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Validation of an Antibody Test for Liver Fluke and Liver fluke distribution in northern Australia

Abstract

The common liver fluke, *Fasciola hepatica*, is an economically important parasite that infects a wide range of livestock species, including sheep and cattle, and has a lifecycle stage in certain species of freshwater snail. A conservative estimate puts the annual cost of fasciolosis to the Australian livestock industries at $90 million. This project was developed to evaluate a new antibody test for diagnosing liver fluke infection and to update information on its distribution in northern Australia. The new test proved to be both sensitive and specific and far superior to current diagnostic technology. Liver fluke remains confined to southeast Queensland but the presence of introduced tropical snails provides potential for the parasite to spread well beyond its current range and ultimately affect the northern beef industry. Recommendations are the adoption of the new diagnostic test and implementation of an ongoing monitoring programme.

Executive Summary

The common liver fluke, *Fasciola hepatica*, is an economically important parasite that infects a wide range of livestock species, including sheep and cattle. A conservative estimate puts the annual cost of fasciolosis to the Australian livestock industries at $90 million. Sheep are particularly susceptible, frequently suffering acute disease, whereas cattle most often develop chronic disease that results in lost production through reduced weight gain (up to 1.4 kg/week), reduced milk production (up to 0.6 l/day in dairy cattle), reduced fertility and condemnation of livers at slaughter.

The life cycle of the liver fluke is relatively complex with stages in a mammalian host and in certain species of freshwater snail. The only native snail host, Austropeplea tomentosa (formerly Lymnaea tomentosa), is a temperate species restricted mainly to wetter areas in NSW, Victoria, Tasmania and South Australia. An abattoir survey in 1985 indicated that liver fluke in Queensland was confined to the northern limit of the New England Tableland around Stanthorpe and a limited area around Maleny and Kilcoy to the north of Brisbane. However, the introduction of two tropical snail species, Austropeplea viridis and Pseudosuccinea columella, that can also act as hosts for liver fluke provides potential for the parasite to spread well beyond its traditional range and eventually threaten cattle industries in northern Australia.

Traditional diagnosis of *F. hepatica* infection in livestock is by detection of eggs in faeces or detection of flukes in the liver post-mortem. Detection of eggs in faeces is the routine diagnostic procedure for live animals but the method is cumbersome and labour intensive, and sensitivity is low (only 30% of infected animals detected in some studies). The alternative is to detect parasite–specific antibodies in serum or milk from infected animals. Antibodies appear in the serum from about two weeks post-infection, which is seven to nine weeks earlier than eggs appear in faeces, and persist indefinitely in most untreated animals. A newly developed antibody test (an ELISA) marketed by a French company (Institut Pourquier) is reported to be both sensitive and specific. Furthermore the use of a purified antigen has apparently overcome the problem of cross-reactions with paramphistomes (stomach flukes), something that has plagued the development of serological tests for *F. hepatica* infection in the past.

The availability of the new diagnostic test and concern about the potential spread of liver fluke prompted the development of this project with the following key objectives:

- Evaluate the sensitivity and specificity of the Institut Pourquier ELISA
- Determine the current distribution of liver fluke and its snail intermediate hosts in Queensland.

Serum and milk samples from cattle and serum samples from sheep in which infection was confirmed by detection of *F. hepatica* eggs in faecal samples were used to estimate the sensitivity of the ELISA. Similar samples collected from animals outside the *F. hepatica*-endemic area were used to estimate specificity. In cattle the sensitivity and specificity of the ELISA were 98.2% and 98.3%, respectively, using serum, and 99.3% and 97.7%, respectively, using milk. In a comparison with the faecal egg count method, 41.4% of serum samples and 41.5% of milk samples from cattle in infected herds were positive in the ELISA, whereas *F. hepatica* eggs were found in only 26.5% of faecal samples. In an uninfected herd there was no evidence of cross-reaction with paramphistomes despite the presence of paramphistome eggs in faecal samples from 46.2% of cattle. In sheep, the sensitivity and specificity of the ELISA were 96.9% and 99.4%, respectively, using serum. In infected flocks, 60.2% on animals were positive in the ELISA and 52.2% were positive by the faecal egg count method.

The ELISA was used to conduct a serological survey to establish the current distribution of liver fluke in Queensland. The parasite is apparently still confined to southeast Queensland although the prevalence of infection in areas like Maleny, Gympie and Kilcoy to the north of Brisbane is much higher than would have been predicted from the 1985 survey, possibly because that survey was confined mainly to beef cattle. The present survey demonstrated that liver fluke is much more prevalent in dairy cattle than in beef cattle, a finding that is not surprising given that the wetter conditions on most dairy farms are more favourable to the intermediate host snails. However, the trend away from dairying and into beef cattle in many traditional dairying areas will inevitably lead to a greater impact on the beef industry in the future. A survey of intermediate host snails present around the northern and western limits of the liver fluke distribution in southeast Queensland identified *P. columella* as the dominant species present on most properties.

