



## Effects of gastrointestinal nematode infection on the ruminant immune system

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### Abstract

Gastrointestinal (GI) nematodes of ruminants evoke a wide variety of immune responses in their hosts. In terms of specific immune responses directed against parasite antigens, the resulting immune responses may vary from those that give strong protection from reinfection after a relatively light exposure (e.g. *Oesophagostomum radiatum*) to responses that are very weak and delayed in their onset (e.g. *Ostertagia ostertagi*). The nature of these protective immune responses has been covered in another section of the workshop and the purpose of this section will be to explore the nature of changes that occur in the immune system of infected animals and to discuss the effect of GI nematode infections upon the overall immunoresponsiveness of the host. The discussion will focus primarily on *Ostertagia ostertagi* because this parasite has received the most attention in published studies. The interaction of *Ostertagia* and the host immune system presents what appears to be an interesting contradiction. Protective immunity directed against the parasite is slow to arise and when compared to some of the other GI nematodes, is relatively weak. Although responses that reduce egg output in the feces or increase the number of larvae undergoing inhibition may occur after a relatively brief exposure (3–4 months), immune responses which reduce the number of parasites that can establish in the host are not evident until the animal's second year. Additionally, even older animals that have spent several seasons on infected pastures will have low numbers of *Ostertagia* in their abomasa, indicating that sterilizing immune responses against the parasite are uncommon. In spite of this apparent lack of specific protective immune responses, infections with *Ostertagia* induce profound changes in the host immune system. These changes include a tremendous expansion of both the number of lymphocytes in the local lymph nodes and the number of lymphoid cells in the mucosa of the

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abomasum. This expansion in cell numbers involves a shift away from a predominant classic T cell population (CD2 and CD3 positive), to a population where T cell percentages are decreased and B cells (immunoglobulin-bearing) and  $\gamma$ - $\delta$  cells are increased. At the same time the expression of messenger RNAs for T cell cytokines (IL2, IL4, IL10 and  $\gamma$ -interferon) is changed to that of increased expression of IL4 and IL10 and decreased expression of IL2 and perhaps of  $\gamma$ -interferon. The reasons for these changes remain to be elucidated, but it is evident that the lack of protective immune responses is not the result of a poor exposure of the host to parasite products, or to the stomach being an immunoprivileged site. In fact, a superficial look at the responses elicited indicates that *Ostertagia* induces responses (the so-called TH2 mediated responses) that are widely considered to be the type of responses necessary for protection against GI nematodes. There are many factors that could lead to this apparent lack of immunity in the face of a strong stimulation of immune responses including: (1) the elicitation of suboptimal responses; (2) the failure of the abomasum to function as an efficient effector organ; (3) active evasion of the functional immune response by the parasite; and (4) that these classic responses are not protective in this particular ruminant-parasite system and that novel protective mechanisms may be required. The strong stimulation of the host gut immune system by *Ostertagia* and perhaps by other GI nematode infections, raises questions about the potential effects of such infections on the overall well-being of the host. A number of authors have indicated that *Ostertagia* infections may diminish the host's ability to mount subsequent immune responses to antigenic challenges such as vaccination against other infectious organisms. In addition, recent studies have indicated that infections with GI nematodes may result in increased circulatory levels of stress-related hormones. The ability of these parasites to inhibit the host immune system through either specific immune mechanisms or by more general means such as the stimulation of increased levels of known immunosuppressives, such as cortico-steroids, could significantly impair host health and productivity especially in times of marginal nutritional status. To date, much of the data attesting to such parasite-mediated effects are largely anecdotal or, are restricted to carefully controlled experimental studies and as such, much remains to be defined in this area of study. © 1997 Elsevier Science B.V.

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## 1. Introduction

Gastrointestinal (GI) nematode infections remain a major constraint on the efficient production of ruminant livestock throughout the world. In the US alone, losses incurred as the result of GI nematodes, including the costs of treatment, are likely to exceed \$2 billion per year. While a number of parasite genera are involved in this interaction, the species that are of the greatest economic importance are those species that elicit weak protective immune responses; *Ostertagia ostertagi* in cattle and *Haemonchus contortus* in sheep. Although other species can be very pathogenic when they become established in the host, their impact on livestock production is lessened by the fact that after a moderate level of exposure, the host is able to resist further establishment of the parasites, thus diminishing their effects on the herd. Interestingly, the parasites that are most pathogenic and most difficult to develop protective immune responses against, reside in the abomasum.

