can survive on the pasture is important for pasture management programs that aim to control parasitism. Nematodes are covered by a tough semi-permeable outer layer called the cuticle. The cuticle is replaced with each moult, but the L_3 larva retains the cuticle of the second-stage larva as a sheath. Although the sheath gives these larvae greater resistance to environmental conditions, it also prevents them from feeding. Once an L_3 uses its stored metabolic reserves, it dies (Zajac, 2006). Consequently, the best conditions for survival of infective larvae are cool, dry weather, because dry weather reduces movement of the larvae. Cool, moist weather also supports survival of L_3 for many months. L_3 survives for much shorter periods in hot weather (Barger, 1999).

The epidemiology of important species of GIN is also strongly influenced by aspects of host–parasite biology after infection occurs. Larvae of important GIN are able to undergo a period of arrested development (hypobiosis) in the host. Following infection, larvae may become metabolically inactive for a period that may last several months and then they can resume development. Although the immune status of the host also has an influence on rates of hypobiosis, the greatest proportion of larvae usually arrest at times of the year when conditions in the environment are least favourable for development and survival of eggs and larvae (Eysker, 1997). In areas where winter arrest of parasite larvae occurs, emergence and development of adult worms in late winter and spring are followed by an increase in faecal eggs counts. The rise in egg counts is magnified in periparturient animals by a relaxation of immunity that increases survival and egg production in existing parasites and also increases susceptibility to further infection. The periparturient egg rise (PPR) can make an important contribution to L_3 populations on pastures as young, susceptible animals begin grazing (Gibbs, 1986; Mandonnet *et al*., 2005)

1.2. Diagnosis of gastrointestinal strongylid parasites

The primary laboratory test used for the diagnosis of strongylid nematodes is a faecal flotation procedure for detection of the eggs of the parasites. Strongylid nematode genera produce eggs that are similar in appearance and cannot be easily discriminated. In faecal samples they are all identified in general terms as strongyles or trichostrongyles eggs. These eggs range in size from approximately 65 to 100 μm in length. Genus identification cannot accurately and consistently be made from observation of eggs in faecal examinations. To identify the genera of these nematodes, faecal culture and identification of L_3 are required. The exceptions is eggs of *Nematodirus* spp. that are much larger (~150–250 μm) and contain distinctive dark cells. A useful and modern reference tool for larval identification of the main gastrointestinal and respiratory nematodes of ruminants can be found in van Wyk *et al*. (2004).

Because virtually all grazing small ruminants are infected with some gastrointestinal nematodes, a simple flotation procedure that simply detects the presence of strongylid parasites is of limited value. The most useful herd or flock test is a quantitative egg-counting technique. Faecal egg counts (FEC) can be used to evaluate the efficacy of parasite control programs, monitor levels of potential pasture contamination, and monitor drug efficacy. FEC can also be used in young animals as a tool for genetic selection for resistance to GIN (Zajac and Conboy, 2006). For flock or herd monitoring, samples should be collected from a proportion of animals representing all age, gender, and reproductive categories. It is preferable to analyze samples from individual animals, even if some results suggest that measurements of FEC relying on pooled faecal samples seems to be a valuable tool to increase the frequency of data in epidemiological surveys (Manolaraki *et al*., 2012).

The most used quantitative technique is the Modified McMaster Test, that requires the use of a special counting chamber (MAAF, 1986). New multivalent techniques are available since 2006 relying on the use of the FLOTAC apparatus, that has demonstrated to be a more sensitive and specific tool than the McMaster chamber; it also allows the analysis of a faecal sample with two different flotation solutions at the same time (Cringoli *et al*., 2010) and it is the most precise tool for the faecal egg count reduction test (Levecke *et al*., 2012).

1.3. Anthelmintics and anthelmintic resistance in small ruminants

Anthelmintic drugs commonly used for sheep or goats belong to one of the following three major groups of broad-spectrum anthelmintics.

Benzimidazoles/pro-benzimidazoles: their primary mode of action is to inhibit microtubule formation. These drugs are all active against nematodes and some of them is active against trematodes at higher dosage. Main drugs of this group most used for small ruminants in Italy are fenbendazole, oxfendazole, albendazole, mebendazole and netobimin.

Macrocyclic lactones: they are an extremely powerful group of anthelmintics with activity against nematodes and arthropods (endectocides). The drugs in this group interfere with chloride channel neurotransmission increasing GABA release and causing paralysis; most used in sheep and goats are ivermectin, moxidectin and doramectin.

Cholinergic agonists: this group of anthelmintics includes levamisole, pyrantel, and morantel. All work on the parasite nervous system as nicotinic agonists and cause paralysis of nematodes. Although levamisole (imidazothiazole) is chemically different from pyrantel and morantel (hydropyrimidines), they share the same mode of action and resistance to one compound is expected to cause resistance to other members of the group (Taylor *et al*., 2010).

Goats, in comparison to sheep, can better tolerate and detoxify natural toxins, in particular plant secondary metabolites (PSMs). This could be partly explained by the caprine browsing habit resulting in evolutionary processes which have favoured the development of physiological and metabolic adaptive mechanisms counteracting the potential toxicity of PSMs (Silanikove, 2000). Experimental studies have shown that these metabolic adaptations to natural PSMs also have consequences for the pharmacology or pharmacokinetics of other xenobiotics, including therapeutic drugs (Short, 1999). It has been shown repeatedly that goats metabolize anthelmintics faster than do sheep and such differences have been described for the three principal broad-spectrum anthelmintic families (Galtier *et al*., 1981; Dupuy *et al*., 2001). Consequently, for years, treating goats at the recommended sheep dose rate has resulted in anthelmintic underdosing thus causing a reduced efficacy.

Since the advent in the 1960s of modern broad-spectrum anthelmintics with wide safety margins producers have relied on dewormers for control of GIN in the developed countries. The outcome of this intensive anthelmintic use is widespread resistance in GIN nematode populations. "Resistance is present when there is a greater frequency of individuals within a population able to tolerate doses of compound than in a normal population of the same species and is heritable" (Prichard *et al*., 1980). When a population of worms becomes resistant to one member of a drug group, it effectively becomes resistant to the other members of the group. Tests are required to permit the resistance status of farms to be determined to aid in planning the optimal use of the remaining effective anthelmintics. Several tests are available to evaluate the efficacy of anthelmintic compounds, in vivo and in vitro. The major method for the detection of resistance remains the faecal egg count reduction test (FECRT), an in vivo test that can be used with all anthelmintic groups. Nematode eggs are counted in faeces at the time of treatment and at defined times after treatment, the time depending on the anthelmintic group used. Either naturally or experimentally infected animals can be used and the dose given should be the registered label dose rate. Where the efficacy is expected to be 99%, anthelmintic resistance is confirmed if efficacy is <95% when calculated by comparing arithmetic means, provided that sufficient animals are in treatment groups to yield statistically significant results (Coles *et al*., 2006).

Undoubtedly anthelmintic resistance in nematode populations is an increasing trouble widespread around the world. Recent studies in Europe report resistance prevalence levels very different from area to area, even if always lower than in most other regions of the world. In the UK multiple resistance to all 3 major anthelmintic classes has been reported in Scotland (Sargison *et al*., 2007), and recently a Scottish sheep flock was identified with resistance in *T. circumcincta* to benzimidazole, levamisole, ivermectin and moxidectin (Sargison *et al*., 2010). In France several cases of benzimidazole resistance have been reported in goats (Cabaret *et al*., 1995; Chartier *et al*., 2001), one case of double resistance to benzimidazole and levamisole has been recently confirmed in a dairy goat farm in the western part of the country (Paraud *et al*., 2009), while ivermectin resistance was absent (Paraud *et al*., 2010). In the Slovak Republic, resistance to albendazole was found in 4% and to ivermectin in 23% of the farms tested (Cernanska *et al*., 2006). Resistance to benzimidazoles and ivermectin was found to be present in sheep farms also in The Netherlands (Borgsteede *et al*., 2010). In Norway resistance was found only to benzimidazole (10.5-31% flocks) and not to ivermectin in sheep flocks, while no resistance was found in goat flocks (Domke *et al*., 2012). In Sweden resistance still remains low (5%) and restricted only to *H. contortus* and to the benzimidazoles (Höglund *et al*., 2009). In Germany benzimidazole resistance is widespread in sheep flocks and resistance to avermectins and milbemycins has been reported (Scheuerle *et al*., 2009), including a very recent case of triple anthelmintic resistance (Voigt *et al*., 2012). In Switzerland avermectin-resistance in gastrointestinal nematodes of Boer goats and Dorper sheep has been reported recently (Artho *et al*., 2007). In Greece, over 16% strains of *Teladorsagia spp*. studied were found to be benzimidazole-resistant strains (Papadopoulos *et al*., 2001). In Spain, presence of flock resistance, under field conditions, to benzimidazoles varied from 18 to 35% and to macrocyclic lactones from 3 to 16%; in all cases, *Teladorsagia* spp. and *Trichostrongylus* spp. were the main genera involved (Alvarez-Sanchez *et al*., 2006; Diez-Banos *et al*., 2008). In Italy, in a goat farm with multiple and repeated introductions of animals, resistance to anthelmintics was recently evidenced for the first time (Cringoli *et al*., 2007): resistance was detected for benzimidazoles only, and *Trichostrongylus colubriformis* was the only resistant strongyle species. In the authors' opinion single drug and single species resistance suggested that resistance was on its beginning and that measures for reducing the spread of resistance were of interest and should have been promoted. In the same year, in sheep farms levamisole resistance was found in all the farms and ivermectin resistance in two out of the three farms examined in *Teladorsagia circumcincta* and *Trichostrongylus* spp., suggesting a potential problem to sheep industry (Traversa *et al*., 2007).

For most producers, parasite control continues to include treatments with anthelmintic drugs, but any veterinarian working with small ruminant producers must stress the importance of alternative methods of parasite control that can be used in combination with anthelmintics. A further incentive for veterinarians to look beyond anthelmintic control programs is the increased interest of producers in sustainable and organic production practices (Zajac, 2006).

1.4. Host response to GIN in small ruminants and peculiarities of the goat

The usual description of a goat as 'the cow of the poor' underlines its importance in small farming systems. Goat is the most highly consumed meat in the world, and more goats' milk is consumed worldwide than cows' milk (Hoste *et al*., 2010). Worldwide, goat production is increasing because of the economic value of goats as efficient converters of low-quality forages into quality meat, milk and hide products for specialty markets.

Helminth parasitism of the digestive tract remains a major threat affecting goat health and production. Despite the similar number of goats and sheep in the world and the similar consequences of GIN parasitism in both hosts, the majority of studies on host–nematode interactions and control of GIN species have been carried out in sheep, leading to severe consequence for the goat industry and impairing parasite control programs in this species (Hoste *et al*., 2010).

Following domestication, sheep and goats developed different feeding behaviours. Sheep are usually described as grazers, preferring to feed on grass and forbs (a broad-leafed plant other than grass). In contrast, goats are classified as browsers or intermediate browsers, ingesting substantial amounts of browse (woody plants, vines and brush) even if other nutritional forage is available (Silanikove, 2000; Provenza, 2003). Compared to grass and forages, bush and tree foliages, which compose a considerable part of the goat diet under browsing conditions, represent a minor risk of nematode transmission. Thus it has been hypothesized that such differences between the two host genera should result in distinct strategies against nematode infections, with major consequences to host–parasite relationships. These two divergent strategies rely on a balance between either the development of an immune response (sheep) or the existence of behavioural responses which limit contact with the infective larvae present in the environment (goats) (Hoste *et al*., 2001). It is also hypothesized that this caprine behaviour to feed on a high diversity of plants has led to three other differences which might be involved in the regulation of parasite populations, i.e. a subdued immune response, a more rapid metabolism of xenobiotics and an ability for self-medication (Hoste *et al*., 2010).

Comparative information through various experimental studies has been obtained on the processes involved in the development of the host response against GIN in sheep and goats. These have been well-described in sheep. After a phase of acquisition of immunity, the expression of the immune response affect the nematodes at different stages of their biological cycle by:

- reducing the larval establishment (measured by the number of worms at necropsy);
- reducing the growth of parasitic larvae (measurements of worm length);

- decreasing the fertility of female worms (measurements of egg excretion, assessment of female egg production in utero)
- expulsing adult worms (worm counts at necropsy)

(Coop and Kyriazakis, 1999; Balic *et al*., 2000; Hoste *et al*., 2008). Several variations affecting these different steps are suspected in goats. Whereas it is usually acknowledged that sheep can acquire an appreciable resistance to nematodes from 6 to 8 months of age, the acquisition of a fully expressed immune response appears delayed in goats (~12 months) (Pomroy and Charleston, 1989a; Vlassof *et al*., 1999). Evidence has been obtained in different goat breeds indicating that the immune regulation of nematode populations occurs by reducing the larval growth and/or by affecting the egg production (Patterson *et al*., 1996a; 1996b; Chartier and Hoste, 1998). On the other hand, data obtained on the response to a larval challenge mainly suggest a poor ability to modulate the initial establishment of larvae (Pomroy and Charleston, 1989b; Huntley *et al*., 1995; Perez *et al*., 2001). Moreover, it has been recorded that the expulsion of adult worms is not a common feature in goats (Watson and Hosking, 1993). One of the main possible differences in goats, in contrast to sheep, is that the presence of an existing worm population seems to be required to stimulate the immune mechanisms regulating the newly incoming ingested larvae. This has been illustrated by the major disturbing effects on immunity recorded with anthelmintic treatments in goats, indicating that the reduction in challenge establishment of *H. contortus* was much more pronounced when the previous infection was not abbreviated (Watson and Hosking, 1993). The conclusion that the immune memory after anthelmintic treatment in goats does not last as long as in sheep was also supported by other results obtained in outdoor or indoor studies (Hadas and Stankiewicz, 1997; Hoste and Chartier, 1998). In addition, in dairy goats, similar levels of GIN infections between adult and young animals have frequently been reported in contrast to sheep where adult ewes are usually much less heavily infected than young animals (Vlassof *et al*., 1999; Hoste *et al*., 2008).

Divergences in the expression of immunity between the two hosts have also been observed. When grazing, a strong regulation of egg excretion has been described in flocks of adult sheep. Conversely, in goats, a trend for the accumulation of parasites, correlated with higher and constantly increasing egg excretion has generally been found over the whole grazing period (Pomroy *et al*., 1986). These results support the hypothesis of a low immune regulation of nematode biology in grazing goats compared to sheep. In contrast, under shared browsing conditions, the levels of parasite infections were much less intense in adult goats than in sheep (Vercruysse, 1983; Chiejina, 1986). The two reported host strategies (behaviour and immune response) are not mutually exclusive. In sheep, despite the predominance of regulatory immune processes, some behavioural mechanisms have been described which contribute to reduced contact with the infective L_3, resulting in limited host infections (Hutchings *et al*., 2000; Cooper *et al*., 2000). By contrast, in goats, evidence of a continuum in the propensity to browse, with associated consequences for GIN infections, has been found (Hoste *et al*., 2001).