We conclude that the Institut Pourquier ELISA is both sensitive and specific and is clearly superior to the faecal egg count method for diagnosing liver fluke infection. The test is expensive but it can be used with pooled blood or milk samples, making it a convenient and affordable management tool for monitoring the prevalence of infection. Liver fluke remains confined to southeast Queensland but the presence of *P. columella* along the northern limits of the distribution suggests that the parasite is poised to spread northwards along the coast. At the moment liver fluke affects mainly dairy herds (and sheep in parts of southern Queensland) but the impact on the beef industry is likely to increase as the parasite spreads northwards and as producers in traditional dairying areas switch to beef.

We recommend the adoption of the Institut Pourquier ELISA in place of the faecal egg count method as the standard test for diagnostic and disease monitoring applications, and for certification of animals being sent to Western Australia. We also recommend the implementation of an ongoing surveillance programme to monitor the spread of liver fluke.

Contents

1 Background

Fasciola hepatica is an economically important parasite that infects a wide range of livestock species including sheep and cattle (Boray, 1999; Rolfe et al., 1997). A conservative estimate puts the annual cost of fasciolosis to the Australian livestock industries at $90 million (Boray 1999). An added concern is that Australian native animals are particularly susceptible to fasciolosis so any spread of the disease into new areas could have a serious impact on wildlife populations. Only one case of human infection with *F. hepatica* has been recorded in Australia but infection in humans is relatively common in some Asia countries.

Sheep are particularly susceptible to liver fluke disease whereas horses are relatively resistant and quickly develop immunity. Cattle fall into the mid-range, rarely suffering from acute disease (except when young) but often developing chronic disease that can have a major impact on production. In cattle production losses can be in the form of reduced weight gain (up to 1.4 kg/week), reduced milk production (up to 0.6 l/day in dairy cattle), reduced fertility and condemnation of livers at slaughter (Rolfe et al., 1997).

The life cycle of the liver fluke is relatively complex with stages in a mammalian host and in certain species of freshwater snail. Infected cattle shed parasite eggs in their faeces. Larvae emerge from the eggs, invade an intermediate snail host and undergo a series of reproductive cycles before being released from the snail and encysting on vegetation. Grazing animals become infected when they ingest this infective stage. The immature worms migrate through the gut wall to the liver and then migrate through the liver for about seven weeks before entering the bile duct where they mature and begin producing eggs.

Until recently, the distribution of liver fluke In Australia had been governed by the distribution of the only native snail host, Austropeplea tomentosa (formerly Lymnaea tomentosa), which is a temperate species restricted mainly to the wetter areas of NSW, Victoria, Tasmania and South Australia. However, two exotic snails, Austropeplea viridis and Pseudosuccinea columella, that can also act as hosts for liver fluke, were introduced in fish tank weed and are now well established in many areas. The introduction of P. columella in New Zealand led to a rapid expansion of the range of liver fluke in that country (Pullan and Whitten, 1972), and both A. viridis and P. columella are hosts for liver fluke in New Guinea and Indonesia. There is therefore potential for liver fluke to spread well beyond its traditional range and eventually threaten cattle industries in northern Australia. The last survey of liver fluke in Queensland was conducted by Baldock and Arthur (1985) using data collected from post-mortem examinations of livers at abattoirs. That survey indicated that the parasite was confined to the northern limit of the New England Tableland around Stanthorpe and an area around Maleny and Kilcoy to the north of Brisbane.

Traditional diagnosis of F. hepatica infection in livestock is by detection of eggs in faeces or detection of flukes in the liver post-mortem. Detection of eggs in faeces is the routine diagnostic procedure for live animals but the method is cumbersome and labour intensive, and sensitivity is low (only 30% of infected animals detected in some studies) (Happich and Boray, 1969). In recent years a number of sensitive and specific serological tests for liver fluke have been described (Ibarra et al., 1998; Cornelissen et al., 1999), culminating in the commercial availability of ELISA kits. The indirect ELISA marketed by Institut Pourquier is based on purified “f2” antigen (Levieux et al., 1994). Kits are available for testing both serum and milk samples from cattle and serum from sheep. An early version of the serum assay underwent a limited evaluation in NSW (Reichel, 2002) and was found to be both sensitive and specific. Reichel (2002) adapted the assay for testing ovine serum and reported similarly high sensitivity and specificity. Most groups

working with ELISAs report the appearance of fluke-specific antibodies in serum from about two weeks post infection, which is seven to nine weeks earlier than eggs are usually detected in faeces. Antibodies appear to persist indefinitely in most untreated animals (Cornelissen et al., 1999; Ferre et al., 1997; Reichel, 2002). Institut Pourquier reports that the ELISA detects antibodies in milk and serum of infected cattle for up to 12 weeks after treatment with an anthelmintic. Furthermore they claim that the use of a purified antigen has overcome the problem of cross-reactions with paramphistomes (stomach flukes), something that has plagued the development of serological tests for liver fluke in the past.

