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ASSESSMENT OF THE EFFICACY OF HELMINTH VACCINES

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ABSTRACT: Progress in the development of vaccines against the major parasitic infections has been slow. However, this situation is likely to change in the future because there is a great desire to move away from the use of antiparasitic drugs. Several characteristics have routinely been cited as important for the ideal parasitic vaccine, e.g., it should be safe, be able to compete favorably with chemotherapy, and have a high efficacy, and ideally its activity should extend to a wide range of parasites. However, 1 characteristic that often receives the least attention is the ultimate step in the process, i.e., how do we assess how good a vaccine has to be in order to be used? The determination of the relative success of a vaccine is a complex task because it will not only depend on the parasite species and circumstances but also on those measurements of efficacy put forward by parasitologists, epidemiologists, immunologists, clinicians, and economists as being essential, all of whom may have different views about the merits of a particular parasite vaccine. It is important to stress that monitoring of parasite vaccines should consider more than just the immediate effect on worm burdens and egg output. The present review focuses on the evaluation criteria for vaccines for 3 groups of helminth infections, i.e., gastrointestinal nematodes in cattle, liver flukes in ruminants, and schistosomes in humans, and aims especially to highlight some of the misunderstandings on efficacy assessments of such vaccines.

VACCINE EFFICACY

Despite increasing knowledge of the immune responses generated during parasite infections and the identification and characterization of many crucial parasite molecules (e.g., Lightowlers, 1994; Emery, 1996; Waine and McManus, 1997; Dalton and Mulcahy, 2001; Knox et al., 2001), progress in the development of vaccines against the major parasitic infections, e.g., malaria, has been slow, and commercially available parasite vaccines are still rare items, e.g., the vaccines for ovine cysticercosis and dictyocaulosis. However, this situation is likely to change in the future because there is a great desire to move away from the use of chemical antiparasitic drugs. This is because the use of chemicals to treat parasitic infections is not sustainable in the long term due to the increasing emergence of drug-resistant parasites. There is also concern about the undesirable environmental effects of chemicals and the presence of their residues in food.

Several characteristics have routinely been cited as important for the ideal parasitic vaccine, e.g., it should be safe, compete favorably, e.g., in cost, with chemotherapy, have a high efficacy, and ideally extend to a wide range of parasites, different gastrointestinal (GI) nematodes and *Schistosoma* spp., for example. However, 1 of the characteristics that often receives the least attention is the ultimate step in the process, i.e., how do we assess the overall value of the vaccine? The determination of the relative success of a vaccine is highly complex because it will not only depend on the parasite species and circumstances but also on those measurements of efficacy used variously by parasitologists, epidemiologists, immunologists, clinicians, and economists.

For far too long, the search for vaccines has been performed using laboratory animals. These are attractive models because of the availability of assays for immunological studies. However, insufficient attention has been paid to the fact that different immune rejection and evasion mechanisms may exist for each host–parasite system. In addition, laboratory animals may provide misleading information on the actual interaction between natural hosts and their parasites. Even for closely related animal species, such as cattle and sheep, there may be significantly different effects, i.e., the 2 species may have different responses to infection with the same liver fluke species (Dalton et al., 1996). It is, therefore, important for the development of effective parasite vaccines that vaccination studies be performed only in natural host–parasite systems. Also, researchers have overestimated the importance of immunological markers, e.g., parasite-specific antibodies, in anticipating vaccine efficacy, and one should not forget that it was not necessary to fully understand immunity when the first viral and bacterial vaccines were produced. The immune responses necessary for protection may, moreover, differ from those generated during natural infections because it is becoming clear that many parasites can manipulate the host immune responses to their favor (Mulcahy et al., 1999). Finally, helminth vaccine efficacies and the levels of protection deemed necessary for commercial release are too often assessed by the percentage reduction in number of parasites (compared with an unvaccinated group) they induce; however, in the absence of field studies, these data may not be of much significance.

Helminth infections have fundamentally different population dynamics from the viruses, bacteria, and microparasites because they are primarily determined by the number of worms present (the intensity of infection) rather than the number of hosts infected (Bundy et al., 1995). Intensity is also believed to be the major determinant of morbidity (alleviation of pathology being a major component of treatment against macroparasitic infections). The major aim in controlling helminth infection by vaccination should be to change the host–parasite relationship by reducing the infection level altogether, or to reduce transmission by decreasing the number of viable eggs that enter the environment, or both. Each helminth has specific epidemiological characteristics, and these will largely determine efficacy requirements of any vaccines against them, e.g., (1) host-protective immunity against helminths is rarely absolute or complete, and decrease in parasite establishment (immune exclusion) and changes in the mortality rate of established worms as a consequence of the host's exposure to infection will vary considerably according to the helminth species involved; (2) hosts often live in contaminated environments and may have been infected from an early age and will be further infected after vaccination; and (3) climatic and environmental conditions will profoundly influence transmission patterns.

