Chapter II: Veterinary Parasitology

Section 1. Helminthology

The dilemma that the Zerasca sheep farmers often encounter is represented by the economic losses associated with gastrointestinal parasitism, almost endemic in sheep husbandry and especially in breeds that are raised under extensive systems.

Gastrointestinal parasites complete their biological cycle on pastures and if they survive they can re-infest animals grazing (Benvenuti et al., 2005). Gastrointestinal parasitism is very serious in sheep because it results in lowered animal productivity (reduced nutrient utilization, growth rate, milk and wool production) while severe infections can be mortal (Papadopoulos et al., 2007). The cost of infection includes reduced productivity, increased mortality, anthelmintics and increased management requirements (Liu et al., 2005).

In taxonomy, nematodes or “roundworms” are known as one of the most diverse of all animal species, of which over 15,000 are parasitic. They are characterized by having a cylindrical-shaped body, a buccal apparatus, a digestive tract, a reproductive system and a complex tegument. A non-taxonomic division of Nematoda defines two groups named Non-Bursate and Bursate, where the former embraces those in which the male copulatory bursa is only a skin fold containing no fleshy ribs, as seen in the hookworms.

Considering other important anatomical structures, the Class of Nematoda is divided between ten Super families which are the following: Trichostrongyloidea, Strongyloidea and Metastrongyloidea which belong to the Bursate Nematodes; Rhabditioidea, Ascaridoidea, Oxyuroidea, Spiruroidea, Filarioidea, Trichuroidea and Dioctophymatoidea belong to the Non-Bursate nematodes (Urquhart et al., 1998). In order to fully comprehend this paper, the species of main interest are described below and belong to only two of these ten Super families, in particular Trichostrongyloidea and Strongyloidea. The species that belong to these Super families are all characterized by having a direct cycle, meaning that there are no intermediate hosts.

Nematodes include opposite sexes and they undergo metamorphosis by carrying out several larval stages, five to be exact, which are indicated as L1, L2, L3, L4 and L5. L3 larvae represent the infesting stage for the host. They then proceed in transforming into the adult worms which can reproduce successfully.

Hypobiosis signifies the ceasing of the nematode larval development and this phenomenon, which occurs only in predisposed larvae, has been interpreted as a sort of defense mechanism towards unfavourable circumstances. It consists in blocking its normal growth at a sexually immature phase. Once the conditions allow the completion of the larval development, the nematode soon grows into an adult and sexually mature worm. It has been established that hypobiosis often occurs at the beginning of the cold season (in the north hemisphere); for example in southern U.K. 50-60% of parasites sustain hypobiosis in the wintertime (Urquhart et al., 1998). Consequently, the Fecal Egg Counts were found to increase during the springtime leading to the expression spring rise, which was reflected on the epidemiology of this parasitic infection. However, the close association was seen to be between FEC and the parturition date, rather than with the onset of spring per se, thus leading to the term periparturient rise in ewes. This particular relation is strikingly evident during late
pregnancy and throughout lactation (2 weeks beforehand until 6 weeks after), which is why it is also identified as the periparturient relaxation of immunity (PPRI) (Houdijk, 2008).

The cause of this periparturient relaxation of immunity has been linked to an increase of prolactin plasma levels, even though recently, the role of IgA serum levels which have been noticed to increase during lactation, may be the cause of reduced local gut immunity levels (Houdijk, 2008). It is important to underline how the parasite, in exploiting the host’s nutritional supplies in the period that delimitates parturition, facilitates its own survival and optimizes its life cycle. In this way, there is a greater probability that the eggs will infect the younger lambs which represent a more susceptible host and consequently will shed larger numbers of eggs, contributing to the contamination of the environment (Urquhart et al., 1998).

The “self cure” phenomenon is quite self-explanatory, accomplished in hosts whose immune system is able to expel gastrointestinal worms thanks to the synthesis of antibodies during primary infestation such as IgE (binded to mast cells and basophils in the gut tract), IgM and IgG (in the blood). When certain hosts re-encounter a second infestation, fluid from the cyst rupture of L3 reacts with the IgE antibodies located in the gut causing degranulation of mast cells and basophils. Histamine and other vasoactive peptides are released causing increase of blood permeability which leads to an increase of IgG and IgM antibodies which are capable of interfering with the parasitic metabolism. Worms are thus expelled due to an increase of intestinal flow (Ambrosi, 1995).

Section 2. Most common gastrointestinal helminthes in sheep

2.a) Super family TRICHOSTRONGYLOIDEA:

(i) Ostertagia (Teladorsagia) circumcincta & Ostertagia (Teladorsagia) trifurcata

These ubiquitous nematodes, frequently found in temperate and subtropical regions of the world, are about 1 cm long and live in the abomasum feeding off chyme. After mating and once the eggs are eliminated, L3 merge directly from the faeces and if ingested, will settle in the gastric glands of the abomasum and carry out their larval development.

The most frequent clinical sign linked to this infection is a distinct weight loss due to malabsorption, lack of appetite, nitrogen deficiency, hypoalbuminemia and hypoimmunoglobulinemia caused by the protein loss which occurs across the gastrointestinal lumen. Consequently, the protein metabolism attempts at restoring this deficiency by augmenting protein synthesis and deducting these from the muscle tissue and lipid deposits. Nutrition must be of the best quality to avoid detrimental effects caused by this metabolic mechanism responsible for scarce carcass productivity and meat quality.

In studying Ostertagia in sheep, it has been noticed that there are two clinical forms which appear in two different times of the year: Type 1 has been described to be seen from August to October whilst Type 2 is generally ascribed to the ingestion of a large quantity of larvae in hypobiosis, causing a rise of the FEC in late winter or early spring, seen mostly in young animals. Type 1 is associated with ewes that eliminate eggs onto pastures, due to periparturient relaxation of immunity during springtime, which inevitably determines the origin of the larval population on grasslands registered from August to October. In addition, we have
to consider that the elimination of eggs from lambs takes place at the same time. Before the end of October, a portion of the larvae ingested will then arrest their development during winter, which generates the Type 2 clinical form (Urquhart et al., 1998).

(ii) *Haemonchus contortus*

This particular nematode is the most dreaded of all, considering that it is a notorious haematophagous parasite and is common in the abomasum of all ruminants. The adult worm is about 2-3 centimeters long and the life cycle is similar to that of the other Trichostrongyloidea. Each individual is capable of extracting up to 0.05 ml of blood per day which means that if a sheep is infested with 5000 parasites, the loss of blood per day counts to a maximum of 250ml (Urquhart et al., 1998). The pathogenicity of Haemonchus leads to emaciation, anemia, lethargy and wool loss not to mention that it can lead to death in heavily infected animals (Burke et al., 2007). Haemonchosis can manifest itself under various clinical forms that go from causing sudden mortality in sheep flocks, to weight loss and prostration.

(iii) *Trichostrongylus colubriformis, capricola & axei*

These are very small histiotrophic worms, around 7mm long, which tend to penetrate across the abomasal mucosa, into the lamina propria and reside between the gastric glands, causing loss of blood and plasma fluids. This is clinically manifested by malabsorption, weight loss and diarrhoea.

(iv) *Cooperia curticei*

Cooperia is another example of a blood-sucking nematode species which shows a similar epidemiology with Ostertagiasis in sheep, described by causing loss of weight, loss of appetite and diarrhoea.

(v) *Nematodirus battus & Nematodirus fillicolis*

In northern Europe, *Nematodirus battus* is the most studied species of the two, since it causes devastating outbreaks of spring disease in temperate areas and appears to have spread, following its sudden emergence in the UK some 50 years ago (Van Dijk and Morgan, 2009). Most common clinical signs are diarrhea and loss of appetite. Flocks of sheep that are raised extensively are largely exposed to eggs eliminated by periparturient ewes. Lambs are thus infected by *Nematodirus* before having developed an efficient immune response.