The availability of the new diagnostic test and concern about the potential impact of liver fluke on the northern beef industry prompted the development of this project. A sister MLA project that was completed early last year provided complementary information on the performance of Institut Pourquier ELISA in NSW.

2 Project Objectives

1. By June 2003 DPI&F will complete a comprehensive evaluation the Institut Pourquier ELISA for monitoring the distribution and prevalence of liver fluke in beef herds.
2. By August 2003 DPI&F will determine the approximate distribution of liver fluke in Queensland by testing a minimum of 750 sera from each of the five administrative regions (SE, S, Central, W and N) represented in an existing bank of sera collected in 1997 from beef herds throughout the state and a minimum of 250 bulk milk samples from the state's dairy farming areas.
3. By December 2003 DPI&F will complete an intensive investigation of the prevalence and distribution of liver fluke, focussing on beef herds in any newly infested or "at risk" areas identified on the basis of the distribution determined in Objective 2.
4. By June 2004 DPI&F will complete a survey of the intermediate snail hosts present both within the established distribution of liver fluke and in any newly infested areas, identified by the survey. The threat posed by liver fluke to the northern cattle industry will be assessed on the basis of the potential distribution of the intermediate snail hosts that are identified.

3 Methodology

3.1 ELISA validation

The broad approach taken was to use serum and, where possible, matching milk samples collected from animals in which infection had been confirmed by detection of eggs in faecal samples as "gold standard" positives for the purpose of estimating assay sensitivity. Similarly, serum and milk samples collected from animals in herds or flocks outside the established liver fluke endemic area, and with no history of fasciolosis, were used as "gold standard" negatives for the purpose of estimating assay specificity. Additional serum samples were obtained from cattle and buffaloes experimentally infected with *F. hepatica* and *F. gigantica*.

3.1.1 Cattle

All available cattle in six herds were sampled. Five herds were from the known *F. hepatica*-infected area of southeast Queensland and had previously tested positive for antibodies to liver fluke in the Institut Pourquier ELISA conducted on bulk milk samples. One herd was from a *F. hepatica*-free, but paramphistome-endemic, area in central Queensland. In four of the infected

herds, individual serum, milk and faecal samples were collected from a total of 301 milking cows, and individual serum and faecal samples only from an additional 102 cattle. In the fifth infected herd, serum and faecal samples only were collected from 243 cattle. In the uninfected herd, individual milk, serum and faecal samples were collected from 135 milking cows, and serum and faecal samples only from an additional 36 cattle.

3.1.2 Sheep

Serum and faecal samples were collected courtesy of Bruce Jackson (DPIWE, Tasmania) from a total of 249 sheep in three flocks that were within the Tasmanian *F. hepatica*-infected area and had a history of fasciolosis. Serum samples from 160 sheep in the *F. hepatica*–free area of western Queensland were obtained from an existing collection. In addition, paired serum and milk samples were collected from ten ewes in a flock with a history of fasciolosis.

3.1.3 Buffaloes and cattle infected with F. gigantica

Serum samples from buffaloes and cattle with natural and experimental *F. gigantica* infections were obtained from Bruce Copeman and Elizabeth Molina (James Cook University, Townsville). The sera were collected immediately prior to infection and then at weekly intervals for 16 weeks post-infection from animals experimentally infected in the Philippines with a local isolate of *F. gigantica*. Additional serum samples were collected at a Philippine abattoir from two animals in which *F. gigantica* infection was confirmed by examination of the liver post-mortem.

3.1.4 Faecal egg counts

Counting of eggs of *F. hepatica* and paramphistome species in faecal samples was conducted using a standard sedimentation/flotation technique based on that of Breza and Corba (1973). Sample weights of 6 g for cattle and 3 g for sheep were used.

3.1.5 ELISA method

The ELISA was obtained from Institut Pourquier (Montpellier, France) and used according to the manufacturer's instructions except that a commercially available peroxidase labelled anti-bovine IgG conjugate (KPL, Gaithersburg, MD) was substituted for the monoclonal antibody conjugate provided with the ELISA kit when testing buffalo sera. The assay is an indirect ELISA with alternate wells coated with purified f2 antigen or uncoated. Test samples are added to wells with and without antigen and the absorbance of the uncoated well subtracted from that of the coated well. Results for individual samples are then calculated as a percentage of a positive control provided with the ELISA kit and included on every plate. The recommended positive threshold of 30% was applied in all cases.

3.2 Serological survey in Queensland

Sera from an existing bank collected in 1997 as part of a DPI&F structured surveillance programme were tested to establish the distribution of liver fluke in Qld beef herds. A total of 5103 sera from 142 herds representing the five Queensland administrative regions (South-east – 1133 cattle in 28 herds, Central – 1027 cattle in 32 herds, South – 1030 cattle in 26 herds, North – 1095 cattle in 36 herds, West – 818 cattle in 20 herds) were tested. In addition, 523 vat milk samples from dairy herds distributed throughout the major dairying regions in southeast Queensland and on the Atherton Tableland were tested. Milk samples were accessed through the enzootic bovine leucosis accreditation programme operating in Queensland. All 113 dairy herds on the Atherton Tableland were sampled. GPS coordinates of all herds tested were plotted using a GIS system to provide a map of the distribution of liver fluke in Queensland.

