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PARADIGM OF COMMERCIAL IRRADIATED VACCINES FOR HELMINTH CONTROL IN LIVESTOCK AND COMPANION ANIMALS- A REVIEW

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Abstract: Three methods of irradiation are described in the literature: X-rays, gamma rays or U.V. light. X-Irradiation was the method used in the original bovine lungworm vaccine; however, gamma irradiation, being faster, has been found to be more convenient and has been used by most authors. Despite substantial research into the use of irradiated larval vaccines in many host/parasite systems and the fact that in a number of systems, good levels of protection have been shown, only three vaccines have been applied as commercially available products. First, bovine lungworm vaccine has been produced commercially since 1959. Second, vaccine against *Dictyocaulus filaria* has been produced in a number of countries in eastern Europe, the Middle East and Northern Asia since it was first described in 1965. Third, canine hookworm vaccine against *Ancylostoma caninum* was commercially launched in the US in 1973. However, despite being a technical success, for a range of commercial reasons, it did not become a profitable product and production ceased in 1975.

Keywords: Irradiation, lungworm, *Dictyocaulus*, *Ancylostoma caninum*, canine hookworm, vaccine.

Introduction

The advent of anthelmintic resistance has led to renewed interest in alternative means of controlling helminth infections of livestock (Waller, 1997). Among the methods under investigation are genetic host resistance, biological control, grazing management and vaccination. Correctly integrated combinations of these approaches, along with occasional use of anthelmintics, may provide the best approach to sustainable helminth control. Vaccination could be applied either to protect the most susceptible animals in a flock/herd or to help minimize the build-up of larvae on pasture and so, reduce the rate of infection in susceptible animals. Current research on helminth vaccines has generally concentrated on producing synthetic vaccines using either natural or hidden antigens (Munn, 1997). While this research is showing promising results (and one suspects that much of the real progress

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may be as yet unpublished for patent reasons), the commercial availability of these products is difficult to predict and may still be some years away.

However, using attenuated larvae to stimulate immunity is another approach to vaccination and may be worthy of renewed investigation. While much of the research in this area was conducted up to 59 years ago, the criteria by which success should be judged may have changed. For example, by combining vaccination with improved management, lower vaccine efficacy (in terms of worm burden reduction) may be acceptable and more emphasis placed on reductions in egg count and, hence, rates of pasture reinfection (Barnes *et al*., 1995). Further, it may not be necessary to concentrate on vaccination in very young animals if immunization of older stock can prevent the build-up of pasture infectivity and reduce the exposure of younger stock. It has been suggested (Newton, 1995) that the practicalities of producing live attenuated vaccines make this form of product too difficult or costly to produce and distribute. However, most of the technical problems can be overcome using existing knowledge and without recourse to methods using high technology.

Commercially available irradiated larval vaccines

Despite substantial research into the use of irradiated larval vaccines in many host/parasite systems and the fact that in a number of systems, good levels of protection have been shown, only three vaccines have been applied as commercially available products. These are the vaccines against the bovine lungworm, *Dictyocaulus viviparus* (Jarrett *et al.,* 1957); the large lungworm of sheep and goats, *Dictyocaulus filaria* (Jovanovic *et al.,* 1965); and the canine hookworm, *Ancylostoma caninum* (Miller, 1978). The bovine lungworm vaccine has been produced commercially since 1959. The *D. filaria* vaccine has been produced in a number of countries in eastern Europe, the Middle East and northern Asia since it was first described in 1965 (Jovanovic *et al.,* 1965; Sharma *et al.,* 1988). It is not clear which countries currently produce the vaccine or whether it has ever been applied as a strictly commercial product rather than provided by government agencies for subsidized delivery. The canine hookworm vaccine was commercially launched in the US in 1973. However, despite being a technical success, for a range of commercial reasons, it did not become a profitable product and production ceased in 1975 (Miller, 1978).