Although *O. ostertagi* in cattle and *H. contortus* in sheep share a common site of infection, there are marked differences in the biology of these infections. While *H. contortus* infections in sheep demonstrate a distinct age-related immunity (Knight and Rodgers, 1974; Barger, 1988) and a clear peri-parturient egg rise (O'Sullivan and Donald, 1970), these phenomena are much less-clearly discernable in *O. ostertagi* infections in cattle (Armour, 1989; Kloosterman et al., 1991). In addition, *O. ostertagia* shows a much more intimate contact with the host as a result of its penetration into the gastric glands. The focus of this paper will be the interaction of *O. ostertagi* and the host immune system. In some cases the comments will be applicable to haemonchosis in sheep, but because of obvious differences between the infections, care must be taken in extrapolation from one system to the other.

Protective immune responses against *O. ostertagi* in cattle are comparatively weak and require a prolonged exposure period before they are discernable. Although immune responses that reduce egg output (Michel, 1963) or result in morphological changes in the worms (Michel et al., 1972) are evident soon after a primary infection, immune responses that reduce the number of parasites developing after subsequent infection require several months of repeated exposure before they are evident (Michel, 1963, 1970; Michel et al., 1973). The central questions that arise concerning immunity to *O. ostertagi* in cattle are: (1) why are protective immune responses against *Ostertagia* so weak; and (2) why do they take so long to become effective? These major questions then invoke a series of more specific questions dealing with the interaction of the parasite and the host immune system. These include: (1) do *Ostertagia* antigens have an adequate opportunity to interact with the host immune system; (2) does *Ostertagia* elicit immune responses that are appropriate; (3) is the abomasum a poor immune effector organ; (4) does *Ostertagia* actively evade or suppress protective immune responses; and (5) If *Ostertagia* suppresses host immunity, does the immunosuppression compromise the host to a degree such that vaccinations are less effective or that the animals become susceptible to other infectious agents?

## **2. Do *Ostertagia* antigens have adequate opportunity to interact with the host immune system?**

Much has been written concerning the uptake of foreign antigens from the gut and about the specialized cells involved in this phenomenon (Neutra et al., 1996), but the vast majority of these studies have concentrated on the large and small intestine. Procedures that normally result in the recovery of large numbers of lymphocytes from the mucosa of the intestine yield very few lymphocytes when applied to the bovine abomasum (Almeria et al., in press). The possibility arises that the abomasum is a poor site for presentation of parasite antigens and as such, the weak and delayed immune responses are a result of a suboptimal exposure of the host to relevant antigens. Although this is one possible explanation for the weak immune responses elicited, the preponderance of evidence argues against this interpretation. It has been repeatedly demonstrated that within the first 3–4 days

of infection, the regional lymph nodes draining the abomasum show marked increases in size. Within 4–5 weeks of infection, the weight of these nodes reaches 20–30 times that of nodes taken from uninfected age- and size-matched calves (Gasbarre, 1986, 1994; Canals et al., in press). This increase in size is a result of an increase in number of both parasite-specific lymphocytes and lymphocytes that do not recognize parasite antigens (Gasbarre, 1986). In addition, the overall increase shows higher percentages of B lymphocytes and T cells bearing the  $\gamma$ - $\delta$  T cell receptor and decreases in conventional  $\alpha$ - $\beta$  receptor-bearing T cells (Gasbarre, 1994, Canals et al., in press).

In addition to these changes in the draining lymph nodes, there is a concomitant increase in lymphocytes in the mucosa of the abomasum. Within 4 days of infection the number of lymphocytes recoverable from the abomasal mucosa increases eightfold (Almeria et al., in press). The changes in cell populations of the mucosal lymphocyte populations mirror those described for the draining lymph nodes with the exception that the shifts to higher percentages of B cells and  $\gamma$ - $\delta$  T cells take place even sooner than observed in the regional lymph nodes. These results indicate that very soon after infection, *Ostertagia* antigens are presented to the host in the draining lymph nodes and that within the first 3–4 days of infection these cells have egressed from the nodes and have become established in the tissues immediately surrounding the parasite.