2.(b) **Super family STRONGYLOIDEA**

(i) *Chabertia ovina*

The nematodes belonging to this Bursate category are divided into two large groups: large strongyles which infect the large intestine and ancylostomatidae which infect the small intestine. *C. ovina* is a strongyle located in the large intestine of ruminants, long about 2 cm and
it feeds off the cells that constitute the mucosa, determining loss of blood and plasma fluids. Diarrhoea is the most signaled symptom.

(ii) Oesophagostomum columbianum

This parasite is long 1-2 cm and the adult worm inhabits the large intestine after having mutated in nodules formed amidst the mucosa bed. Chronic infections are often found in sheep, causing loss of appetite, striking loss of weight, anemia and intermittent diarrhoea. The faeces may contain excess mucus as well as streaks of blood. As the diarrhoea progresses, sheep become emaciated and weak.

(iii) Bunostomum trigonocephalum

This is the only gastrointestinal strongyle documented in small ruminants which belongs to the Family Ancylostomatidae, also known as the strongyles which infest the small intestine. Bunostomum can be identified by its distinctive hook and relatively large size (1-3cm). Bunostomum is another haematophagous parasitic species, highly pathogenetic since 100 worms are capable of establishing anemia, hypoalbuminemia, loss of weight and occasional diarrhoea. Moreover, they have the ability to penetrate across the skin, usually described in intensive farming. Following infection, the larvae are transported via the blood to the pulmonary parenchyma before proceeding to their site of predilection where they mature.

Past monitoring of the Zerasca flocks has illustrated that the genera Ostertagia (Teladorsagia), Trichostrongylus, Oesophagostomum and Haemonchus are the predominant nematode populations (Bianchi, 2005).

Section 3. Economic losses due to GI parasitism

Small ruminant farming with the prevailing tendency towards milk production, is typical of Mediterranean regions. The contribution of sheep to meat production is significant in the south part of the basin (Ronchi and Nardone, 2003). The Food and Agriculture Organization (2007) has estimated that in the year 2007, sheep stocks in Italy summed up to 8,227,000, the number of slaughtered sheep was 6,561,170, yielding 59,093 tonnes of sheep meat. The small ruminant enterprises of Europe are numerically insignificant among world standards (especially in comparison to Australia and New Zealand). However, they provide valuable quality products predominantly for local markets and consequently the value of animals is high (Waller, 2003).

Gastrointestinal nematode parasitism and the subsequent host immune response have important productivity consequences for sheep meat and wool production (Jacobson et al., 2009). In Italy, it represents one of the most important health problems in sheep (Perrucci et al., 2006). Nematode infection threatens the health and welfare of livestock and compromises the efficiency of livestock production. Nematodes are possibly the major disease challenge facing ruminants. The severity of disease and the loss of production depend upon the intensity of infection, immunity and resistance of the host and its nutritional status (Becker, 1997; Stear et al., 2006).
In particular, endo-parasitic diseases are known to decrease voluntary food intake, cause malabsorption of nourishment, reduce efficiency of minerals and cause loss of diet proteins. These phenomena impair meat, milk, wool production and growth rate, not to mention that pathological lesions often lead to the confiscation of certain organs of slaughtered heavily-infected animals (Perrucci et al., 2006). Parasitized sheep spend less time grazing, are less active than uninfected sheep and have reduced herbage intakes. At early stages of endo-parasitic infestations, sheep display disturbed behavioural patterns: restlessness, disturbed lying behaviour, intense rubbing of area of the fleece, biting at the flanks. As the disease progresses, infected sheep become increasingly disturbed and agitated by the presence of the allergens (Sevi et al., 2009).

In considering the correlation between GI parasitism and carcass productivity, Jacobson investigated how an infection caused by Teladorsagia circumcincta and Trichostrongylus colubriformis affects gastrointestinal tract size and dressing percentage of sheep fed lucerne diets (Medicago sativa). The author found a difference in dressing percentage of 1,3% in the larval challenged lambs which was small but significant on a large scale. The difference resulted from an increase in the weight of gastrointestinal tract (observed either when full and empty) due to the presence of larvae which caused a decrease in dressing percentage when compared to unchallenged sheep (Jacobson et al., 2009).

Gastrointestinal parasites are known to be a major cause of sheep production losses in the world. In Argentina, Suarez calculated that losses may amount to 5%, 24% and 25% for wool, meat and mortality, respectively. Haemonchus contortus is indeed the most dangerous nematode for sheep production (Suarez et al., 2009).

Section 4. Parasite-environment relationship

Gastrointestinal parasite burden is closely linked to the environment, including factors such as climatic conditions (temperature, rainfall and relative humidity), husbandry practices as well as the nutritional and the physiological status of the animals. The development of grazing management systems to reduce parasitic nematode infection requires detailed knowledge of the population dynamics (Papadopoulos et al., 2007). In employing simple management routines, it is possible to reduce significantly the number of Anthelmintic (AH) treatments (Benvenuti et al., 2005).

The L3 survival rate is influenced by climatic conditions and it is dramatically expanded in temperate weather. The survival rates vary from 6 to 12/18 months. However, L3 are susceptible to severe prolonged drought and/or cold conditions such as frost or snow (Torres-Acosta and Hoste, 2008). The dispersal of larvae from faeces is largely dependent on sufficient water from dew or rainfall to allow larvae to migrate. Larvae have the ability to perform a horizontal movement onto pasture but may also move vertically up grass or stems of other plants to increase their chances of being ingested. The type of forage species available influences the rate of vertical migration while soil may harbor nematodes from adverse weather. Infact, it may be useful to keep in mind that browsing on bushes or shrubs limits the ingestion of L3 by the animals, due to its limited climbing activity (Torres-Acosta and Hoste, 2008). The free-living stages have acquired the capacity to adapt to the predominant local
conditions. Therefore, the creation of effective strategies requires local studies and local knowledge (Stear et al., 2006).

Several factors influence the viability of Gastrointestinal nematodes (GIN); some of these are intrinsic to the host: age, previous contact, breed and individual characteristics; other are related to environmental or managerial aspects: nutrition status, presence of other parasites etc.; together they justify the disparate responses sheep manifest towards GIN. There are fundamentally three ways to “break” the biological cycle of GIN:

1. by eliminating the worms in a host (AH treatments)
2. by improving the host resistance and/or host resilience (see Lexicon)
3. by reducing the contact between host and L3 parasite (grazing management)

**LEXICON**

**Host resistance**: is defined as the ability of the host to limit FEC during a heavily challenge of GIN (Burke and Miller, 2008).

**Host resilience**: is defined as the ability of the host to maintain productivity during a heavy challenge (Baker, 1997).

These mechanisms belong to the host’s immune response (Torres-Acosta and Hoste, 2008).

**Host tolerance**: is defined as the capacity to maintain survival in spite of parasitic diseases (Baker, 1997).

**Section 5. Anthelmintic treatment schemes**

Currently in Italy, sheep breeding is largely based on traditional extensive farming, where nutrition is mainly supplied by grazing. As mentioned above, Gastro-Intestinal Nematodes (GIN) complete their biological cycle upon pastures where they re-infest animals and this explains why farmers rely on pharmacological treatment as a prophylactic strategy, even without laboratory investigation (Benvenuti et al., 2005; Liu et al., 2005).

Each farm has to apply its own tailor-made anthelmintic treatment scheme according to its own features (Torres-Acosta and Hoste, 2008). This is justified knowing that several factors affect the level on pasture contamination in a given geographical region (Papadopoulos et al., 2007).

However, some principles can be used as criteria to determine when to treat.
5.a) Strategic treatment schemes

This scheme indicates treating animals in a strategic manner hence all animals at a specific moment of the physiological cycle. It is recommended where parasitic challenge on grasslands is absent or scarce. Instead, where animals reproduce at different times of the year and with well-defined dry and wet seasons, some authors recommend the use of AH treatments at the beginning of the wet season to avoid the build-up of infection. It is also possible to treat at the end of the wet season to reduce the parasite burden passing to the next wet season inside the animals (Torres-Acosta and Hoste, 2008).

Another author advises to treat ewes during late pregnancy in a pasture different to the one where ewes are raised with the newborn, in order to eradicate as much as possible the larvae who survived the winter by arresting their development (Corrazza, unpublished material).