The present review aims especially to highlight some of the misinterpretations on efficacy assessments of helminth vaccines and to discuss which efficacy criteria could be used and why

we should go as quickly as possible from the well-controlled, and often biased, experimental test to the real-life field trial. We will consider 3 groups of helminths, i.e., GI nematodes in cattle, liver flukes in ruminants, and schistosomes in humans because of our own experience with these parasites and the abundance of information dealing with each of them. For each helminth species, we will discuss, after a brief overview of some of the important epidemiological characteristics, those points that we consider should be in the design of vaccine trials with artificial and natural challenge infections.

EFFICACY REQUIREMENTS FOR VACCINES AGAINST GI NEMATODES IN CATTLE

Their life cycles, in brief

Gastrointestinal nematodes of great economic importance in the dairy and beef cattle industry are *Ostertagia ostertagi* (temperate regions) and *Haemonchus placei* (tropical regions) (Reinemeyer, 1994; Shaw et al., 1998a). Both parasites have a similar direct life cycle. The eggs are passed in the feces and develop in the fecal pat to the infective third stage (L3), within a couple of weeks if temperature and humidity are optimal. When moist conditions prevail, the L3 migrate from the feces on to herbage. After ingestion, the L3 exsheath in the rumen, and further development takes place in the mucosa of the abomasum. After 2 molts, adults emerge on the surface of the mucosa, about 3 wk after infection.

It would be expected that due to the close taxonomic affinity between the 2 parasite species (Trichostrongylidae) and because both are localized in the abomasum and use the same host, the efficacy requirements for a vaccine would be similar. However, even between these closely related parasites, the requirements for a vaccine diverge significantly because the 2 parasites differ in their epizootiology and the way they are affected by the host's immune response.

Efficacy requirements for vaccines against *Ostertagia ostertagi* (Europe)

Cattle operations in western Europe breed and raise both milk- and meat-producing animals, and in most cases, there is a well-defined grazing period (May–October) and a winter-housing period (October–April). First grazing season (FGS)-calves are the most susceptible to infection with GI nematodes; clinical parasitic gastroenteritis (PGE) is mainly seen in this age class when no appropriate preventive measures against GI nematode infections have been taken. The overwintering larval population that FGS calves encounter when they are turned out is mostly small. On the basis of tracer worm burdens in the beginning of the FGS (Shaw et al., 1998a), weekly larval uptake can be estimated as described earlier (Claerebout, Vercruysse et al., 1998). The estimated uptake of *O. ostertagi* larvae early in the FGS in western Europe varies between 700 and 6,100 L3/wk. The role of the surviving L3s is to infect calves at a level that produces patent subclinical infections and ensures amplification of the pasture contamination for the rest of the grazing season. The eggs deposited in spring develop slowly; all reach the infective stage from July onwards. If sufficient numbers of L3s are ingested, PGE can occur any time from July until October.

Control treatments such as vaccination should thus consider that FGS calves must be vaccinated before turnout, i.e., they are worm-free when vaccinated, and they must be protected against a relatively low parasite challenge. Reduction in egg excretion should be the target because the number of worm eggs shed during the first part of the grazing season determines the number of infective larvae on the pasture in the second half of the grazing season. Duration of protection should be approximately 2–3 mo because in western Europe the peak egg output occurs around 2 mo after turnout (Shaw et al., 1997, 1998a). Geometric mean egg counts 2 mo after turnout have been shown to be a good parameter to predict pasture contamination and whether or not calves will suffer from clinical PGE on a set-stocked pasture (Shaw et al., 1997, 1998b).