1.5. Traditional goat breeding and parasite control in Lombardy

Goat breeding in Italy is quite diffused, with great differences in the production objectives, consequently with different breeds reared and differing farm managements adopted from an area to another. In particular in Lombardy the main products related to goat breeding are milk and cheese and with the increasing knowledge about the optimal qualities of goat milk, especially for those people with low tolerance to cow milk or specific dietetic necessities (overall kids and old people), demand for this healthy product is also increasing. Therefore recently some goat big farms are developing in the flat area of the region; these farms are often characterised by intensive systems and modern management of the flock (e.g. zero grazing, out-of-season mating, etc...) to meet the demand for goat products throughout the year. However, the traditional goat farming, which can be found in the hilly or mountainous areas, still remains an important way of breeding goats in the region. This system is usually characterised by semi-extensive breeding, with great use of the high alpine pastures during the hot months, the lower pastures in spring and autumn and the coming back to the fold at the bottom of the valley during the winter months before kidding season. In this context of traditional farming a lot of traditional products have developed, related to the use of milk or meat, some of them really peculiar from specific areas, thus deserving a protection trademark (e.g. D.O.P.). This particular kind of breeding is also very important for the landscape of the mountain areas which suffered of territory abandon in the past decades, with nowadays great damage of the landscape (reforestation) and higher risk of negative natural events such as fires or landslides. In fact, goat breeding is complementary to other herbivores breeding, as goats feed with plants scarcely used by other species, playing an important role in limiting the reforestation. Besides, it is a breeding system that usually needs much lower economic inputs compared with other large animal species' breeding.

Although the traditional goat breeding is based on the exploitation of large plots of land, where nematode eggs are well dispersed on the ground, parasitic nematodes of the gastrointestinal tract pose a great problem. Analysing faeces from 110 goat farms in Lombardy, Manfredi *et al*. (2010) found that almost all the examined goats (96%) were positive to the coprological exams. Data collected on the prophylaxis measures adopted by the farmers showed that in most of the farms, they performed anthelmintic treatment at least once a year, but the dosage used by veterinarians or farmers themselves was often incorrect for goats, as they usually adopted the same protocol as for sheep treatment, whose dosage needs to be lower. Moreover, the calculation of the correct dose of drugs was not always performed by an accurate evaluation of the animals' weights. It has been generally considered that such inaccurate practices are widespread among farmers and even among veterinarians (Jackson and Coop, 2000; Cabaret, 2000). Although the different response to the anthelmintic drugs by the two species has been well substantiated in scientific literature (Attili *et al*., 2004; Genchi, 2006), drug dosages are officially calculated for sheep and translated to goat. In particular, there is a lack of drug protocols specifically registered for the goat, as the dosage indicated by producers usually refers only to sheep or even cattle. In Italy anthelmintics officially registered for goats are fenbendazole, oxfendazole, morantel and ivermectin. In all the products there is no difference in the recommended dosage between goat and sheep.

Furthermore, treatments are not usually preceded by parasitological exams in order to adopt a target therapy on infected animals and then to reduce anthelmintic resistance of parasites (Manfredi *et al*., 2010). On the contrary, a periodical examination of the faeces is useful to monitor parasitic diseases in domestic animals, without killing them. Particularly, the FEC is considered a valid procedure to value eggs excretion in small ruminants, even though it does not estimate the worm burden directly. As to the validity of FEC method in diagnosis of parasitic infections, recently Cringoli *et al*. (2008a) added data on the validity of FEC for the in vivo diagnosis of gastrointestinal strongyles in dairy goats, assessing a significant positive correlation between FEC and adult worms.

In goats as well as in other animal species, the distribution of worm populations in the host is aggregated, corresponding to negative binomial distributions (Hoste *et al*., 2002). This fact reflects in differing immunologic and/or genetic resistance to parasites among individuals within the same breed and has led to exploration of some control methods relying on the development of immune mechanisms, for example genetic selection for resistance to GIN species (Mandonnet *et al*., 2001). Phenotypic evaluation of resistance to worms can be based on FEC and it has been shown to be a heritable trait, with substantial differences identified between different breeds as well as within the same breed (Dominik, 2005) In fact, results from surveys that compared response to GIN infection among goat breeds, suggested that some breeds are more resistant and/or resilient than others. For example, 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 lower reduction in blood values (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 the current context of the worldwide spread of resistance to chemical anthelmintics, selection of animals which are genetically resistant to infection by parasite worms is an attractive approach and it can provide long-term solutions, especially when an integrated control relying on different strategies will be applied (e.g. grazing management, nematophagous fungi, vaccination, feed complementation, mineral or plant drugs against helminths, etc...).

2. OBJECTIVES

The research work of the Ph.D. project was aimed at the investigation of the interaction between gastrointestinal nematodes and goat. The work was developed through different approaches, in particular we studied:

- the effects of gastrointestinal nematode infection on qualitative and quantitative productivity of milk, hypothesizing that productivity might increase with the number of lactations and that indigenous breeds might be affected by GIN to a minor extent than more selected breeds;
- the effect of differing levels of GIN natural infection on milk yield and quality, hypothesizing that high levels of GIN infection might affect more strongly the milk yield and contents and the length of lactation and taking also into consideration the breed effect;
- the trends of FEC in natural infections in order to observe the variations throughout the year and to compare the egg emission between a local goat breed (Nera di Verzasca) and a more selected one (Alpine) to find out if these breeds were characterised by different resistance to nematodes;
- the differences in the egg emission in experimental infection, after a single larval challenge, between goats of Alpine and Nera di Verzasca breed, in order to confirm and to strengthen the data on resistance obtained under field conditions, avoiding the influence of possible differences due to feeding behaviour;
- the levels of parasite-specific circulating antibodies to investigate if the differing susceptibilities to nematode infection expressed by FEC in the two goat breeds were due to differences in the humoral immune response.

3. GASTROINTESTINAL NEMATODE AND MILK PRODUCTION IN THE GOAT

Gastrointestinal nematode infections are common in grazing ruminants worldwide and are still one of the main constraints to ruminant production, since they can depress food intake causing tissue damage and reduction in skeletal growth and live-weight gain (van Houtert and Sykes, 1996) as well as reduction of milk yield. In dairy cattle, anthelmintic treatment showed to be economically beneficial, increasing milk yield by 0.6 kg/cow per day (Charlier *et al*., 2009). Also Reist *et al*. (2011) in dairy cattle found highly significant positive effects of treatment with eprinomectin on milk yield (+1.90 kg and +2.63 kg at day 62 and 131 post-treatment, respectively) and a modest decrease in somatic cell counts (SCC) compared with the control animals. In sheep, the administration of an anthelmintic to grazing animals has positively improved milk yield showing a significant increase that ranged from 19% to 44% (Cringoli *et al*., 2008b). These results are supported also by Sechi *et al*. (2010) who found a significant effect of anthelmintic treatment on lactation milk yield of treated adult ewes, that produced 19.2 l/year more than control ewes, and this effect was markedly greater in high producer multiparous ewes (+32.7 l/year); in that study the authors did not observe any significant variation in the milk constituents. On the other side Cruz-Rojo *et al*. (2012) found that ewes with low level of GIN infection can produce 11.1% more milk than high infected ewes, with significant differences also for the milk constituents, in particular for the protein content. Also in dairy goats, Rinaldi *et al*. (2007), reviewing several trials, reported similar results: in fact, goats receiving an anthelmintic treatment had a significant milk yield increase of 12% and a better quality regarding fat (+29.9%), protein (+23.3%) and lactose (+19.6%) contents. Moreover a strong interaction between lactation, production level and stage, and parasite challenge does exist. Epidemiological studies in French dairy goat flocks showed that either animals in their first lactation or high milk-producing multiparous animals have the highest faecal egg counts (FEC) (Hoste *et al*., 2002). Additionally, during the lactation period a high level of milk production can lower effectiveness in the response against parasites, which does not occur in dry goats (Etter *et al*., 2000).

3.1. EFFECTS OF GASTROINTESTINAL NEMATODES ON MILK PRODUCTION - I

Few comparative studies have evaluated differences in response to GIN infection among goat breeds so far; nonetheless there are evidences suggesting that some breeds are more resistant and/or resilient than others (Pralomkarn *et al*., 1997; Baker *et al*., 1998; Chiejina and Behnke, 2011). Such results highlight the different susceptibility of each distinct goat breed to GIN infections. Therefore, it can be hypothesized that the parasite effect on milk production might be different among goat breeds. No study was found where the combined effects of impact of parasite infection, breed and lactation number had been evaluated in relation to milk production. Thus, the aim of this study was to test the effect of GIN on qualitative and quantitative productivity of milk, hypothesizing that productivity might increase with the number of lactations and that indigenous breeds might be affected by GIN to a minor extent than more selected breeds.

3.1.1. Materials and methods

Goat breeds

The effect of parasitism and lactation number on milk productivity was compared in three goat breeds: Alpine, Saanen and Nera di Verzasca. The Alpine dairy goat is a breed originated in the French Alps characterized by a medium to large size. They are known for their high milk productivity and described as hardy, adaptable animals that thrive in any climate while maintaining good health and excellent production. The large-sized Saanen goats are named after the Swiss valley from where they have originated. They usually pass any average milk production, and their product tends to have a low fat content. Both Alpine and Saanen goats are widespread in Europe and the USA; they have been selected for milk production under intensive farming for years. Nera di Verzasca represents a goat breed originated in Switzerland and now reared also in some provinces of northern Italy, though still showing only few animals. These goats are black, large in size and produce less milk if compared to Alpine and Saanen; they are known for their high adaptability to extensive farming even on poor grasslands.

Data collection

The survey was carried out from November 2006 to July 2007 in the northern Italian region of Lombardy. In particular, the sampled farms were in the step-hill provinces of Varese (VA), Como (CO) and Bergamo (BG), characterized by continental climate, i.e. hot summers and cold winters. The survey involved 379 goats from the named three goat breeds: 216 Alpine, 130 Saanen and 33 Nera di Verzasca, respectively. These goat breeds were sampled from 13 farms, homogeneous as regards their management and grazing regime with the following distribution; Alpine derived from seven different, Saanen from five and Nera di Verzasca from two different farms. One farm reared both Alpine and Nera di Verzasca. Individual faecal samples were collected once per goat and directly from the rectum of each animal. The sampling was performed from November to December before administration of anthelmintic treatments. Milk production data were collected monthly and evaluated by the Milk Standard Laboratory of A.I.A, an Italian Farmer Association, and accounted for milk quantity, fat, protein and lactose contents, following the routine infrared method (Laboratorio Standard Latte – Associazione Italiana Allevatori (LSL–AIA) website: http://www.aia.it/lsl/index.htm). A problem had to be considered; in fact the traditional goat breeding has peculiar features which lead to uneven duration of lactations between goat individuals, a key factor influencing the total lactation milk yield (Crepaldi *et al*., 1999). Thus, for statistical analyses the mean daily milk yield and the mean percentage of fat and protein content were estimated dividing the lactation total milk yield and the percentage of fat and protein by the number of lactating days. This method allowed to standardize milk productivity among those individuals with a different number of days of lactation.

Parasitological techniques

Qualitative and quantitative parasitological exams were performed by flotation techniques following standard procedures. The identification of eggs was up to genus or order level and to species only for cestode. The individual faecal egg counts were performed by a modified McMaster method (MAAF, 1986; Raynaud, 1970) using 40.5 ml of a sodium nitrate and sucrose-based flotation solution (specific gravity = 1.300) and 4.5 g of faeces. The EPG (eggs per gram) was considered as the number of any parasite eggs, except for *Skrjabinema* and the cestode *Moniezia*, found in the McMaster chamber. *Eimeria* spp. infection was only evaluated by qualitative analysis. Composite faecal cultures were made for each farms; third-stage larvae were identified using the morphological keys proposed by Gevrey (1971) and Van Wyk *et al*. (2004).

Statistical analyses

Epidemiological indices were calculated for each parasite according to Bush *et al*. (2001). Prevalence (P) was considered as the ratio between the parasitized animals and the total of examined animals, expressed as a percentage. Mean intensity (I) of egg emission was calculated as the arithmetic mean of EPG only of the infected animals. Effects of the parasites (EPG), breed and lactation number on mean daily milk yield (measured in litres) and the mean daily percentage of fat and protein contents were analyzed through Generalized Linear Models (GLM) with Gaussian distribution. The effect of goat breed and lactation number on EPG was analyzed through GLM with negative binomial error distribution in order to consider the aggregated distribution of parasites

(Wilson and Grenfell, 1997; Shaw *et al*., 1998). Confidence level was held at 95% and $p < 0.05$ was set for significance; all analyses were undertaken in R (R Development Core Team, 2011).

3.1.2. Results

Total prevalence of GIN was 58.31% with a distribution among breeds as shown in Table 1. The following gastrointestinal parasites, with their relative prevalence and mean intensity of egg emission, were identified: Strongylida (P = 47.76%; I = 1291.07 EPG), *Strongyloides* spp. (P = 15.83%; I = 68.37 EPG), *Trichuris* spp. (P = 7.12%; I = 83.99 EPG), *Nematodirus* spp. (P = 0.26%; I = 33.35 EPG). Further *Moniezia benedeni* (P = 10%), *Skrjabinema* spp. (P = 25.4%) and the coccidian *Eimeria* spp. (P = 88.5%) were found. Strongylida larvae were isolated from fecal culture and the following genera were identified: *Teladorsagia* (60%), *Haemonchus* (23%), *Trichostrongylus* (10%) and *Nematodirus* (7%).

Breed	1° Quartile	Mean	3° Quartile	Prevalence
Alpine	0	755	975	62.04 %
Saanen	0	191	66	42.31 %
Verzasca	466	1576	2001	100 %

Table 1 - EPG values and distributions in goat breeds

Factor	Deviance	d.f.	*p*
Breed	52.14	2	<0.001
Lactation N°	0.02	1	0.878
Breed : Lactation N°	46.99	2	<0.001

Table 2 - Effect of goat breeds and lactation number on EPG

Emission of parasite eggs was influenced by goat breed and number of lactations (Table 2). In particular, Verzasca showed the highest EPG while Saanen had the lowest values (Fig. 1). Although the number of lactations had a different quantitative effect on the three breeds, in each of them it led to an increase of EPG, an effect more pronounced in Verzasca (Fig. 1).

Mean daily milk yield (litres) was influenced by parasite infection, breed and lactations. Moreover, a significant third order interaction was observed evidencing that in the breeds the effect of parasites changed on different lactations (Table 3).

