WORMS' WORLD - 21st CENTURY OUTLOOK

Professor Lord Soulsby
Department of Clinical Veterinary Medicine
University of Cambridge, Cambridge, U.K.

PAST ACHIEVEMENTS:

When looking to the coming century it would be unrealistic to ignore the very many and important achievements in the field of ruminant helminthology during the 19th and 20th Centuries. The role of such infections as a cause of ill health and production loss in domestic livestock was beginning to be generally accepted in the late 19th Century and in 1890 the U.S. Department of Agriculture Bureau of Animal Industry codified the information in a report on "Animal Parasites in Sheep" (3). From then the study of parasitic disease of livestock expanded enormously and continued in full spate into the 20th Century. In particular the 1920's and 1930's were a flourishing time for animal parasitology, especially in the U.S.A. The Department of Agriculture produced numerous pamphlets on the morphology and life cycles of animal, especially ruminant, parasites.

One might say, the first half of the 20th Century was a parasitologist's paradise with the discovery of new species, life cycles and pathogenic effects. Many names became household terms in the veterinary parasitological world, such as Ackert, Chitwood, Gordon, Hall, Hassall, Otto, Ransom to mention a few.

One of these notables of the 1920's whom I had the honour and pleasure to know was Norman Stoll. Working with *Haemonchus contortus* and other helminths he made many important observations, still pertinent to the modern day situation. But a signal contribution to parasitology was Stoll's (1947) "This Wormy World" (19) where he attempted to quantify the importance of parasitic infections in man and livestock. This was the first realistic measure of loss due to parasites. The more detailed analysis of pathophysiological events has culminated in Dr. Symon's (20) volume on the pathophysiology of gastrointestinal parasitism which lays out very clearly the complex situation as a result of intestinal parasitism. Other contributors in this field (15) clarified the pathophysiology of infection of cattle with the nematode *Ostertagia ostertagi* and the loss of appetite, diarrhoea, loss of weight gain and the presence of pepsinogen and gastrin in the blood plasma.

The studies of *Ostertagia* spp. further revealed biological behaviours of parasites which allowed them to synchronise their developments with physiological events of the host, such as lactation, in the phenomenon of *hypobiosis.* This phenomenon is well recognised in several of the trichostrongylids of ruminants and plays an integral part in the epidemiology of such infections as discussed below.

Similar studies on the pathophysiology of trematode infection of ruminants, especially for *Fasciola hepatica*, showed that anaemia and hypoproteinaemia were essential components of the disease process but also confirmed that the plane of nutrition was important (4) in pathogenesis.

The complexity of the factors involved in the pathogenic process indicates that changes in the parasitised intestine, for example, affecting the fate of proteins, may change the bacterial component with a consequent effect on the physical environment. With mixed species parasitic infections, as occurs in the field, the complications and potentiations are greatly magnified from those of single experimental infections (12).

An area of research which received, and continues to receive, much study during the 20th Century is that of immunity to parasites.

The idea that animals could become resistant to parasitic infections and that one may create such resistance artificially by vaccination was somewhat too advanced thinking even in 1949, when I took up the subject. Immunity to parasites was a debatable issue and vaccination was considered an unrealistic conclusion. However, in the 1950's a first practical commercial vaccine against a parasitic helminth infection was developed, consisting of X-irradiated infective larvae of *Dictyocaulus viviparus*, the bovine lungworm (11).

There was the hope some 30 years ago that similar approaches might be used for several other major parasitic diseases of man and animals. However, in no case has an effective practical vaccine been produced for a variety of reasons including: immune unresponsiveness of young animals, immunosuppression, lack of sufficient immunising stages and their preservation in a viable immunising form. However, with the advent of molecular biological techniques, the outlook for practical field vaccines for parasitic infections has changed dramatically and there are a number of parasitic infections where vaccines constitute realistic approaches to control.

But perhaps the greatest development in veterinary, especially ruminant, parasitology in the 20th Century has been the progress in therapy.