It is known at the present time that these schemes directly compromise: "refugia" (see Lexicon) and select heavily for Anthelmintic Resistance (AR). To improve the strategic scheme an alternative is that of treating some animals within the flock instead of all, in limiting selection for resistance. However, it has been suggested that this approach may have prolonged the useful life of some anthelmintic groups by reducing drench frequency (Van Wyk, 2001).

5.b) Suppressive treatment schemes

This approach constitutes a regular AH treatment, every 2-3 weeks, aiming at an almost total elimination of worms from the environment. This scheme is non-desirable and will rapidly select for resistance (Torres-Acosta and Hoste, 2008).

This program is time and money-wasting, not to mention that negative effects it causes (Corrazza, unpublished material). If a farmer is obliged to adopt such a program because of excessive stock losses, it is an indication of Anthelmintic (AH) failure (Torres-Acosta and Hoste, 2008).

LEXICON

Refugia: in a flock, the part of the worm populations which is not exposed to anthelmintic drugs. This occurs during the parasitic stages of the external phase of the life cycle (i.e. present on pastures). In addition, the size of refugia can also be increased by allowing a proportion of the flock to remain untreated (Van Wyk, 2001). Worms in refugia that remain unselected by drug treatment provide a pool of alleles sensitive to anthelmintics, thus diluting the frequency of resistant alleles in that population of worms (Burke et al. 2007).
5.c) Tactical treatment schemes

These involve drenching all or some animals in the flock when pastures are already contaminated with worm larvae i.e. in refugia. In mild temperate climates, this typically occurs during spring and summer. The worms already present on pastures are responsible for diluting resistant parasites, thus reducing the risk of causing AH resistance.

It will be necessary to monitor early clinical signs of GIN infections which are unfortunately easily confused with other health problems causing anemia, undernourishment, diarrhea and chronic wasting. Farmers often recognize GIN infections once they have already caused considerable losses in productivity. To overcome this, farmers can obtain the recognition of sub-clinical infections by observing productivity of animals (growth rate, milk production, feed conversion etc.), and by identifying the production threshold. The animals which reach the threshold of non acceptable productivity must be treated to avoid major economic losses (Torres-Acosta and Hoste, 2008).

5.d) Selective treatment schemes

It has been known that within a flock, nematodes are not evenly distributed among the hosts thus creating a situation where a small number of animals are heavily infected while most individuals present a moderate worm burden. By acknowledging such concept, it is evident that in treating only heavily infected subjects, leaving the others untreated, is a procedure which reduces pressure selections considerably, as well as the cost of AH treatment for the farm. It is currently being investigated whether the selective treatment scheme should be based on the clinical signs or the production losses. Any scheme, naturally, will need to be efficient, economically viable, practical and safe (Torres-Acosta and Hoste, 2008).

Section 6. Anthelmintic drugs

Anthelmintics offer a simple and cost-effective method of controlling nematodes. They kill existing parasites and reduce the production of eggs. Therefore they can prevent disease in infected animals and reduce the intensity of future infection in infected animals and in their offspring. The widespread application of anthelmintics has transformed the livestock enterprises, especially cattle and sheep. Indeed, without effective anthelmintics the livestock industry could not exist in its current form (Stear et al., 2006).

There are three major drug classes used to control nematodes in livestock: Benzimidazoles (such as Albendazole), Imidazothiazoles-tetrahydropyrimidines (such as Levamisole and Pyrantel) and Avermectin-milbemycins (such as Ivermectin and Moxidectin). Respectively, these drugs were first approved for commercial use in the 60’s, 70’s and 80-90’s (Kaplan, 2004). Unfortunately, there are reports of anthelmintic resistance (AR) that date back to the early 60’s which marked the beginning of the modern chemical assault on nematode parasites (Kaplan, 2004). Presently, multiple-drug-resistance (MDR) has been revealed towards all 3 categories in H. contortus, T. circumcincta and T. colubriformis throughout the world, not to mention the resistance of Cooperia spp. towards Avermectin-milbemycins (named also
macro cyclic lactones) detected in some areas of the world (Kaplan, 2004). Although severe, in Europe and Canada, MDR worms have only been infrequently reported and resistance is of less concern when compared to certain tropical areas of the world, such as South America, South Africa, Malaysia and southeast USA (Kaplan, 2004). On the bright side, at the World Association for the Advancement of Veterinary Parasitology (WAAVP) Congress 2007, data on what is potentially the first new livestock anthelmintic class in 25 years was presented: the amino acetronitrile derivatives (AADs) (http://mdsheepgoat.blogspot.com/2007/09/first-new-anthelmintic-in-25-years.html) (Torres-Acosta and Hoste, 2008). The new class of parasiticides has a potentially novel mode of action, which has shown promising results against all sheep and cattle gastro intestinal nematodes including those resistant to existing treatments. While further testing is required, early in vivo research suggests a kill rate of greater than 95 percent in key economically important nematodes. Currently, no drug from this class is available on the market.

It has been suggested that better results can be achieved by using AH in combination, which should reduce the development of resistance, but combination drenches are more expensive than single drenches. However, in pragmatic terms, combination therapy has only ever been applied once Anthelmintic Resistance (AR) is evident towards one of the substances of the mixture thus precluding the possibility to verify its full benefit. Van Wyk (2001) previously stated that Levamisole drenching may select against Benzimidazole resistance, bringing some relief for resistance to this latter class of drugs.

Another valid recommendation is that of alternating anthelmintic drugs at annual intervals. This will select less intensively for resistance compared to when alternation is within a given worm generation (Van Wyk, 2001).

6.a) Negative aspects associated with AH treatments

Naturally, there are several downsides associated with the copious use of AH treatments, the most costly of all being the Anthelmintic Resistance (AR) that these worms have developed over the decades. Another imperative for change is given by the increasing demand by consumers that agricultural products should be uncontaminated with substances that can have tragic adverse embryogenic effects and contribute to the development of resistant human microbial pathogens (Waller, 2003). Product quality is primarily dependant on farm management therefore, epidemiological and on-farm studies are needed to evaluate risk factors. There is an increasing awareness of the potential drug residues in edible products and possible ecological toxic effects of drug excretion on the environment (Ronchi and Nardone, 2003). In fact, consumers now manifest a growing concern and call for the production of meat with minimal chemical input (Burke and Miller, 2008).

(i) Anthelmintic resistance

It is expected that during anthelmintic treatments, a small number of worms survive, these being the most resistant population. These worms contaminate the pasture with a majority of resistant larvae for subsequent generations, leading gradually to the selection pressure of AR. This selection rate depends on the percentage contribution to the next generation between nematodes surviving treatment and other ones not exposed to it (in
refugia). Any action increasing the percentage contribution that survivors of treatment make to the next generation, will contribute to development of resistance, whilst any action increasing the prevalence of the untreated population will slow down its development (Papadopoulos, 2008).

Within a population of nematodes there is a genetic diversity, which is obtained by mutations that take place. If the mutations include receptor sites where anthelmintics work or differences in enzymes or mechanisms that may affect the metabolism or transport of the anthelmintics, a proportion of nematodes may become genetically resistant, equipped with dominant or recessive alleles. These nematodes with a modified genome have an evolutionary advantage as opposed to the rest of the population. Resistance will develop faster if genes are dominant rather than recessive. Finally, development of resistance depends upon whether resistant worms are as fit, including life cycle completion, egg production, pasture survival and infectivity, or less fit than susceptible ones (Coles, 2005).

The initial reports of AR were to the drug phenotiazine in the late 50’s and early 60’s, first in *Haemonchus contortus* of sheep. Reports then appeared of benzimidazole resistance in other major trichostrongylid nematodes of sheep such as *Teladorsagia (Ostertagia) circumcincta* and *Trichostrongylus colubriformis*. The same pattern repeated itself in the 70’s and 80’s following the introduction of the newer imidazole-tetrahydropyrimidine and Avermectin-milbemycins classes of anthelmintics and by the early 80’s, reports of multiple-drug resistant (MDR) worms appeared for the first time (Kaplan, 2004). At present time, the global rate of development and the extent of anthelmintic resistance in helminthes of sheep and goats indicates that the numerous strategies developed over the course of the previous century have been incorrectly applied (Van Wyk, 2001).