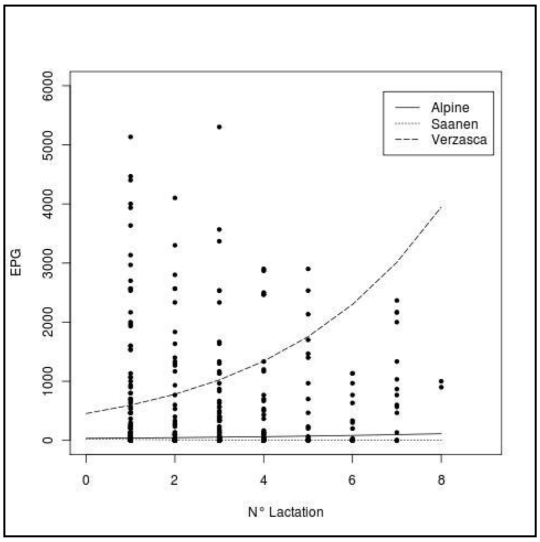

Figure 1 - Effect of breed and lactation number on intensity of infection (estimated by EPG)

Factor	Deviance	d.f.	F	*p*
Mean daily yield				
EPG	7.961	1	21.064	<0.001
Breed	56.287	2	74.467	<0.001
Lactation N°	22.067	1	58.388	<0.001
EPG : Breed	0.332	2	0.439	0.644
EPG : Lactation N°	0.855	1	2.272	0.133
Breed : Lactation N°	2.021	2	2.673	0.070
EPG : Breed : Lactation N°	3.428	2	4.535	0.011
% of protein				
EPG	0.100	1	1.736	0.188
Breed	0.853	2	7.339	<0.001
Lactation N°	0.982	1	16.892	<0.001
EPG : Breed	0.121	2	1.042	0.353
EPG : Lactation N°	0.117	1	2.028	0.155
Breed : Lactation N°	0.012	2	0.104	0.900
EPG : Breed : Lactation N°	0.018	2	0.159	0.853
% of fat				
EPG	0.337	1	2.365	0.124
Breed	2.629	2	9.209	<0.001
Lactation N°	0.236	1	1.654	0.20
EPG : Breed	0.388	2	1.362	0.257
EPG : Lactation N°	0.213	1	1.497	0.221
Breed : Lactation N°	0.283	2	0.992	0.371
EPG : Breed : Lactation N°	0.255	2	1.421	0.234

Table 3 - Effect of the factors EPG, goat breed and lactation number on milk production parameters (mean daily yield, protein and fat content)

As to production values, Saanen showed the highest mean value, Alpine intermediate and Verzasca the lowest one (milk litres mean±SE; Saanen = 3.14±0.06; Alpine = 2.72±0.04; Verzasca = 1.60±0.09). The model (Table 3) predicted that milk production generally changed with the number of lactations, increasing of 13.8 cl each lactation, with the greatest increment in Saanen and Verzasca and the smallest one in Alpine (milk increase per lactation in breeds; Saanen: 18.1, Verzasca: 16.0, Alpine: 7.4 cl/lactation) (Fig. 2A). Such increases were estimated from the model considering with null intensity of infection since the parasite had a generalized negative effect on milk production (Table 3).

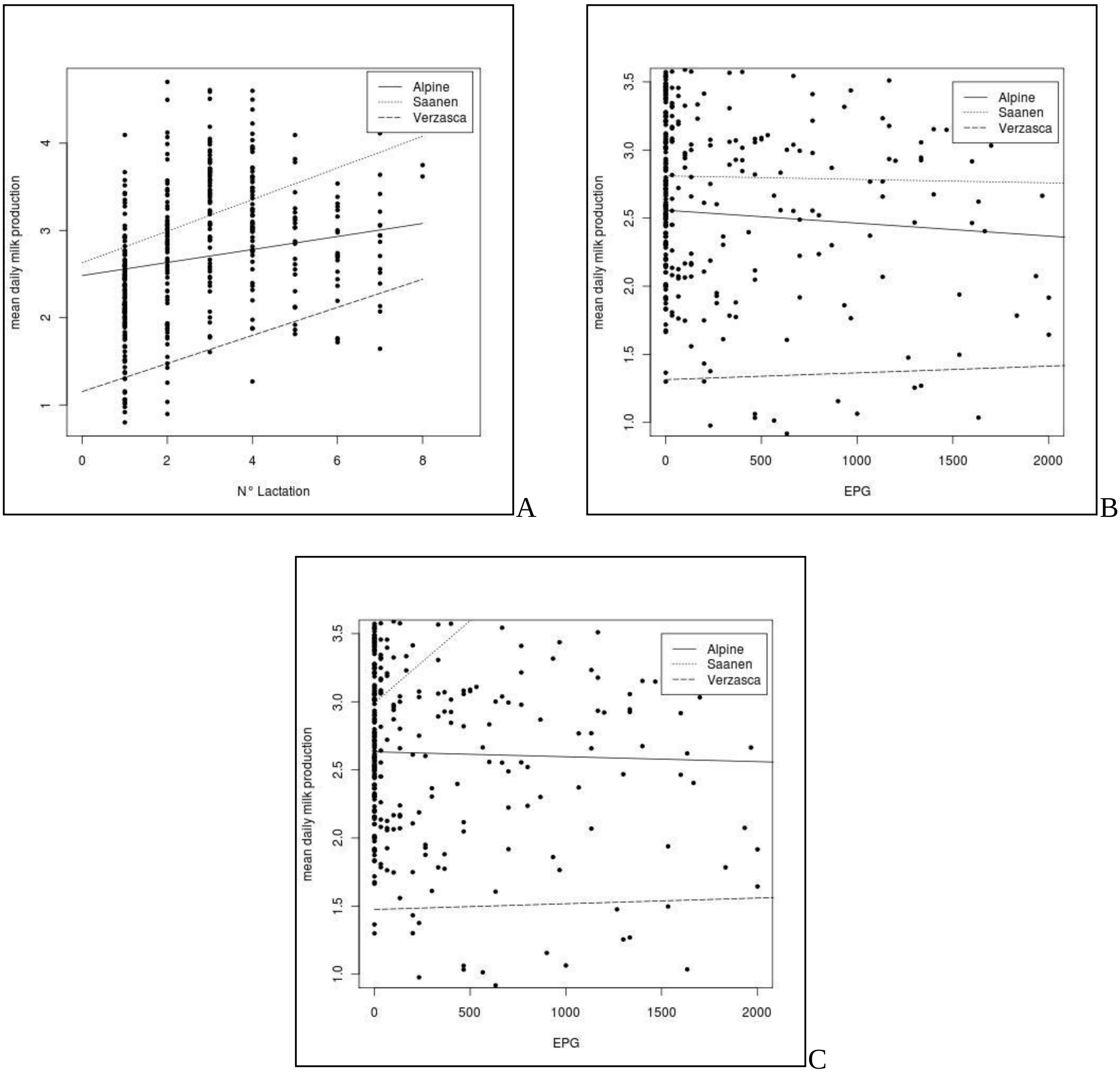

Figure 2 - Influence of factors affecting milk production (measured in mean litres per day): A) Effect of number of lactation, B) Effect of the intensity of infection (estimated by EPG) during first lactation, C) Effect of the intensity of infection (estimated by EPG) during the third lactation.

The parasite effect on milk production resulted to be different among lactations and goat breeds. In fact, during first lactation parasites had a negative effect on milk production in Alpine and caused a slight reduction in Saanen, but they did not affect Verzasca's production (Fig. 2B). On the following lactations the negative effect of parasites decreased with more pronounced changes in Saanen than in the other breeds (Fig. 2C). The composition of milk showed a reduced variability. In particular, the percentage of protein content was different among goat breeds and decreased with the number of lactations, but no effect of the parasites was observed (Table 3). At the same time the percentage of fat content was influenced only by the breed, while lactation and parasites produced no effect (Table 3).

3.1.3. Discussion

The present study highlighted that milk yield was affected by parasite burden, breed and lactation number. In fact, analyzing the effect of parasite and lactation number on milk production from different goat breeds, it was observed that if the parasite burden affected the amount of milk produced on first lactations by the most selected breeds, this did not occur with the most rustic native breed. This effect was evident despite Nera di Verzasca goats revealed the highest FECs in the study. Actually, this result is divergent from those of Pralomkarn *et al*. (1997) and Baker *et al*. (1998), where the native breeds showed the lowest EPG and the best pathophysiological parameters. Additionally, higher emissions of eggs was expected from the most productive goats (Saanen): in fact, Chartier and Hoste (1997) had reported that goats with the highest milk yield were more susceptible to the infection and showed the highest losses in production, with a depression in milk production of −21.5% versus a depression of −6.25% in the lowest producing goats. Their survey was conducted on 100 goats of the same breed (Alpine); it is likely that their observations are valid only if applied to animals within the same breed, and cannot be used while comparing different breeds. As regards milk composition, it has been reported that anthelmintic-treated goats have a significantly higher milk production than naturally infected ones, with an average milk yield increase of 12% (7.4–18.5%) and a better quality regarding fat (+29.9%), protein (+23.3%) and lactose (+19.6%) contents (Rinaldi *et al*., 2007). The results of the present study partially diverged from these observations; in fact, parasite infection had no significant effect on the chemical composition of milk, but only on milk yield. Even Chartier and Hoste (1997) observed a reduction in milk production caused by gastrointestinal nematodes with no significant change in protein and fat contents. Many factors can generate great differences in the response to GIN infections among goats. Undoubtedly, goats may have many parasites and yet they can manage with contrasting the parasite effects without suffering from serious damages. According to Woolaston and Baker (1996), we can define resistance as "the ability of a host to initiate and maintain responses to suppress the establishment of parasites and/or eliminate the parasite load" and resilience as "the ability of the host to maintain a relatively undepressed production level under parasite challenge". Definitions suggesting that high parasite intensities harboured by goats can be associated with their ability to cope and control the parasite pathogenic effects. In our study, an increase of EPG with lactations was observed, confirming that, compared with sheep, goats show a diffused lower ability to develop a resistance to GIN during their lives (Le Jambre, 1984; Huntley *et al.*, 1995). On the other hand, the results led to hypothesize some resilience in goats; in fact, though EPG increased with lactations, the negative effect of parasite infection on milk production was important on first lactation (though differently in the three considered breeds) (Fig. 2B) and became weaker during following lactations (Fig. 2C). Besides, Nera di Verzasca seemed to be characterized by a higher resilience to GIN than the other investigated breeds, showing minor effects of GIN on their productivity, as mentioned above. A peculiar fact that, if confirmed by further studies, could stimulate farmers with increasing their rearing these goats, which would help to preserve a breed now undergoing low diffusion and to enhance breed biodiversity. To date we hope that future surveys can clear up whether in this goat breed infection tolerance is accompanied by higher resistance thanks to their genetics or to their lower milk production affecting with minor intensity their immune response to parasites.

3.2. EFFECTS OF GASTROINTESTINAL NEMATODES ON MILK PRODUCTION - II

In the previous survey, we looked into the effects of gastrointestinal nematodes on milk production in three goat breeds from different farms. What we found was that milk yield was affected by parasite burden, breed and lactation number and that Nera di Verzasca goats seemed to be characterized by a higher resilience to GIN than the other investigated breeds (Saanen and Alpine), showing minor effects of GIN on their productivity (Alberti *et al*., 2012). Aim of the present study was to test the effect of differing levels of GIN natural infection on milk yield and quality, hypothesizing that high levels of GIN infection might affect more strongly the milk yield and contents and the length of lactation, and that these effects might be different between the two breeds of goat investigated (Alpine and Nera di Verzasca).

3.2.1. Materials and methods

Farm and animals

The study was carried out on 61 adult female goats of two different dairy breeds: 31 goats of Nera di Verzasca breed and 30 goats of Alpine breed. These animals were reared in the same farm, well-integrated with each other and with no difference in management regime between the two breeds. The farm is located at 980 m of altitude on the mountains around the lake Verbano, in the Province of Varese, northern Italy. Goats are reared in semi-extensive way: they are kept in the fold during the winter months, before kidding, fed with hay *ad libitum* and an increasing concentrate supplementation (from 300g/day before kidding to 600g/day in the early lactating period). Kidding occur from January to March. Goats are milked twice daily (morning and evening) from kidding to September; from March to November they are free to graze and browse in a large mountain area (~200 ha) from 900 to 1550 m above sea level during the day (or the night in the hotter months) and are kept in the fold during the night (or the day in hotter months). The area is mainly characterised by the three following climax plant associations: *Quercum-Betuletum insubricum*, *Luzulo niveae-Fagetum*, *Alnetum viridis*. Other associations of the area (*Molinietum*, *Sarothamnetum scopariae*, *Vaccinio-Rhododendretum*) are to be considered as temporary stages leading to one of the above-mentioned main associations (Zanatta, 1996). In this context goats showed to prefer spending their time eating mainly *Molinia arundinacea* (27.8%), *Betula pendula* (22.9%), *Sarothamnus scoparius* (20%), *Vaccinium myrtillus* (10.9%), *Alnus viridis* (8.2%) and other species (10.2%) (Maggioni *et al*., 2004).

Samples and techniques

Anthelmintic treatment (netobimin 15 mg/kg) was administered to the whole herd in November 2009. The 61 lactating goats selected for the study were from 1 to >7 years old.

From February to September 2010 monthly collection of milk samples was carried out; milk qualitative and quantitative parameters were evaluated by the Milk Standard Laboratory of A.I.A, the Italian Breeder Association, and accounted for milk quantity, fat, protein and lactose contents, following the routine infrared method (Laboratorio Standard Latte – Associazione Italiana Allevatori (LSL–AIA) website: http://www.aia.it/lsl/index.htm); somatic cell counts (SCC) were determined by fluoro-opto-electronic counting (Bentley SOMACOUNT 150, Bentley Instruments, USA).

Faecal samples were also collected monthly during the lactating period (±7 days from day of milk collection) to evaluate the gastrointestinal parasitic load expressed by the emission of eggs and measured as eggs per gram of faeces (EPG). Faecal egg counts were performed using FLOTAC double technique with NaCl solution (s.g. 1.200) (Cringoli *et al*., 2010).

Statistical analysis

Before analysing parasitological data, animals were divided in five classes according to their level of egg emission: Le0: <100 EPG; Le1: 101-200 EPG; Le2: 201-600 EPG; Le3: 601-1500 EPG; Le4: >1500 EPG. EPG values were then log-transformed to achieve a normal distribution of the data. Somatic cell counts were transformed in somatic cell scores (SCS) as follows: SCS = $\log_2$(SCC/100)+3). Data were analysed using ANOVA (GLM for repeated measures) to test the effects of the variables month, age, days in milk (linear, quadratic and cubic transformed) and breed on the EPG and the effects of the variables month, age and the interactions between days in milk (linear and quadratic transformed), breed and infection level on the production parameters (daily milk yield, fat and protein contents, SCS). Goat was used as random effect in the models. Data were analysed by the SAS software (SAS Institute Inc. 2008. SAS OnlineDoc® 9.1.3. Cary, NC).

3.2.2. Results

Very significant differences in EPG ($p < 0.0001$) were found due to the effect of the interaction between month and breed (Fig. 3).