In a larger context, there are increasing numbers of reports of strong *Ostertagia*-specific immune responses in the systemic circulation. Within 3–4 weeks of experimental infection (Canals and Gasbarre, 1990; Mansour et al., 1990) and 2 months of exposure to infected pastures (Gronvold et al., 1992, Gasbarre et al., 1993, Nansen et al., 1993), previously naïve calves show significant rises in anti-*Ostertagia* antibodies in the peripheral circulation. These antibody responses have been detectable using a wide range of parasite-derived antigens and involve all major immunoglobulin isotypes. The strong local immune responses in the draining lymph nodes and mucosal tissues, coupled with the evidence of significant peripheral sensitization, indicates that cattle infected with *O. ostertagi* have ample opportunity for exposure to parasite antigens and that the lack of protection observed in cattle through the first few months of grazing is not due to an insufficient exposure to parasite antigens.

### **3. Does *Ostertagia* elicit immune responses that are appropriate?**

Assuming that cattle have ample opportunity for antigenic exposure and subsequent responses to *Ostertagia* antigens, the next logical explanation for the weak protective responses is that the immune responses elicited are inappropriate for protection. Recently, much attention has been given to the concept that soluble mediators released by immunocompetent lymphocytes are important in determining the overall type of response after exposure to infectious organisms. These mediators, called lymphokines, control the growth and differentiation of cells of the immune system and thus control the make-up of the cell populations responding to the infectious agent. Work in murine models has demonstrated that

response to infection can vary significantly depending upon the activation of different types of lymphokine-secreting cells. In mice these two subsets are referred to as Th1 (standing for T helper cell 1) and Th2 cells. These distinct subsets of T helper cells produce distinct arrays of lymphokines that drive immune responses into one of two defined patterns. The Th1 cells produce, among other lymphokines, Interleukin 2 (IL2) and  $\gamma$ -interferon (IFN) and are responsible for immune responses of a type referred to as Delayed Type Hypersensitivity (DTH). In contrast the Th2 subset produces, among other lymphokines, IL4, IL5 and IL10 and the resulting immune response is termed an Immediate Type Hypersensitivity response. In addition to driving the immune response in these directions, the two T-helper subsets tend to inhibit each other. It is generally accepted that Th1 type responses are necessary for protection against intracellular parasites, while Th2 responses are involved in protection to extracellular parasites, most notably parasitic helminths (Urban et al., 1992). Although these T-helper cell subpopulations are well-documented in mice, T-cell clones in cattle show a much less restricted lymphokine profile (Brown et al., 1993, 1994). Even though T cells from cattle may not differentiate into the terminal Th1 and Th2 subsets as in mice, it is still possible that the overall lymphokine response after infection resembles a Th1 or Th2 response when looked at in its entirety. As such, it is important to assess the lymphokine response in the local tissues after a primary infection. If protective mechanisms are the same in the *Ostertagia*-bovine system as those reported for murine intestinal nematodes, one would expect that a primary *Ostertagia* infection elicits little in the way of a Th2-like lymphokine response. When examined by the detection of increases in messenger RNA for the bovine lymphokines, one finds that *Ostertagia* infections like nematode infections in mice (Svetic et al., 1993) elicit very strong IL4 responses, which are the hallmark of the Th2 response (Canals et al., in press). In fact, IL4 has been demonstrated to be directly involved in protective immunity to intestinal nematodes in mice (Urban et al., 1991, 1995). The fact that a primary infection with *O. ostertagi* results in large amounts of messenger RNA for IL4, but does not seem to result in protective immunity, appears to contradict to some degree existing thoughts on the nature of protective immunity against GI nematodes. There are several ways to explain this apparent contradiction. The first possibility is that the expression of lymphokine message does not correlate with the secretion of a functional product and as such is an inappropriate assay. While this remains a possibility, with the exception of IL15 (Bamford et al., 1996), the induction of lymphokine messages appears to correlate well with the level of secreted product (Hutchinson et al., 1994; Prud'homme et al., 1995; Schulze-Koops et al., 1995). A second possibility is that the Th2 responses elicited are suboptimal. Urban et al. (1995) have shown that in murine model systems, the addition of IL4 to a primary infection will help clear the primary infection and they theorize that clearance requires high levels of IL4. In spite of the fact that additional IL4 will aid the clearance of primary nematode infections in mice, the fact remains that in these nematode-model systems, mice are normally refractory to reinfection. In contrast, such levels of protective immunity are not observed in *O. ostertagi*-infected cattle in spite of a strong induction of IL4 message during a