Vaccine trials with artificial challenge *Ostertagia ostertagi* infections

In the following paragraphs, we suggest some points to be considered in the design of an *Ostertagia* sp. vaccine trial with artificial challenge infection. It is clear that besides these specific suggestions for the evaluation of a vaccine for *O. ostertagi*, any vaccine trial should fulfill the critical requirements for a clinical trial, such as randomization, the use of an appropriate control group, the use of single or double blind techniques, and the use of a sufficient number of experimental units in the experimental groups (Elbers and Schukken, 1995). Because the buildup of a protective immune response against *O. ostertagi* is influenced by host factors such as gender (Herd et al., 1992), age (Michel et al., 1979), and nutritional status (Mansour et al., 1991, 1992), these confounding variables should be controlled in the experimental design by either using animals of the same age and sex that are given the same nutrition or by making sure (by blocking) that the treatments (vaccine vs. control) are balanced for occurrence of the confounding variables.

For statistical analysis of differences between the vaccinated animals and control animals, the sample size should be determined before the trial to ensure that the number of animals used is large enough to detect the level of differences required with a high probability. The number of animals that should be included in an *O. ostertagi* vaccine trial is affected by the skewed parasite frequency distribution. Typically, these skewed distributions have a high variance to mean ratio and can be described by a negative binomial distribution. However, in most circumstances, transformation of parasite data to $\log(x + 1)$ can more or less normalize the frequency distribution. Given a confidence level of 95% ($\alpha = 0.05$), a level of power of 80% ($\beta = 0.2$), an expected difference of 60% between the mean of the transformed fecal egg counts of the vaccinated group and the control group, and a standard deviation that is similar to the difference between the groups, the number of animals that is needed per group can be calculated (e.g., Bratcher et al., 1970) to be at least 16. In most cases, it is impossible to use this number of cattle in a vaccine trial. Results obtained from trials with insufficient numbers of calves should be interpreted carefully.

In most *O. ostertagi* vaccine studies, calves received a large, single challenge infection, ranging from 26,100 L3 to 200,000 L3 (Herlich and Douvres, 1979; Hilderson et al., 1995; Siefker and Rickard, 2000b; Smith et al., 2000). The intensity of such experimental single-point challenge infection exceeds natural

infection levels by several orders of magnitude, and therefore, the epidemiological relevance of such a challenge is unclear (Quinnell and Keymer, 1990). Rather, a natural challenge infection should be mimicked by a prolonged, low-trickle infection. An infection model with 1,000 *O. ostertagi* L3/day, 5 days/wk for 5 wk was recently used by Geldhof et al. (2002) to assess the protective efficacy of 2 *O. ostertagi* antigen fractions. This infection level corresponds to a weekly uptake of 5,000 L3, which is within the infection levels observed under natural conditions. It could perhaps be advised to extend this challenge infection period to 2–3 mo to examine the performance of the vaccine under constant infection pressure.

The animals should be monitored for at least 2 mo after the first challenge infection to allow the fecal egg output to be assessed during this period. This is crucial because reduction of egg shedding is the primary goal of the vaccination protocol. In most previous vaccine trials, calves were killed earlier to perform worm counts (Herlich and Douvres, 1979; Hilderson et al., 1995; Siefker and Rickard, 2000b; Smith et al., 2000). However, the worm fecundity of *O. ostertagi* is highly regulated by host immunity (Smith et al., 1987), and fecal egg output can be greatly reduced without a reduction in worm numbers (Gasbarre, 1997; Claerebout and Vercruysse, 2000). Therefore, postmortem worm counts shortly after the challenge infection are not the best parameter for evaluation of an *O. ostertagi* vaccine.

Fecal egg counts are usually monitored at successive time points. Because repeated measures are made from each host through time, these measures are correlated and cannot be treated as independent data points, as is often done incorrectly (e.g., Siefker and Rickard, 2000b; Smith et al., 2000). The use of repeated measure linear modeling has been suggested as a possible solution (Paterson, 2001), and more frequently, generalized linear models (Wilson and Grenfell, 1997), particularly mixed-effect models, are being utilized. The negative binomial frequency distribution of parasite data complicates such analyses; however, statistical routines that allow for such data are becoming more readily available. A simpler, though not very elegant, way to quickly examine such data is to compare the cumulative fecal egg counts between the groups (e.g., Geldhof et al., 2002).

Furthermore, it is as yet unclear which level of efficacy should be obtained by an *O. ostertagi* vaccine in an artificial challenge infection trial to be considered effective. Shaw et al. (1998b) showed that, whether a geometric mean fecal egg count taken 2 mo after turnout was greater than 200 EPG or not, successfully indicated whether or not PGE was observed during the FGS. However, this threshold cannot be readily extrapolated to experimental infection studies because in natural infections the fecal egg output is composed of eggs from different nematode species, i.e., *Cooperia* spp. and *Trichostrongylus* spp.