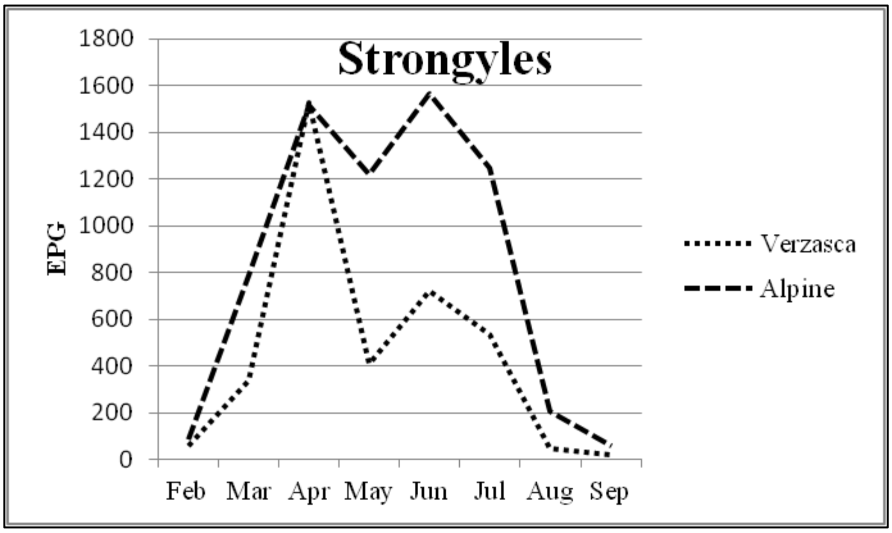

Figure 3 – EPG values observed in the two breeds during the lactation period

Breed	N° kidding	EPG: Mean – (SD)
Nera di Verzasca	2	266,75 – (569,73)
	3	277,48 – (585,21)
	4	307,41 – (562,42)
	6	572,00 – (707,11)
	7	512,80 – (1057,08)
	8	677,11 – (907,57)
	10	24,00 – (33,94)
Alpine	1	1009,29 – (1710,05)
	2	515,10 – (772,14)
	3	904,67 – (1430,60)
	4	526,23 – (699,38)
	5	853,72 – (1657,10)
	6	681,24 – (972,99)

Table 4 – Mean and standard deviations (SD) in the two breeds based on the number of kidding

No significant influence of the number of kidding on EPG was found. FEC was influenced significantly by the age of the animal only when comparing: between breeds 2 years old goats (p = 0.0276) and 2 years old Verzasca vs. 6 years old Alpine goats (p = 0.0213); or within Alpine breed 1 year old vs. 2 years old (p = 0.0242) or vs. 6 years old goats (p = 0.0111) (Table 4).

The trend of the EPG was increasing-decreasing, partly similar to the milk production curve; in fact it was observed an increase of egg emission with the progress of the lactation and the reaching of the maximum level of EPG when goats were at their $90^{th}/100^{th}$ day of lactation (Fig. 4).

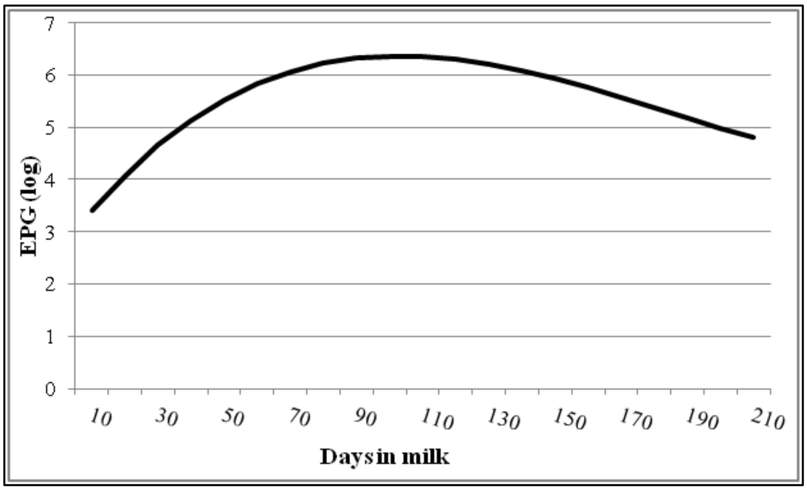

Figure 4 – Curve of the emission of EPG during the lactation period

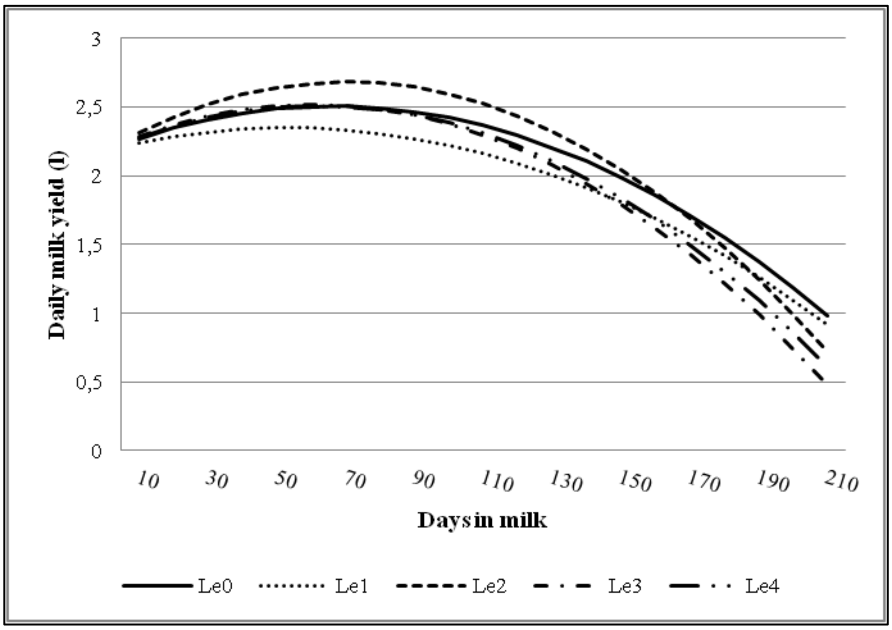

Figure 5 – Lactation curves in goats with different levels of GIN infection

The third order interaction among breed, class of infection and days in milk resulted very significant in influencing the production parameters ($p < 0.0001$). Some difference in milk yield due to the level of infection were noticed (Fig. 5): in particular, milk yield decreased in both breeds with the increasing of the infection level, but in Alpine goats production seemed affected in Le3 (601-1500 EPG) yet, while in goats of Nera di Verzasca it did not seem affected before Le4 (>1500 EPG) (Table 5). The greatest decrements were observed from Le2 to Le4 (-10.8% in Verzasca and -7.9% in Alpine).

Breed	Class of infection	EPG	Days in milk	Daily milk yield (l)	Protein (%)	Fat (%)	SCC (10^3/ml)
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Verzasca	Le1	63,93 (50,28)	113,13 (70,56)	1,73 (0,82)	3,30 (0,42)	3,48 (0,99)	720,97 (1538,25)
	Le2	379,44 (117,58)	123,51 (52,04)	1,76 (0,77)	3,08 (0,25)	3,03 (0,92)	563,51 (547,32)
	Le3	986,32 (234,42)	124,64 (54,25)	1,70 (0,94)	3,01 (0,32)	3,24 (0,83)	543,56 (590,29)
	Le4	2385,91 (815,39)	110,91 (28,79)	1,57 (0,494)	3,14 (0,37)	4,26 (0,96)	1478,09 (3588,41)
Alpine	Le0	0 (0)	117,17 (66,95)	1,83 (0,96)	3,28 (0,64)	3,51 (1,06)	1029,06 (1255,78)
	Le1	62 (47,93)	132,81 (81,02)	1,68 (1,05)	3,58 (0,65)	3,93 (0,87)	1860,32 (2453,96)
	Le2	379,37 (82,57)	108,84 (66,98)	2,29 (0,96)	3,22 (0,41)	3,60 (1,06)	750,21 (872,98)
	Le3	1033,48 (254,26)	102,44 (51,25)	2,18 (1,13)	3,09 (0,63)	3,44 (1,15)	1055,18 (1497,82)
	Le4	2762,6 (1309,31)	113,57 (50,99)	2,11 (0,97)	3,15 (0,55)	3,36 (1,18)	1238,89 (1712,78)

Table 5 – EPG, days in milk, milk quantity and quality with different levels of infection in the two breeds

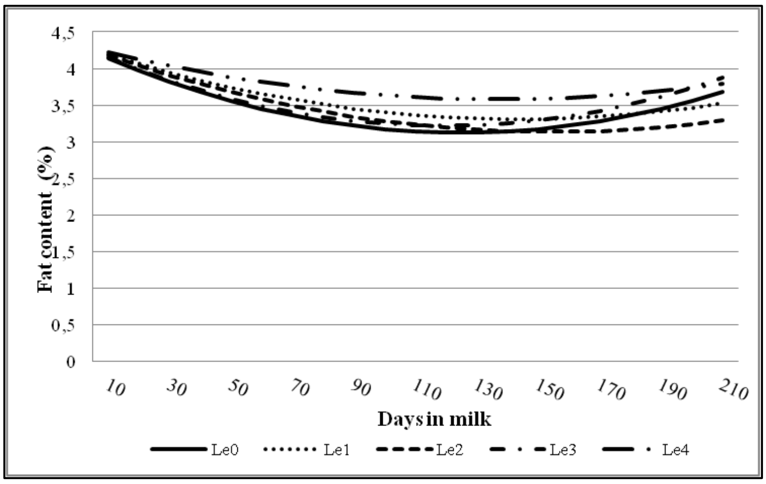

Figure 6 – Variations in fat content (%) during lactation in goats with different levels of GIN infection

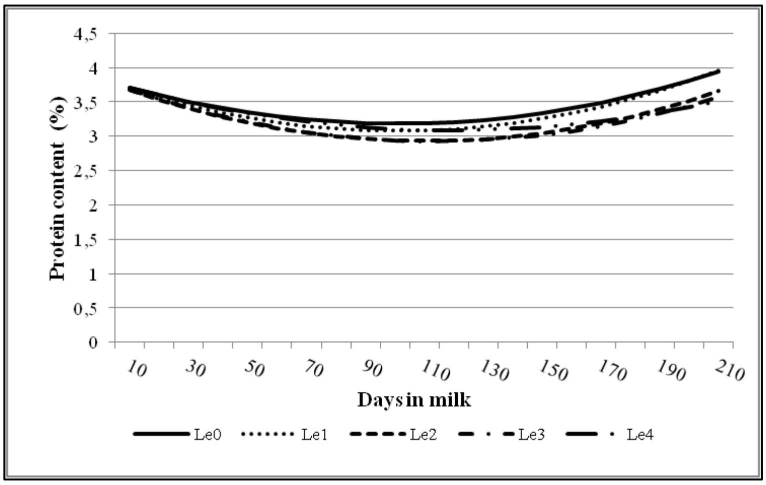

Figure 7 - Variations in protein content (%) during lactation in goats with different levels of GIN infection

Also fat (Fig. 6) and protein (Fig. 7) content showed some variations due to the class of infection: in particular protein content showed the greatest decrease from Le1 to Le3 (-8.8% in Verzasca and -13.7% in Alpine). No significant influence of nematodes on SCC values was detected, even if an important increase could be observed in the highest class of infection (Table 5).

3.2.3. Discussion

In this study we analysed the effects of the different level of Strongylida eggs emission on production throughout one lactation period in two breeds of dairy goat (Alpine and Nera di Verzasca) reared in a same farm. The trends of FEC were strongly different between the breeds and among the months of sampling. It can be hypothesised that these variations were due to climate conditions and to differences in the genetic resistance to nematodes between the breeds; both factors were not unexpected, in fact we knew that faecal egg counts 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) and that some authors report differences in resistance to GIN among goat breeds (Chiejina and Behnke, 2011).

The scarce influence of the number of kidding, that more or less coincides with age, on the emission of nematode eggs was a confirmation that goats do not develop a strong and complete immune response against nematodes, as instead occurs in sheep (Huntley *et al.*, 1995).

The similarity between curves of lactation and curve of egg emission was an interesting result that could be explained in different ways: it is possible that the reaching of the peak of egg emission 20/30 days after the peak of milk yield was related to a lower effectiveness in the immune mechanisms responsible of the control of the infective larvae ingested, with subsequent higher development of adult nematodes and increased FEC; another possibility is that, since kidding took place mainly in January, the peak of FEC for most goats was in April and it could be related to the increase in the environmental temperatures and development of larvae on pastures.

Testing the effect of class of infection, breed and days in milk on milk yield and quality, our study showed a high significance of the interaction of these factors. Even if in the present study we did not use an anthelmintic treatment to test the effect of parasites on production, our data confirmed clearly that high levels of GIN infection can lead to production losses. This finding was in agreement with results from other studies either on cattle (Charlier *et al*., 2009; Reist *et al*., 2011), sheep (Cringoli *et al*., 2008b; Sechi *et al*., 2010; Cruz-Rojo *et al*., 2012) or goats (Hoste and Chartier, 1998; Rinaldi *et al*., 2007).

Moreover, the substantial decrease in protein content caused by high level of gastrointestinal parasitism was very remarkable. It is worth-mentioning that in Italy a large amount of goat milk is used to make cheese (more than 2250 ton in 2008; FAOSTAT); in particular in the study area where this work has been carried out, the earnings of goat farmers mainly rely upon cheese production and kid meat (only for Easter), thus a so substantial but not showy decrease in the protein content, like above-reported, could be very important for their economy.

Finally our study showed that the level of EPG necessary to cause the loss in production may be different from one breed to another, probably because of different potential levels of milk production as well as different abilities of resilience to GIN infection consequences.

4. RESISTANCE OF NERA DI VERZASCA GOAT TO GASTROINTESTINAL NEMATODE INFECTION UNDER FIELD CONDITIONS

In the section “Effects of gastrointestinal nematodes on milk production – I” we found that Nera di Verzasca goats seemed to be characterized by a higher resilience to GIN than the other investigated breeds (Saanen and Alpine), showing minor effects of GIN on their productivity. The purpose of the present survey was to observe and compare the trend of GIN infection (measured by FEC) throughout one year between two goat breeds (Nera di Verzasca and Alpine) reared in semi-extensive way in the same flock, to find out if these breeds were characterised by different resistance to nematodes.

4.1. Materials and methods

Farm and animals

This study was carried out on 61 adult female goats of two different dairy breeds: 30 goats of Alpine breed and 31 goats of Nera di Verzasca breed. These animals were reared in the same farm, on the mountains around the lake Verbano. For farm management information see the materials and methods of the section “Effects of gastrointestinal nematodes on milk production – II”.

Samples and techniques

Anthelmintic treatment (netobimin 15 mg/kg) was administered to the whole herd in November 2009. The 61 lactating goats selected for the study were homogeneously distributed within the groups as regards their age from 1 to >7 years old. Faecal samples were collected monthly from January to December 2010 to evaluate the gastrointestinal parasitic load expressed by the emission of eggs and measured as eggs per gram of faeces (EPG). Strongylida faecal egg counts were performed using FLOTAC double technique with NaCl solution (s.g. 1.200) (Cringoli *et al.*, 2010).

From April to December 2010 (except for June) serum samples were obtained from all the goats by monthly puncture of the jugular vein and the level of pepsinogen in samples was determined using the micromethod proposed by Dorny and Vercruysse (1998).