The anthelmintics now available often are highly effective against the larval stages as well as the adult forms of parasites. Indeed with efficacy so good, other concerns now are cost, delivery systems, drug resistance, operator safety, residues and environmental issues. There have been major advances in delivery systems with increasingly ingenious ways for the application of antiparasitic compounds. These consist of slow release, pulse-release, pour-on preparations, etc., and no doubt others are being developed. These delivery systems also make mass medication easier but they tend also to encourage overuse of drugs, leading to anthelmintic resistance. Resistance does not pose as serious a threat in cattle as in sheep, nevertheless there are reports of oxfendazole, levamisole and morantel tartrate resistance to trichostrongyles in cattle. While ivermectin resistance has been demonstrated for *H. contortus* of sheep on several continents, the phenomenon is infrequent in cattle but its occurrence in calves infected with *H. contortus* has been reported in Texas (5).

One further development to be mentioned in this incomplete list of achievements is that of our understanding of the environmental factors which influence the epidemiology of helminth infection in ruminants. A fuller understanding of this would undoubtedly assist in the strategies for control of parasites and it may well be that the basic factors of transmission are being ignored because of the highly effective antiparasitic compounds.

Maturation and survival of eggs, of pre-infection larval stages, derived from them and the infective stage, play important roles in the parasitic process. They represent that portion of the parasite population outside the host which is often ignored as an integral part of the whole. But where intermediate hosts are concerned, as with trematodes and cestodes, there have been many studies, delineating the ecology and the role of the snail in the transmission. Such work has led to the ability to forecast dangerous meterological periods of infection by *Fasciola hepatica (8).*

For many years it was believed that data derived from *Haemonchus contortus* was applicable to ruminant nematodes in general and that development to the infective stage was rapid and survival relatively short. However, studies of pasture conditions and larval requirements for different species now indicate that only a few generations of parasite may occur within one year (1 or 2) and infective larvae may survive several months and perhaps over winter in temperate zones. Further, while much attention was paid to the faeces and herbage, little was paid to the soil and its role as a reservoir for infective larvae. It is now established that the soil can serve as a reservoir for larvae, increased larval counts from herbage occurring in spring time and the surface soil (top 7.5 cm) is now regarded as important in the epidemiology of, for example, the bovine lungworm *Dictyocaulus viviparus.* Indeed survival in the soil may be prolonged and larvae deposited the previous Fall may emerge on to an aftermath following the removal of hay or silage in mid-July (2).

Finally, with respect to environmental issues and their influence on parasitism, mention must be made of larval inhibition or hypobiosis. This phenomenon is a mechanism adopted by nematode parasites to improve survival during periods when external conditions are unfavourable to development of free living stages. It is synchronised in onset with environmental change, (e.g. declining temperature perhaps also photo-period) but where rainfall fluctuations produce adverse conditions the trigger factor may well be dessication (21). The physiological changes in the larval stages undergoing hypobiosis are, as yet, unclear and the proportionate role of environment and host in the phenomenon has yet to be clearly defined. The mechanisms for release from hypobiosis are similarly unclear.

Resumption of development of *Ostertagia* spp. for example may be synchronous or asynchronous. In the former it is frequently associated with serious disease (Type II ostertagiosis in cattle) though the triggers for such development are unclear. Lactation clearly plays an important role in resumption of development and in sheep is closely associated with the periparturient rise in faecal egg counts. Lactation is also associated with a temporary relaxation of immunity, influenced by endocrinological changes, of which levels of prolactin have received much attention.

PRESENT CONCERNS:

Target for Antiparasitic Compounds:

The antiparasitic compounds used at present are directed against the parasites within the host and frequently against the mature stages, though there is an increasing number effective against larval stages. However, the majority of a parasite population is outside the host, either in the form of developing or infective larvae or eggs; few, if any, antiparasitic compounds are directed against these.