Vaccine trials with natural *Ostertagia ostertagi* infections

Proteins or protein fractions that have proven their protective properties in an artificial infection trial then need to be tested in natural field conditions to determine whether or not vaccination with the selected antigen significantly suppresses worm egg shedding in the face of a natural challenge infection. It also needs to be determined whether worm egg shedding is sufficiently reduced to prevent an increase of the larval pasture contamination, thereby protecting the calves against PGE-induced production losses.

Vaccinated and control calves are grazed on a pasture that is known to be contaminated with infective larvae from GI nematodes, preferably a pasture that was grazed by FGS calves in previous years. In a first natural infection trial, both vaccinated and control calves can be grazed together to exclude the confounding effect of the pasture. In this experimental design, the statistical significance of differences in fecal egg output between the groups can be analyzed with generalized linear mixed effect models–repeated measures if a sufficient number of animals are used. Alternatively, vaccinated and control calves can be grazed on different plots of the pasture, which allows one to investigate the effect of a reduced egg excretion on the pasture infection level. In this experimental design, the number of animals per group is less important. Because the plot has a confounding effect on the results of the group of animals that are grazing on it, the plot is the experimental unit (Elbers and Schukken, 1995) and replicate plots are needed to allow statistical analysis of differences between the treatments. If there is only 1 experimental unit per treatment group (each treatment group grazed on 1 plot) the statistical significance of differences in parasitological parameters between the groups cannot be estimated. Therefore, results from such a field vaccination trial need to be interpreted with caution and should be confirmed in subsequent field trials.

The calves should be monitored during the entire grazing season. The main parameters to be monitored are fecal egg output, pasture larval contamination, and final worm burdens at housing. Body weight gains can be measured to evaluate a reduction in parasite-induced production losses (Shaw et al., 1998a). Fecal egg counts are particularly important early in the grazing season. Geometric mean peak egg counts in the vaccinated group should stay below 200 EPG, with mean *O. ostertagia* egg counts below 100 EPG, considering that at least half of the worm eggs excreted during the first 2 mo of the pasture season belong to *Cooperia* sp. (Claerebout, Dorny et al., 1998; Claerebout et al., 1999).

If parasite transmission is effectively reduced by vaccination, pasture larval counts on the plot grazed by the vaccinated calves should be reduced in the second half of the grazing season. In western Europe, mean pasture larval counts on pastures grazed by untreated calves varied between 2,707 L3/kg dry herbage (calves with subclinical PGE) and 8,535 L3/kg dry herbage (calves with clinical PGE) (Shaw et al., 1998a). If only *O. ostertagi* larval counts were considered, all 'clinical' control pastures exceeded 1,000 *O. ostertagi* L3/kg dry herbage, and over three quarters of the studies exceeded 5,000 *O. ostertagi* L3/kg dry herbage (Shaw et al., 1998a). Mean pasture larval counts on pastures grazed by chemoprophylactic-treated calves at the end of the grazing season varied between 350 L3/kg dry herbage (farms with subclinical PGE in the untreated calves) and 1,600 L3/kg dry herbage (farms with clinical PGE in the untreated calves) (Shaw et al., 1998a). On the basis of these data, it can be suggested that vaccination should reduce the total pasture contamination to below 2,000 L3/kg dry herbage and the *O. ostertagi* pasture contamination to below 1,000 L3/kg dry herbage. The pasture infection level can be estimated by using tracer calves or by performing pasture larval counts. Worm burdens obtained from pairs of tracer calves that have

grazed for 2 wk are the most sensitive for estimating pasture larval contamination (Shaw et al., 1998a). Nevertheless, there is a significant positive relationship between pasture larval counts and mean tracer worm burdens, and this relationship still holds if just geometric mean *O. ostertagi* burdens and pasture larval counts are compared (Shaw et al., 1998a).

The final goal of reducing the parasite transmission by vaccination is to decrease the number of adult worms in the calves below a threshold level that is associated with production losses. Untreated calves harbor large worm burdens of 40,000–80,000 *O. ostertagi* in the second half of the grazing season as a result of the high pasture infection (Hilderson et al., 1987). According to worm counts from 25 field trials from western Europe, untreated calves harbor on average 57,000 worms at housing, of which around 87% are *O. ostertagi* sp. (data not shown). Adult *O. ostertagi* burdens can also be estimated without the requirement for postmortem by determination of the calves' serum pepsinogen levels (Dorny and Vercruysse, 1998). Serum pepsinogen levels, particularly in the second half of the FGS, show a very good and highly significant correlation with the number of adult worms present (Dorny et al., 1999; Eysker and Ploeger, 2000; Vercruysse and Claerebout, 2001). Adequate prevention of ostertagiasis is indicated by mean serum pepsinogen levels of below 3 units tyrosine at housing (Dorny et al., 1999).