Meteorological data

Data on daily mean temperature and daily cumulative rainfall were recorded at the nearest weather station (Luino) by the regional meteorological service of the Regional Environmental Conservation Agency of Lombardy (A.R.P.A. Lombardia) and downloaded from the archive of the weather data (http://ita.arpalombardia.it/meteo/meteo.asp).

Statistical analysis

EPG values were log-transformed and data were analysed by the SPSS software (SPSS 17.0, SPSS Inc., USA)

4.2. Results

Looking at the whole parasitological data throughout the year, a large variability emerged both in the prevalence of infected animals and in the range of EPG values (Table 6). This variability appeared different between the two breeds: the minimum prevalence was higher in the Alpine breed and in these goats the average value of EPG on the whole sampling period was more than double than in goats Nera di Verzasca. This difference was highly significant (p = 0.0037). Differences were also noticed in the distribution of the nematode egg emission: in fact, in both breeds EPG values were over-dispersed following a negative binomial pattern (adjustment to binomial distribution Nera di Verzasca: χ^2=8.315152, p = 0.0039342; adjustment to binomial distribution Alpine: χ^2=9.517596, p =0.0020368), but 87.1% of Verzasca goats showed EPG ≤ 600 while only 30% of Alpine goats did (Fig. 8).

	EPG: Mean – (SD)	EPG: Min - Max	Prevalence (Min - Max)
Total	568,85 - (1119,98)	0 - 10452	51,52 - 100%
Nera di Verzasca	334,40 - (663,65)	0 - 4300	51,52 - 100%
Alpine	818,94 - (1415,97)	0 - 10452	68,75 - 100%

Table 6 – EPG and prevalence of infection in the two breeds throughout the year

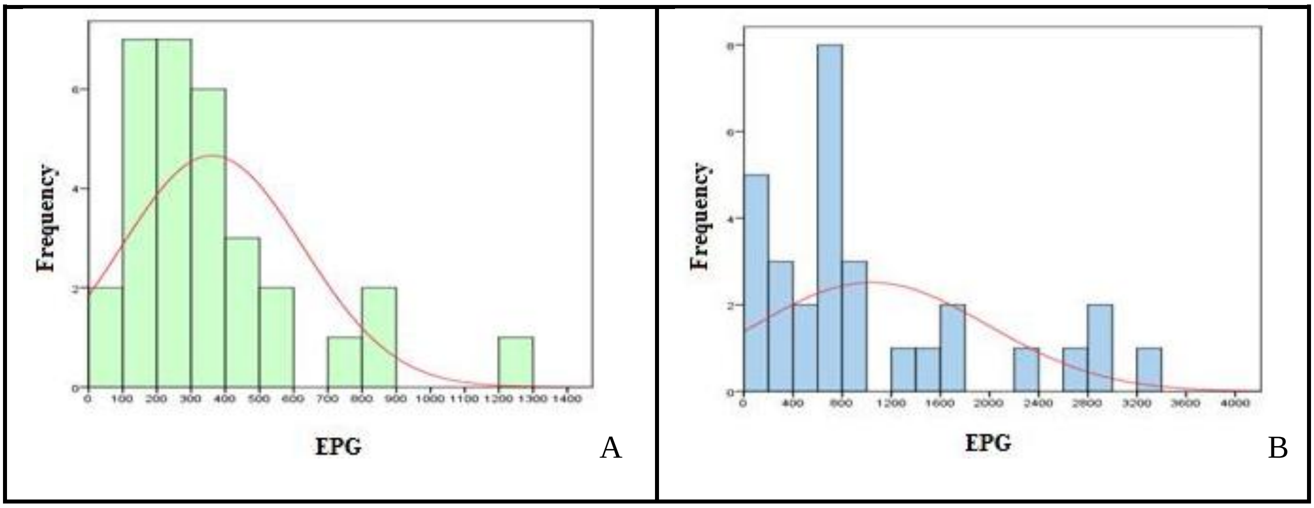

Figure 8 – Distribution of EPG emission in goats of Nera di Verzasca (A) and Alpine (B) breed

At the beginning of the survey EPG values were low and slightly increasing in all goats; then in March and April EPG rised up to very high levels in both breeds. From May onwards the monthly average EPG became very different between breeds, with goats of Verzasca showing lower EPG than Alpine till the end of the year. In fact Verzasca goats showed a rapid and persistent decrease in EPG values, opposite to Alpine goats that showed high levels of EPG till July, then a rapid decrease in August and September, when the breeds had similar EPG counts; during the last months of the year Alpine goats showed again an upward trend in EPG values, while Verzasca goats kept very low levels (Fig. 9). Very significant differences in EPG ($p < 0.0001$) were due to the effect of the interaction between month and breed.

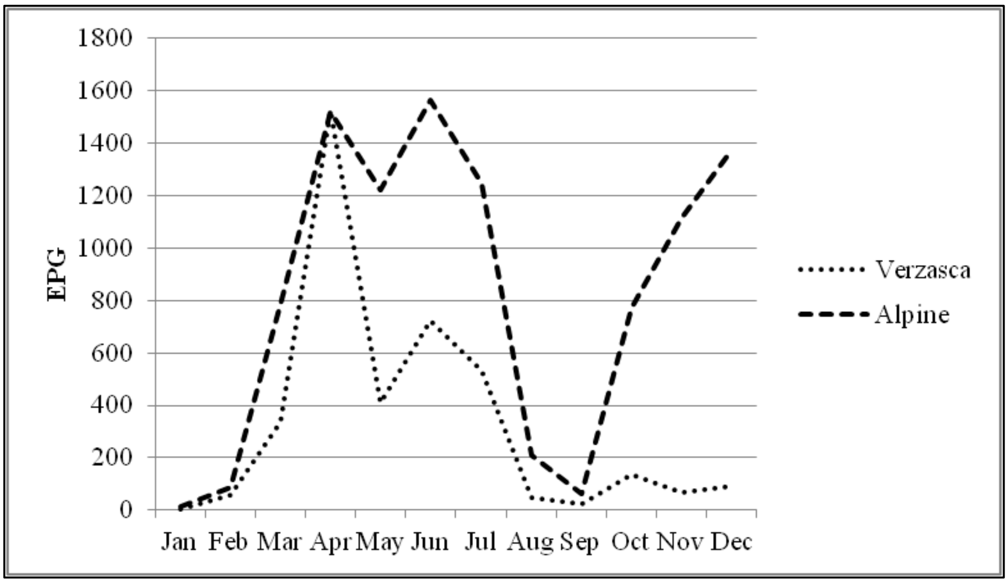

Figure 9 – EPG levels during the year in the two breeds

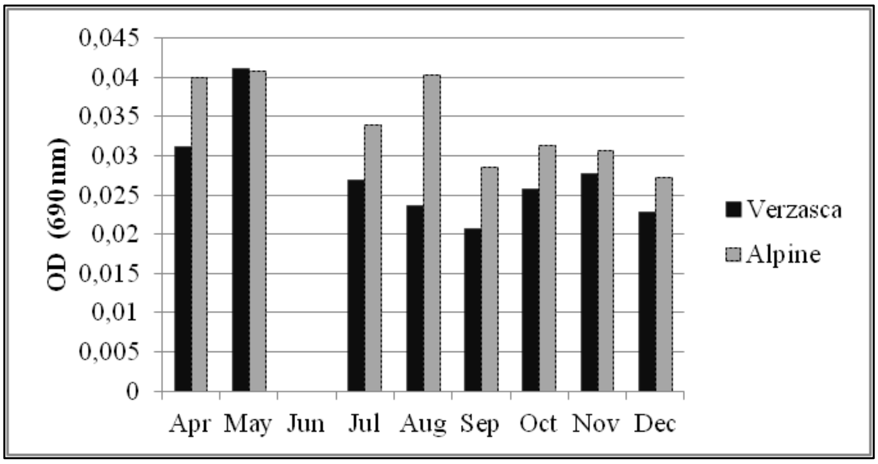

Figure 10 – Pepsinogen levels (in OD) in the two breeds

Pepsinogen levels varied significantly with the sampling month (Kruskall-Wallis test, $p < 0.0001$) and were different between the breeds, with the Alpine goats showing higher values almost throughout the year (Kruskall-Wallis test, $p < 0.0001$). The levels of this pro-enzyme varied during the year with a trend very similar to that of the EPG (Fig. 10), showing a significant correlation with EPG (Spearman correlation $p < 0.0001$). This mild positive correlation ($r = 0.19$) can be appreciated comparing the graph of EPG (Fig. 9) and pepsinogen (Fig. 10): for example, the increase in pepsinogen levels (mainly in Alpine) seen in July and August was probably due to the increase of the intra-mucosal larval population, sign of a gradual increase in parasitic load.

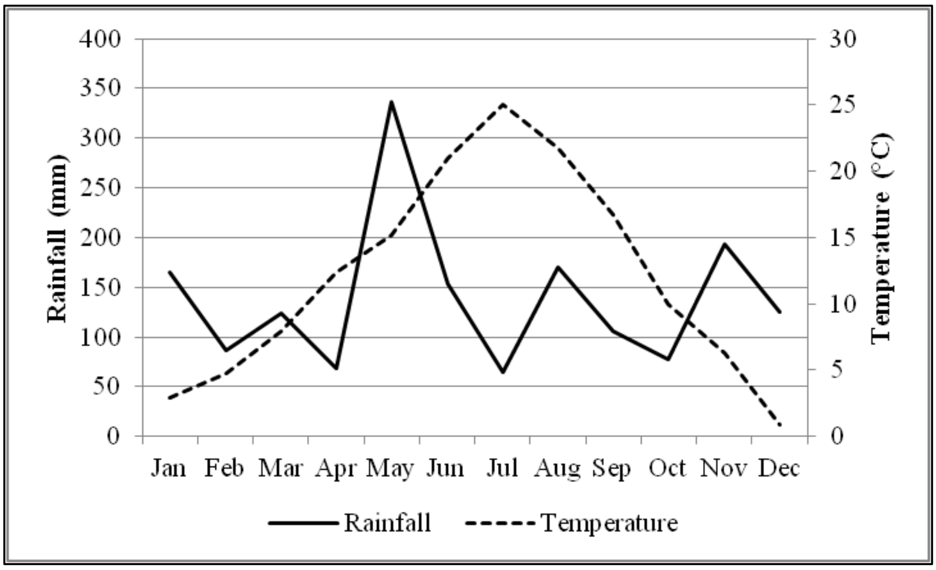

Figure 11 – Rainfall and temperatures in the area of study during the year of sampling

The mean of temperatures and cumulative rainfall data recorded during the year of the study are shown in the graph in fig. 11. The most rainy month of the year was May (336 mm H_2O), followed by November (193 mm H_2O) August (170 mm H_2O) and January (165 mm H_2O), while the driest months were July (65 mm H_2O) and April (69 mm H_2O). The hottest months were June (21°C), July (25°C) and August (22°C), while the coldest were December (1°C) and January (3°C).

4.3. Discussion

In this study we analysed the emission of Strongylida eggs throughout one year in two breeds of goat (Alpine and Nera di Verzasca) reared in a same farm. The FEC started at very low level in the first trimester, then there was a peak in the month of April, followed by a decrease until August and September and a second rising only for the Alpine breed during the last trimester of the year. This trend was probably related to climate condition and management of the farm (very high significance of the month effect). In fact, after the anthelmintic treatment (in November before the starting of the survey) the EPG kept low because in the winter months goats were indoor for large part of the day and did not have high risk to get infected. Then in the spring season goats were free to increase their time spent on pasture, there were higher temperatures and rainfall and probably the reactivation of quiescent larvae; these factors may be relevant in explaining the peak in EPG emission observed in both breeds. The subsequent falling of FEC in our opinion was partly due to the development of the immune response of the goats (also as a result of a better nutrition on pastures) and partly to the hotter and dryer climate of summer months that inhibited the larval development on pastures. The second rising in FEC in Alpine breed might be explained indeed by a new generation of nematodes developed after summer and not well hindered by these goats; after summer in fact, average temperatures decreased and rainfall slightly increased, thus providing a more suitable environment for the survival of nematodes infective larvae, which consequently became a source of infection for the grazing animals. Very similar observations about the trends of FEC and the weather conditions throughout the year were reported in Greece (Papadopoulos *et al*., 2003).

From a previous study Nera di Verzasca was found to be a more resilient breed than Alpine; furthermore other authors published evidences of greater resistance to nematodes in local breeds of goat, less selected by man than other more susceptile breeds (Pralomkarn *et al*., 1997; Baker *et al*., 1998; Chiejina and Behnke, 2011). The trends of FEC of the two breeds were strongly different in the present study and the local breed Nera di Verzasca showed a higher resistance to gastrointestinal nematode infection. In fact, except for the month of April in which the average EPG were comparable, FEC of Nera di Verzasca goats were constantly lower for the remainder of the year. The serum pepsinogen level is widely accepted as a useful parameter for monitoring gastrointestinal nematode infections in first-season grazing calves (Charlier *et al*., 2011) and it is related to the abomasal modifications induced by the nematodes. Pepsinogen levels in our study well reflected the difference between the two breeds in infection level expressed by FEC, complementing these data and suggesting that Nera di Verzasca goats were less affected by the parasite infection.

Moreover the prevalence of infected Nera di Verzasca goats was lower in the first months of the observation period, suggesting a slower rate of infection for this breed. In goats as well as in other animal species, the distribution of worm populations in the host is aggregated, corresponding to negative binomial distributions (Hoste *et al*., 2002). The different distribution of EPG emission between the breeds in the present study, with Verzasca showing lower maximum levels and a more narrow range of EPG values, are key-points demonstrating the diffused ability to control nematode infection in this breed. This capacity is probably not related to a differing feeding behaviour in Nera di Verzasca, as we never detected differences between the two breeds during observations on pasture circuit (personal observations) and other authors that made similar observations before us never mentioned differences as well (Maggioni *et al*., 2004).

These results suggest that Nera di Verzasca breed could be genetically endowed with a greater resistance and resilience to trichostrongyles, whose mechanisms have to be deeper investigated.

5. RESISTANCE OF NERA DI VERZASCA GOAT TO GASTROINTESTINAL NEMATODE INFECTION UNDER EXPERIMENTAL CONDITIONS

This study was carried out with two main purposes: first, to investigate the interaction between goat and gastrointestinal nematodes under experimental infection and so to widen knowledge about mechanisms of resistance and resilience to GIN in goats; second, to highlight differences in the egg emission after a single larval challenge between goats of Alpine and Nera di Verzasca breed, in order to confirm and to strengthen the data obtained under field conditions, avoiding the influence of possible differences due to feeding behaviour.

5.1. Materials and methods

The study was carried out in the experimental farm of the School of Veterinary Medicine of Milan; the farm is located in Borgo Adorno, Cantalupo Ligure (AL) and it is a teaching and experimental farm in which dairy goats breeding and cheese production are the main activities.