primary exposure. This difference argues against the concept that the very weak protection elicited by a primary *Ostertagia* infection is the result of a suboptimal response. In fact, in murine model systems a second effect of exogenous IL4 is a reduction in worm fecundity (Urban et al., 1995). It is possible that the reduced fecundity of *Ostertagia* infections is the result of the stereotypic Th2 response, but that protective immunity requires other immune mechanisms, a possibility that is gaining more attention in GI nematode systems (Finkelman et al., in press). The third possibility is that in the murine model systems investigated, all infections were in the intestine, but *O. ostertagi* inhabits the stomach. Perhaps, in this milieu, the appropriate effector mechanisms are not in place to result in killing or expulsion of the parasites. If this is the case, then an IL4-mediated response is appropriate in the intestine, but is not an appropriate response in the abomasum. A third possibility is that *O. ostertagi* has developed means to actively suppress or evade the typical protective immune response and that other aspects of immunity must be invoked. This concept will be further explored in subsequent sections.

#### **4. Is the abomasum a poor immune effector organ?**

If we return for a moment to the stomach as a site where immunity to *Ostertagia* must take place, most studies dealing with immunity to GI nematodes have dealt with model systems and employed parasites of the large or small intestine. While there are many common features between the linings of the intestines and the stomach there are also major physiologic and structural differences between these organs. The possibility exists that a given type of immune response could function very well in the intestine, but remain ineffective in the stomach. Earlier we indicated that *Ostertagia* infections strongly stimulate host immune cells and that the resulting cytokine responses are those assumed to be protective in murine model systems. In addition (Baker et al., 1993a,b) have shown that abomasa infected with *O. ostertagi* are characterized by an influx of effector cells considered necessary for the expression of an immediate hypersensitivity response. These results indicated that *O. ostertagi* is capable of stimulating immune responses that have been demonstrated to result in protection in a number of other nematode–host systems. Why these responses are weakly protective in the *Ostertagia*-host system remains to be determined.

One possibility is that although the initiation of the immune response is appropriate, the stomach is not very effective in ridding itself of GI nematodes. Numerous authors have demonstrated that infections by intestinal nematodes cause profound physiological and structural changes in the gut (Manson-Smith et al., 1979; Castro and Harari, 1982; Bell et al., 1984) and at least some of these changes take place in *Ostertagia*-infected abomasa (Murray, 1969). Most authors believe these changes lead to rejection of the parasites by making the environment of the gut less suitable for the parasite's development, or by being directly involved in expulsive mechanisms. While it is well-documented that *O. ostertagi* developing in a previously infected host are stunted and show distinct morphological changes

(Michel et al., 1972), to date no-one has demonstrated an expulsive mechanism directed at *Ostertagia* in the bovine abomasum. On the other hand, cattle do appear to become resistant much more quickly to other abomasal dwelling nematodes. For example, we have shown that a 3–4-month grazing period is sufficient to significantly reduce the numbers of *Haemonchus* sp. and *Trichostrongylus axei* upon subsequent pasture exposure. This same primary exposure did not reduce the numbers of *O. ostertagi* during the re-exposure period. These results indicate that the abomasum can mount protective immune responses against other abomasal-dwelling nematodes after a relatively brief primary exposure, but that *Ostertagia* is refractory to these responses. A second explanation for the exceptional ability of *Ostertagia* to reinfect previously infected cattle, is that *Ostertagia* actively evades or suppresses immune responses.

##### **5. Does *Ostertagia* actively evade or suppress protective immune responses?**

A number of authors have indicated that *Ostertagia* infection can exhibit non-specific suppressive effects. The bulk of these studies have focused on transient, but significant reductions in responses to mitogens of peripheral blood lymphocytes from infected cattle (Klesius et al., 1984; Cross et al., 1986; Snider et al., 1986; Wiggan and Gibbs, 1990), but a similar suppression was not observed when the source of cells was the draining lymph nodes (Wiggan and Gibbs, 1990). A second demonstration of non-specific immunosuppression caused by *Ostertagia* was the demonstration that infection reduced the level of circulating IgG antibodies in calves immunized with an unrelated protein antigen (Mansour et al., 1991, 1992).