Conclusions

An *O. ostertagi* vaccine in cattle needs to be an antifecundity vaccine. Its primary objective is to prevent the accumulation of a large worm burden in the second half of the FGS by reducing the excretion of worm eggs in the first 2–3 mo after turnout. Because fecundity of *O. ostertagi* sp. is highly density dependent, an anti-infection vaccine, i.e., a vaccine that reduces the number of worms, would only be successful if it has an extremely high efficacy. It is important to stress that these efficacy requirements considered to be useful in western Europe may differ in other continents and should be adapted to local epidemiological situations.

EFFICACY REQUIREMENTS FOR VACCINES AGAINST *HAEMONCHUS PLACEI* (SOUTHERN HEMISPHERE, SOUTHEASTERN UNITED STATES)

In the Southern Hemisphere, as well as in the southeastern United States, the most important GI nematode in cattle is *Haemonchus placei*. Haemonchosis forms a major constraint to cattle production in tropical and subtropical areas. The pathology of haemonchosis is the result of anemia and hypoproteinemia caused by its bloodsucking activity. In contrast to *O. ostertagi*, the fecundity of *H. placei* is not regulated by the intensity or the duration of the infection and there is a very good correlation between total daily fecal egg counts and mature female worm burden (Coyne and Smith, 1994). Consequently, to prevent the buildup of a high pasture infection level, a vaccine needs to reduce the number of adult worms present in the animals early in the grazing season, either by reducing the establishment of infective larvae (immune exclusion) or by increasing the mortality of established worms. Efficacy requirements for a vaccine against *H. placei* will thus differ from *O. ostertagi*.

On the basis of the assumptions of constant fecundity of female worms, decreases in parasite establishment, and changes in the mortality of established worms as a consequence of the host's exposure to infection, a model for the closely related *Haemonchus contortus* in lambs was developed by Coyne and Smith (1994) and subsequently adapted for predicting the effects of vaccination against *H. contortus* (Meeusen and Maddox, 1999). Different effects of vaccination against *H. contortus* in a grazing population of lambs were simulated, with a larval vaccine giving 50 or 80% protection against larval forms and an adult vaccine giving 80% protection against adult worms incorporated in the model. As expected, the model predicted that the use of vaccines that confer higher levels of immunity will have greater effect than those conferring lower levels of immunity, but a threshold of protection that is needed to prevent the animals from acquiring harmful worm burdens during the entire grazing season was not determined (Meeusen and Maddox, 1999). Barnes et al. (1995) modified the model of Barnes and Dobson (1990) on the population dynamics of *Trichostrongylus colubriformis* in sheep to predict the effect of different vaccines on worm population dynamics in grazing lambs. They concluded that with vaccines based on 'conventional' antigens, i.e., antigens that provoke the same immune responses that are stimulated by infection, substantial benefits can be obtained with 60% efficacy in 80% of the flock. These simulations have been referred to in several papers to argue that it is not essential for a vaccine against GI nematodes in ruminants to approach 100% efficacy in all animals (e.g., Siefker and Rickard, 2000a). However, these predictions cannot be readily extrapolated to all other nematode species in ruminants. For example, in the model of Barnes et al. (1995), the efficacy of the vaccines was expressed as percentage decrease in establishment of incoming infection. Decreased establishment of ingested larvae as a measure for vaccine efficacy can also be used for *Haemonchus* spp. in sheep and cattle but makes the model less suitable for *O. ostertagi*.

In conclusion, the differences in population dynamics between different worm species and their epidemiology should be accounted for in the design of vaccination experiments for GI nematodes, e.g., a reduction in worm numbers is the most important parameter in *Haemonchus* sp. vaccine trials; however, for *O. ostertagi* a reduction in worm fecundity during the first 2 mo of the grazing season is more important.