3.3 Snail survey

Snails were collected from infected properties along the northern and western limits of the liver fluke distribution established by the serological survey. Specimens were identified to species level on the basis of morphology and preserved in ethanol. The preserved specimens were then sent to Dr Winston Ponder at the Australian National University for confirmation of identity.

3.4 Extension activities

Extension activities undertaken in the latter part of the project included the following:

- Three producer information sessions at Gympie, Kilcoy and Warwick
- Liver fluke information displays at five local shows and Farmfest
- Distribution of pamphlets and fact sheets
- Media releases
- Radio interview
- Publication of project results in appropriate refereed journals
- Presentation at a national conference.

4 Results and Discussion

4.1 Sensitivity and specificity of the ELISA

4.1.1 In cattle

Specificity estimates for the ELISA, based on testing 171 sera and 135 milk samples from the uninfected herd, were 98.3% (95% confidence limits: 94.6 and 99.6) using serum, and 99.3% (95% confidence limits: 95.3 and 100) using milk. Paramphistome eggs were found in faecal samples from 46.2% of cattle in the herd. Sensitivity estimates for the ELISA, based on testing 167 sera and 86 milk samples from those cattle in which infection with *F. hepatica* had been confirmed by detection of eggs in faecal samples were 98.2% (95% confidence limits: 94.4 and 99.5) using serum, and 97.7% (95% confidence limits: 91.1 and 99.6) using milk. In the infected herds overall, 41.4% of sera and 41.5% of milk samples were positive in the ELISA, whereas *F. hepatica* eggs were found in only 26.5% of faecal samples.

Table 1. Cross tabulation of ELISA and faecal egg count data for cattle in infected herds

		Faecal egg count	
		+	-
Serum ELISA	+	168	98
	-	3	377
Kappa 0.659 (0.599, 0.719)*			
Mc Nemar's chi sq# 89.4			
P <0.01			

		Faecal egg count	
		+	-
Milk ELISA	+	84	41
	-	2	174
0.692 (0.608, 0.776)			
35.4			
P<0.01			

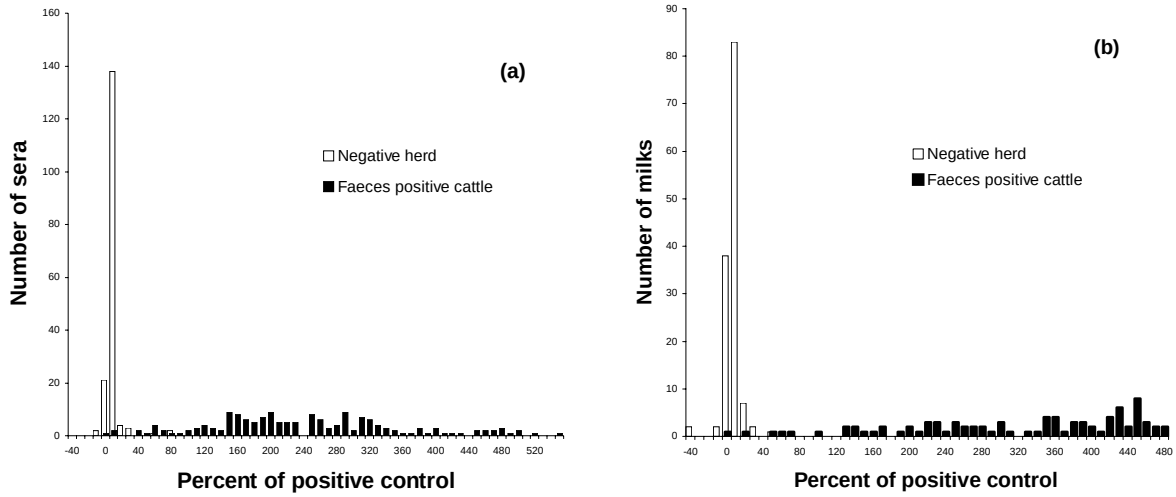
		Serum ELISA	
		+	-
Milk ELISA	+	123	4
	-	8	166
0.919 (0.874, 0.964)			
1.3			
P not significant			

* Approximate 95% confidence limits for Kappa

Uncorrected chi-square statistic on one degree of freedom, necessary value for significance (P=0,01) = 6.6.

Comparison of data from infected herds (Table 1) demonstrates the superior sensitivity of the ELISA over the faecal egg count method. Agreement between the serum and milk ELISAs was excellent although the serum ELISA detected marginally more infected animals, probably reflecting the higher antibody concentration in serum. Given the demonstrated high specificity of the ELISA the discordance between faecal egg counts and ELISA ($P<0.01$) is almost certainly due to the superior sensitivity of the ELISA.