The final host is the collecting point through which a parasite population must pass in order to progress to the next generation of parasites. Present day antiparasitic compounds attack the parasite when it is established in the host and there have been few attempts to stop parasite development at the entry point of infection. There are many triggers required to convert a free-living or an infective stage and in gastrointestinal nematodes, exsheathment or hatching are such events. No exsheathment, no parasite! Would an anti-exsheathment compound for example be as effective as one of the compounds highly effective against an adult parasite stage? Similarly, triggers for site finding and functional development are activated in the early stages of parasitism within the host, the inhibition of these events would limit development to only the early stages. Such an approach would recognise not only that there are various key points which might be the targets for control strategies but also that a parasite population consists of that mass within a host and the generally larger mass of organisms outside it. Other than pasture management and grazing practice, there have been few developments in the control of this external population.

Distribution of Parasites Within Host Populations:

The over dispersed frequency distribution of worm burdens in a population of hosts is now a familiar phenomenon in parasitology. This represents a negative binomial distribution (1) and is simply stated that the majority of worms occur in the minority of hosts. The heavily parasitised animals are predisposed to such infections (17) and in human hookworm after treatment, those patients who were lightly infected initially tended to remain so and those heavily infected previously tended to become heavily infected again. As in man, so in ruminants! One is familiar with the "wormy animal" almost determined to be heavily parasitised despite repeated anthelmintic treatments. This situation can be modified and is done so by selective breeding in animals. Previous examples are the "violet" factor in sheep (23) so named after a ram, and sheep have been separated into 'responders' and 'non-responders' on the basis of their ability to respond to an X-irradiated vaccine (24). This has been demonstrated to be associated with the presence of an ovine lymphocyte antigen (SY1) present in high frequency - 73% in responder rams(vs. 22% in low responder rams) and 65% in responder ewes (vs. 33% in low responder ewes) (16). It has been concluded that the SY1 antigen is likely to be part of the sheep's major histocompatibility complex (MHC) (16). It is clear, therefore, that host populations can be manipulated by selective breeding and merino sheep have been bred for resistance to *Trichostrongylus colubriformis* (25) and to *H. contortus* (26).

In man, mass chemotherapy at a level less than that required to eradicate the parasites can significantly reduce the level of herd immunity, and raise the average worm burden above the levels existing prior to anthelmintic 'control' (1). But, of course in veterinary parasitology also we adopt the strategy of mass medication while there is every evidence that this too may lead to greater susceptibility of the flock. Studies on the effect of discontinuity of infection on resistance to *Haemonchus contortus* in sheep, (6) concluded that the use of anthelmintics on a flock basis for the general control of *H. contortus* populations should be approached with caution and a case could be made for limiting their use to flock or individuals within flocks showing clinical evidence of haemonchosis.

More recent studies (9) however, indicate that genetically resistant lambs are able to maintain their immunity while subject to strategic anthelmintic treatment, a fact which lends hope to the future control approaches as discussed below.

Consumer Pressure:

One of the major issues on the future development of parasitic control will be consumer pressure. Frequently this is totally unscientific, but nevertheless may be a very powerful force in the determination of the use of therapeutic substances in general. Examples of where consumer pressure has caused concern are the use of anabolic steroids and somatotrophins in cattle. Similarly, environmental concerns are increasingly apparent as certain compounds are shown to affect the natural fauna and flora, e.g. their effect on earthworms and dung beetles. The case for mass medication of animals especially by the use of slow or sustained release or timed release devices may be challenged both by groups concerned with animal welfare and those so called organic farming zealots.

Whether it be consumer pressure or drug resistance or over dispersion of parasites within hosts, new approaches are necessary; a 21st Century outlook.

FUTURE OUTLOOK:

Response to the Concerns of Chemotherapy:

A major approach must be the enhancement of resistance either by active vaccination or by selective breeding of resistant animals. While substantial progress has been made in breeding sheep resistant to parasitic infection little work has been done in this direction in cattle. Sheep have been separated into responders and non responders as indicated above and lambs of a resistant genotype were able to sustain genetic differences in resistance to gastrointestinal nematodes in the face of strategic anthelmintic medication (9). Sheep bred for resistance to one species of nematode may also express resistance to a different species and such information, if applicable to cattle, will be important in the long-term development of resistance to infectious diseases in ruminants. There is some indication that resistance to parasitic infections is broadly based in cattle since the N'dama trypanosome tolerant bovine of West Africa is also more resistant to other infections than its non-tolerant counterpart.

