
Animal health links to recording systems. Resistance to internal parasites in sheep

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Traits related to health have been given limited attention by ICAR. Gastro-intestinal parasites are a major burden to sheep production especially under extensive grazing conditions. One important trait is fecal egg count (FEC) used both to measure resistance of helminths to drugs, and resistance of sheep to internal parasites. There is ample evidence that resistance of internal parasites to drugs is a growing world-wide problem. Integrated parasite control (IPC) including genetically resistant sheep seem to be a sustainable strategy in sheep production systems. FEC is a trait with a skewed distribution and logarithmic transformations are used to analyze the data. Its heritability is of the order of 0.25 to 0.30 under artificial challenge, and seems to be lower under natural challenge. Repeatability is of the order of 0.40. Protocols for measuring resistance of sheep to internal parasites have been developed but must be subject to further research. The relationship of FEC to production traits must also be clarified. There are ample research opportunities for the genetics of resistance to endoparasites in sheep, both in genomics and in breeding strategies. FEC and protocols for measuring resistance are areas in which ICAR could expand its research and development activities at the international level.

Key words: *health recording, drug resistance, genetic resistance, endoparasites, integrated control, fecal egg count.*

Los caracteres relacionados con la salud animal han tenido limitada consideración por parte de ICAR. Los parásitos gastrointestinales son una carga muy importante para la producción ovina especialmente bajo condiciones de pastoreo extensivo. Un carácter importante es HPG (huevos por gramo de materia fecal) que se usa para medir tanto la resistencia de los helmintos a las drogas, como la resistencia de los ovinos

Summary

Resumen

a los parásitos internos. Existe amplia evidencia de que la resistencia de los parásitos internos a las drogas es un problema mundial en aumento. El control parasitario integrado (CPI) incluyendo el uso de ovinos genéticamente resistentes, parece ser una estrategia sustentable para los sistemas de producción ovina. HPG es un carácter con una distribución asimétrica que requiere de transformaciones logarítmicas para el análisis de los datos. Su heredabilidad es del orden de 0.25 a 0.30 bajo desafío artificial, pero parece ser menor bajo desafío natural. La repetibilidad es del orden de 0.40. Protocolos para medir resistencia de los ovinos a los endoparásitos han sido desarrollados pero deben ser sujetos a más investigación. La relación entre HPG y caracteres de producción debe ser estudiada. Existen amplias oportunidades para la investigación de la resistencia genética a los endoparásitos en los ovinos, tanto en genómica como en estrategias de selección. HPG y protocolos para medir resistencia son áreas en las cuales ICAR podría expandir sus actividades de investigación y desarrollo a escala mundial.

Palabras clave: control sanitario, resistencia a las drogas, resistencia genética, endoparásitos, control integrado, huevos por gramo.

Introduction

The International Committee on Animal Recording (ICAR) has as its motto *quod scriptum est manet*, what is written remains, thus expressing the idea that modern animal production and its components like management, feeding, health and breeding are only possible to be conducted rationally and economically if proper recording is done. ICAR has long experience in recording production traits, and it has recently expanded its scope to include also some functional traits. Although recognized as very important, there has been however little progress in recording health related traits.

It would be far beyond the scope of the present paper to review the wide range of health related traits to consider as possible candidates for recording. The focus will be on fecal egg count (FEC) as an important health related trait in sheep. The case study presented here originates from an FAO technical cooperation project on genetic resistance to gastrointestinal nematodes in sheep, conducted by the Uruguayan Wool Secretariat (SUL) and the Uruguayan Veterinary Services (DILAVE). This paper will also include a general description of the problem and some related results from experiences in other countries.

The problem

The vast majority of sheep in the world are raised under grazing conditions and are subject to internal parasites. The most common and important internal parasites affecting sheep in temperate South America - in this case Uruguay - are: *Haemonchus contortus*, *Trichostrongylus spp.*, *Ostertagia spp.*, *Cooperia spp.*, and *Nematodirus spp.* The measurement of

resistance requires diagnosis and adequate recording. Two types of resistance must be considered: resistance of sheep to internal parasites and resistance of internal parasites to drugs (Box 1).

Box 1.

Two types of resistance to be considered

- **Resistance of internal parasites to drugs.**
depends on the genetics of the parasite, it arises from selection pressure due to the use of anthelmintics, it is a growing problem, it has reduced options in drugs.
- **Resistance of sheep to internal parasites.**
depends on the genetics of the sheep, it is basically a polygenic trait with medium to low heritability, with selection programs for resistance to gastrointestinal nematodes being conducted, some breeds have naturally higher resistance.

