YERSINIOSIS : WORKSHOP REPORT

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A workshop on Yersiniosis was held in Dunedin in May in conjunction with the New Zealand Microbiological Society and New Zealand Society for Immunology Combined Scientific Meeting. It was attended by 20 people from around New Zealand working with Yersinia spp. in various animals.

The objectives were:

a) to review the current state of knowledge of these diseases.
b) to develop an awareness of other workers' current research efforts and objectives.
c) to identify research priorities.
d) to discuss areas of co-operation between research groups.

CURRENT SITUATION AND RESEARCH

Yersiniosis - an overview

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For the purposes of this workshop yersiniosis is defined as the clinical disease caused by Yersinia pseudotuberculosis (Y.pstb.) and Y. enterocolitica (Y.e.). In NZ it is reported to occur most commonly in red deer calves but also affects cattle, goats, sheep, guinea pigs and cage birds, as well as being a Zoonosis.

The majority of reported cases of yersiniosis in domestic animals have been due to Y.pstb., although Y.e. is reported to have caused enterocolitis in sheep and goats. In some of those cases Campylobacter jejuni has been isolated in conjunction with Y.e. Both have been isolated from clinically normal animals (deer, sheep, goats, cattle, pigs, cats, dogs and feral animals) on a number of occasions.

Clinically, yersiniosis in livestock usually presents as a diarrhoea which is often watery, smelly and tinged with blood. Untreated cases often die. Other conditions, including sporadic abortions in sheep, and cattle, mastitis in goats, pneumonia in cattle etc, have been attributed to Y.pstb. In humans, Y.pstb. generally causes mesenteric lymphadenitis with abdominal pain similar to appendicitis, while Y.e. usually causes enterocolitis and diarrhoea.

Until quite recently yersiniosis was overlooked as an important cause of wastage in domestic animals. Hagan and Bruner's "Infectious Diseases of Domestic Animals" published in 1981 states that "the organism has little importance in animal pathology except in stocks of guinea pigs." The Merck Veterinary Manual (1986) only refers to it as a disease of rabbits and as a zoonosis.

The disease in deer alone is likely to cost the country over $750,000 in 1988 from the death of calves (based on a mortality rate of 1%, 2500 x $300). There will also be losses associated with the cost of treating sick calves, necropsies and possibly reduced production from affected animals that recover. When the losses with sheep, goats and cattle, together with the costs associated with human cases are considered the total cost to the country is quite substantial.

The epidemiology of yersiniosis in various species has been investigated by a number of workers (for a summary see Deer Branch Proceedings No. 1, 1984). Surveys of wildlife in New Zealand and overseas have demonstrated that birds, rodents, lagomorphs and feral cats can act as carriers. Domestic animals, especially pigs, can also act as symptomless carriers to varying degrees. It appears that most livestock are exposed to Y.pstb. and Y.e. in their first year of life and experience a subclinical infection resulting in the development of a degree of immunity. However, if the animals are stressed at the time of infection then clinical disease will result in a proportion of animals. The most common stresses include under-nutrition, exposure to severe weather conditions, intercurrent disease such as parasitic enteritis or bronchitis and transport.

The majority of cases of yersiniosis occur in winter and the organism appears to be able to persist in the environment in cold wet conditions. This is also the time of year when animals are under the greatest feed and environmental stresses.

At Invermay we have monitored a group of red deer calves throughout the autumn-winter spring period together with taking environmental samples. Blood, faeces, grass and surface water samples were taken at 3 to 4 week intervals from March to October. Subclinical infections spread through the group slowly between May and August. On any one sampling occasion 10-20% of calves yielded Y.pstb. isolates. By August 97% of calves had seroconverted and 53% had had culture positive faeces on at least one occasion. However, all these deer remained in good condition and there were no cases of clinical yersiniosis in this group. Soil and pasture samples yielded Y.pstb. isolates between April and July.

Control

The most appropriate control procedures involve

(a) minimising stress by management
(b) augmenting natural immunity

Yersiniosis in deer is almost entirely a disease of young or recently captured animals. Because all animals are exposed to Y. pstb in the farming environment it is assumed that older animals have developed a strong immunity to infection. Therefore we have been working on the principle that a possible means of preventing yersiniosis is to immunise young deer before they receive natural field challenge in their first autumn/winter.