History of lungworm vaccination

The bovine lungworm vaccine, Dictol, was rapidly brought to market by Allen and Hanburys Ltd of Hertfordshire, UK, and was released in 1959 (Anon, 1959). In time, vaccine manufactured at a single site came to be marketed by two different companies under two

brand names, Dictol (Schering Plough Animal Health) and Huskvac (Intervet), and the vaccine is now marketed only as Bovilis®Huskvac. It was marketed under Intervet label by 2013, but currently, it is marketed by MSD Animal Health. It is manufactured in the UK and marketed in Switzerland, Holland, and Belgium as well as in UK. Bain and Urquhart (1988) demonstrated that the Dictol vaccine was effective if injected subcutaneously. Use of vaccine does not stimulate sterile immunity to a natural exposure to *D. viviparus*, and although they show no clinical signs, vaccinated animals may shed small numbers of larvae after natural exposure.

Dictol was first launched following a series of research studies by workers in Glasgow on the use of irradiated larvae to stimulate immunity. The disease was studied by a young veterinary graduate in Glasgow, William Fleming Hoggan Jarrett (1928-2011) who, died on August 27, aged 83, was 'one of the most outstanding veterinary pathologists of all time'. Two doses of 1000 infective larvae, X-irradiated at 40 krads and given 4 weeks apart, had been found to give a very high level of protection against subsequent challenge. This experimental system was scaled up to a commercial operation (Jarrett *et al.,* 1958).

Parasitic bronchitis caused by *Dictyocaulus* was one of the most important diseases for the UK dairy industry in late 1950s. There were no drugs that were effective for treatment or prophylaxis, knowledge of the epidemiology was scanty, and the fact that clinical disease could be caused by low numbers of worms meant that outbreaks occured rapidly and were uncontrollable. In this context, the advent of a vaccine was a very welcome addition to the animal health armory and the product's early commercial success was assured (Bain, 1999).

Over the three ensuing decades, modern anthelmintics became available (Parker *et al.,* 1959; Gordon, 1961; Thienpont *et al.,*1966; Chabala *et al.,* 1980) and the advantages of using the vaccine were reduced. Where the drug-based control programme has been effective, animals may not experience sufficient low-level challenge to develop and maintain immunity and lungworm cases can occur in adult cattle. Despite the recent decline in product sales, lungworm vaccine has been one of the most successful and profitable products in the animal health industry. The vaccine has survived virtually technically unchanged for almost 59 years and there was sufficient business for two competing products to co-exist for years. Even today, there are very good technical reasons for farmers to use the vaccine rather than relying on drug prophylaxis.

Radiation attenuated lungworm, *D. filaria,* vaccine in India

Parasitic bronchitis is one of the most important diseases affecting sheep and goats in Himalayan and other hilly parts of India and is responsible for heavy mortality in young animals. Even those that survive the acute infection to become chronic carriers usually suffer ill effects including blood dyscrasia, which causes high morbidity. Such animals also act as a source of pasture contamination. The disease is caused by several species of trichostrongyloid and metastrongyloid nematodes, of which *D. filaria* is the most pathogenic and most widely prevalent representing a major economic threat to the development of the sheep and goat industry in this region (Dhar *et al.,* 1981).

Historical Background

After development of Dictol, a strong impetus arose for development of radiation attenuated vaccine against *D. filaria* on similar lines. With financial assistance and technical expertise from United Nations Development Program (UNDP) and International Atomic Energy Agency (IAEA), a gamma radiation-attenuated vaccine against *D. filaria* was developed during 1970-71 at the Nuclear Research Laboratory (NRL) of the Indian Veterinary Research Institute. The vaccine was successfully tested under laboratory and field conditions and an irradiated vaccine laboratory was established at Srinagar (Kashmir) in 1973 for large scale production. Also, it appeared to induce a degree of acquired resistance against other nematode lungworms such as *Varestrongylus pneumonicus, Protostrongylus rufescens* and *Muellerius capillaris*.

In field trials, a high proportion of lambs may be infected at the time of vaccination. Such animals do not respond so efficiently to vaccination. Investigation showed that lambs exposed to single or trickle infections with normal *D. filaria* larvae prior to vaccination, developed a poor acquired resistance (55%) compared to previously uninfected controls (98%). Dhar and Sharma (1981) therefore recommend routine survey for infected lambs before vaccination, and advised that the vaccination should be done in very young lambs (six weeks old), before they receive a natural infection.