The continuous and intensive use of drugs has resulted in high frequency of resistant gastrointestinal nematodes all over the world, according to an FAO survey (Nari and Hansen, 1999). This study was conducted mainly to determine the importance of the problem of resistance of ecto- and endoparasites to the most commonly used chemical compounds. The definition of resistance used in the report is the detection by sensitive tests of a significant increase of individuals of one species and population of parasites that are capable of tolerating doses of drugs proven to be lethal for most individuals of the same species. The main conclusion of the study is that 86% of 77 OIE (*Office International des Epizooties*) member countries, representing 52% of membership, have reported the occurrence of resistance to anthelmintics.

During the past decade this problem has been given increasingly more importance, but its control has not been altered substantially. Countries taking some measures to prevent parasite resistance to drugs do so almost exclusively by rotating or changing antiparasitic agents. The situation is worsened by the fact that no permanent supply of new drugs is foreseen (Nari and Hansen, 1999) due to high cost of research, long development time to reach the market, and the existence of more profitable areas for drug development, like drugs for human use. In many parts of the world

**Resistance of
internal
parasites to
drugs**

veterinary services are no longer a totally public service, have lowered their vigilance status, and do not have the means or infrastructure necessary to carry out tests for drug resistance. Therefore, the fact that a country has not reported resistance does not mean that it does not occur. However, there seems to be a trend towards less frequency of resistance of internal parasites to drugs in countries with low or no use of anthelmintics.

A survey conducted on 252 farms randomly distributed over the sheep raising areas of Uruguay and representing 80% of the total sheep population of the country (Nari *et al.*, 1996) concluded that in 92% of sheep farms in Uruguay some degree of resistance to anthelmintics exists. The drug groups tested were benzimidazoles, levamisole and avermectins (Table 1). In many cases multiple resistance was detected: 28% of farms showed resistance to one anthelmintic group, 64% to two anthelmintic groups and 1% to all three groups tested.

Table 1. Anthelmintic resistance in sheep nematode parasites in Uruguay (Nari et al., 1996)

	Percentage resistance		
	Benzimidazoles	Levamisole	Ivermectin
<i>Trichostrongylus</i>	91.7	95.0	0.0
<i>Haemonchus</i>	61.3	28.5	1.2
<i>Ostertagia</i>	18.4	12.8	0.0

The lack of response of a flock to anthelmintic treatment does not necessarily mean that there is a resistance problem. After other possible causes have been discarded, a test for anthelmintic resistance (Nari, 1987) can be conducted based on measurements of reduction in FEC (RFEC), as summarized in Box 2. Groups of at least 10 animals each from the problem flock are formed. There will be two basic groups: non treated control (C) and treated with the problem drug (T). There may be additional groups formed with sheep from the same problem flock; for example, animals treated with a different product, other means of administering the drug, or given different doses. During the test all animals must be in the same paddock under the same rearing conditions. The sampling of fecal matter has to be done individually at day=0 and day=10 after treatment. All animals have to be kept in the collective pens the same amount of time so as to be equally affected by the fasting period conducted previous to the test. If any statistical analysis is to be valid, egg counting techniques must have at least a sensitivity of 50 eggs/g and there must be an acceptable realized repeatability of measurements. The nematode

species can be ascertained by an identification of larvae in pools originated from the different groups. The raw FEC data are generally transformed logarithmically (Box 2) and the RFEC is then calculated by the formula: $RFEC(\%) = [1 - T_2/T_1 \times C_1/C_2] \times 100$, where C and T are the geometric means of the control and treated groups respectively, and 1 and 2 identify the FEC measurements done respectively at day 0 and 10 post-treatment. Arbitrary values of less than 95% are taken as indicating anthelmintic resistance. Confirmation is further sought by autopsy on 2 to 4 animals in each group, 10 to 14 days after treatment. Possible next steps are in-vitro experiments using reference strains and experiments with controlled infestation.

Box 2. Protocol for determining drug resistance (Nari, 1987).

**Determination of resistance
Measurement: reduction in FEC
Field experimental design (standard)**

- Control sheep=C (N>10).
- Drug treated sheep=T (N>10).
- Sample at day 0 (C1 and T1).
- Sample at day 10 (C2 and T2).
- Autopsy at day 14 (N=3).
- $RFEC(\%) = [1 - T_2/T_1 \times C_1/C_2] \times 100$
(C, T = geometric means).
- $RFEC < 95\%$ indicates likely resistance.
- *In vitro* experiments with reference strains.
- Controlled infestation.