There have been few attempts to demonstrate specific suppression of the anti-*Ostertagia* response during infection. Cells collected from the draining lymph nodes were found to have high numbers of *Ostertagia*-reactive cells early in the infection, but as time progressed these cells were lost, even if the calves were previously immunized (Gasbarre, 1986, 1994). One explanation for this loss of parasite-specific T cells was that *O. ostertagi* infections cause a polyclonal activation of cells with specificities different from *Ostertagia* (Gasbarre, 1986). Recent studies have indicated that *Ostertagia* infections cause an increase in the percentage of B cells in the local tissues (Baker et al., 1993c; Almeria et al., in press; Canals et al., in press). Polyclonal B cell activation has been postulated to be a potent cause of immunosuppression in a number of parasitic diseases caused by both protozoa (Gasbarre et al., 1980; Sacks et al., 1980) and helminths (Crandall et al., 1978). Although it remains to be conclusively demonstrated that immunosuppression is a consistent and important feature of *O. ostertagi* infections, it is plain that at least at certain periods of the parasite life cycle, or in very heavy infections, a transient reduction in the immune reactivity of the host takes place. Future studies need to define the magnitude and mechanisms of this immunosuppression in order to measure its impact on herd health. This is especially true with the current movement towards more intensive management systems.

## **6. If *Ostertagia* suppresses the host immune system, does this immunosuppression compromise the host to a degree that leads to production losses?**

If the assumption is made that *O. ostertagi* infections result in significant immuno-suppression of the host, the next question becomes: what is the effect of this immunosuppression on productivity? It is difficult to measure production losses resulting from parasite-induced immunosuppression. However, two qualitative indicators have been employed: (1) the assessment of the efficacy of vaccination against unrelated pathogens, in parasitized vs. non-parasitized cattle; and (2) the measurement of infection levels or sick days attributed to other pathogens. The second approach is difficult to accomplish without extremely large animal populations or catastrophic illness. As a result the only studies to date that have explored the effects of *Ostertagia* infection on the overall health of the host deal with the response to vaccination in parasitized and unparasitized cattle. In one study Yang et al. (1993b) found no difference in serum antibody titer after vaccination with *Brucella abortus* and bovine rhinotracheitis vaccines in calves experimentally infected with *Ostertagia* and *Cooperia* when they compared infected, non-drug treated to infected, then drug treated groups. In a second study by the same authors (Yang et al., 1993a), it was reported that the antibody response to vaccination with *B. abortus* arose more slowly in infected and infected-treated groups when compared to uninoculated controls. Although there was a slight delay in the circulating antibody response to vaccination, the level of circulating antibodies against *Brucella* were not different in infected or uninfected calves. At this point in time attempts to show that *Ostertagia* infections compromise the overall health of the host by immunosuppression are largely inconclusive. This is not surprising given the complexity of the host–parasite system involved. It is likely that *Ostertagia* will be shown to have very deleterious effects on host immunity, especially when compounded by other stressors such as poor nutrition and management practices, but these studies have not yet been attempted. Similarly, the role of the intensity of infection in the animals and the genetic make-up of the test populations have not been evaluated.

## **7. Direction of future research**

Much new information has been accumulated over the past several years regarding the interaction of *Ostertagia* and the host immune system, but much remains to be accomplished. The primary focus of future research should cover three areas. The first is to identify protective immune mechanisms in economically important GI nematode species. This will most probably entail studies in protected vs. unprotected individuals or groups. As a corollary to these studies there is the need to identify easily discernable markers for the protective immune responses, to allow producers to utilize host immunity as part of their management programs. The use of the host immune systems may encompass traditional means such as anti-parasite vaccination, or may utilize more complex programs such as strategic



anthelmintic treatment to boost immune responses, or the use of genetically superior animals. A second area of focus is the identification of mechanisms by which the parasites evade protective immune responses; this area is of paramount importance in *Ostertagia* in cattle and *Haemonchus* in sheep. Finally, definitive studies are required to assess the importance of parasite-induced immunomodulation in animals on production programs and to ascertain the importance of these infections as stressors in modern production systems.

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### **Discussion: Effects of gastrointestinal nematode infection on the ruminant immune system (Gasbarre)**

**(Coyne — USA)** Considering the size of the nematodes for which investigators are trying to develop vaccines, it would seem that they would be difficult to kill with antibody alone. It would seem that the only way of getting a lethal effect would also entail fixation of complement. Has it even been established whether parasite cell types are vulnerable to mammalian complement systems after antibody production?