Presently, MDR to all three major class drugs has been documented all around the world. Such concern is predicated on the fact that there are few anthelmintics currently being developed. Another consideration focuses on the small or absent evidence that susceptibility has the capability of being a reversible phenomena, and essentially AR is everlasting. Theoretically, reversion may be plausible if resistant pools of parasites suffer a decrease in fitness however there is little evidence (Kaplan, 2004).

In the past few decades, the arsenal of highly effective and relatively inexpensive drugs led to recommendations for parasite control that were based almost solely on the frequent use of anthelmintics, the goals of which were to maximize livestock health, productivity and profitability. Though this approach was highly successful, history clearly suggests that this approach was short sighted and unsustainable (Kaplan, 2004). The problem of AR has reached a stage where the efficiency of this mode of chemical control is dramatically challenged.

This situation implies that it is now imperative to change our general concept for use of AHs in farm conditions, and to seek alternative or complementary solutions to conventional, chemical treatments (Torres-Acosta and Hoste, 2008). This crisis situation has pushed many farmers and parasitologists to form an integrated worm management using anthelmintics plus alternative methods of control. Needless to say, total eradication or annihilation of any parasitic population is a realistic goal we can try to attain. However, the inevitable losses in productivity and problems of animal welfare that result from a failure to control MDR worms adequately, should generate serious efforts to preserve the potency of the few drugs that remain effective (Kaplan, 2004). This last concept is strengthened by the awareness that none
of the alternative methods for parasite control is sufficiently competent without anthelmintic support (Papadopoulos, 2008).

Factors affecting selection of AR are principally: refugia, under-dosing and frequent drenching.

The term “refugia”, as seen in the previous pages, represents the portion of a parasite population unexposed to a particular given measure, thus escaping the selection for resistance. Gastrointestinal nematodes primarily in refugia are those of the free-living stages on pasture. In the host’s body, the parasitic stages of some worm species such as the histiotrophic larvae in the intestinal mucosae, also escape the effect of a drug. Selection is greater in sheep drenched on a clean pasture than on a contaminated pasture, in other words, refugia plays a role in reducing selection pressures (Van Wyk, 2001). Worms in refugia originate from three sources: larvae on pasture; untreated animals and stages of nematodes in the host that are not susceptible to treatment (usually) inhibited larvae (Coles, 2005). If the treatment is given at a time when, or in circumstances in which all the worms on the farm are in the treated hosts, selection will be rigorous (Van Wyk, 2001).

Under-dosage is defined as the medicinal treatment at a quantity lower than the therapeutic level recommended by the manufacturer, and it is a common assertion that it will quickly select for resistance. It is probable that every compound that is less than 100% effective will select for resistance. In the present World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines, aimed at international standardization of anthelmintic testing for ruminants, it was outlined that “highly effective” is referred to as 98% killing-capability whereas 80% denominates the “effective” drugs. Drugs with less than 80% defines the “effective” drugs. Drugs with less than 80% efficacy should not be registered. In that view, under-dosing is considered as all levels of drenching less than the dosage rate required to kill 80% of a worm strain not previously exposed to the drug concerned. If dosage rates are high enough to kill the heterozygous resistant worms, resistance genes may be rendered effectively recessive. On the other hand, sub-therapeutic doses make the resistance genes effectively dominant (Van Wyk, 2001).

That drenching frequency is in relation with AR does not cast a shadow of a doubt. Although on the field, it is probably subordinate particularly to refugia, and it should not be viewed independently of other factors, such as the degree of control attributed to the anthelmintic. Selection pressure does not depend on the frequency of treatment such as, but on the circumstances in which it is given (Van Wyk, 2001).

In nature, “low-worm” pastures result from seasonal cycling and nomadic movements of animals where this is still possible. One of the most common methods used to reduce the rate of re-infection of these “low-worm” pastures has been the “drench-and-move” strategy. Subsequently, the possibility that the drench-and-move system would select for resistance was considered. If sheep are moved to clean pasture after a moxidectin drench, all the H. contortus that develop will be the progeny of resistant worms. Obviously, the advantage of reducing exposure to re-infection and the risk of selecting for resistance must be considered in light of all other factors on a case-by-case basis before dose and move strategies are used (Van Wyk, 2001). Van Wyk suggests that farmers should refrain themselves from drenching an entire flock prophylactically, when they are about to be moved to a “safe” pasture, and should try to
obtain a treatment scheme with minimum frequency of treatments, unless there is a continuous monitoring of the Fecal Egg Counts.

Another common management flaw which favours AR is the lack of quarantine of newly introduced animals (Torres-Acosta and Hoste, 2008). Few farmers make a serious attempt to quarantine animals, ensuring that resistant nematodes are not brought on to the farm (Coles, 2005).

AR can be detected using various laboratory methods of analysis; the most widely used being the Fecal Egg Count Reduction Test (FECRT). Generally, it compares the egg count before and after treatment with an anthelmintic: nematode eggs are counted in fecal samples at the time of treatment and at defined times thereafter, these depending on the specific anthelmintic tested. Naturally, an untreated control group of animals should also be included, in order to record any natural changes to egg counts, which may occur during the test period. Ideally, 10 animals with an EPG (eggs per gram) count of >150 should be included per group (Papadopoulos, 2008). Fecal samples should be collected 8-10 days after treatment if using Benzimidazoles; when using Tetrahydropyrimidines and Imidazothiazoles, the interval should be of 3 to 7 days and when using macrocyclic lactones, 14-17 days are sufficient (Coles, 2005). No nematode egg should be found in fecal samples after these periods. Leaving sheep for up to 3 weeks can pose the risk of larvae picked up shortly after treatment developing to egg laying adults before 21 days. A small proportion of worms surviving treatment may indicate a resistance problem, which could further develop under drug pressure and thus, should be monitored. If reduction in fecal egg counts is over 95%, then the anthelmintic can be considered highly effective and its use may be continued (Papadopoulos, 2008).

An alternative method is the Egg Hatch Test (EHT) which is based on the nematode ovicidal activity of Benzimidazoles. It requires fresh eggs which alternatively can be anaerobically stored for up to 7 days after collection. Discriminating doses can be used as a threshold in detecting the resistant strains. The percentage of eggs hatching in the discriminating dose corresponds to the percentage of benzimidazole-resistant eggs in the sample (Papadopoulos, 2008). With a change to a discriminating dose, this test is very much more sensitive detecting down to a 2% of resistant eggs (Coles, 2005). Instead, the FECRT is reliable if more than 25% of the worms are resistant, when it may be too late to interfere (Papadopoulos, 2008).

Resistance surveillance is extremely useful because it provides data which can be used to relate the extent of anthelmintic resistance to management practices, and thus adjust the way AH are used. In addition, data can give a warning in terms of productivity and animal welfare issues that may be seriously compromised if different actions for worm control are not taken (Coles, 2005). Finally, molecular assays have been developed for detecting single point mutations which have brought out resistance, but suffer from the potential problem that resistance may have resulted from more than one mutation. Real time PCR is very valuable even though it requires the use of very expensive equipment but it is time-saving (Coles, 2005).

\( \text{(ii) The need for sustainability:} \)

When not well managed, livestock production may specifically reinforce land degradation, the decline and pollution of water resources, the emission of greenhouse gases
and the loss in biodiversity. On the other hand, with good management, animal agriculture can make a positive contribution to the natural resources by enhancing soil quality, increasing plant and animal biodiversity and substituting for scarce, non-renewable resources such as fossil fuels. Animals are an integral part of the rural systems and can contribute to the sustaining of soil fertility and promote the effective use of land. Therefore, there is a need to develop models of sustainable systems for various soil types and environmental conditions (Boyazoglu, 2002), especially considering that production systems are largely based on traditional transhumance activities and shepherding small flocks, which graze on different pastures from one season to another (Waller, 2003). In this view, quality assurance programs of products deriving either from conventional or organic farming, must focus on the quality of the environment (water, soil and air) (Ronchi and Nardone, 2003).