Frequency distributions of ELISA results for serum and milk samples from the uninfected herd, and serum and milk samples from cattle with confirmed infections are shown in Figures 1a and 1b. Discrimination between animals in *F. hepatica*-free herds and animals with confirmed infections was excellent and the 30% positive threshold prescribed by the manufacturers of the ELISA appeared to be appropriate. The sensitivity and specificity estimates obtained in this study using serum and milk samples from cattle are consistent with those obtained by Reichel (2002) and Hutchinson (2003) using sera and should be more reliable because larger numbers of animals were tested. It could be argued that the estimates of sensitivity provided by all three studies are unrealistically high because, like Reichel (2002) and Hutchinson (2003)), we based our estimates on results from animals with mature infections, as demonstrated by the presence of parasite eggs in faeces. Confirmation of infection by examination of livers post-mortem would undoubtedly have been better but was impractical. The frequency distribution of ELISA results for all 646 cattle in the infected herds (Fig. 1c) provides some reassurance that our estimates of sensitivity and specificity are a reasonable reflection of the performance of the test in the field. Presumably those herds contained a mix of uninfected animals and animals with early and mature infections, yet there was still clear evidence of discrimination between infected and uninfected populations similar to that observed with animals from uninfected herds and animals with confirmed infections. Paramphistomes are a common cause of cross-reactions in serological tests for *F. hepatica* but there was no evidence of cross-reactions in either the serum or milk ELISAs. Paramphistome eggs were found in 46.2% of cattle in the *F. hepatica*-free herd, but the actual prevalence of infection was probably higher.



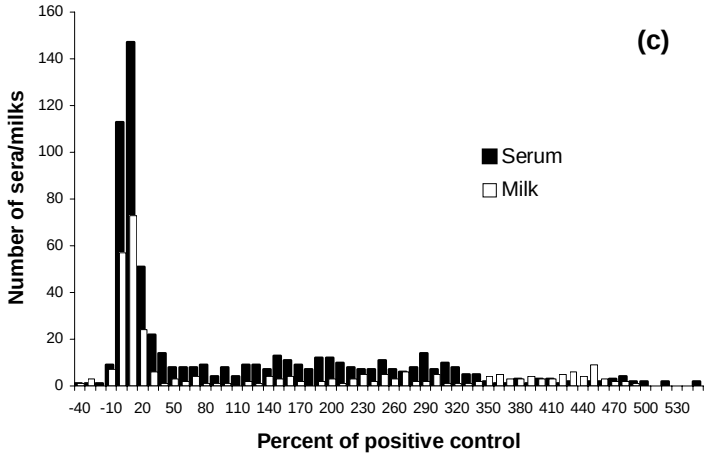


Figure 1: Frequency distributions of ELISA data for (a) serum samples from 171 uninfected cattle and 167 cattle with confirmed *F. hepatica* infections, (b) milk samples from 135 uninfected cattle 86 cattle with confirmed *F. hepatica* infections and (c) all 646 serum and 301 milk samples from cattle in *F. hepatica*- infected herds.

4.1.2 In sheep

Sensitivity, estimated using sera from 130 sheep with confirmed infections, was 96.9% (95% confidence limits: 91.8 and 99.0). Specificity, estimated using sera from 160 sheep in uninfected flocks, was 99.4% (95% confidence limits: 96.0 and 100). Of a total of 249 sheep in the infected flocks, 60.2% were positive in the ELISA and 52.2% were positive by the faecal egg count method.

Table 2. Cross tabulation of ELISA and faecal egg count data for sheep in infected flocks

		Faecal egg count	
		+	-
Serum ELISA	+	126	20
	-	4	99

Kappa 0.806 (0.732, 0.879)*
Mc Nemar's chi sq# 10.7
P <0.01

* Approximate 95% confidence limits for Kappa
# Uncorrected chi-square statistic on one degree of freedom, necessary value for significance (P=0,01) = 6.6.

Cross tabulation of ELISA and faecal egg count data again demonstrated the superior sensitivity of the ELISA although the difference was not as great as observed with cattle.