Various studies of the immune response of deer to natural infection, immunisation with bacterins and experimental challenge have been undertaken at Invermay in association with Dr Frank Griffin and his group at Otago University and the results are summarised in Deer Branch Course No. 3 (1986). Natural infection results in moderate M.A. titres averaging 1:80 to 1:160 and lasting for 1 to 3 months. The administration of oil adjuvanted formalin killed Y.pstb. bacterins resulted in similar titres which also persisted for around the same time, whereas aluminium hydroxide adjuvanted bacterins only produced a low transient response. The lymphocyte transformation test (LTT) and skin tests showed variable CMI response in immunised animals. The results of subcutaneous challenge of immunised deer with Y.pstb. suggest that humoral immunity may be more

important than CMI for protection against systemic Y.pstb. infection in deer. However, this may not necessarily be the case in enteric infections. Because we experienced considerable difficulties in producing experimental infection of deer under controlled conditions most of our recent work at Invermay has been based on the field trialling of killed bacterins.

This obviously has many shortcomings but was worth pursuing as a possible shortcut to produce a commercial vaccine for which there was an existing demand. The costs of running these field trials was originally going to be funded by a vaccine company. Unfortunately, they withdrew their support when their batch of experimental vaccine failed the QC testing and they have subsequently decided that the size of the market does not justify the cost of development of a yersiniosis vaccine for deer. Since then the value of deer has dropped further making it even less likely a commercial company will support R & D work.

Therefore, if further work on yersiniosis is to continue, it must be largely supported by MAF funding. If a commercial vaccine is developed then there should be a return on the investment, even if the vaccine production is contracted out to a commercial company.

So far, work at Invermay has only touched on a few aspects of the immune response of animals to infection and immunisation, but there are many areas which need considerable investigation to support the development of a useful vaccine. They include studies of the:

(a) Organism - plasmids/virulence factors etc
- optimal growth requirements for virulence Ag expression

(b) Deer - pathogenesis of infection and disease
- immune response, both CMI and humoral

(c) Laboratory animal models - mouse model for virulence and vaccine tests

(d) Laboratory tests - ELISA
- Lymphocyte transformation tests
- monoclonals, antibodies, etc

Yersiniosis in goats
Bryce Buddle, MAFTech, Wallaceville

Most of the work at Wallaceville to date has been on yersiniosis in goats and more recently some work has been done in conjunction with Invermay on Y. pstb. strains from yersiniosis in deer.

A goat mortality study was carried out with goats and the strains isolated from the 22 cases were characterised.

The goats were predominantly feral x angora types, less than 12 months of age and all were from flocks of more than 100 goats. Losses were spread throughout the year and predisposing factors appeared to be heavy rain, recent transport, inadequate feeding, shearing, etc. The isolates were identified as 21 Y.e. and one Y. pstb. All were diagnosed on histopathy plus isolation as there are dangers of diagnosis on isolation alone.

The characterisation of Y.e. isolates was difficult using the standard API20E system. A number of assays to indicate virulence of Yersinia organisms were carried out.

They included:

a) HeLa cell invasiveness - probably coded by Yersinia chromosomes and not related to virulence plasmids

b) Ca dependency - more reliable than HeLa cell invasiveness with virulent strains being Ca^{++} dependent at 37°C

c) Serum resistance - virulent strains resistant to bactericidal properties of human serum at 37°C, but are susceptible at 25°C, whereas non-virulent strains are susceptible at 37°C and 25°C. All the goat isolates of Y.e. were neither Ca dependent nor serum resistant and therefore, were not classed as "virulent" by the above tests; nevertheless they were isolated from fatal cases.

d) Autoagglutination - virulent strains autoagglutinate in tissue culture medium at 37°C but no at 25°C - non-virulent strains do not autoagglutinate.

e) Mouse model - Because the above tests were not really satisfactory a mouse model has been developed to measure virulence of Y.e. Mice which have been pretreated with an iron chelator are inoculated intraperitoneally with Y.e. and then 2 to 7 days later they are euthanased, the spleen removed and the number of bacteria in the spleen are counted.

f) Plasmid analysis - All "virulent" Y.e. strains contain 2 plasmids of 40 to 60 mega Daltons and all virulent Y. pstb. have 1 plasmid of 40 mD. All the goat isolates had "virulence" plasmids.

Future work

The aim of future work will be to assist Invermay research in the development of a Y. pstb. vaccine for deer. The following questions need to be answered before a vaccine can be developed.