EFFICACY REQUIREMENTS FOR VACCINATION AGAINST *FASCIOLA HEPATICA*

Fascioliasis is an important parasitic disease of cattle. Briefly, the life cycle of *Fasciola hepatica* consists of 5 phases, i.e., (1) development of eggs in the environment, (2) hatching of miracidia and penetration in the intermediate snail host (usually *Lymnaea truncatula*), (3) development and multiplication of the parasites inside the snail, (4) emergence of the cercariae and their encystment, and (5) ingestion of infective metacercariae by the final host and development to adult worms (prepatent period of approximately 8 wk) (Andrews, 1999). *Fasciola hepatica* is responsible for clinical disease but even more important for substantial subclinical losses (Vercruysse and Claerebout, 2001). First grazing season calves, as well as older cattle, are susceptible to the effects of infection. Earlier studies suggested that cattle can acquire resistance to challenge infection

with *F. hepatica* when previously sensitized with a primary homologous infection (Hope Cawdery et al., 1977). However, recent studies (Clery et al., 1996) indicate little evidence for immunity in preventing reestablishment of new infections. Therefore, no age group of cattle can be ignored from the point of view of disease or as a potential source of pasture contamination. In Europe, cattle are mainly infected from late summer as a result of an early infection of snails. This "summer" infection of snails results from the hatching of overwintering eggs or eggs passed in the spring. A smaller infection may occur in the spring. This "winter" infection of snails is due to infection of snails in the previous autumn.

The first aim of vaccination should be the reduction of worm burdens to reduce pathology. Fluke burdens above 30–80 are sufficient to cause weight loss (Hope Cawdery et al., 1977; Dargie, 1986; Vercruysse and Claerebout, 2001). Therefore, because naturally acquired fluke burdens are usually in the 4–140 range (Malone et al., 1982; Dargie, 1986), a vaccine should produce a reduction in worm burden of at least 50%. In cattle, vaccination with glutathione S-transferase (GST) in Quil A/SM resulted in a mean reduction in fluke numbers of 43% (range 19–69%) (Morrison et al., 1996). From the above, it was suggested that the control of fascioliasis by immunological intervention appears to be an achievable goal (Spithill et al., 1999). However, caution is needed because the severity of disease may also vary considerably between animals and with the nutritional condition of the animals.

Another specific aim of a control program should be the reduction of pasture contamination, i.e., vaccinated animals should excrete as few eggs as possible because of the great reproductive capacity of *F. hepatica*. To achieve this by vaccination, the vaccine will need to provide a high level of protection against challenge infections over a long period of time (1 grazing season). To reduce transmission, it was shown that only regular treatments at 12- to 13-wk intervals with flukicides effective against both mature and immature flukes, i.e., efficacy higher than 90%, were able to reduce the intensity of infection in a flock or herd over time (Boray et al., 1985; Fawcett, 1990; Maes et al., 1993; Taylor et al., 1994). Dalton et al. (1996) observed that cathepsin Ls in cattle exhibited a high antiembryonation–antifecundity effect (>98%) on parasites that survived in vaccinated animals. Field vaccine trials will need to confirm that these levels of protection are sufficient to reduce parasite transmission below a threshold level that results in harmful fluke burdens.

In contrast to GI nematodes, there will not be a continual boosting of immunity after vaccination by field exposure to *F. hepatica*. Vaccination should thus be repeated annually as it is unlikely that vaccination will induce a life-long immunity. Finally, wildlife reservoirs (rabbits, deer) or infected irrigation or watercourses may recontaminate the pastures with eggs. Thus, it may well be that significant decreases in pasture contamination cannot be achieved by vaccination alone.

Vaccine trials with artificial *Fasciola hepatica* infections

In most vaccine trials, a high single-point challenge infection of 500–1,000 cercariae has been used (e.g., Dalton et al., 1996; Morrison et al., 1996). In only 2 studies were trickle infections used. Morrison et al. (1996) infected the animals twice weekly with 125 cercariae for 4 successive weeks, i.e., a total of 1,000 metacercariae. De Bont et al. (2003) used a less stringent trickle challenge infection: starting 4 wk after the last vaccination, all calves were challenged orally with 20 metacercariae/day, 3 days/wk for 6 wk (a total of 360 metacercariae). To our knowledge, there are no data available on how many cercariae are ingested by grazing cattle in different parts of the world, but worm counts in the control animals from vaccine trials are consistently higher than the low fluke burdens observed in natural conditions. Clearly, more trials are required.