In the longer term, transgenic approaches to resistance to parasites are envisaged though such development will be hampered initially by the absence of basic knowledge of the mechanisms of immunity to parasites in ruminants.

Active Immunisation:

In the absence of such long term developments and to attend to the more immediate concerns of chemotherapy, active vaccination offers the logical immediate approach. It addresses the negative binomial problem; it does not suffer from drug resistance; there are no residues or environmental concerns or consumer pressures with respect to welfare. Also it has the attribute that limited handling of the animal is required, such as once or twice to inject the immunogen, or possibly the immunogen can be given in the feed.

Earlier attempts to produce antiparasitic vaccines have been frustrated either by the inability to identify the important antigens or the limited availability of antigen responsible for resistance. Organisms were and still are not readily cultivated in vitro and in some cases the source of such material (e.g. man) made vaccine exploitation impossible. However, gene cloning techniques now offer a unique opportunity to overcome these problems. Specific monoclonal antibody probes are being developed to identify protective antigens, and these in turn will be used to produce protective epitopes in unrelated microorganisms by recombinant DNA molecules coding for the protective antigens.

For sheep, significant advances in the isolation and cloning of enzymes and antigens of *H. contortus* and *T. colubriformis* have been achieved. With the former, a protective antigen "contortin" has been isolated (14) which induces significant protection in lambs. This is a polymonic helical structure present in the luminal membrane of the intestinal epithelium of the parasite. A second protective antigen, H11, is also associated with the parasites intestine. In both cases these are covert or concealed antigens not normally "seen" by the host and not boosted by normal exposure. The immune response generated by the injection of these antigens is presumed to be an antigut antibody which when ingested by the parasite affects the worm's survival.

Reviews of the present status of vaccines against gastrointestinal parasites of ruminants (7, 13) indicate there is much to be optimistic about. More long term developments will be synthetic vaccines and possibly anti-idiotype vaccines. The latter approach has been shown to be feasible in human schistosomiasis (10).

Responsiveness of Young to Vaccines:

An important handicap in the development of vaccines against gastrointestinal nematodes of ruminants is the poor immunological response of the young. Thereby, animals are at substantial risk to infection for several months prior to the acquisition of protection, which when acquired is strong (13).

Lambs may not respond effectively to *H. contortus* or *T. colubriformis* until they reach 3-6 months of age, and strong protection may require several more months (18). A similar unresponsiveness occurs in calves, but this is not well documented. Both parasite and host factors probably are concerned. For example, whereas young lambs are unable to develop protection against re-infection with most gastro-intestinal nematodes, in the case of infection with *Nematodirus battus* lambs can mount a prompt and effective immune response. In addition, lambs can mount an effective immune response to a wide range of bacterial and viral antigens (13). The mechanisms of this immunological unresponsiveness are not fully understood ànd various hypotheses on the defect(s) include feedback inhibition by maternally transferred anbitody, colostral transfer of soluble antigen or soluble suppressor factors and the generation of suppressor cell populations in the neonate. Until recently neonatal unresponsiveness to *H. contortus* in lambs was attributed to an immature immune system. However, now it has been demonstrated to be functional and intact in the young lamb and the most likely explanation is a plasma factor that prevents either the immune recognition of *H. contortus* antigen or the proliferation of antigen sensitised T lymphocytes (22).

The infection status of the ewes may be an important factor in the induction of immunity in lambs. Thus, immunisation of 4-week-old lambs against infection with *H. contortus* with soluble antigens induced a degree of protective immunity, but only if the lambs had been born of non-infected ewes. This degree of protection was abrogated if the ewes carried an infection with *H. contortus* (13). Although there is an early failure of young ruminants to develop an effective immunity to gastrointestinal nematodes, early infection of such animals does not seem to prevent this subsequent development of protective immunity when animals are reinfected at a later date.