1. Do the vaccine strains of Y. pstb. need the virulence plasmid which codes for a number of outer membrane proteins which may be essential for the development of protective immune response?

2. What are the optimal growth conditions needed for the expression of these outer membrane proteins? They are only expressed during growth at 37°C under stringent nutritional requirements.

3. Is there cross-protection between strains of Y. pstb.? There are 3 main serotypes.

4. What is the optimal method of preparing a killed vaccine, eg, formalin or heat killing?

5. What is the most appropriate adjuvant?

6. Would a live vaccine be more effective and is it feasible?

A mouse model may be very useful to investigate some of these questions. The mice are vaccinated subcutaneously and challenged three weeks later orally or intraperitoneally with Y. pstb. and a few days later the number of bacteria are counted in the spleen. An iron chelator is not required for Y. pstb. The aim of protection is to prevent or reduce systemic infection which results in bacterial colonies in the spleen. A correlation between protection in the mouse model and protection in deer (and goats) would have to be demonstrated by experimental or field challenge trials.

The mouse model is considered to be appropriate because in both Y.e. infection in goats and Y. pstb. in deer there appears to be systemic spread to local lymphatic tissues associated with clinical yersiniosis whereas subclinical infection in deer is probably restricted to the gut.

Haematology of deer challenged with Y. pstb.
John Cross, Microbiology Department, Otago University

The haematology of the deer challenged with Y. pstb. in the Invermay experiments was examined. The most important features were:

a) Immediately post challenge the neutrophils in peripheral blood increased markedly (up to 7 times the control values).

b) There was an eosinopaenia within a few hours of challenge.

c) Toxic cells appeared in circulation immediately after challenge. There were also elevated fibrinogen levels.

d) By day 2 there were fewer toxic cells and the neutrophils were returning to normal levels which were reached in 5 to 6 days post challenge.

During this time the deer appeared to have few abnormal clinical signs and none developed yersiniosis.

Yersiniosis in goats
Stan Fenwick and Eugene Lanada, Dept Vet Pathology & Public Health, Massey University
They have carried out 2 studies, namely a prevalence survey and a cohort study of yersiniosis in goats in the Manawatu.

Prevalence survey
This involved 30 farms with 20 to 1200 goats. Preliminary results showed 18 of 24 farms had Yersinia species isolated from samples of goats, mostly being Y.e. and "environmental" species. Kids less than 1 year had the highest prevalence of Y.e. Hoggets and adults had mostly environmental Y. species.

The Y.e. strains were very difficult to biotype because all the current systems are based on human biotypes of which there are 5 or 6. Most of the goat Y.e. were biotype 5, but with minor variations. They are probably specifically goat biotypes.

Cohort study
Four farms with Y.e. were selected. Ten animals of 3 age groups are currently being sampled monthly through the year, together with a monitor of disease occurrence.

Yersiniosis in humans and pig
Stan Fenwick, Dept. Vet Pathology and Public Health, Massey University
A letter to the editor of the NZ Medical Journal asking for information on yersiniosis in humans got a response from a private medical lab in Auckland which had 60 isolates of Y.e. These have been typed as mostly Biotype 4. Few other labs are isolating Y.e. which is probably because they are not using appropriate selective media or cold enriching samples.

In pigs, 60-80% have been found to carry Y. pstb. and 20% carry Y.e., predominantly biotype 4. It is thought that pigs may be a major source of human Y.e. infections, especially biotype 4.

Reflections on yersiniosis
Tom Henderson, formerly MAFQual, Invermay, currently with Xentech, Dunedin
It is highly likely that there are a number of animal factors other than organism factors (eg, virulence) which determine whether a weaner deer got yersiniosis or subclinical infection. Although up to 80% of deer carry Y.e. and "environmental" Yersinia sp as normal gut flora, Y. pstb. is almost always the cause of yersiniosis in deer. Isolation alone may not be significant for diagnosis which must be supported by clinical evidence and histopathology.

Characterisation of Yersinia isolates was a problem especially Y.e. with the API system not always adequate. Different animals tend to have different Y.e. biotypes and these may also vary from region to region, while New Zealand probably has unique strains. There was a definite need to co-ordinate results from different laboratories and what was really needed was a Yersinia **Reference Laboratory**. The question was, should New Zealand have its own or should we try to use the Australian one run by Dr Prpic in Melbourne?