How does selection for parasite resistance to drugs work? The enormous reproductive potential of the parasites and the repeated use of drugs will increase the frequency of genes for resistance in the parasite populations. The genetic basis of this resistance is still not well known, and it may be due to polygenes or quantitative trait loci (QTL), and also to the action of single major genes. The parasite cycle involves two sub-populations: the parasitic phase population and the free phase population which includes eggs and larvae in three stages (L1, L2, L3) and that is not affected by anthelmintics. Both populations are interdependent and connected through contamination rates from animals to pastures, and contamination rates from pasture to animals. The efficiency of the selection process towards parasite resistance to anthelmintics will depend

on the drug (type, dose and method of application) and on the environmental conditions (pasture, season, weather conditions, flock management). For example, many individuals of the free parasite sub-population may be lost due to exposure to sun, heat, dry conditions, predators, or simply because they did not reach the appropriate host.

Preventive measures against the development of nematode resistance to anthelmintics have been recommended. They include less frequent use of anthelmintics or their differential use in highly infected animals, the use of drugs with reduced spectrum, high doses as to ensure total kill, the use of anthelmintics of different groups in slow rotation, the alternate use of drugs with high and low spectrum (wormkill) and, probably the most efficient and logical strategy, the so called integrated parasite control (IPC). This last one is based on the use of a combination of prevention and parasite control measures: high nutritional levels (well fed sheep are more resistant), strategic use of drugs (this requires good professional guidance), pasture management (to decrease contamination) which includes alternate grazing of different species (cattle and sheep) and grazing by age groups (young sheep are less resistant to parasites). Methods of biological control, for example a fungus that predaes on parasite larvae, are being developed but their practical use is still in the experimental phase. Finally, another component that is sought as part of helminth IPC is the genetic resistance of sheep to internal parasites. A parallel reason to think of genetic resistance of sheep to parasites as a major component of IPC is the realization that chemical residues of antiparasitic drugs may accumulate in the tissues of host animals or in products like animal fibers like wool and hair, and hides. This may be detrimental both for human health and for international trade.

Resistance of sheep to internal parasites

Time old field observations indicated that there are individuals within a flock that show more resistance to internal parasite infections. This has led to experiments to study the distribution and genetic parameters of sheep resistance to helminths, and to possible selection plans for this trait within populations. The same type of observations has been made over many breeds, ascertaining that there are breeds that seem to be more resistant to internal parasites than others. There are therefore two main lines of research in the area of sheep resistance to internal parasites, namely seeking breeding for resistance within breeds (Woolaston and Baker, 1996) and the study of resistant breeds (Baker, 1998).

The main within breed selection strategies have been: selection for resistance, selection for resilience, and selecting for reduced number of treatments (Woolaston and Baker, 1996). Resistance is defined as the ability of the host to initiate and maintain responses to suppress the establishment of parasites and/or to eliminate the parasite load. Resilience is the ability of the host to maintain a relatively undepressed production level under parasite challenge. Selecting for a reduced number of

treatments relies on the ability of breeders to detect those animals that require more treatments, and treating them differentially as to achieve good production levels. This last strategy seems rather difficult to apply in practice, especially under extensive grazing conditions. The choice seems therefore to be between selecting for resistance or selecting for resilience, although probably both traits are genetically correlated. For conceptual reasons based on the physiology of the relationship between host sheep and parasites, and the evidence that the heritability is higher for resistance, this trait has been the preferred selection strategy. The selection criterion used is FEC. The average heritability of FEC over many studies and populations is 0.25 and the average repeatability 0.35. However, analyzing data from Uruguay, Swan (2001) obtained preliminary estimates of 0.09 and 0.27 respectively. This may be an indication of lower genetic parameters due to more environmental variation under the extensive grazing conditions of temperate South America, or also due to the fact that natural parasite challenge was used instead of artificial challenge. This last type of challenge is under better experimental control and is supposed to achieve higher FEC heritabilities and repeatabilities.

There are important between breed differences in resistance to internal parasites in sheep. Many studies have been and are being carried out for identifying and characterizing this resistance, of which two recent ones are mentioned as examples (Baker *et al.*, 2001 and Rege *et al.*, 2002). It is not the scope of this paper to review these studies. One of the basic ideas that represents a logical work hypothesis is that over time different breeds have evolved different mechanisms for resistance. At a later stage, by crossing resistant breeds a synthetic population of highly resistant sheep can be obtained.