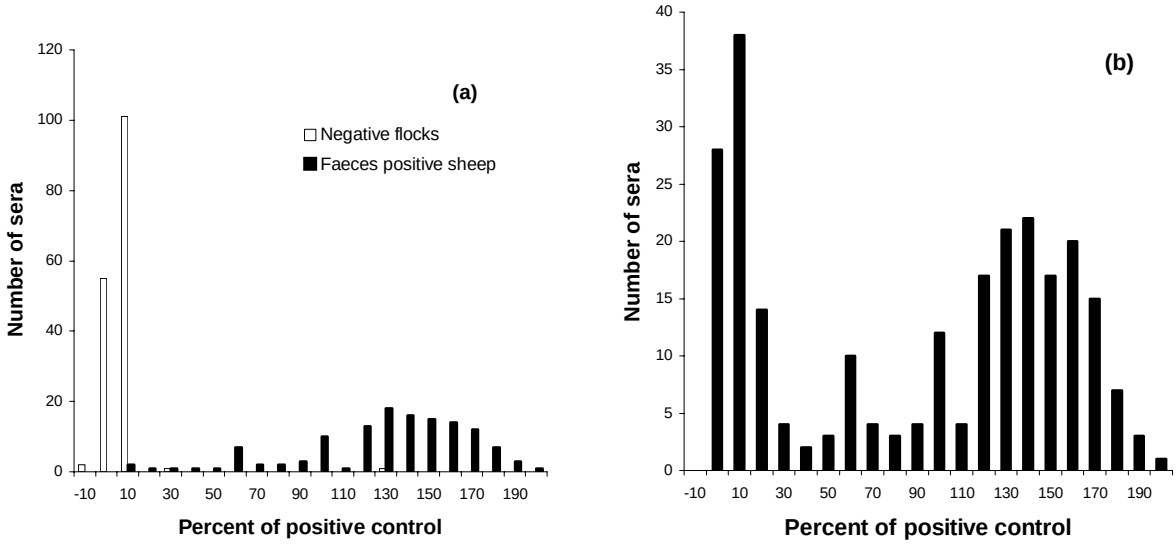


Figure 2: Frequency distributions of ELISA data for (a) sera from 160 uninfected sheep and 130 sheep with confirmed *F. hepatica* infections and (b) all 249 sera from sheep in *F. hepatica*-infected flocks.

Frequency distributions of ELISA results for sera from sheep with confirmed infections and sheep from uninfected flocks (Fig. 2a), demonstrated discrimination of infected and uninfected animals similar to that observed with cattle. Likewise, the frequency distribution for all sheep in the infected flocks (Fig. 2b) suggested discrimination between infected and uninfected populations. The 30% threshold value again appeared to be appropriate.

Of the ten ewes in an infected flock from which paired serum and milk samples were collected, eight were positive in both the serum and milk ELISAs and the remaining two were negative in both assays. This small-scale evaluation suggests that the ELISA might also be used with ovine milk samples. Milk samples can be easier to collect than serum samples and testing individual or pooled milk samples from ewes post-lambing could provide a convenient means of assessing the prevalence of infection in a flock.

4.1.3 Detection of antibodies to F. gigantica in cattle and buffalo

The bovine and buffalo sera from animals with natural *F. gigantica* infections were both positive in the ELISA using the 30% threshold prescribed for cattle infected with *F. hepatica*. Using the same threshold value, the bovine and buffalo sera collected sequentially from animals experimentally infected with *F. gigantica* were positive by two weeks post-infection (Fig. 3), which is consistent with the results obtained by Reichel (2002) and Hutchinson (2003) with cattle infected with *F. hepatica*.

This small-scale evaluation complements a similar evaluation reported by Hutchinson (2003) and suggests that the Institut Pourquier ELISA could be a useful surveillance tool in northern Australia. *Fasciola gigantica* is not present in Australia but is an important parasite of livestock in southeast Asia and therefore could present a threat to cattle industries in northern Australia should suitable snail hosts become established in the region.

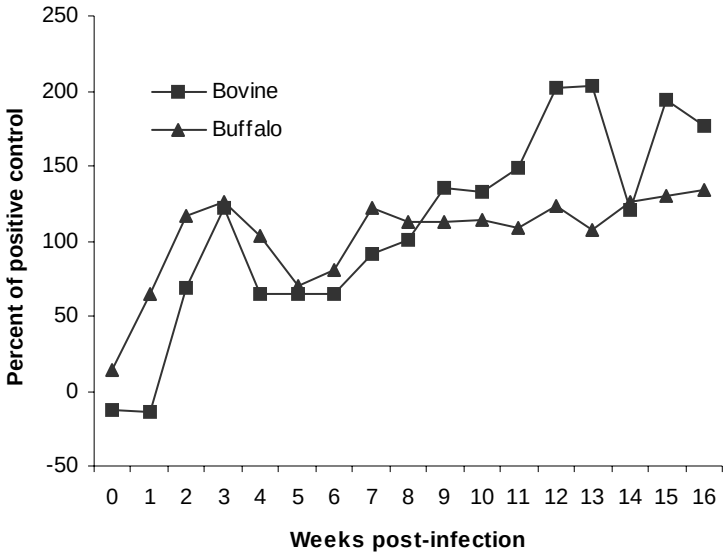


Figure 3: Increase in f2-specific antibodies over time in one buffalo and one cow experimentally infected with *F. gigantica*.

4.2 Distribution of liver fluke

The distribution and approximate prevalence of liver fluke in Queensland beef and dairy herds are shown in Figure 4. Dairy herds were included in the survey because of the ability to readily access milk samples through the enzootic bovine leucosis accreditation programme, the ability to test vat milk samples to provide and estimate of the prevalence of infection in the herd and the consideration that liver fluke was likely to be more prevalent in dairy herds.