There was general dissatisfaction with the Australian laboratory because there has been no response to enquiries over the last twelve months. The problem with setting up or running a Yersinia reference laboratory is the cost and who would pay for it. An alternative would be an Yersinia **Information Exchange Group** should would allow free and regular interchange of information and culture between workers in this field.

WHO IS WILLING TO RUN SUCH AN INFORMATION EXCHANGE GROUP?

Developing an ELISA for Yersinia
Sarah Hook, Microbiology Dept, Otago University
This project involves development of an ELISA test for detecting Yersinia antibodies in deer (in collaboration with Invermay).

Lipopolysaccharides extracted by a hot water/phenol method are initially being used as the antigen. The V antigen will also be investigated as an antigen for this test. Sera from naturally and experimentally challenged deer will be used in the development of the ELISA. Serotype specificity and sensitivity will be investigated. Currently anti sheep reagents are used because they cross-react with deer sera.

Yersiniosis - an antigenic nightmare
Frank Griffin, Microbiology Dept, Otago University
Frank commented that the yersiniosis area was an antigenic nightmare with variable immunological response and much cross reactivity. Naturally and experimentally infected animals and animals immunised with various bacterins gave dramatically different cell mediated and humoral responses. The current serological test based on agglutination is not very satisfactory.

In order to more accurately define the immune response of deer to infection and immunogens it is necessary to:

a) Work with pure antigens (eg, virulence antigens, LPS, etc) and define responses with these.

b) Use an ELISA which has better sensitivity and specificity than the agglutination test. Various antigens can be used for different purposes.

c) Measure cell mediated immune responses.

Plasmid analysis of organisms should be done to ensure that the organisms used are virulent.

A striking feature of experimental challenges carried out at Invermay was the lack of endotoxic reaction when 10^9-10^{10} organisms were injected into deer.

We must study the factors that predispose deer to yersiniosis. What are those factors or "triggers"?

Field diagnosis
Noel Beatson, Veterinary Practitioner, Timaru
Noel was involved with the first recognised outbreak of yersiniosis in New Zealand in the winter of 1978. It occurred in 2 year old hinds and there were 60 deaths. Eventually, the connection between the isolation of *Y. pstb.* from samples from these animals and the clinical syndrome in deer was made.

In the field, diagnosis of yersiniosis is based on history, clinical signs and response to treatment. Laboratory confirmation usually comes when the animal has either died or has recovered.

Treatment
Peter Wilson, Massey University
With respect to the treatment of yersiniosis, Peter recommends isolation of the affected animals under cover and injections with tetracyclines or trimethoprim-sulphonamide combination and fluids. Mass treatment of all the animals in a mob requires injection with long acting tetracyclines or a 2 to 3 ml dose of oral neomycin. Prevention involves minimising stress, and particularly feed and climatic stress.

CONCLUSIONS

The workshop was useful in that it made everyone aware of what Yersinia work was going on in different species around New Zealand

The work is important because yersiniosis not only causes economic loss and wastage in the livestock industry but it is also a zoonosis.

Clinical yersiniosis, which usually occurs in only a proportion of a mob of animals, is usually caused by Y.e. in goats and Y. pstb. in deer and cattle.

Pigs naturally carry both Y. pstb. and Y.e. without clinical disease.

The diagnosis of yersiniosis should be based on history, clinical signs, (and/or post mortem examination), culture and histopathology.

In the past serological responses of deer to Y. pstb. infection have been measured by the agglutination test which is not very satisfactory because of high background and cross reactivity. It is hoped that the development of an ELISA will provide better sensitivity and specificity, using various purified antigens.

Future work should be directed at more precisely defining the immunological response of deer to infection, and to inoculation with purified antigens and vaccines.

For the development of a vaccine we must:

a) Determine the optimal growth conditions for the expression of virulence antigens for which assays must be developed.

b) Investigate the cross-protection between strains and serotypes.

c) Develop optimal adjuvant systems.

d) Develop mouse models for verifying the virulence factors which convey protection and for potency testing vaccines. Obviously the results from these models must equate with virulence and protection in the target species, namely deer, goats, etc.

This workshop should lead to better understanding and co-operation between the major groups based at Massey University, Wallaceville, Otago University and Invermay.

The ultimate goal of a vaccine against yersiniosis is still perceived as worthwhile and achievable.