Resistance cannot be measured directly. FEC is a good indicator trait and can be used as the selection criterion for resistance (Baker, 1998). It has been suggested that counting techniques should use improved sensitivity McMaster, since one egg in the counting represents 50 eggs/g upon extrapolation from the sample to the animal. Diverse protocols have been proposed. The Milk and Livestock Commission of the UK (MLC) in association with CBS Technologies, UK (Nieuwhof and Evans, 2002) does sample at scanning (20 weeks) plus 4 weeks no deworming. The logistics include labelled bags, and data quality and checks by controlling FEC within flock mean and variance. The International Livestock Research Institute (ILRI) has extensive experience in the area of measurement of sheep resistance and uses similar protocols for FEC (Baker *et al.*, 2001; Rege *et al.*, 2002).

The Commonwealth Scientific and Research Organization, Australia (CSIRO, 1992) utilizes a standard operating procedure (CSIRO SOP No. PR/02) for sheep management during worm challenge, which has been

**Fecal egg
count as
measurement
of resistance**

adopted by the FAO sheep resistance project in Uruguay (Swan, 2001). It states that during worm challenge sheep should be managed in such a way as to minimize pasture contamination. Then to monitor the health of the animals so that the infection, either artificial or natural, does not compromise the welfare of the flock. All the sheep in the flock are drenched prior to artificial infection, using a chemical that will kill all the parasitic nematodes present, e.g. Ivomectin. Sheep are then infected with a known dosage of stage III larvae of the required worm species. The day of infection depends of the clean up drug used. With Ivomectin, currently recommended by the CSIRO laboratories as a clean up drug, sheep should not be infected within 7 days after drenching. The dose of larvae depends on factors such as the species of worm, age and size of sheep, pasture conditions, body condition of sheep and the length of the planned infection. For example, for *Haemonchus contortus* it is recommended to carry out infections of 10000 larvae per head for a 3 to 4 week challenge. Sheep can be run under normal conditions for the first 18 days of the infection, after which time the larvae will have reached maturity and begin laying eggs. From this time on the pasture will be contaminated with large numbers of eggs and larvae. At the completion of the fecal sampling, the sheep should be drenched with a drug appropriate to the parasite species used in the challenge, leaving them 24 hours before moving them to a fresh pasture.

FEC has an asymmetrical distribution (Figures 1 and 2) with significant indexes of skewness and kurtosis. A logarithmic transformation is performed on the data to allow statistical analyses that rely on a normal

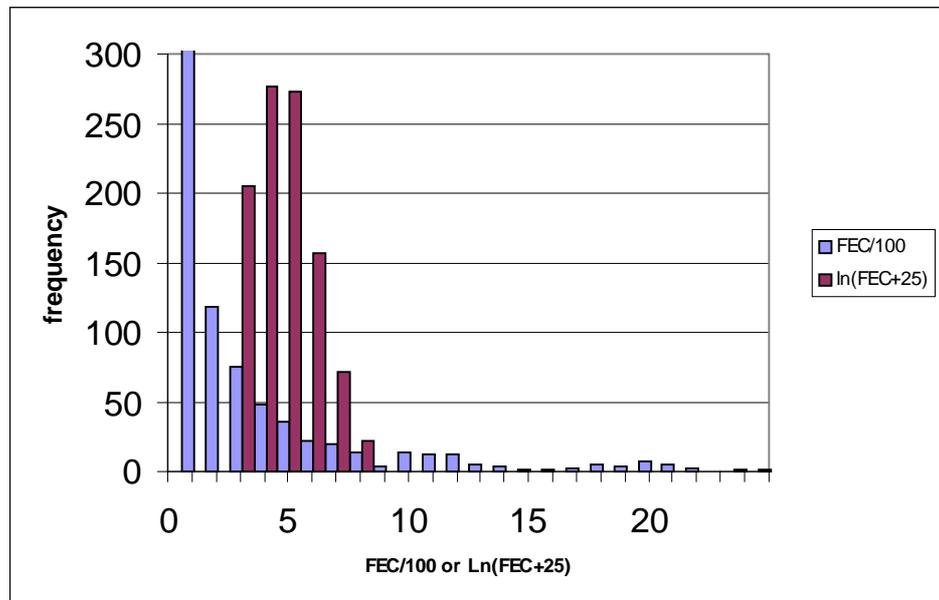


Figure 1. Example of the phenotypic distribution of fecal egg count in sheep in the UK (Nieuwhof and Evans, 2002).