Larvae preparation

Third-stage infective larvae of mixed gastrointestinal nematodes were cultured from fresh faeces of highly infected goats (3000-9000 EPG). The faeces were collected directly from the animals' rectum and then put in a large vessel in the lab; the vessel with faeces was incubated for 10 days at 25°C, ensuring a good level of oxygen and moisture (50-80%). At the end of the coproculture, larvae were recovered by a modified Baermann method; then concentrated by a sucrose-based solution and finally washed by several passages in water and stored in flasks with water at 4°C (MAAF, 1986). A little volume of this suspension were fixed with formalin (final concentration 2%) and 200 larvae were identified following the keys by van Wyk *et al*. (2004) in order to know the genus of the larvae in the suspension and their relative prevalence: the most prevalent genus was *Teladorsagia* spp. (84%), followed by *Haemonchus* spp. (15%) and *Trichostrongylus* spp. (1%). Two days before challenge infection, larvae were put out of the fridge at room temperature, so that they could reactivate.

Goats' preparation

In this experiment 15 goats were involved; all animals were homogeneous for their age and production stage (2-3 years old, dry and non pregnant goats). Animals were divided in two groups according to the breed: a group with 7 goats Nera di Verzasca and the other with 8 goats of Alpine breed. For the whole duration of the study the two groups of animals were separated in two different pens with no contact between breeds; goats were fed with gramineous hay and a little supplementation of pelleted concentrate and they did not have access to any pasture. The goats were treated with netobimin at the dose of 15 mg/kg to make them free from parasites. After 3 days they were moved into two pens with clean straw and at the 11th day after treatment individual faecal egg counts were performed to make sure of the efficacy of the anthelmintic treatment. After that, at day 0, all the goats were infected (*per os*) with ~6000 infective larvae (L_3) of mixed gastrointestinal nematodes. From the 15th day post-infection individual FEC were performed on all goats, initially twice per week, then after the 50th day post-infection once a week until 76 days post infection. At the end of the experiment all the animals were treated with netobimin at the above mentioned dosage and came back to their farms to re-start production.

All faecal egg counts were performed with FLOTAC double technique with NaCl flotation solution s.g. 1.200 (Cringoli *et al.*, 2010).

5.2. Results

Data obtained from the faecal egg counts are synthesised in the figure below (Fig. 12). Briefly, starting from the third week post-infection the egg emission increased quite constantly reaching a peak at day 63, then showing a minimum decrease. This trend was observed in both breeds, but goats of Nera di Verzasca showed average EPG values lower than Alpine goats throughout the experiment, with Alpine showing values of EPG 1.87 times higher at the peak (63rd day post-infection) (Alpine 588 EPG vs Verzasca 314 EPG).

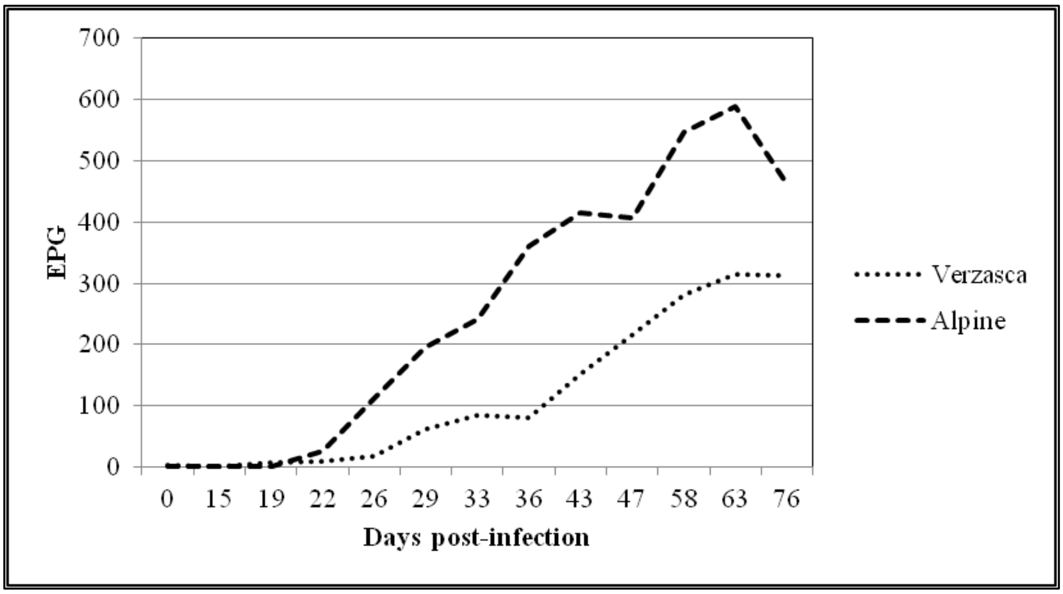

Figure 12 – FEC in the two breeds after the single challenge with ~6000 infective larvae of *H. contortus*

5.3. Discussion

The study confirmed that goats of Nera di Verzasca are less susceptible to the infection with gastrointestinal nematodes; furthermore, it demonstrated that such difference in resistance it was not attributable to differences in feeding behaviour between the two breeds at pasture, in fact in this experiment all the goats received the same food. Looking at the FEC, the data suggested that goats of Nera di Verzasca probably have a greater ability to inhibit the establishment of incoming infective larvae and/or they are more able to reduce the fertility of the adult nematodes than goats of Alpine breed. Anyway that ability leads to a fewer contamination on the ground (almost always pasture in the case of Nera di Verzasca) with nematode eggs and to a less frequent need of anthelmintic treatment.

Although the study was carried out with a little number of animals, such results were very promising and worth mentioning. Nonetheless it must be noticed that this small number of animals and the farm of common origin were factors that could partly explain the similar response to the infection in Nera di Verzasca; in other words, it is possible that the common genetic of this small group of goats (scarce genetic variability) determined the fact that all these goats had a similar responsiveness to the infection. In the other hand, it is also true that the total amount of goats belonging to this breed reaches a few thousands of animals, so it is very likely that the genes of resistance to parasites are quite diffused.

In order to eliminate these confusing factors, it would be interesting to promote studies with a larger number of animals originated from several different farms. Furthermore it would be worth to increase the parameters measured in the animals (e.g. blood cell count, serum biochemistry, etc...) and to slaughter some of the goats at the end of the experiment, if possible; that would give the possibility to count the adult worm populations established after the challenge infection and to evaluate the modifications of the tissues and cells involved in the local immune response against the nematodes.

6. SPECIFIC ANTIBODY RESPONSES TO *TELADORSAGIA CIRCUMCINCTA* IN NERA DI VERZASCA AND ALPINE GOATS

After the interesting results reported in the section "Resistance of Nera di Verzasca goat to gastrointestinal nematode infection under field conditions", about the different susceptibility to GIN infection expressed by FEC values between goats of Alpine and Nera di Verzasca breeds, we wanted to deepen the study in order to understand the mechanisms at the basis of this diversity. As we had stored (-20°C) double serum samples of all the goats sampled from April to December 2010 and as we knew from previous surveys that *Teladorsagia circumcincta* was the most prevalent gastrointestinal parasite of goats in this area (Zanzani, Ph.D. thesis, 2010; Manfredi *et al*., 2010), we decided to focus our attention on the interaction between this parasite and the humoral immune response of the goats. Thus, the purpose of this study was to investigate if the differing susceptibilities between the two goat breeds expressed by FEC were due to differing levels of circulating *T. circumcincta* specific antibodies.

6.1. Materials and methods

This study was carried out on 60 adult female goats of two different dairy breeds: 30 goats of Alpine breed and 30 goats of Nera di Verzasca breed. These animals were reared in the same farm, on the mountains around the lake Verbano. For farm management information see the materials and methods of the section "Effects of gastrointestinal nematodes on milk production – II".

Faecal examinations

The 60 goats selected for the study, 30 per breed, were homogeneously distributed within the groups as regards their age from 1 to >7 years old. Individual faecal samples were collected once per month from April to December 2010 to evaluate the gastrointestinal parasitic load expressed by the emission of eggs and measured as eggs per gram of faeces (EPG). Trichostrongyle faecal egg counts were performed using FLOTAC double technique with NaCl solution (s.g. 1.200) (Cringoli *et al*., 2010).

ELISAs

Monthly collection of serum samples from April to December (except for June) was also carried out; sera were stored in 1.5 ml Eppendorf tubes at -20°C until analysis. Indirect ELISAs for the detection and quantification of *Teladorsagia circumcincta* specific immunoglobulin(Ig) A and IgE in the goat sera were performed using *T. circumcincta* L_3 complex somatic antigen, following the Moredun Research Institute (MRI) protocol. Briefly, ELISA plates (Immulon 2HB, thermoelectron 3455) were incubated overnight (4°C) with (50 µl/well) *T. circumcincta* L_3 antigen (2µl/ml) in carbonate/bicarbonate buffer pH 9·6. Wells were washed five times in PBS containing 0.05% Tween 20 (PBS/T20), a blocking buffer (PBS + 3% fish gelatine, Sigma G7765) was added to each well (100 µl/well) and plates incubated for 1 hr at 37°C before receiving 50 µl/well (finale volume) of serum samples diluted in PBS containing 0.5% Tween 80 and 0.5 M NaCl (PBS/T80). Plates were then sequentially incubated with 50 µl of: a 1/1000 dilution in PBST80 of the anti-ovine/bovine IgA (AbDSerotec MCA628) or a 1/100 dilution in PBST80 of the anti-sheep/goat IgE (clone 2F1, culture supernatant MRI); after that with 50 µl (1/1000 dilution in PBST80) of rabbit anti-mouse Ig-HRP (Dako P0260). Each incubation was for 1 hr at room temperature and plates were washed five times in PBS/T20 between incubations. Colour reaction was developed with ortho-phenylenediamine (OPD) substrate (SigmaFast OPD, P9187) 100µl/well at room temperature for 5-10 minutes and stopped by the addition of 25 µl of 2.5M H_2SO_4. Reading of OD was at 492. The optimum dilution of serum samples was determined following serial two-fold dilutions from 1/5 to 1/2560; for IgA established dilution was 1/20 and for IgE 1/10.

Statistical analysis

The effect of IgA, IgE, goat breed, month and age (n. of kidding) on the nematode infection (EPG) was analyzed through GLM with negative binomial error distribution in order to consider the aggregated distribution of parasites (Wilson and Grenfell, 1997; Shaw *et al.*, 1998). Confidence level was held at 95% and $p < 0.05$ was set for significance; all analyses were undertaken in R (R Development Core Team, 2011).

6.2. Results

Effect of the factors tested on EPG are shown in the table below (Table 7). EPG values (Fig. 13) were higher in the Alpine breed, with a statistical difference between the breeds ($p < 0.001$). FEC were also influenced by the effect of the month of the year ($p < 0.001$). Also the interaction between the breed and the month showed very important effect on EPG values ($p < 0.001$). No direct effect of the age on FEC was found, but the effect of the interaction between age and month ($p < 0.001$) and between age and breed ($p = 0.021$) showed statistical significance.

Although IgA levels were higher in the Alpine breed throughout the sampling period (Fig. 14), the interaction between IgA and goat breed did not show any statistical effect on the EPG ($p = 0.385$). The effect of IgA levels was not important on the FEC when considered alone ($p = 0.574$), but the interaction between IgA and age showed a mild effect ($p = 0.049$) and the interaction between IgA and month showed a strong effect ($p = 0.007$) on the EPG.

Factor	Wald statistic	n.d.f.	F statistic	*p*
IgA	0.32	1	0.32	0.574
IgE	7.04	1	7.04	0.009
Breed	34.88	1	34.88	< 0.001
Age	2.80	1	2.80	0.100
Month	544.96	7	77.85	< 0.001
IgA : IgE	2.81	1	2.81	0.095
IgA : Breed	0.76	1	0.76	0.385
IgE : Breed	0.19	1	0.19	0.661
IgA : Age	3.90	1	3.90	0.049
IgE : Age	2.70	1	2.70	0.104
Breed : Age	5.64	1	5.64	0.021
IgA : Month	19.87	7	2.84	0.007
IgE : Month	1.89	7	0.27	0.965
Breed : Month	33.75	7	4.82	< 0.001
Age : Month	32.98	7	4.71	< 0.001

Table 7 – Effect of the factors IgA, IgE, goat breed, age and month on the EPG

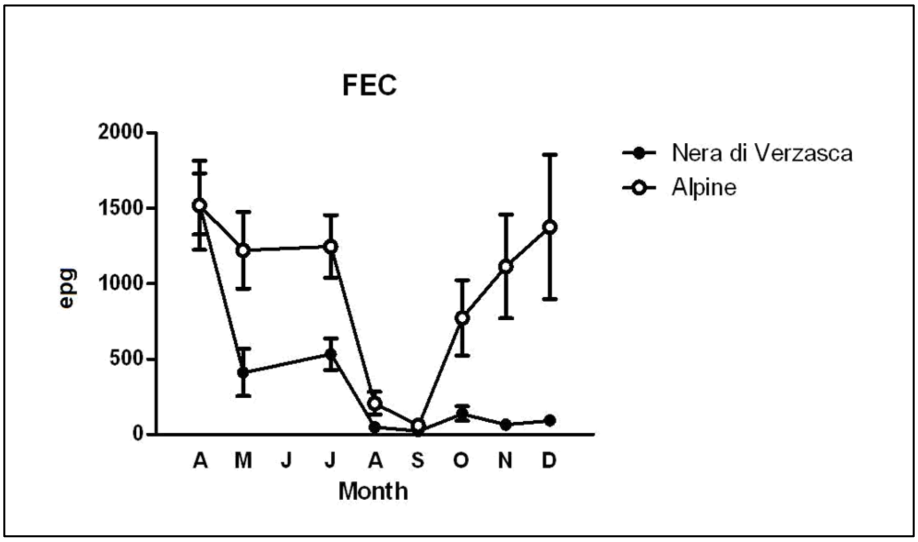

Figure 13 – FEC in the two breeds during the study

IgE levels were a little higher in Nera di Verzasca breed in the first half of the study, then they overlapped in the two breeds in the second part of the year (Fig. 15). IgE showed a significant direct effect in reducing the FEC ($p = 0.009$), while no significant effect of the interactions between IgE and breed ($p = 0.661$), age ($p = 0.104$) or month ($p = 0.965$) was seen.