**(Gasbarre — USA)** For these systems I don't know, some of the people here who have worked with *Haemonchus* may know. There were a number of studies back in the 1950s and 1960s looking into these questions and I think they were relatively inconclusive. Most people who will speak about an antibody response are not necessarily talking about a binding of antibody and a lysis of the worm. What they are talking about is something like an immediate hypersensitivity response that will call in a number of other potential effector cells and basically change the overall environment (adverse) for the worm. Dave Smith (Moredun) has published several papers about some of the potential effector mechanisms in the gut. There is evidence that gut peristalsis increases and other non-specific effects. To go along with that, there were papers published in the 60s and 70s involving multiple infections with *Trichostrongylus*, showing that if an immune response was elicited in the neighborhood of one species you could clear other species from that area. I think the key is that the responses are specific in their trigger, but non-specific in their effector mechanisms.

**(Coyne — USA)** Earlier, were you suggesting that there might be an overwhelming decoy antigen release or shedding.

**(Gasbarre — USA)** I think this is a possibility. We think that the adults are very good stimulators of the local responses, but at the same time we feel that the

protective mechanisms function mostly against the larval forms. The adults may not be decoys, but may be inaccessible to the immune effector mechanisms.

**(Coles — UK)** One of the interesting experiments that was done some years ago at the Central Veterinary Laboratory was the inter-reaction between coccidia and *Nematodirus battus*. They gave a dose of *Nematodirus battus* which did not affect the growth of the lambs. They gave a dose of coccidia which did not affect the growth of the lambs, but when they gave the two together, they killed half the lambs. I wonder within this kind of system whether work has been done to look at possible inter-reactions between coccidia and *Ostertagia*, in which case, you might see whether there's an inter-reaction in either direction.

**(Gasbarre — USA)** I think those relationships have been somewhat examined, I'm not sure to what degree and perhaps some of the other people that know those systems better than I do can comment on that.

**(Williams — USA)** Back in our 1986 workshop it was largely anecdotal unless I missed something, but Bob Bohlender the practitioner from Nebraska in cow/calf programs and so forth, talked about his experiences with fenbendazole treatment in young cattle. He spoke of numerous instances in which worm removal by fenbendazole treatment had appeared to alleviate coccidiosis problems and also seemed to enhance vaccination effects against a variety of infectious agents. To my knowledge, these observations were not reported in a journal paper or otherwise. But, there may be someone else who has some evidence or indication about this type of relationship.

**(G. Smith — USA)** Lou, given the sort of thing that I do, most of the way I view immunity is in terms of a final consequence, the numbers of worms that you see and count. So I'd like to come back to a comment that you made in which you expressed the belief that immunity, when it operates, probably doesn't operate particularly against the adult worms. Is that what you did say?

**(Gasbarre — USA)** That's what we believe.

**(G. Smith — USA)** Well, lets imagine the consequence of that. Suppose you have a series of single infection experiments where you infect animals with just a single dose of larvae, but you have increasing numbers of larvae in the dosing series and then you follow those animals with serial slaughter, on your hypothesis, you would not expect any difference at all in the survivorship curve of the adult worms. Well, in fact, that's the reverse of what happens if you look at Anderson (1977), you get a very clear exponential decay curve and the slope of that curve is directly proportional to the magnitude of the initial infecting dose. So, clearly in those instances the higher the, let's say the antigen challenge, the greater the mortality. Now the mortality surely is an expression of the immune system. And it's actually operating on those adult worms.

**(Gasbarre — USA)** Well, yes and no. In our thinking the worms are damaged prior to becoming adults. If you look at a secondary infection in animals that show some level of immunity, besides the arrestment that Jozef has talked about, the worms are delayed in their development. If you look at naïve animals and animals that have had a lot of previous exposure, experimental infections given at the same time result in larvae that lag behind in their development. Larvae and adults are both stunted in growth and subsequent adult female worms will not develop a vulval flap. I think they have been damaged during development. Whether clearance is due to an active response against adults or whether the adults are less robust from having undergone a previous negative environment remains to be demonstrated. I don't know how you separate those two alternatives, perhaps by the passive transfer of normal adults into immune animals and maybe that's been done.

**(Klei — USA)** Lou, consider the question relative to non-specific effectors in the abomasum. You said all the studies in mice indicate L<sub>4</sub> larvae are sort of central to protective mechanisms. Are there any studies done to show cross protection in first exposure to, say *Ostertagia*, following immunity to *Haemonchus*? There are some published studies on field trials like that. I don't remember all the details, but there is some suggestion that there is cross protection one way, but not the other. Maybe someone in the audience could answer that and if, in fact it happens, that would support your idea that you would get expulsion.

**(Gasbarre — USA)** That's an interesting question. One of the things we would like to do is look at effector mechanisms in the cattle stomach to abomasal nematodes other than *Ostertagia*. We haven't done it, but if anyone here has, I would sure like to hear about it.