Vaccine Production

An infection dose of 150 *D. filaria* larvae per kg body weight per lamb was found to be ideal for raising a donor animal under laboratory conditions. On average, each donor lamb during useful patency daily passed out first-stage larvae in the faeces sufficient to produce 55 doses of the vaccine. The survival rate of such producer lambs was 45-55% during the period. Administration of betamethasone during early patency further reduced the dose of infection

to 75 larvae per kg body weight without compromising the faecal larval output and survival rate during useful patency of such donors. However, this drug is expensive, and its use was not economical for vaccine production (Bhat *et al.,* 1989).

First-stage *D. filaria* larvae were isolated by modified Baermann's technique and cultured at 26°C for developing into infective larvae which were then maintained at 4°C. Fresh batches of infective larvae were exposed to gamma radiation (50 krad) for attenuation. The vaccine was standardized in guinea-pigs for infectivity, attenuation and immunization potential-following IAEA standards. It was also cultured to test for the presence of *Salmonella* spp., *Brucella abortus* and *Mycobacterium johnei*, before issuing for field use. The vaccine had a shelf life of two weeks at 4°C and was recommended for oral administration in 6-10-week-old lambs. Two doses of vaccine were given over a 4-week interval. This produced effective immunity for about one year in the absence of further challenge. However, in endemic areas, good immune status may be maintained for longer due to repeated natural challenge (Dhar *et al.,* 1981).

Impact of Vaccination

In India, there are so many breeds of sheep. It remained to be ascertained, to what extent these breeds may differ in susceptibility to *D. filaria* infection or how different breeds will respond to the vaccine. A further problem concerned sheep flocks in remote and inaccessible highland pastures. Transportation of the vaccine to these places was difficult and could take over a week (Bhat *et al.,* 1989). Hence, the two week shelf-life of the vaccine at 4°C was inadequate and needed to be increased. Sheep and goats are often grazed and housed together. Goats have been reported to be more susceptible than sheep to *D. filaria* infection, and there is a need for simultaneous control of parasitic bronchitis in both types of animals. In goats, the *D. filaria* vaccine conferred a high degree of protection against experimental challenge, and a large scale field trial of the vaccine in goats needed to be undertaken before it was recommended as a regular part of the vaccination programme (Dhar *et al.,* 1981).

Of the two irradiation doses used for the attenuation of the larvae, the 50 kR-irradiated larvae stimulated a better immune response in the host as compared to the 40 kR irradiated larvae (Bhat *et al.,* 1989).

Promising irradiated larval vaccines

Following the early success of the bovine lungworm vaccine, there was great hope that similar vaccines would soon be available for use in controlling other parasites of livestock. Many host parasite systems were investigated, including *Haemonchus contortus,*

Trichostrongylus colubriformis, *Strogylus vulgaris*, *Schistosoma japonicum* and *Schistosoma bovis*. In terms of worm burden reduction against controls, irradiated *S. vulgaris* larvae gave a 92% protection in horses and irradiated *Haemonchus* and *Trichostrongylus* larvae showed 95% and > 88% protection, respectively, in sheep.

Unfortunately, early hopes of the production of an irradiated *H. contortus* vaccine were dashed when it became clear that high levels of protection could be achieved only in animals over about 7 months of age, and even then only if they remained uninfected prior to vaccination. This meant that the most susceptible stock (lambs under 1 year old) could not be protected in *Haemonchus* endemic areas where the vaccine would be most likely to be useful.

History of hookworm vaccination

In 1964, T. A. Miller showed that *A. caninum* larvae could be attenuated using 40,000 R of X-ray. As the amount of radiation increased, the larval infectivity decreased, and the pathogenicity was also reduced. He also observed that female larvae irradiated at 40,000 R or more, were consistently sterile. A single subcutaneous vaccination in dogs 3-4 months old using 1,000 *A. caninum* larvae exposed to 40,000 R of X-ray, resulted in a high degree of resistance to the challenge burden of normal worms in terms of hematological, clinical and coprological changes. Miller then conducted a series of experiments to investigate the effect of double vaccination with irradiated *A. caninum* larvae (irL_3) and to determine if the route of vaccine administration [subcutaneous (s.c.) or oral] had any effect on the immunogenic efficiency (Miller, 1964; 1965). He concluded that double oral vaccination was not as effective as double s.c. vaccination in dogs that were 3-4 months of age, by measuring the establishment of adult hookworms after challenge with infective larvae. Both routes of vaccination seemed equally effective in terms of resistance to the pathogenic effects of hookworm infection, involving hematologic, coprologic (eggs per gram of feces), and clinical observations after challenge with infection with L_3.