Because a reduction of parasite transmission is a major objective of a *F. hepatica* vaccine, animals need to be monitored for several months, and therefore, postmortem worm counts shortly after the challenge infection may be, as for *O. ostertagi* not the most appropriate parameter for evaluation of an *F. hepatica* vaccine. Animals should be monitored not only for reductions in worm burdens but also for reductions in fecal egg counts or fertility of the eggs or both. However, little is known about the correlation between fecal egg counts and the intensity of infections with *F. hepatica*. Furthermore, the reduction in viable egg counts needed to significantly lower parasite transmission is unknown. In most cases egg counts in natural infections are low, commonly less than 5 eggs/g (EPG), even in heavily infected herds. Possible reasons for this are (1) the marked fluctuations in the EPG for *F. hepatica* in the course of the day, making multiple samplings a necessity; (2) herd egg counts vary widely among animals because as for so many other parasites, flukes are overdispersed, most flukes and egg shedding being related to a few highly susceptible animals; and (3) coprological techniques are not very efficient. This makes it difficult in practice to have sufficiently representative EPG values that can be related to actual infection levels (Vercruysse and Claerebout, 2001).

Vaccine trials with natural *Fasciola hepatica* infections

We are not aware of any vaccine trials with *F. hepatica* in natural field conditions, and it is particularly difficult to predict whether the current experimental vaccines can significantly reduce parasite transmission in the long term because the experimental conditions used to test candidate vaccines differ widely from the field situation. Also, parasite distribution in the environment is extremely variable; however, forecasts can be made or models of infection are available (Malone and Yilma, 1999).

Because there are no data available on how many cercariae are ingested by grazing cattle in natural conditions and because the level and the duration of the reduction in worm numbers or viable eggs excreted that are required to reduce parasite transmission to such a degree that production losses are minimized are unknown, it remains difficult to design *F. hepatica* vaccine experiments and to set criteria for assessment of efficacy of a vaccine. It may be that for fascioliasis the best parameter to monitor parasite transmission is the final worm burdens measured 8 to 10 wk after housing. As a diagnostic aid to measure worm burdens in cattle, gamma Glutamyl Transpeptidase (GT) may be a useful indicator. Normal values of GT in cattle should be below 50 units/L. In cattle infected with 1,000 metacercariae, values increased up to 317 units/L; in cattle receiving 360 cercariae (over a 6-wk period, resulting in a mean of 30

worms), mean values of 150 units/L were observed (Vercruysse and Claerebout, 2001).

To conclude, although it has been suggested that the control of fascioliasis by vaccination appears to be an achievable goal (Spithill et al., 1999), on the basis of the high levels of efficacy in experimental conditions, field vaccine trials will need to confirm that these levels of protection are sufficient to reduce infection levels or parasite transmission below a threshold level that results in harmful fluke burdens.

EFFICACY REQUIREMENTS FOR VACCINATION AGAINST HUMAN *SCHISTOSOMA* SPP.

Schistosomiasis is 1 of the most significant parasitic diseases in humans. *Schistosoma* spp. inhabit the veins of the mesentery and urinary bladder, and eggs pass through the vessel wall and other tissues to reach the gut or bladder lumen. Excreted eggs hatch in the water and develop further in a snail. Infection of the host is by penetration through the host's skin by the cercariae. The prepatent period for most species is 6–8 wk. The World Health Organization (WHO) estimates that over 200 million people are currently infected worldwide, mainly in rural agricultural and periurban areas. There has been particularly good progress for vaccines against schistosomiasis; the WHO has listed 6 candidate vaccine antigens (Bergquist, 1995), and the first helminth vaccine ever evaluated in humans in field conditions was against schistosomiasis. Clinical trials related to the safety and the immunological responses with a recombinant 28-kDa *Schistosoma haematobium* GST (Bilhvax-Pasteur Institute, Lille, France) have been conducted in Senegal (results not yet available).

In the case of human schistosomiasis, the long-term consequences of vaccination are, however, particularly hard to predict because of the complex interrelationships between age-dependent patterns of exposure and natural and vaccine-induced immunity (Chan et al., 1997). Basch (1993) and Chan et al. (1997) have emphasized that there remains great uncertainty concerning the optimal design of schistosomiasis vaccination programs and their potential for disease control in the field. Schistosomiasis is probably the most complex disease for which immunization has been proposed. This uncertainty arises because the impact of a vaccination program must be evaluated in the long term and may not be fully apparent for many years thereafter. The duration of a vaccine is very critical. Chan et al. (1997) calculated, for example, that the effects of increasing duration from 5 to 10 yr has a greater impact than increasing the degree of protection against schistosomiasis from 50 to 75%. Woolhouse (1995) explored the potential outcome of a phase II schistosomiasis vaccine trial using mathematical models that assume some natural immunity and a vaccine providing partial protection for a limited time. Analysis suggests that vaccination may have only a limited impact on lifelong cumulated worm burden and may lead to increased susceptibility and higher worm burdens at some ages, a 'rebound' effect that results from reduced opportunity to acquire natural immunity. Ethical considerations preclude experimental human infection, and natural parasite exposure under field conditions is difficult to accurately assess. Moreover, multiple variables related to genetic predisposition, earlier infections, and large disparities between transmission patterns, e.g., low, high, seasonal, and permanent, also exist.