In Mediterranean areas, small ruminant farming systems have some specific characteristics: utilization of marginal lands, prevalence of pastoral system, availability of commonage, production of typical cheeses and a low level of mechanization. One of the emerging indicators of unsustainability of small ruminant farming systems in the Mediterranean is precisely the large incidence of parasitic diseases and the necessity of preventive and curative chemical treatments. Thus, a new model of livestock production is needed which is able to satisfy multiple objectives, such as productive efficiency, animal welfare, correct use of the environment and non-renewable resources, animal product quality and safety (Ronchi and Nardone, 2003).

Frequent use of suppressive or therapeutic anthelmintic drugs is no longer considered sustainable. Now and in the future, anthelmintics must be thought of as highly valuable and limited resources to be preserved. The only realistic strategy for sustainable nematode parasite control is to develop novel non-chemical approaches that decrease the need for treatment and use of anthelmintics that remain effective in a more intelligent manner. Therefore, as novel non-chemical control methods become available and widely applied, anthelmintics will still be required for life-saving therapy when other control measures fail (Kaplan, 2004).

The availability of a sustainable control of gastrointestinal parasites along with limited use of chemotherapy could prove to be of great value to farmers and beneficial to the environment (Benvenuti et al., 2005). The farmer must be brought to realize that in contrast to the approach in the past, it is optimum production that should be strived for in grazing animals, and not unsustainable maximum production that is almost completely dependent on effective anthelmintics (Van Wyk, 2001).

For a start, we have to abandon the general concept that parasites are an evil plague that should be maximally suppressed. Breeders have to learn to farm with internal parasites, trying to prevent only the unacceptable production losses while simultaneously breeding animals fit for the environment, rather than making the environment fit for existing animals. The priority should be the attainment of optimum productivity and profitability as opposed to maximum productivity (Bath, 2006). Alternatives need to be adopted to improve parasite control schemes and need to be based on the principles of integrated management which incorporates grazing management; use of plant extracts; homeopathic treatment; special forage crops; vaccines; diet composition; breeding parasite-resistant animals and biological control by applying natural enemies against nematodes (Ronchi and Nardone, 2003).
Moreover, the problem of resistance to AH has become by far the most severe in small ruminants where dramatic changes must be made (Kaplan, 2004). Hence the increasing urge to develop alternative or supplementary methods of nematode control.

Section 7. Alternative methods of parasite control

These methods fall into several categories: grazing management; biological control; nutrition; vaccination; genetic selection and the use of non-conventional anthelmintics (Stear et al., 2006).

7. a) Grazing management

The aim of current grazing schemes is to maximize the use of available pasture, seeing how small ruminant livestock depends upon it (Stear et al., 2006). However, grazing management is a decisive point in reducing the contact between host and the infective stage of the parasite (Torres-Acosta and Hoste, 2008). In achieving a low number of eggs deposited and a low number of larvae on pastures, it is recommended that stocking density be limited to avoid that animals graze near contaminated faeces. However, sheep husbandry is not particularly profitable if the number of animals drops considerably, thus at low stocking densities farmers would find it unsustainable (Stear et al., 2006).

By grazing the same species on a pasture year after year, an increase of larval numbers and thus infection severity will unintentionally be achieved. Alternating crops and livestock would reduce intensity of infection as would the use of different species. Alternate grazing between small ruminants and cattle contributes to reducing anthelmintic resistance, and is an example of dilution strategy (Ronchi and Nardone, 2003). Major changes in the susceptibility of parasites was accomplished during an on-farm study using goats, where the degree of efficiency of the drugs tested rose from 57-70% to 95-98%. The attainment of this goal is on account of the implementation of a strict procedure where for three months any livestock grazing was prevented; followed by harvesting of forage for further three months and finally grazing of cattle for another three months (Sissay, 2006).

Another option is to rotate young and older animals because the latter are generally less susceptible, expelling lower egg counts. This might also avoid the build-up of infected larvae (Stear et al., 2006). It is difficult to determine a threshold under which infections are minimized because of the disparate dynamics of infection depending on the host species, the parasite, the pasture species and the climatic conditions (Torres-Acosta and Hoste, 2008).

Another procedure is to move vulnerable animals onto clean pastures but this has to be discussed about, as we have seen, Van Wyk (2001) does not recommend it. This would be an example of the evasive system (Ronchi and Nardone, 2003). Bath also recommends eliminating the treat-and-move strategy where possible, and states that one either moves the animals 2-3 weeks after treatment, or treats only a portion of the flock (Bath, 2006).

Rotational grazing is a very well-known technique in attempting to reduce the worm burden on pastures. The pasture is divided into different sections that are grazed in sequence,
and ideally the rotation occurs at the time at which most of the infected larvae have died. It has some drawbacks, especially in temperate regions where the infected larvae have a remarkable longevity (Stear et al., 2006). This is an example of exploiting the natural rate at which L3 die (Torres-Acosta and Hoste, 2008).

Improper grazing management can lead to land degradation and this aspect is particularly important when considering environments characterized by a wide complexity due to climate, topography, soil characteristics and peculiarity of agriculture, such as the Mediterranean basin. Here, land degradation in agro-silviculture and pastoral systems is associated with overgrazing and improper grazing management (Ronchi and Nardone, 2003). These two flaws are often correlated to the public use of land, where native citizens exert their rights of grazing. These lands, in Italy, are known as “agrarian communities” and correspond to mountain or hill-areas of rich ecological value (Ronchi and Nardone, 2003). Curiously, mountains are being abandoned more and more, which paradoxically leads to the phenomenon of land degradation as well because of the insufficient animal and human pressures (Ronchi and Nardone, 2003).

7. b) Biological control

Biological control is obtained via two main approaches: natural and applied biological control. The first by effective use of natural enemies whereas the second, by enhancing these native or natural enemies. The latter tends to give better results and an example is described here below (Ronchi and Nardone, 2003).

Factors which control the growth of a certain species population have been the subject of numerous researches, in attempting to apply them as alternative methods in the control of parasites. Many biological agents, under laboratory conditions and in field trials as well, have been proven to be effective in altering the free-living stages of GIN by increasing the larval death rate. In particular, a haematophagous fungi denominated *Duddingtonia flagrans*, has the ability to kill nematode larvae in the faeces (Torres-Acosta and Hoste, 2008). Chlamydospores of this fungus germinate in the faeces and create a peculiar three-dimensional network that effectively traps the larvae. Nevertheless, it is known that nematode stages already present at the beginning of the fungus administration are not influenced, considering that *D. flagrans* does not reduce the egg output but only the infection pressure of pasture (Epe et al., 2009). Therefore, *D. flagrans* contributes to reducing contamination on pasture without causing any damage to non-parasitic soil-dwelling nematodes. The fungal spores are administered daily and for optimal control, a diet supplementation is required during periods of nematode control (Stear et al., 2006).

Biological control methods are highly effective when implemented at times when contamination of pastures produce seasonal peaks on larval numbers. In the winter rainfall regions, this corresponds generally to spring (PPRI), derived largely from contamination during the previous late summer/early fall. This can be catastrophic to the growth of young lambs. In spring, lambing and periparturient ewes invariably excrete a relatively large number of nematode eggs. Young and recently weaned lambs are highly susceptible to parasite infection, thus our objective should be to graze these animals on pastures of low infectivity. Weaning sheep are themselves responsible for contaminating greatly the pastures, since they eliminate
large quantities of eggs. In providing an effective biological control for 2-3 months immediately after weaning, the pastures will remain lightly infected, as long as nutrition is not limiting. Animals are ensured a satisfactory growth and will gradually acquire a natural immunity to parasite infection, during the following months (Waller, 2003). For the fungus, it is crucial that its distribution on pasture must be as homogeneous as possible (Epe et al., 2009).