Three characteristics of attenuation were observed by Miller when irL_3 were used to immunize canines:

(1) a reduction in larval infectivity (Miller, 1964)

(2) a reduction in the pathogenicity of the worms that eventually reached the small intestine (Miller, 1964); and

(3) a sterilizing effect on female worm's fecundity as measured by eggs per gram of feces (epg) (Wilson *et al.,* 1999).

Miller used these "characteristics of attenuation" for the product development, industrial manufacture and USDA licensing of the 1st hookworm vaccine, which consisted of gamma-irradiated infective *A. caninum* larvae (Miller, 1978). The vaccine was field tested by approximately 1,500 practicing veterinarians across the US (Miller, 1971; 1978). While the vaccine proved to be safe for the dogs and even effective (90%) for canine, it had a considerably short shelf-life (6 months) and a unique set of storage condition. The vaccine was discontinued in 1975 due to commercial failure; most veterinarians were unwilling to incorporate it into their vaccination programs because of difficulties in storing the vaccine, the short shelf life, the lack of sterilizing immunity, and economics— it prevented routine de-worming of canines, a routine and rather lucrative source of income for veterinarians (Miller, 1978).

A Chance for Learning

Although the irL_3 vaccine failed commercially, it provided compelling evidence of possible existence of human hookworm vaccine and the expectations of such a vaccine. First, dog owners complained about the appearance of eggs in faeces following vaccination. Miller countered that it was an "unrealistic expectation for successful banishing of all hookworm infection: and that sterilizing immunity to such macro-parasite was probably not an option; a reduction in the number of worms and not complete elimination of the worms was sufficient to have an important clinical effect" (Miller, 1978). Indeed, such expectation on the part of the dog-owners was not unreasonable. The majority of human vaccines are indeed sterilizing in nature, eliciting a rapid and specific immune response that kills the pathogen—a characteristic vital to combating viral and bacterial pathogens that reproduce asexually, such as varicella and measles or the effect of toxins such as from tetanus. Additionally, the success of human vaccines against viral and bacterial pathogens are measured in terms of decreasing the incidence of disease in populations receiving the vaccination and also generate "herd immunity." However, given the size and the life cycle of this blood-feeding parasitic nematode, a human hookworm (or any other helminth vaccine for that matter) is unlikely to have a sterilizing effect (Loukas *et al.,* 2006). Hence, an important lesson learned from Miller is to product profile of a human hookworm vaccine, the indication of which would be to reduce the total worm burden, resulting in an associated decrease in the morbidity from hookworm diseases. For example, Miller claimed that the irradiated canine hookworm vaccine was discontinued due to "the failure of veterinarians to differentially diagnose hookworm infection from hookworm disease". In other words, it failed to induce a sterilizing

immunity in canines. However, as Miller goes on to argue, the degree of pathology generated from "hookworm is directly related to the number of worms residing within the host" (Miller, 1978) and not simply infection alone; i.e., there is a critical difference between "hookworm infection" and "hookworm disease", and it is based on the intensity of infection. The more hookworms infect the human or canine host, the greater the chance for hookworm disease; hence, a human hookworm vaccine should target "hookworm disease" and not "target hookworm infection". More specifically, a human hookworm vaccine should diminish the risk for moderate and heavy worm burdens and the possible clinical sequelae.

Conclusion

Current research on helminth vaccines has generally concentrated on producing synthetic vaccines using either natural or hidden antigens. While this research is showing promising results, the commercial availability of these products barring Barbervax, is difficult to predict and may still be some years away. However, using attenuated larvae to stimulate immunity is another approach to vaccination. While much of the research in this area was conducted up to 59 years ago, the criteria by which success should be judged may have changed. Despite substantial research into the use of irradiated larval vaccines in many host/parasite systems and the fact that in a number of systems good levels of protection have been shown, only three vaccines have been applied as commercially available products. These are the vaccines against the bovine lungworm, *Dictyocaulus viviparus*; the large lungworm of sheep and goats, *Dictyocaulus filaria*; and the canine hookworm *Ancylostoma caninum*.

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