Further developments in vaccine development will include the detection of immunosuppressive epitopes and their elimination from recombinant and sub unit vaccines and the development of suitable expressor systems. Vaccinia virus is most commonly identified as an expressor carrier, its genome is large and capable of receiving several foreign genes, but much developmental work will be necessary before a vaccinia vectored helminth vaccine is available.

The challenge now is to apply molecular techniques to the problem of immunogenicity to identify the synthesis and manipulation necessary to produce an acceptable vaccine.

Other Necessities:

While I have concentrated on genetic resistance and immunisation as the great hopes for the future and it will take us well into the 21st Century to produce products in every day use, I would stress that continued work on the biology, including molecular biology, of parasites is essential. Areas where deeper knowledge should prove useful in control are hatching, infection and host and organ selection mechanisms. Particularly, the nervous system and the role of regulatory peptides of helminths are areas for detailed investigation. In the long term it may be feasible to exploit, mimic and divert the normal functions in a worm and to develop a new approach to control.

CONCLUSION:

Developments in helminthology can be broadly categorised into decades of study of morphology, life cycles, pathology and patho-physiology, chemotherapy and immunity. The emphasis now on control must be environmentally compatible and on physiological events will govern the life cycle of the form.

SUMMARY:

The development of veterinary parasitology is reviewed through the last 100 years. The age of discovery of species and their life cycles set the scene for studies of the biology, pathophysiology and immunological responses directed against them. Anthelmintics have undergone major developments, now have high efficacy but other issues such as the environmental impact are increasingly dominant necessitating considerations of genetic resistance to disease and the production of novel vaccines such as the use of hidden antigenic determinants of the gut of helminths. Future developments include the synthesis of T cell reactive polypeptides and the avoidance of immunosuppressive epitopes. Not withstanding developments in the immunological field it is recognised that major

developments can flow from a deeper understanding of the biology and molecular biology of helminths.

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Impact of Parasites on the Immune System of Cattle. Gary L. Zimmerman, BS,MS,PhD,DVM, College of Veterinary Medicine, Oregon State University, Corvallis, Oregon 97331-4802.

All cattle have parasites. Even low worms burdens, often considered normal, can have an economic impact on productivity. The major effects of parasitism are not from competition for host nutrition, but rather from phenomenon such as damage to host tissues, alteration of physiological functions, host reaction to the parasites, and adverse immune interactions. Consequences of the interactions between the host immune system and parasites can be dramatic. In order to survive in immunocompetent hosts, parasites either modulate or avoid the host immune responses. When parasites actively modulate host immunity, the results can range from the inapparent to severe pathologic changes that compromise the health and survival of the animal. The majority of these changes have some adverse effects on productivity. Suppression of host immune responses can increase susceptibility to other parasites as well as bacteria and viruses. Parasite-induced suppression of host immune systems may reduce responses to vaccines, which can be misinterpreted as vaccine failure. A practical consequence of the immune response to parasites is the production of antibodies that can be detected by various techniques. The complex immune interactions and a wide diversity of parasite transmission patterns require more than a cursory consideration to achieve maximum production efficiency. The astute bovine practitioner must consider the overall parasite-host interface in order to develop a more integrated approach to herd health programs.

Introduction

The theme of this meeting is "Challenges of the Bovine Industry in the Twenty-First Century." Improving herd health and production efficiency are among the greatest challenges facing practitioners. Parasites decrease productivity more than any other disease agent. Although clinical parasitism occurs in many parts of North America, low-level parasitism is more widespread and causes more overall production losses. Parasites burdens, as demonstrated by fecal egg per gram (EPG) counts as low as 10-20 EPG, can reduce productivity.[1] Recommendations for treatment should be based on the producer's return on investment (ROI). This decision is made with consideration of the current burden of parasites, and the potential of