distribution of observations (Figure 1). Nieuwhof and Evans (2002) found an average variance of $\text{Ln}(\text{FEC}+25) = 1.1$ also using in practice a heritability value $h^2 = 0.30$. Genetic correlation (r_g) with production traits is considered very low but negative (-0.1), as well as phenotypic correlation (r_p) with production traits (-0.05). Expected breeding values (EBV) are not additive but multiplicative, ranging from -0.7 to +0.7. An EBV = -1 represents 37% less eggs as compared to EBV=0 and on the other extreme an EBV = +1 represents 2.7 times as many eggs as the base EBV=0.

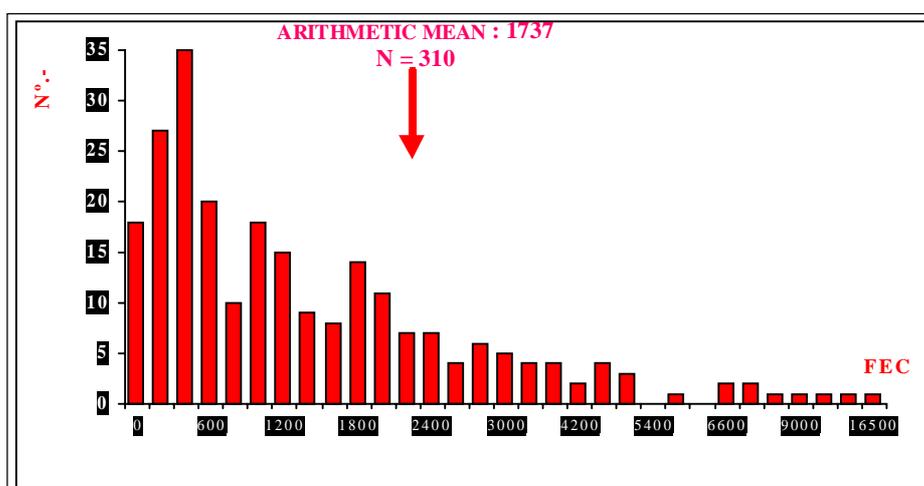


Figure 2. Example of the distribution of fecal egg count in sheep in Uruguay.

Resistance of internal parasites to drugs is both a global problem and a growing problem. It has to be addressed immediately since the options for available and effective new drugs are getting narrower all the time. There are also environmental and human health implications to a worldwide increase in the use of drugs. Integrated parasite control (IPC) based on the use of a combination of prevention and parasite control measures like high nutritional levels, strategic use of drugs, pasture management, including alternate grazing of different species and by age groups, is justified as a sustainable strategy. It is recommended that research efforts be directed towards the study of FEC as a trait to measure resistance. This includes biological aspects, for example, what FEC is really measuring from a physiological and production point of view, as well as statistical aspects like distribution of the trait and better ways to handle data analysis. Another area for research is the development of protocols for internal parasite challenge experiments considering the wide range of species of internal parasites and host species and breeds, and a range of environments. The genetics of resistance, both resistance of sheep

Conclusions and recommendations

Box 3. Conclusions and recommendations

- Drug resistance affects almost all countries.
- Integrated control of gastrointestinal parasites is a sustainable strategy.
- There are research needs concerning:
 - 1) FEC as resistance measurement trait.
 - 2) Protocols for internal parasite challenges.
 - 3) Genetics of resistance (sheep & parasite).
- ICAR should consider FEC and resistance measurement protocols.
- FAO should support breeding strategies for resistance to gastro-intestinal parasites in sheep.

to gastro-intestinal parasites, and resistance of nematodes to anthelmintics is a wide area for research to contribute to practical solutions. The first type of research may include the genomics of resistance by mapping single genes or QTLs, functional genomics by trying to discover how genes work to make animals or breeds resistant to internal parasites, breed comparisons, the determination of genetic parameters like heritabilities for FEC and related traits, and genetic correlation of FEC with production traits, and genotype x environment interactions. Box 3 summarizes the main conclusions and recommendations. From an institutional point of view, ICAR should consider FEC and resistance protocols among its areas for future involvement and FAO should support projects that include breeding strategies for resistance to gastro-intestinal parasites in sheep.

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