Consequently, we are presently not able to define the efficacy requirements of a human schistosomiasis vaccine. The following paragraphs discuss only some desirable characteristics for a schistosome vaccine that should be considered because they may affect the outcome of vaccine trials.

A vaccine against schistosomiasis might take 3 forms: (1) an antiinfection vaccine that would reduce the numbers of worms before they are able to produce eggs, (2) an antifecundity vaccine that would prevent adult parasites from producing eggs, and (3) an antipathology vaccine that would redirect the host immune response, such that it would fail to stimulate production of the harmful fibrous lesions leading to disease. This last approach is aimed at the host and will not be further discussed in this article.

Anti-infective vaccines are typically assessed by comparing worm counts between vaccinated and control animals. WHO suggests that the requirement for candidate *Schistosoma* spp. vaccines is a 40% or more reduced worm burden after challenge, and often this criterion is used as a first selection criteria (Bergquist, 1995). However, it may well be that a schistosome vaccine that uses the schistosomule as a target of action (anti-establishment model) is not an appropriate selection criterion. Moreover, the level of reduction that would be acceptable is unknown and would depend on transmission levels. Finally, there is no practical way to count the adult worms in a living human being and thus evaluate efficacy. The current tools for determining infection levels are the demonstration of eggs in feces or urine; however, egg counts cannot be directly translated into worm burdens. The extensive individual variation in schistosome egg counts translates into a wide range of corresponding worm burdens (Polman, 2000). Epidemiological, mathematical, and biomedical arguments indicate that individual worm burdens in endemic areas number in the hundreds or thousands, instead of the few dozens generally assumed, on the basis of available autopsy data (Gryseels and De Vlas, 1996). Several studies indicate that the determination of circulating worm antigen might provide a better measure of the level of infection (Polman et al., 1998). Polman (2000), however, suggested that the relationship between antigen measurements and worm burden might not be as straightforward as assumed.

The aim of an antifecundity vaccine is to reduce egg production, i.e., reduced pathology induced by eggs and reduced transmission as fewer eggs are secreted. Much work has been done in laboratory animals and cattle with the 28-kDa GST as a candidate vaccine antigen. Immunization with the 28-kDa GST reduces both worm burden and female worm fecundity (Capron et al., 1994). However, efficacy of the 28-kDa GST may also well depend on the level and type of challenge given—the vaccine did not protect calves against a massive single experimental challenge of *Schistosoma mattheei*, but a significant protection was observed (89% reduction in egg counts) under conditions of low to moderate natural infection (De Bont et al., 1997). It will also be very difficult to evaluate an antifecundity effect in humans. In cattle schistosomiasis, immunity development does not result in reduced worm counts, but in reduced worm fecundity (De Bont and Vercruysse, 1998). However, this could only be demonstrated by postmortem examination. Also, to reduce transmission by decreasing the number of viable eggs that enter the environment may well be an impossible task. Vercruysse et al. (2001) showed that in a high

endemic situation, treatments with praziquantel, resulting in a 84% reduction in the index of potential contamination, could not reduce transmission. One should highlight the importance of quantifying the transmission levels before any vaccination trial. The relationship between worm burden and transmission rate is crucial in determining whether vaccination will have an impact on transmission.

Many other factors would also complicate the picture, e.g., the impact of previous infections and chemotherapy, the importance of mothers in inducing tolerance in their children, etc. However, on the basis of this brief analysis, it should be obvious that the actual efficacy levels required to have a useful impact on parasite transmission are unknown and that we have only a limited number of tools to measure it.

CONCLUSIONS

This short review was not intended to suggest that we should not move forward in a direct manner with the development of the candidate vaccines we now have. We must continue with these efforts. However, those involved in vaccine research should (re)consider, together with epidemiologists, parasitologists, and clinicians, how to test helminth vaccines. Until now, many critical questions on helminth vaccine evaluation have not been addressed and, as such, require consideration before trials are conducted.

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