It must be recognized that feeding less often than each day will result in an uneven shedding of fungal spores in animal dung, therefore allowing some nematode eggs to develop to infected larvae if the pasture contains a sub-optimal concentration of D. flagrans (Waller, 2003). But because sheep are often extensively raised, it is not always possible or practical to administer the fungal spores mixed in the daily feed supplement. Waller suggests the employment of supplementary blocks or controlled release devices in such sheep management systems (Waller, 2003). It has also been suggested by Epe (et al., 2009), that a more convenient technique of administration may be obtained by using a bolus or even automated feeding.

Data from assays in field conditions were unfortunately less consistent, but this may be due to the short period of feeding or perhaps the lack of confirmation as to whether all animals obtained a sufficient intake of feed. Epe (et al., 2009) established a reduction in the quantitative larval culture to be of 81.4% in 40 fungus-fed sheep that were naturally infected with GIN during a 3-month feeding period. This is in accordance with other observations of 82-99%. However, it did not show clear effects in reducing the parasite burden, which underlines the fact that this fascinating method can only be regarded as a valid supplemental tool for parasite control, rather than a realistic alternative to anthelmintic treatment (Epe et al., 2009). Infact, it should be used in addition to alternate grazing strategies; use of the FAMACHA system (Coles, 2005) and long-term genetic selection of resistant animals towards parasites, according to Waller (2003).

An alternative method of biological control (BC) is to influence the rate of fecal breakdown or degradation in order to accelerate the exposure of the larvae to hostile or inclement weather. Fecal degradation depends upon the type of forage. Infact, it has been demonstrated that sheep grazing on pasture containing some plant species such as chicory (Chicorium intybus) and birdsfoot trefoil (Lotus corniculatus) have lower egg outputs than sheep grazing on Lucerne (Medicago sativa), for example (Hoste et al., 2006). Birdsfoot trefoil contains condensed tannins, plant polyphenols that have drawn much scientific interest (Stear et al., 2006).

The beneficial properties have been identified as anti-parasitic effects and positive consequences for the host. Certain forages and fodders have plant secondary metabolites (PSM), which are currently the core of many studies suggesting their use as neutraceuticals with anthelmintic properties (Hoste et al., 2006). The term neutraceuticals describes nutrients which are mainly used in farm animals because of their perceived positive properties on health and/or modulator of the digestive flora. Their nutritive value is only considered as a second intention (Torres-Acosta and Hoste, 2008).

Tannins are secondary plant polyphenols with a high affinity to proteins and polysaccharides. They are produced by a different number of plant species, for example; sulla (Hedysarum coronarium); sainfoin (Onobrychis viciifoliae); big trefoil (Lotus pedunculatus); Sericea lespedeza (Lespedeza cuneata) and of course birdsfoot trefoil and chicory, previously mentioned. On the basis of their biochemical structure, they are categorized into two major
groups: i) hydrolysable and ii) condensed tannins. Condensed tannins are further divided as procyanidins and prodelphinidins. One of their most important chemical properties is the ability to form soluble and insoluble complexes with macromolecules, such as protein, starch and fiber. The virtual absence of hydrolysable tannins in the legume forages has focused the attention towards the condensed tannins. Chicory instead, contains sesquiterpene lactones, other plant secondary metabolites attributed to the anthelmintic properties of this plant.

Beneficial effects on host physiology and performance under parasitic challenge have been pinpointed by the evaluation of clinical status of animals; pathophysiological measurements and the assessment of the impact of parasitism on production when infected sheep consume legumes or chicory (Hoste et al., 2006). Reductions on nematode numbers, worm fecundity, nematode egg excretion and egg output have been recorded and overall FE counts substantially decrease. In vivo studies examining the consequences of tannin-rich quebracho (Schinopsis spp.) bark extract given to nematode-infected sheep did indeed result in a decrease of egg output and worm fertility (Hoste et al., 2006). It is however difficult to determine whether the improved physiological response (resilience) is due to improved nutrition or to the role of specific biochemical compounds. Most in vivo studies attempting to confirm the capacity of condensed tannins in having parasitic properties have usually been confirmed by results of in vitro bioassays. Nevertheless, there is a great variability of results gathered during on-farm trials which have been justified by main causes.

Other than the content of tannins in the diet, it has been recognized that their structure and chemical characteristics might also be important in modulating anti-parasitic activity. Infact, highest level of activity against the GIN occurs in plants presenting larger proportions of prodelphinidins than procyanidins. Moreover, effects of the active compounds could vary from one parasite species to the next. Finally, the formation of condensed-tannin-protein bonds is a complex phenomenon that is influenced by the molecular weight of tannins, those of protein and the presence of amino acids such as proline and of course environmental conditions i.e. pH. Infact, the nematode cuticle is known to be rich in proline.

Because of the affinity in bonding with proteins, tannins can protect them from being degraded in the rumen and increase protein flow to the small intestine. This indeed, improves homeostasis and the host’s immune response. As a consequence of their ability to bind with proteins, tannins could also be able to inhibit enzymatic secretion or activity belonging to the worms.

The exact mechanisms remain obscure and undoubtedly vary according to numerous factors. However, excessive consumption of the majority of PSM can detrimentally affect the parasitized host; which is why a threshold of dosage has been determined. At concentrations superior to 70-80 g of condensed tannins per kg of dry matter (7-8% DM), reduction in food intake, growth inhibition and interference with the morphology and the proteolytic activity of microbes in the rumen decreasing production rates, have all been observed negative effects. In contrast, moderate concentrations (3-6% DM) have positive effects on host physiology, growth, wool and milk production.

It cannot be underlined enough that nematode control in ruminants cannot rely on a single solution, but tannin-containing plants have a potential role as a component of an integrated approach in fighting nematode parasitism (Hoste et al., 2006).
7. c) Nutritional supplementation

Nutritional supplementation, along with vaccination and utilization of genetic variation, are methods which aim at boosting the immune response within the host. Enhancing immunological function provides benefits both to the individual and also to the flock, by reducing pasture contamination while requiring little or no chemical input (Greer, 2008). Hosts with an enhanced immune system are those which eliminate fewer eggs, which may be less viable and the larvae require more time to develop into adult parasites (Stear et al., 2006).

How does nutrition enhance the immune system?

Supplementary feeding with additional protein enhances the immune response to *T. circumcincta*, *T. colubriformis* and to *H. contortus* (Stear et al., 2006). Additional protein also enhances the ability of the host to repair tissue damage. Interestingly, dietary supplementation with urea also enhances resistance and resilience to *T. colubriformis* as well as resilience to *T. circumcincta*, presumably because bacteria in the rumen convert urea into amino acids and polypeptides readily absorbed by the host. Finally, a number of trace elements have been demonstrated to have a positive effect on host resistance especially, zinc, iron, copper and molybdenum.

Copper oxide wire particles (COWP) have shown the potentiality to limit parasite infections in sheep, mainly towards the highly pathogenic *H. contortus* species (Torre-Acosta and Hoste, 2008). A dose of COWP as low as 2 g can decrease FEC and worm burdens in lambs. However, there is a drawback: sheep are very susceptible to copper toxicity because of its accumulation in the liver. Hence, not more than one dose every 12 months is recommended. Although some breeds are more susceptible than others and some flocks have more accessibility to copper. Recently, smaller doses of 0.5 and 1 g have been found to be effective for GIN control. The collected data from Burke’s field trial (2006) carried out using 26 naturally infected lambs, suggests that multiple-low dose of COWP administered selectively using FAMACHA system could provide an alternative to chemical control of GIN (Burke and Miller, 2006). Unquestionably, nutrition has the potential to mediate the rate of acquisition or the degree of expression of immunity.