Liver fluke is apparently still confined to southeast Queensland although the prevalence of infection in areas like Maleny, Gympie and Kilcoy to the north of Brisbane is much higher than would have been predicted from the last survey (Baldock and Arthur, 1985). The likely explanation is that their abattoir survey was confined to beef cattle. Baldock and Arthur (1985) also found a low level of infection (0-1%) in western and central Queensland but they rightly concluded that it was probably due to movement of infected cattle. All sera we tested from beef herds were from homebred cattle. We recorded low levels of infection in some herds outside the expected liver fluke distribution, but only at levels that could be explained in terms of assay specificity, and all were weak positive results just above the assay threshold. Nevertheless we undertook some intensive follow up testing of cattle in a suspicious cluster of such herds along the Burdekin River near Charters Towers, but were unable to find any evidence that liver fluke was present. Interestingly, virtually all of the herds in which we recorded weak positive ELISA results in a small number of cattle were along the coast, suggesting low-level cross-reaction with some other agent that is not present in inland areas (possibly stomach fluke). We also followed up on consistent anecdotal evidence of liver fluke infection in cattle near Mackay and Proserpine in North Queensland but concluded that the reports were almost certainly due to confusion with stomach fluke, which is very common in the area. A dairy herd with 20–50% of cattle infected with liver fluke was identified on the Atherton Tableland. However, follow up testing of individual milk samples demonstrated that the infection was confined to a group of cows introduced from Victoria and that there had been no transmission to homebred cattle in the herd, suggesting that no suitable snail host was present. All affected cattle were subsequently treated with flukicide.

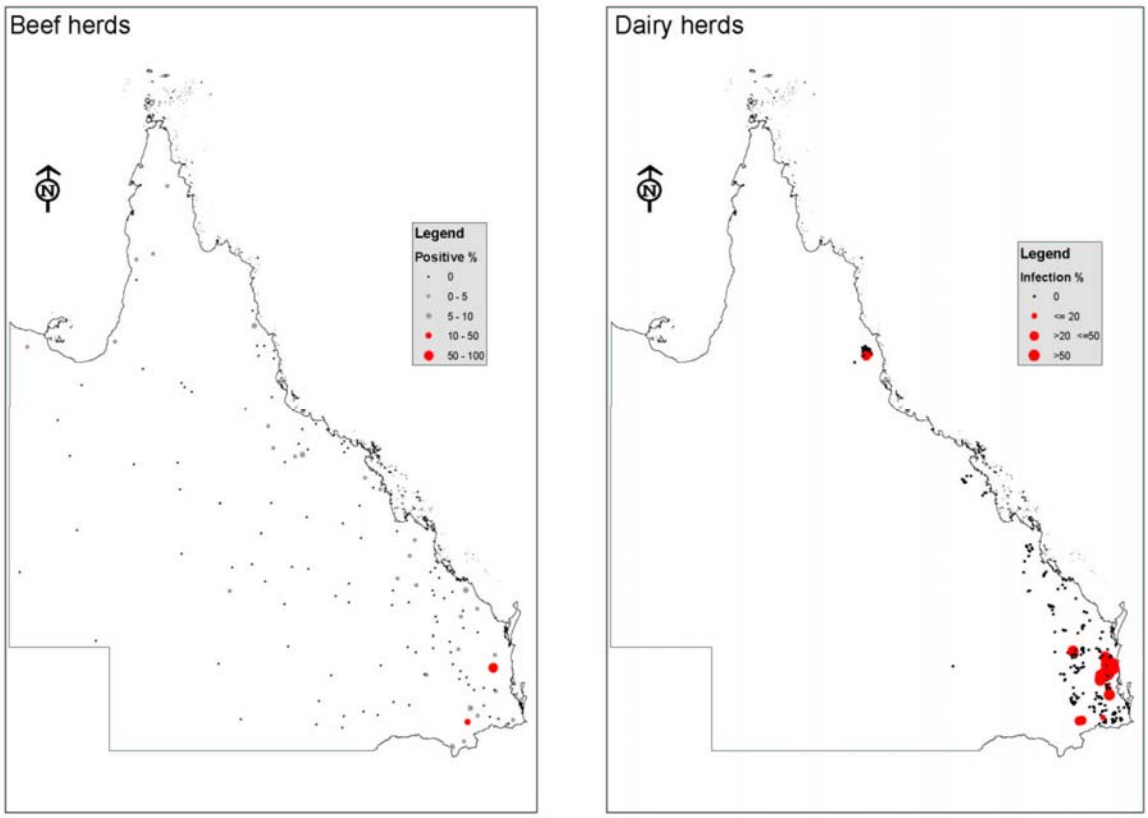


Figure 4: Distribution of liver fluke in Queensland beef and dairy cattle. Prevalence of infection in dairy herds was estimated from the intensity of the response in the ELISA using a table provided by the assay manufacturer. Prevalence of infection in beef herds was calculated as the percentage of animals that were seropositive.