**(Lichtenfels — USA)** Jim Haley did some of that at the University of Maryland with *Nippostrongylus* and found that they did go on and develop.

**(Gasbarre — USA)** But again, that is a murine model in the intestine. I'm not at all diminishing my belief that this is a very good immune response in the intestine, and that is the appropriate response. I guess my question would be, is that the appropriate response in the cow abomasum.

**(Vercruyse — Belgium)** I would like to bring up the subject of *Mecistocirrus*, also a parasite of the abomasum in cattle. I've always thought the abomasum was a bad place for immunity to develop. For those who don't know, *Mecistocirrus* has a long prepatent period of 62 days. We did some studies on immunity and found that with one larval challenge, an immune response of nearly 100% was developed. It is impossible to use trickle infections. If one infects through 10 days, you only get the first infection to develop. Thus, its not only a question of the organ, but also of the parasite.

**(Gasbarre — USA)** We'd like very much to look at some of the cells from those

animals.

**(Vercruyse — Belgium)** Very Well, I will send you some.

**(Gasbarre — USA)** I think you will have to send us RNA, you can't send the whole cells in. We'd be interested in looking at that. That is exactly what is needed to determine whether the abomasum is a good place to elicit a response and what type the response might be.

**(D. Smith — UK)** I was going to ask you what sort of degree of variation did you find with your interleukin levels between individual animals? The reason I'm asking is — we've been looking at a model system with *Ostertagia* in sheep for a number of years and have actually looked at gastric lymph cells and various interleukins therein and in our case, we do have a model system where we get very demonstrable protective immunity in mature animals. When we looked at the various interleukins, we got a mess. Everything was all over the place, nothing correlated whatsoever with protection. I was just curious to know what happened in your system.

**(Gasbarre — USA)** Ana Canals can best address that, she has actually run the assays.

**(Canals — USA)** In an experiment using 18 animals with three killed at days 4, 11, 21 and 28, the slides shown by Lou represent one animal for each day, but all three animals for each day were similar. On the slides shown, we did not actually scan the bands, so I cannot give exact figures, but for the three animals for each day, there was a fivefold increase or decrease. What technique are you using?

**(D. Smith — UK)** This was done by colleagues in molecular biology, not by me, but some kind of PCR, reverse PCR on mRNA. We got huge variation and no correlations.

**(Canals — USA)** Yes, this is the same technique, this is an RT-PCR, but there are different types of PCRs and we may not be talking about the same thing. We use a competitive RT-PCR which is a much more accurate way to measure cytokines than with just RT-PCR alone. On RT-PCR you have to be really careful that you are not working on the plateau phase of the PCR. We have repeated these assays on a number of animals, for example, we were looking at the responses of 12 infected and control animals and we consistently found the same results. After infection with the experiment reported, using the protection assay, one of the animals did not return back to regular or control levels after 28 days of infection. The other two of the group did. But, using the RT-PCR, all animals of the respective groups gave similar results at all times tested.

**(Gasbarre — USA)** I think we need to get a lot more data in terms of protected and non-protected animals, to determine what cytokine profiles look like. This is

still only demonstration of changes, there is no cause and effect here at this point. We haven't looked at the universe of potential kinds of responses. It just looks like in the animals we've tested, we're driving the system the way it is supposed to go. The real question is, what happens in animals that do show protection versus those that don't and maybe in about a month we could tell you.

**(D. Smith — UK)** Perhaps along those lines on a macro level, one of the premises that we are accepting is that an animal which grazes for a period of time, whether first season or not, no matter what breed or its genetic breakdown, size, shape or form it is, the animal develops a solid and reasonably functional immunity to at least three species of nematode parasites in its second season. Anyone who has followed these things into the second season would agree that there is a fairly large degree of variability there and I think we have to be careful about making some sweeping comparisons in studies comparing two or three animals, recognizing of course the constraints, financial and otherwise in generating large replicated studies. But, the pictures of lymph nodes you showed seem to show some considerable variation and I guess you alluded to the fact that a five times increase in the volume of mass of the lymph nodes was indicative of some sort of immune response. Would you just comment on the basic variability that exists among the animals, given the fact that some of those volumes appear to be five times greater than the others to start with.