The periparturient rise plays a critical role in the epidemiology of infection, indeed ewes are seen as a reservoir of infection for the lambs. A nutritional basis for the PPRI has been proposed. The expression of immunity to parasites is a bodily function that competes with reproductive functions that have higher priority for the allocation of scarce nutrients. Nutrients will be scarce when the demand outweighs the supply, which frequently occurs during periparturient period where the demands rise up to six times higher than that of non-reproducing ewes. Thus, the increasing nutrient demand for progressing gestation and subsequent lactation at times of nutrient scarcity will result in a penalty on expression of acquired immunity to parasites. At times of nutrient scarcity, an increased nutrient supply will reduce the degree of PPRI. Infact, this has been put into practice resulting in clear evidence that this postulate does not lack scientific support.

It has been observed that litter size has an effect on PPRI in which FEC and/or worm burdens are modified in relation to the number or lambs reared by the ewe and on the parasitic species involved. Houdijk carried out a field trial where 3 groups of ewes were formed and given different diets: Group 1 was offered a scarce amount of protein (0.8 times their protein
requirements) and was twin-rearing; Group 2 was offered an adequate amount of protein (1.2 times their protein requirements) and was twin-rearing, and Group 3 was fed the same allowance as Group 1 but one of the two lambs was weaned on day 10. After day 10, the FEC of the latter group decreased by 60% within days, and became similar to that of Group 2. Therefore there was a clear response following increased protein availability (Houdijk, 2008). Parasitized sheep would require extra metabolizable protein (MP) to replace damaged tissue and to develop and express immunity. Dose-response experiments in growing lambs as well as periparturient ewes have indicated that MP requirements are increased in the order of 20-25%.

In growing lambs, parasites are encountered for the first time thus prioritization of scarce nutrient allocation has to be made in order to permit the acquisition of immunity over growth and avoid the overwhelming adverse consequences of parasitism before reaching reproductive maturity (Kyriazakis and Houdijk., 2006). Protein supplementation has resulted in an increased concentration of circulating and local inflammatory cells, sheep mast cell proteases and circulating antibodies. Local effector mechanisms such as mucosal antibodies, mucosal mast cell proteases and goblet cells have been demonstrated in a rat lactating model, in being sensitive to nutritional supplementation (Houdijk, 2008).

What is the cost of immunity?

As Greer (2008) cleverly pointed out, a strong immune response does not come without a cost that reflects both the resources required and the consequences of effector cell recruitment and the associated signaling. This response includes components such as immunoglobulins, leukotriens, mast cells, globule leucocytes and lymphokines which represent a considerable nutritional penalty. Infact, it has been estimated that the maintenance of immunity towards nematode parasites in sheep would incur a 15% loss of productivity. The development of immunity imposes a substantial cost as we have seen however, the manifestation of a direct benefit to the animal’s performance may not be apparent until the point at which the animal has been able to recoup the nutritional investment (Greer, 2008).

How does GIN parasitism effect nutrient economy?

Infections with gastrointestinal parasites in sheep are typified by suboptimal animal performance caused by the reductions in both feed intake and nutrient utilization, with lamb growth rates returning to comparable levels to their uninfected controls after the development of an effective immune response and the expulsion of the parasite (Greer, 2008).

Generally, infections with GIN are characterized by: villous atrophy; reductions in both saliva and serum phosphate concentrations (up to 50% during T. colubriformis infections); rise in abomasal pH either through reduced acid production or through an increase in other secretory products and decreased pepsinogen production. Daily Nitrogen balances in sheep has been observed to be 3-5 g less than their uninfected controls, up to 36% of which may be plasma N that leaked as a result of parasite-induced damage to the digestive tract. There is also poor protein deposition in the body of infected animals, probably because it is deviated towards the reparation of tissue. There is evidence that gastrointestinal tissue and liver protein syntheses increase. Infact, in lambs infected with T. colubriformis, the daily fractional synthesis rate of protein in the liver increased from 0.346g/d to 0.724g/d. Leucine is more and more
sequestered from arterial pools in the order of 24%, whereas the oxidative loss is around 22-41%. Lastly, there is a reduction in the live weight gain implying that there is a repartitioning of amino acids from tissue to repair intestinal damage or to develop immune response.

This last consideration has been the core of recent studies to assess the cost/benefit relationship of immunity. Understandably, it is a very complex and intricate series of mechanisms to be analyzed; nevertheless immunosuppressed infected animals have an equal impairment of growth as to the infected non-immunosuppressed controls. In conclusion, the production loss is not a consequence of the host immune response and to which there is an advantage to the host in having immune-mediated resistance (Greer, 2008).

Young grazing sheep consume large amounts of fibrous diets which often are not able to suffice the high demands of their productive functions, alongside with the development of immunity. This calls for a prioritization of limiting nutrients which unquestionably causes repercussions to the productive functions. The higher the priority, the less likely the host nutrition will affect the bodily function (Kyriazakis and Houdijk, 2006). Animals would be expected to give the highest priority to the allocation of scarce resources for maintenance functions, i.e. maintenance of body protein, as this will guarantee survival in the short term.

This nutrient-partitioning framework suggests that a young lamb with excessive nutrients at disposal preferentially diverts the resources to the acquisition of immunity rather than growth. Once immunity is established, the framework is modified and the nutrient requirement for growth is given a higher priority over the expression of immunity. However, in adult ewes, the course of these mechanisms are a little different considering that the immunity is fully matured and the nutrient supply is enough to maintain the immune response; the exception to which is the periparturient period, during which they prioritize reserves to ensure the survival of their offspring (Greer, 2008).

Modifications of nutrient-partitioning are indeed reflected by the correlation between FEC and MP supplementation which has been studied numerous times. Positive effects of MP supply on FEC and worm burdens during gastrointestinal nematode parasitism would be expected to evolve showing fluctuations and not be consistent. Infact, there is a large body of evidence to support that the acquisition but not the expression of immunity takes priority over growth (Kyriazakis and Houdijk, 2006).

This diversion of nutrients, when animals are given a limited supply, is likely to impose a substantial trade-off in the form of reduced performance that may explain the infection-induced reductions of wool growth of up to 11% and in Metabolizable Energy (ME) utilization of 15% in sheep that are resistant to *T. colubriformis*. This trade-off represents an undesirable short-term depression in performance, but in the long-run, it guarantees the establishment of a preserved immune response to parasites (Greer, 2008).

The relative priority accorded to key life processes are influenced by age and experience of infection. This is why in the tropics, where nutritional environment is often poor, nematodoses such as Haemonchosis, Trichostrongylosis and Oesophagostomosis are often a major health issue for lambs and children causing high mortality rates. On the contrary, in temperate regions, where nutritional stresses are of less importance and parasites are often less pathogenic, the major benefit accruing from dietary supplementation usually relates to resilience rather than resistance (Jackson, 2008).
7. d) Vaccination

The only commercially available vaccines against nematode infections are irradiated larval vaccines effective towards bovine and ovine lungworm. They do not induce immunity in lambs against gastrointestinal nematodes presumably because the mechanisms in lung tissue differ. However, they do protect older sheep against *H. contortus*, possibly because in older sheep natural mechanisms to control adult worms are present. Vaccination of older animals is not commercially available (Stear *et al.*, 2006).

An alternative angle for vaccine development was taken which was the search for hidden antigens that can generate immune response but normally the host does not recognize during natural infection. Proteins from the gastrointestinal tract of nematodes are an option but unfortunately the extraction of such molecules in sufficient quantities is impractical. The current aim is to create vaccine based on recombinant molecules which may or may not induce protective immunity (Stear *et al.*, 2006). Viability of vaccines used under filed conditions especially where mixed infections are prevalent, remains profoundly questionable. For now, no commercial vaccines are available on the market for the control of GIN in small ruminants despite great scientific efforts (Torres-Acosta and Hoste, 2008).

7. e) Genetic selection

Breeding sheep able to tolerate nematodes has the advantage of reducing the chemical input but on the other hand there is great concern as to how this can modify the productivity of animals. Examples of sheep selectively bred for resistance include “Rylington Merino”, a *Haemonchus*-resistant Merino sheep flock in New South Wales, Australia and a Romney selection line in New Zealand. The response manifested consisted in lower worm egg contamination on pastures but unfortunately there was a deleterious effect on the wool growth rate where these sheep produced 9% less clean wool than the unselected control. The resistance attained by genetic variation did indeed reduce fleece weight of Merino sheep even though body weight gain was affected only at the beginning of the natural infection in lambs. Infact, the effect on wool production seems age-related in this case: young resistant sheep had slightly low wool growth rate during the early stage of the infection and need more nutritional attention, particularly regarding protein supply. Younger resistant sheep had higher proportion of globulin in relation to albumin, indicating they have earlier development of immune response. This repartitioning of proteins can be expected to influence wool growth (Liu *et al.*, 2005).