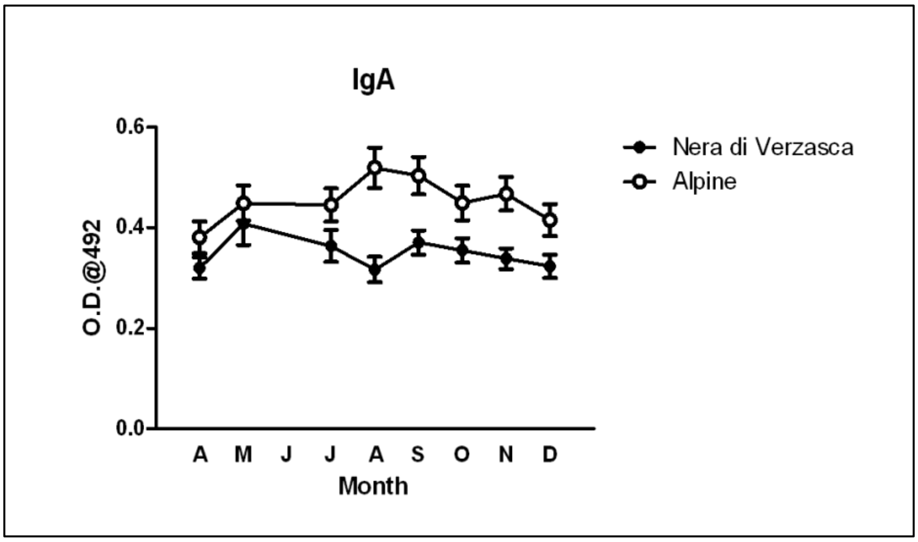

Figure 14 – IgA levels (in OD) in the two breeds during the study

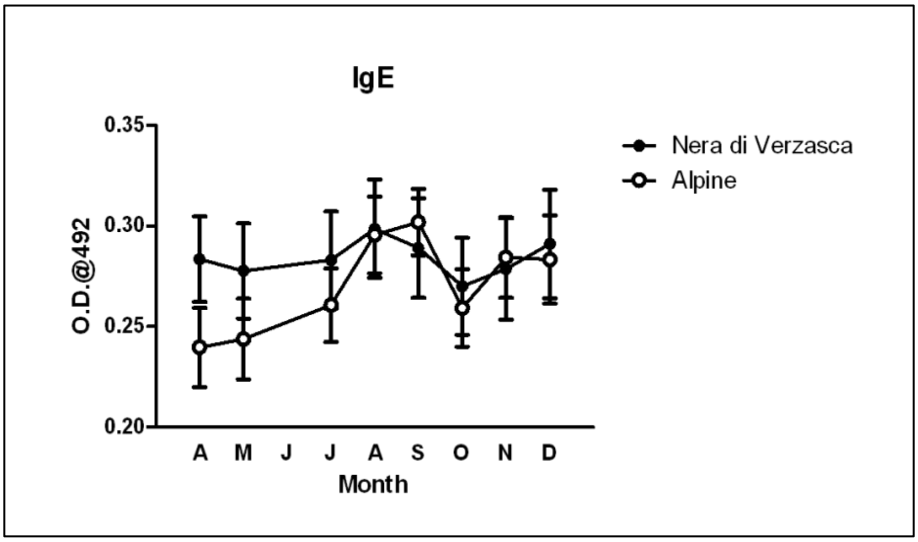

Figure 15 – IgE levels (in OD) in the two breeds during the study

6.3. Discussion

In this study we evaluated the humoral immune response against *Teladorsagia circumcincta* in two goat breeds characterised by differing resistance to trichostrongyle infection; in fact, FEC were strongly influenced by the breed, suggesting that the Alpine breed is more susceptible to trichostrongyle nematode infections than the Nera di Verzasca breed. For the evaluation of the immune response an indirect ELISA was used to quantify the levels of circulating specific IgA and IgE against the L_3 stages of the nematode. Variations found in antibody levels over time could probably be related to the seasonality of parasite life cycle in our region, 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. Moreover, it must be considered that the goats of the study spent very long time on wide pastures, exposing themselves to continuous re-infections by mixed trichostrongyles. Thus egg emission was probably linked also to species other than *T. circumcincta* and this fact could partially explain the discrepancies, when present, between the trends of FEC and levels of immunoglobulin A and E.

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) has shown that in sheep variation in parasite-specific IgA and eosinophilia responses, interacting in the regulation of *T. circumcincta* growth, were strongly related to the variation in adult worm length. In the other hand an association between high plasma IgE activity against a major surface allergen of *T. circumcincta* L_3 and low FEC was clearly demonstrated in naturally infected crossbred lambs (Huntley *et al*., 2001). Scarce data on this topic are available for goats. De la Chevrotière *et al*. (2012) showed that in Creole goats IgA response against L_3 or escretory/secretory products of adults *H. contortus* was positively correlated to FEC, in contrast with the negative correlation between IgE against L_3 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. Even if based on the immune response to a different nematode (*T. circumcincta*), the results of the present study are quite similar to those of De la Chevrotière *et al*. (2012); in fact, we found a negative effect of IgE levels on the EPG, but no effect of the IgA.

In our study no statistical difference was seen in the levels of IgA and IgE between the breeds. 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 to increased IgA and IgE circulating levels; after that period EPG rised again, suggesting that a turnover of the nematode population took place despite the increased antibody levels. It must be noticed that in this last trimester the FEC of the Alpine breed showed a higher variability among individuals than before, as demonstrated by the greater S.E. (Fig. 11). That could mean that some goats achieved a sufficient immune response to control the infection, while some others did not.

The Nera di Verzasca goats gave evidence of a more rapid and 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. In these goats neither IgA nor IgE levels were statistically different from those of Alpine goats. Therefore the higher resistance to trichostrongyle nematode infection seen in the Nera di Verzasca breed does not appear to be related to circulating levels of *T. circumcincta*-specific IgA or IgE against L_3 stage. Nonetheless IgA were a little lower throughout the study and IgE a little higher in the first half of the study; 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, at this stage there are still some hypothesis to verify in order to better understand the mechanisms involved in the immune response against *T. circumcincta* in goats and in Nera di Verzasca breed in our particular case. As we did not find a significant role either of the IgA or the IgE directed against L_3 crude antigen in the more resistant breed, it is possible that different antigens are targeted between the resistant and the susceptible breed. Another hypothesis is that our results could be different if we analysed the response against L_4 or L_5 (immature adult stage) antigens. 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). Probably the two breeds of our studies induce the immune response to GIN by two different mechanisms. We know in fact that the immune response to parasites is quite complex and involves many components: parasite-specific antibodies, effector cells (e.g. mast cells and eosinophils), cytokines (many interleukins and γ-interferon) and other molecules for examples of the family of galectins (Meeusen *et al*., 2005). With no doubt these are interesting points to verify and develop in further investigations.

7. CONCLUSIONS

With this work we add some knowledge on the nematode–host interactions in the goat. In particular our research showed that the milk production in goats was affected by the parasite burden and that this effect could be stronger in the first lactation and decreased in the following lactations. This resilience was also found to be different among goat breeds, with the local breed showing fewer effects on production than the more selected breeds. Despite the decreasing effect of parasites on production in the years, in the other hand the work confirmed that with the increasing of the age, goat do not develop a sufficient immune response against nematodes, as instead occurs in sheep. Testing the effect of different classes of infection on milk yield and quality, our study showed a high significance of the interaction of the class of infection with breed and days in milk, giving evidence that high levels of GIN infection can lead to production losses and that the level of EPG necessary to cause the loss in production may be different from one breed to another, probably because of different potential levels of milk production, as well as different abilities of resilience to GIN infection consequences.

After those results we decided to focus the attention on the differences between breeds, exploring the higher potential resistance in a local breed reared in northern Lombardy, the Nera di Verzasca goat. Comparing the nematode egg emission for a whole year between this local breed and goats of Alpine breed reared together, we found resistance to nematodes in the Nera di Verzasca goats, expressed by lower FEC throughout the year. Moreover, the different distribution of EPG emission between the breeds gave evidence of the diffused ability to control nematode infection in the Nera di Verzasca breed. This breed confirmed its resistance also under experimental infection, after a single challenge with mixed GIN infective larvae. Finally, investigating the specific antibody response against *Teladorsagia circumcincta* L_3, our research confirmed the role of IgE in the protection against gastrointestinal nematodes. Nonetheless, through that study, we did not find a clear explanation of the higher resistance of Nera di Verzasca goat to nematodes and some hypothesis still have to be verified and developed in further investigations.

The losses of production caused by the infection with internal parasites in grazing animals, the cost of anthelmintics and the increasing frequency of anthelmintic resistance, increasing environmental concerns and consumer demand for animal products and pastures free of chemical residues are all good reasons to invest in research aimed at finding some alternative solutions in the parasite control.

Because of the considerable importance that parasitic diseases still have in small ruminant breeding and of this increased need for new approaches in the control of these infections, studies on the topic of this research must be seen as additional supports to the control of parasites with methods alternative to conventional anthelmintic therapy. In fact breeds with greater resistance to parasites require fewer treatments, consequently these breeds contribute to a lesser extent to the onset of anthelmintic-resistance in parasites. Thus, although the breeding of local goat breeds often provides fewer profits, it can be economically advantageous as well, when the lower productions are rewarded with lower expenses in veterinary services and lower management costs.

Finally, this kind of breeding must be taken into great consideration in today's society, as it plays an important role related to its ethical value, to the preservation of biodiversity, of the cultural and historical heritage and of the landscape and territory.

8. REFERENCES

Alberti, E.G., Zanzani, S.A., Ferrari, N., Bruni, G., Manfredi, M.T., 2012. Effects of gastrointestinal nematodes on milk productivity in three dairy goat breeds. *Small Rum. Res*. 106, Suppl 1, S12–S17

Alvarez-Sanchez, M.A., Perez-Garcia, J., Cruz-Rojo, M.A., Rojo-Vazquez, F.A., 2006. Anthelmintic resistance in trichostrongylid nematodes of sheep farms in Northwest Spain. *Parasitol. Res*. 99, 78–83

Amarante, A.F.T., Bricarello, P.A., Huntley, J.F., 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. *Vet. Parasitol*. 128, 99–107

Artho, R., Schnyder, M., Kohler, L., Torgerson, P.R., Hertzberg, H., 2007. Avermectin-resistance in gastrointestinal nematodes of Boer goats and Dorper sheep in Switzerland. *Vet. Parasitol*. 144, 68–73

Attili, A.R., Ayala, C., Traldi, G., Furbetta, R., Habluetzel, A., 2004. Endoparasitic infection patterns in angora goats and merino sheep in a fibre animal farm in Central Italy. *Parassitologia* 46 (suppl. 1), 23

Baker, R.L., Mwamachi, D.M., Audho, J.O., Aduda, E.O., 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

Balic, A., Bowles, V.M., Meeusen, E.N.T., 2000. The immunobiology of gastrointestinal nematode infections in ruminants. *Adv. Parasitol.* 45, 181–241

Barger, I.A., 1999. The role of epidemiological knowledge and grazing management for helminth control in small ruminants. *Int. J. Parasitol.* 29, 41–47

Borgsteede, F., Verkaik, J., Moll, L., Dercksen, D., Vellema, P., Bavinck, G., 2010. How widespread is resistance to invermectin among gastrointestinal nematodes in sheep in The Netherlands? *Tijdschr. Diergeneeskd*. 135, 782–785

Bush, A.O., Fernandez, J.C., Esch, G.W., Seed, R., 2001. Parasitism: The Diversity and Ecology of Animal Parasites. Cambridge University Press, Cambridge, UK

Cabaret, J., 2000. Anthelmintic resistance in goats: from fiction to facts. In: *Proceedings of the 7th International Conference on Goats*, Tours 15–18th May, pp. 793–794

Cabaret, J., Baudet, H.M., Devos, J., Hubert, J., Cortet, J., Sauvé, C., 1995. Studies on multispecific resistance of gastrointestinal nematodes to benzimidazoles on dairy-goat farms. *Vet. Parasitol.* 60, 331–337

Cernanska, D., Varady, M., Jorba, C., 2006. A survey on anthelmintic resistance in nematode parasites of sheep in the Slovak Republic. *Vet. Parasitol*. 135, 39–45

Charlier, J., Dorny, P., Levecke, B., Demeler, J., von Samson-Himmelstjerna, G., Höglund, J., Vercruysse, J., 2011. Serum pepsinogen levels to monitor gastrointestinal nematode infections in cattle revisited. *Res. Vet. Sci*. 90, 451–456

Charlier, J., Höglund, J., von Samson-Himmelstjerna, G., Dorny, P., Vercruysse, J., 2009. Gastrointestinal nematode infections in adult dairy cattle: impact on production, diagnosis and control. *Vet. Parasitol.* 164, 70–79

Chartier, C., Hoste, H., 1997. Response to challenge infection with *Haemonchus contortus* and *Trichostrongylus colubriformis* in dairy goats. Differences between high and low-producers. *Vet. Parasitol*. 73, 267–276

Chartier, C., Hoste, H., 1998. Repeated infections with *Haemonchus contortus* and *Trichostrongylus colubriformis* in dairy goats: comparison of resistant and susceptible animals. *Parasitol. Res.* 84, 249–253

Chartier, C., Soubirac, F., Pors, I., Silvestre, A., Hubert, J., Couquet, C., Cabaret, J., 2001. Prevalence of anthelmintic resistance in gastrointestinal nematodes of dairy goats under extensive management conditions in southwestern France. *J. Helminthol*. 75, 325–330

Chiejina, S.N., 1986. The epizootiology and control of parasite gastroenteritis of domesticated ruminants in Nigeria. *Helminthol. Abstract (Serie A)* 55, 413–419

Chiejina, S.N., Behnke, J.M., 2011. The unique resistance and resilience of the Nigerian West African Dwarf goat to gastrointestinal nematode infections. *Parasites & Vectors* 4, 12

Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M.A., Vercruysse, J., 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. *Vet. Parasitol*. 136, 167–185

Coop, R.L., Kyriazakis, I., 1999. Nutrition–parasite interaction. *Vet. Parasitol.* 84, 187–204

Cooper, J., Gordon, I.J., Pike, A.W., 2000. Strategies for the avoidance of faeces by grazing sheep. *Appl. Anim. Behav. Sci*. 69, 15–33

Crepaldi, P., Corti, M., Cicogna, M., 1999. Factors affecting milk production and prolificacy of Alpine goats in Lombardy (Italy). *Small Rumin. Res*. 32, 83–88

Cringoli, G., Rinaldi, L., Maurelli, M.P., Utzinger, J., 2010. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. *Nature Protocols* 5, 503–515

Cringoli, G., Rinaldi, L., Veneziano, V., Pennacchio, S., Gorgoglione, M., Santaniello, M., Schioppi, M., Fedele, V., 2008a. Gastrointestinal strongyle faecal egg count in goats: circadian rhythm and relationship with worm burden. *Vet. Res. Commun*. 32, S191–S193

Cringoli, G., Veneziano, V., Jackson, F., Vercruysse, J., Greer, A.W., Fedele, V., Mezzino, L., Rinaldi, L., 2008b. Effects of strategic anthelmintic treatments on the milk production of dairy sheep naturally infected by gastrointestinal strongyles. *Vet. Parasitol*. 156, 340–345

Cringoli, G., Veneziano, V., Rinaldi, L., Sauvé, C., Rubino, R., Fedele, V., Cabaret, C., 2007. Resistance of trichostrongyles to benzimidazoles in Italy: a first report in a goat farm with multiple and repeated introductions. *Parasitol. Res*. 101, 577–581

Cruz-Rojo, M.A., Martínez-Valladares, M., Álvarez-Sánchez, M.A., Rojo-Vázquez, F.A., 2012. Effect of infection with *Teladorsagia circumcincta* on milk production and composition in Assaf dairy sheep. *Vet. Parasitol.* 185, 194–200