**(Gasbarre — USA)** They were greater, because they were taken at different times. That was a series of animals killed from day 0 through the infection. In a primary infection, there is really very little variation between the animals. You'd be surprised at how close the lymph node masses are, maybe within a couple of grams of each other. Again, these are from experimental infections, in the barn and in animals that have never been infected before. In terms of the abomasal lymph nodes in pastured animals, because of the work we're doing in genetics, we field test our animals by putting them on infected pastures for in excess of 120 days. So, they get about 4 months of exposure. We feel that is a sufficient time to begin to see some of the differences taking place. And, in those animals the one strong correlation that we always find is that the abomasal lymph nodes, their size is correlated with the numbers of worms that the animals have in the abomasum. I don't have the figures with me, but the *R* value is somewhere in the neighborhood of 0.8 to 0.9. It is an extremely good correlation.

**(D. Smith — UK)** The size of the lymph node in that context, if one were to consider it in that fashion, the mass of the lymph nodes is inversely correlated to the immunity of the animal really, or what I'm saying is inversely related to the worm burden.

**(Gasbarre — USA)** Yes, exactly. We're not saying that a large lymph node results in a protected animal. At first, we might say, the better stimulation of the immune system you have, the better protective immunity you're going to have. In fact, all that size of the lymph node means is probably an infection dose effect, indicating

more parasite antigen being exposed to the host. There was no implication intended of lymph node size and protection, it is a positive, not a negative correlation.

**(Klei — USA)** Lou, how much difference is there between peripheral blood responses, either peripheral blood versus abomasal lymph nodes, for example, proliferation to antigens and cytokines? Do you see a big difference?

**(Gasbarre — USA)** We did that quite a few years ago when we first started working with *Ostertagia* and to be honest with you I haven't done peripheral blood in 10 years, although Ana is doing some now, in looking at some of the cytokine questions. When we did this years ago, what we found in the local lymph nodes was that the response was measurable in 3 or 4 days and anything in the peripheral blood responses was not seen until 3 or 4 weeks had passed. Also in the blood, the changes are relatively insignificant and quite variable.

**(Barger — Australia)** Lou, getting back to your earlier question of an example of an effective immune response against a nematode in the abomasum of cattle, it may be worth looking at *Haemonchus contortus* from sheep, because many years ago when we were doing our work with alternate grazing of sheep and cattle, we noticed that while we could get and regularly did get patent *H. contortus* infections in young calves, we didn't ever find any, not one single worm in older animals. We have no evidence that it's due to an immune response, but it could be worth looking at.

**(Gasbarre — USA)** I agree that might be a potential. We have focused on cattle and I guess I have a real bias against the sheep model for cattle. I'm not sure at this point that you will see the same effector mechanisms. For instance, concerning TH1, TH2 responses, cattle really don't have TH1 and TH2 cells, not if you use the definition from murine systems. No one has ever isolated a cloned bovine T-cell that shows a cytokine pattern like that of mice. In cattle the patterns are much less distinct. You have cells that can produce a variety of different cytokines. What we're looking at is the overall effect, when referring to TH1 and TH2.

**(Lima — Brazil)** What do we know about IgE in the immune response of cattle to nematodes?

**(Gasbarre — USA)** We haven't looked at IgE at all. Dave Baker did out at Davis, California and he had some correlations with *Ostertagia* numbers and IgE, but they tended to change with the level of infection. For instance, I think at low level infections he had a negative correlation, but then once the size of infection reached a certain level, they changed and were positively correlated. So, again, I'm not exactly sure what that means. There aren't many reagents available for bovine IgE right at this point.



**(Vercruysse — Belgium)** Just to answer some of Tom Klei's question, we looked to the lymphocyte responses and also to the peripheral lymphocytes, messenger and abomasal lymphocytes. We saw some proliferation with abomasal lymphocytes and we also looked at the L<sub>3</sub> stages, the L<sub>4</sub> and the L<sub>5</sub> and what we noticed was for the L<sub>3</sub>, you get a proliferation and with the L<sub>4</sub> and L<sub>5</sub> you get suppression. But you could only notice that in the lymphocytes from the abomasum lymph nodes and not in the peripheral or mesenteric lymphocytes.

**(Gasbarre — USA)** Yes, if you worked with standard lymphocyte proliferative assays using *Ostertagia* antigens you have a real problem. There are some ways around it, but they're pretty time consuming. David Smith referred to looking at some cytokines in sheep. Has anyone else had that experience in *Haemonchus*? As Ian Barger pointed out, some protection is afforded at least in older sheep and also some protection against *Ostertagia* in older sheep