Tendentiously, resistant animals expel lower FEC than the susceptible controls and selection of sire using this criterion may result in more resistant lambs. Over time, as susceptible animals with higher FEC are culled, pasture contamination should decrease and overall GIN infection in flocks should lessen (Burke and Miller, 2008).

Not so surprisingly, genetically resistant Romney sheep lines have reported greater numbers of mast cells and globule leucocytes in the abomasal and intestinal mucosa. Moreover, production of parasite-specific antibodies in these sheep was seen to be of higher amounts in the intestinal lymph nodes during infection compared to their susceptible counterparts (Greer, 2008).
Another option is that of selecting for resilience, aimed at better production criteria in animals challenged by parasite infections. Burke for example, used the FAMACHA system, PCV and FEC parameters in order to distinguish between resistant and resilient animals. Resilient animals have higher fecal egg counts but do not show clinical signs of anemia therefore PCV numbers are higher, demonstrating that in these animals erythropoiesis is increased, and thus they may not be identified by the FAMACHA system (Burke and Miller, 2008).

Often farmers prefer to substitute native resistant breeds with more productive but more susceptible breeds imported from exotic areas of the world. This trend has already placed many livestock breeds under threat of extinction leaving a few breeds of high producing animals to express their production traits under highly specialized intensive conditions (Torres-Acosta and Hoste, 2008).

The breed described at the beginning of the introductory chapter is indeed an example of a local breed. The majority of autochthonous breeds are indeed more genetically resistant towards almost all parasitic species. Even those parasites that survive within the hosts and reproduce, need to find a balance with the genetic traits of resistance expressed by the organism. However, livestock husbandry has shifted the equilibrium with the intent of reaching maximum productivity, leading undoubtedly to an alteration of relationship between parasites and their host, in favour of the parasites. The usually difficult systems of raising sheep has required that these animals develop a significant adaptability towards the environment conditions of their territory, therefore enhancing a natural resistance towards whichever parasitic infections are characteristic of such terrains (Cianci and Ambrosini, 2001).

To conclude this second chapter, it must be stated that in reviewing the existing methods of nematode control, it should be crystal clear how alternative methods must be more and more implemented, and in combination is by far the best way to fight the ever so risky anthelmintic resistance nematodes are manifesting around the world.

7. f) Non conventional anthelmintics

Despite the continued efforts for more than 50 years of the pharmaceutical industry to develop efficient, low cost, non toxic and easy-to-apply chemical Ahs, traditional veterinary pharmacopeia, mainly based on phytotherapy or plant remedies, remains the principal resource to treat animals against helminthes in a large part of the world. This is justified by the low cost combined with the local availability of such resources. Since the onset of AR, the interest of such natural anthelmintics is rising (Torres-Acosta and Hoste, 2008).

The effectiveness of herbs or plant extracts against helminths is traditionally known and many products are available on the market. Results from a field trial on dairy ewes in an organic farm located in Central Italy indicate a double positive effect of herbal extract treatment. The first is the reduced incidence of toxic effects of the anthelmintics on animals and the second is the decrease in parasite infection in a similar way to conventional treatment. Veterinary phytotherapy compounds administered in this case contained extracts of Cardus, Eucalyptus, Gentiana, Urtica, Mallotus and Dryopteris (Ronchi and Nardone, 2003).

In another field trial, herbal commercial dewormers were supplemented to 6 goats. Recommendations involved the use of two formulas where formula 1 contained Artemisia absinthium (wormwood), Allium sativum (garlic), Foenicum vulgare (fennel), Juglans nigra (black
walnut) and Stevia rebaudiana Bertoni. Formula 2 contained Cucurbita pepo (field pumpkin), Artemisia vulgaris (mugwort), Allium sativum, Foenicum vulgare, Hyssopus officinalis (hyssop), Thymus vulgaris (thyme) and Stevia rebaudiana Bertoni. These different mixtures of dried plants or plant products have demonstrated various levels of activity against nematodes (Burke et al., 2009).

Moreover, some of the most popular European phyto-pharmaceutical preparations contain an American herb called Echinacea and the first commercial European preparation of this plant was made over 50 years ago. Echinacea has been used for its anti-inflammatory effects: antibacterial; antiviral; bacteriostatic; fungistatic and insecticidal in particular. Today, Echinacea is a widely sold plant especially on US and Europe’s market. The chemical and pharmacological researches demonstrate that the plant is a stimulant of the immune system. Therefore, the future of Echinacea in the international phyto-pharmaceutics has been assured (Ichim, 2005).

However, due to the variability in biochemical content and the lack of standardization, these herbal drug products have variable effects (Torres-Acosta and Hoste, 2008).

Among the use of non-conventional methods of parasite control, homeopathy plays a role in overcoming the problems linked to AH treatments (Benvenuti et al., 2004). Homeopathy is a natural therapeutic method which stimulates the organism to fight a disease by taking advantage of its own recovery potentials. Therefore, it does not eliminate the cause but simply beckons the immune system in doing so.

Homeopathic medicine is a therapeutic method which utilizes substances, deriving from the animal, vegetal and mineral kingdoms, which undergo certain procedures of succession and potentization. This created the homeopathic remedy or medicinal which permits the interaction with the organism (Pisseri, 2009).

The homeopathic remedy does not interact on a chemical or biochemical basis and although its operating mechanism has not yet been fully understood, it can be estimated that it brings about certain information and consequently generates a series of reactions in the body. This triggers a chain reaction which improves the equilibrium existing between various parts of the organism and between the individual and its environment. Naturally, the organism will subsequently possess a better adaptability to the environmental conditions and therefore be more resistant to illnesses it can come across.

The cure is prescribed based on Hahnemann’s principle dictating that “like cures like”, which entails the global approach with which a homeopathic doctor gathers the symptoms and interprets them. These homeopathic symptoms constitute the pathogenesis of the remedy and are referred not only to the physical function, but include the emotional and mental aspects as well. When the framework of symptoms collected is the most similar to the symptoms listed in the pathogenesis of the remedy, the better the results expected shall be (Hahnemann, 1920).

Homeopathy can be applied to livestock but it is essential that the medical history includes an assessment of the environmental aspects, climate conditions, nutrition, the facilities, human-animal relationship and animal-animal dynamics. The usage of homeopathy in animal husbandry allows us to obtain certain important advantages:
• absence of drug residues in animal products
• no environmental impact
• overcoming the microbial and anthelmintic resistance to medicines
• increase in animal welfare
• low costs of therapy
• implementation of a preventive strategy reducing the production losses due to subclinical forms
• increasing consumers’ request for organic food products.

The entire flock is usually studied as if it were a single individual and the various pathologies encountered are interpreted as the expression of the pathological tendency of the farm (Benvenuti et al., 2004).

Homeopathic treatment is known to stimulate the immune system, increasing the resilience capacity oh hosts without directly eradicating the parasitic population (Benvenuti et al., 2004). Treatment with homeopathic preparations such as *Arsenicum album* and *Ferrum phosphoricum*, alternately, and *Calcarea carbonica*, has been seen to cause favourable responses against worm challenges in twenty naturally infected lambs (Zacharias, 2008).

In another field trial, *Sabadilla MK* was administered in a group of ewes throughout a year, in the attempt of comparing the FEC with the control group left untreated. Results demonstrated how FEC was kept under the threshold which corresponds to the arise of the health damage and production loss. This proves that an effective monitoring program can avoid unnecessary anthelmintic treatment where an optimum equilibrium among host, parasite and environment has been reached (Benvenuti et al., 2008).