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

Diez-Banos, P., Pedreira, J., Sanchez-Andrade, R., Francisco, I., Suarez, J.L., Diaz, P., Panadero, R., Arias, M., Painceira, A., Paz-Silva, A., Morrondo, P., 2008. Field evaluation for anthelmintic-resistant ovine gastrointestinal nematodes by in vitro and in vivo assays. *J. Parasitol.* 94, 925–928

Dominik, S., 2005. Quantitative trait loci for internal nematode resistance in sheep: a review. *Genet. Sel. Evol*. 37, S83–S96

Domke, A.V.M., Chartier, C., Gjerde, B., Höglund, J., Leine, N., Vatn, S., Stuen, S., 2012. Prevalence of anthelmintic resistance in gastrointestinal nematodes of sheep and goats in Norway. *Parasitol. Res.* 111, 185–193

Dorny, P., Vercruysse, J., 1998. Evaluation of a micro method for the routine determination of serum pepsinogen in cattle. *Res. Vet. Sci.* 65, 259–262

Dupuy, J., Chartier, C., Sutra, J.F., Alvinerie, M., 2001. Eprinomectin in dairy goats: dose influence on plasma levels and excretion in milk. *Parasitol. Res*. 87, 294–298

Etter, E., Hoste, H., Chartier, C., Pors, I., McKellar, A., Huntley, J., De La Farge, F., 2000. Susceptibility of high and low producer dairy goats to single experimental infection with *Trichostrongylus colubriformis*. *Parasitol. Res*. 86, 870–875

Eysker, M., 1997. Some aspects of inhibited development of trichostrongylids in ruminants. *Vet. Parasitol.* 72,265–283

Galtier, P., Escoula, L., Camguilhem, R., Alvinerie, M., 1981. Comparative availability of levamisole in non lactating ewes and goats. *Ann. Rech. Vet*. 12, 109–115

Genchi, C., 2006. Schemi terapeutici e antielmintico-resistenza. *Parassitologia* 48, 423–431

Gevrey, J., 1971. Les coprocultures: réalisation, interprétation en vue de la diagnose des strongles digestifs des ruminants et du porc. *Recl. Med. Vet*. 147, 287–317

Gibbs, H.C., 1986. Hypobiosis and the periparturient rise in sheep. *Vet. Clin. N. Amer.: Food Anim. Prac.* 2, 345–353

Hadas, E., Stankiewicz, M., 1997. The results of anthelmintic abbreviated infections of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* on fecal egg counts in goats on pasture. *J. Parasitol.* 83, 532–533

Henderson, N.G., Stear, M.J., 2006. Eosinophil and IgA responses in sheep infected with *Teladorsagia circumcincta*. *Vet. Immunol. Immunopathol*. 112, 62–66

Höglund, J., Gustafsson, K., Ljungstrom, B.L., Engstrom, A., Donnan, A., Skuce, P., 2009. Anthelmintic resistance in Swedish sheep flocks based on a comparison of the results from the faecal egg count reduction test and resistant allele frequencies of the beta-tubulin gene. *Vet. Parasitol*. 161, 60–68

Hoste, H., Chartier, C., 1998. Response to challenge infection with *Haemonchus contortus* and *Trichostrongylus colubriformis* in dairy goats. Consequences on milk production. *Vet. Parasitol.* 74, 43–54

Hoste, H., Le Frileux, Y., Goudeau, C., Chartier, C., Pors, I., Broqua, C., Bergeaud, J.P., 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

Hoste, H., Leveque, H., Dorchies, Ph., 2001. Comparison of nematode infections of the gastrointestinal tract in Angora and dairy goats in a rangeland environment: relations with the feeding behavior. *Vet. Parasitol.* 101, 127–135

Hoste, H., Sotiraki, S., Landau, S.Y., Jackson, F., Beveridge, I., 2010. Goat–Nematode interactions: think differently. *Trends Parasitol.* 26, 376–381

Hoste, H., Torres-Acosta, J.F.J., Aguilar-Caballero, A.J., 2008. Nutrition–parasite interactions in goats: is immunoregulation involved in the control of gastrointestinal nematodes? *Parasite Immunol.* 30, 79–88

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. *Res. Vet. Sci.* 58, 5–10

Huntley, J.F., 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

Hutchings, M.R., Kyriazakis, I., Papachristou, T.G., Gordon, I.J., Jackson, F., 2000. The herbivore dilemma: trade-offs between nutrition and parasitism in herbivore foraging decisions. *Oecologia* 124, 242–251

Jackson, F., Coop, R.L., 2000. The development of anthelmintic resistance in sheep nematodes. *Parasitology* 120, S95–S107

Krecek, R.C., Hartman, R., Groeneveld, H.T., Thorne, A., 1995. Microclimatic effect on vertical migration of *Haemonchus contortus* and *Haemonchus placei* third-stage larvae on irrigated Kikuyu pasture. *Onderstepoort J. Vet. Res.* 62, 117–122

Le Jambre, L.F., 1984. Stocking rate effects on the worm burdens of Angora goats and Merino sheep. *Aust. Vet. J.* 61, 280–282 (abstract)

Levecke, B., Rinaldi, L., Charlier, J., Maurelli, M.P., Bosco, A., Vercruysse, J., Cringoli, G., 2012. The bias, accuracy and precision of faecal egg count reduction test results in cattle using McMaster, Cornell-Wisconsin and FLOTAC egg counting methods. *Vet. Parasitol.* 188, 194–199

MacKinnon, K.M., Zajac, A.M., Kooyman, F.N.J., Notter, D.R., 2010. Differences in immune parameters are associated with resistance to *Haemonchus contortus* in Caribbean hair sheep. *Parasite Immunol*. 32, 484–493

Maggioni, L., Mondellini N., Corti M., 2004. Utilizzazione di formazioni vegetali miste nelle Prealpi lombarde occidentali mediante circuiti di pascolo con capre da latte. In: Il sistema delle malghe alpine: aspetti agro-zootecnici, paesaggistici e turistici; *Quaderno SoZooAlp* n.1, 139–147

Mandonnet, N., Bachand, M., Mahieu, M., Arquet, R., Baudron, F., Abinne-Molza, L., Varo, H., Aumont, G., 2005. Impact on productivity of periparturient rise in fecal egg counts in Creole goats in the humid tropics. *Vet. Parasitol*. 134, 249–259

Manfredi, M.T., Di Cerbo, A.R., 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

Manolaraki, F., Boukary, M., Pardo, E., Pommaret, A., Le Frileux, Y., Hoste, H., 2012. Comparison of pooled vs individual faecal egg counts to evaluate infections with gastrointestinal nematodes in goats. *Abstract book from the 4th CAPARA WGs Workshop & MC Meeting*, Las Palmas de Gran Canaria, Spain, 19–21 Sep. 2012

Meeusen, E.N.T., Balic, A., Bowles, V., 2005. Cells, cytokines and other molecules associated with rejection of gastrointestinal nematode parasites. *Vet. Immunol. Immunopathol*. 108, 121–125

Ministry of Agriculture, Fisheries and Food (MAAF), 1986. Manual of Veterinary Parasitological Laboratory Techniques. London, HMSO

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

Papadopoulos, E., Himonas, C., Coles, G.C., 2001. Drought and flock isolation may enhance the development of anthelmintic resistance in nematodes. *Vet. Parasitol*. 97, 253–259

Paraud, C., Kulo, A., Pors, I., Chartier, C., 2009. Resistance of goat nematodes to multiple anthelmintics on a farm in France. *Vet. Rec*. 164, 563–564

Paraud, C., Pors, I., Rehby, L., Chartier, C., 2010. Absence of ivermectin resistance in a survey on dairy goat nematodes in France. *Parasitol. Res.* 106, 1475–1479

Patterson, D.M., Jackson, F., Huntley, J.F., Stevenson, L.M., Jones, D.G., Jackson, E., Russel, A.J.F., 1996a. Studies on caprine responsiveness to nematodiasis: segregation of male goats into ‘responders’ and ‘non-responders’. *Int. J. Parasitol*. 26, 187–194

Patterson, D.M., Jackson, F., Huntley, J.F., Stevenson, L.M., Jones, D.G., Jackson, E., Russel, A.J.F., 1996b. The response of breeding does to nematodiasis: segregation into ‘responders’ and ‘non-responders’. *Int. J. Parasitol*. 26, 1295–1303

Perez, J., Garcia, P.M., Hernandez, S., Martinez-Moreno, A., Martin de las Mulas, J., Camara, S., 2001. Pathological and immunohistochemical study of the abomasum and abomasal lymph nodes in goats experimentally infected with *Haemonchus contortus*. *Vet. Res*. 32, 463–473

Pomroy, W.E., Charleston, W.A.G., 1989a. Development of resistance to *Trichostrongylus colubriformis* in goats. *Vet. Parasitol*. 33, 283–288

Pomroy, W.E., Charleston, W.A.G., 1989b. Failure of young goats to acquire resistance to *Haemonchus contortus*. *N. Z. Vet. J.* 37, 23–26

Pomroy, W.E., Lambert, M.G., Betteridge, K., 1986. Comparison of faecal strongylate egg counts of goats and sheep on the same pasture. *N. Z. Vet. J.* 34, 36-37

Pralomkarn, W., Pandey, V.S., 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

Prichard, R.K., Hall, C.A., Kelly, J.D., Martin, I.C.A., Donald, A.D., 1980. The problem of anthelmintic resistance in nematodes. *Aus. Vet. J.* 56, 239–250

Provenza, F. (2003). Behavioural mechanisms influencing use of plants with secondary metabolites by herbivores. *Proceedings. of the Satellite Symposium: Secondary compounds and browse utilization. Matching herbivore nutrition to ecosystems biodiversity. VI International Symposium on the Nutrition of Herbivores*. Merida, Mexico, 19–24 Oct. 2003

R Development Core Team, 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Raynaud, J.P., 1970. Etude de l’efficacité d’une technique de coproscopie quantitative pour le diagnostic de routine et le contrôle des infestations parasitaires des bovins, ovins, équins et porcins. *Ann. Parasitol. Hum. Comp*. 45, 321–342

Reist, M., Forbes ,A.B., Bonfanti, M., Beretta, W., Pfister, K., 2011. Effect of eprinomectin treatment on milk yield and quality in dairy cows in South Tyrol, Italy. *Vet. Rec.* 168, 484–487

Rinaldi, L., Veneziano, V., Cringoli, G., 2007. Dairy goat production and the importance of gastrointestinal strongyle parasitism. *Trans. R. Soc. Trop. Med. Hyg*. 101, 745–746

Sargison, N.D., Jackson, F., Bartley, D.J., Wilson, D.J., Stenhouse, L.J., Penny, C.D., 2007. Observations on the emergence of multiple anthelmintic resistance in sheep flocks in the south-east of Scotland. *Vet. Parasitol.* 145, 65–76

Sargison, N.D., Jackson, F., Wilson, D.J., Bartley, D.J., Penny, C.D., Gilleard, J.S., 2010. Characterisation of milbemycin-, avermectin-, imidazothiazole- and benzimidazole-resistant *Teladorsagia circumcincta* from a sheep flock. *Vet. Rec.* 166, 681–686

Scheuerle, M.C., Mahling, M., Pfister, K., 2009. Anthelminthic resistance of *Haemonchus contortus* in small ruminants in Switzerland and Southern Germany. *Wien. Klin. Wochenschr.* 121 (Suppl. 3), 46–49.

Sechi, S., Giobbe, M., Sanna, G., Casu, S., Carta, A., Scala, A., 2010. Effects of anthelmintic treatment on milk production in Sarda dairy ewes naturally infected by gastrointestinal nematodes. *Small Rum. Res*. 88, 145–150

Shaw, D.J., Grenfell, B.T., Dobson, A.P., 1998. Patterns of macroparasite aggregation in wildlife host populations. *Parasitology* 117, 597–610

Short, C.R., 1999. Comparative pharmacokinetics: sorting the sheep from the goats. *Vet. J*. 158, 159–161

Silanikove, N., 2000. The physiological basis of adaptation in goats to harsh environment. *Small Rumin. Res.* 35, 181–193

Taylor, M.A., Coop, R.L., Wall, R.L., 2010. Parassiti degli ovini e dei caprini. In: *Parassitologia e malattie parassitarie degli animali*, 1[a] edizione italiana basata sulla 3[a] inglese curata da Garippa, G., Manfredi M.T., Otranto, D., Roma, EMSI

Traversa, D., Paoletti, B., Otranto, D., Miller, J., 2007. First report of multiple drug resistance in trichostrongyles affecting sheep under field conditions in Italy. *Parasitol. Res*. 101, 1713–1716.

van Houtert, M.F., Sykes, A.R., 1996. Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. *Int. J. Parasitol.* 26, 1151–1167

van Wyk, J.A., Cabaret, J., Michael, L.M., 2004. Morphological identification of nematode larvae of small ruminants and cattle simplified. *Vet. Parasitol*. 119, 277–306

Vercruysse, J., 1983. A survey of seasonal changes in nematode faecal egg count level of sheep and goats in Senegal. *Vet. Parasitol*. 13, 239–244

Vlassof, A., Bisset, S.A., McMurtry, L.W., 1999. Faecal egg counts in Angora goats following natural or experimental challenge with nematode parasites: within-flock variability and repeatabilities. *Vet. Parasitol.* 84, 113–123

Voigt, K., Scheuerle, M.C., Hamel, D., 2012. Triple anthelmintic resistance in *Trichostrongylus* spp. in a German sheep flock. *Small Rum. Res*. 106, 30–32

Watson, T.G., Hosking, B.C., 1993. Establishment of resistance to *Haemonchus contortus* on Saanen kids. *Proc. N. Z. Soc. Anim. Prod*. 53, 243–246

Wilson, K., Grenfell, B.T., 1997. Generalized linear modelling for parasitologists. *Parasitol. Today* 13, 33–38

Woolaston, R.R., Baker, R.L., 1996. Prospects of breeding small ruminants for resistance to internal parasites. *Int. J. Parasitol*. 26, 845–855

Zajac, A.M., 2006. Gastrointestinal nematodes of small ruminants: life cycle, anthelmintics, and diagnosis. *Vet. Clin. N. Amer.: Food Anim. Prac*. 22, 529–541

Zajac, A.M., Conboy, G.A., 2006. Fecal examination for the diagnosis of parasitism. In: *Veterinary Clinical Parasitology*, 7th edition, Ames, Blackwell Publishing

Zanatta, G., 1996. Studio dei pascoli della Valle Veddasca. Tesi di laurea in Scienze Agrarie, Università degli Studi di Milano, a.a. 1995–1996

Zanzani, S.A.G., 2010. Il parassitismo gastrointestinale nella capra da latte: aspetti epidemiologici, ricadute sulle produzioni e problematiche di farmacoresistenza negli allevamenti Lombardi. Ph.D. Thesis, Corso in Igiene Veterinaria e Patologia Animale, XXIII ciclo, Università degli studi di Milano