The survey showed that liver fluke is much more prevalent in dairy cattle than in beef cattle, a finding that is not surprising given that the wetter condition on most dairy farms are more favourable to the intermediate host snails. However, beef properties can still be severely affected. For example, repeated testing of an infected beef herd near Jimna to the northeast of Brisbane consistently demonstrated 100% seroprevalence. The trend away from dairying and into beef cattle in many traditional dairying areas will inevitably lead to a greater impact on beef cattle in the future.

4.3 Distribution of intermediate host snails

The survey of intermediate host snails was focused on the northern and western limits of the liver fluke distribution in southeast Queensland. Snails were identified on the basis of morphology at the site of collection and the identity confirmed by experts at the Australian National University. We collected snails on a total of 15 infected properties and identified suitable intermediate hosts on four properties. *Pseudosuccinea columella* was the only species identified on three properties to the north of Brisbane. To the south of Brisbane, *A. tomentosa* was found as far west as Yelarbon (near Goodiwindi), albeit in a fairly unique habitat (the overflow from a sewerage treatment facility).

The snail survey was hampered by the drought conditions that prevailed in southeast Queensland virtually throughout the project. In most cases, follow-up testing of herds by ELISA revealed a reduced prevalence of infection indicating a limited number of new infections,

probably as a result of diminished snail populations. Whether *P. columella* was the only snail present along the northern edge of the liver fluke distribution, or whether it was simply present in larger numbers because of its greater ability to adapt to dry conditions is uncertain. Nevertheless, the survey clearly demonstrates that at least one of the exotic snail species is present in the area and is no longer confined to local creeks in Brisbane where it was identified in 1977 (P.E. Green, personal communication). Given the dry conditions and the extreme difficulty we experienced locating snails, even on properties where we knew liver fluke was established, we did not attempt to survey areas outside southeast Queensland. We cannot therefore rule out the possibility that isolated populations of snails exist elsewhere in the state.

4.4 Adoption of project outcomes

4.4.1 Diagnostic technology

The Institut Puorquier ELISA is now in routine use in DPI&F laboratories. Apart from routine diagnostic applications, a number of pharmaceutical companies are submitting samples to DPI&F laboratories and bearing the cost of testing to provide a free liver fluke monitoring service to customers in Queensland, NSW and Victoria. Negotiations are also under way with the Western Australia Department of Agriculture with a view to modifying that state's animal importation regulations to allow use of the ELISA in place of the faecal egg count method for testing cattle being transported from the eastern states to Western Australia. A preliminary trial with a group of 269 animals was completed successfully in 2004 and permission has been obtained from Western Australian authorities to use the test on another consignment of approximately 300 animals in 2005. A paper describing the validation of the assay has been accepted for publication in Veterinary Parasitology and a presentation at the recent Australian Parasitology Association conference in Perth, stimulated considerable interest and discussion.

4.4.2 Extension effort

In conversations with producers, it became very apparent that there was a great deal of misinformation circulating about liver fluke and often a lack of knowledge about the potential impact on production and the available management options. This prompted us to negotiate a variation to allow us to put more effort into extension activities in the latter part of the project.

Media releases (Appendix 1), radio interviews, posters and attendance at local agricultural shows and Farmfest were all used to disseminate information on liver fluke and to promote information sessions at Gympie, Kilcoy and Warwick. We estimate that we spoke to between 750 and 1000 producers and distributed similar numbers of brochures and fact sheets on snail identification and treatment options (Appendix 1). The producer information days were sponsored by the project with financial support from several pharmaceutical companies that displayed their products. Approximately 100 producers attended to hear presentations by Dr Glenn Anderson (DPI&F), Dr Glen Coleman (UQ) and John Molloy (DPI&F) on the distribution, economic impact, diagnosis, life-cycle and management of liver fluke. Feedback at all sessions was very positive. Information on liver fluke was also available at the DPI&F stand at the Brisbane RNA show.

5 Success in Achieving Objectives

All project objectives were achieved. The new diagnostic test has been fully validated and is now in routine use in DPI&F laboratories. The serological and snail surveys were completed and demonstrated that liver fluke is still confined to southeast Queensland and that *P. columella* is the dominant intermediate snail host on most properties to the north of Brisbane. A more extensive snail survey may have revealed that the exotic snails were present outside the southeast corner but the drought conditions that prevailed almost throughout the project made finding snails extremely difficult, even on properties where liver fluke was established.

6 Conclusions

The Institut Pourquier ELISA is both sensitive and specific and is clearly superior to the faecal egg count method for diagnosing liver fluke infection. The test is expensive, but the ability to pool milk and, potentially, blood samples makes it a convenient and affordable management tool for monitoring the prevalence of infection.

Liver fluke remains confined to southeast Queensland but the presence of *P. columella* along the northern limits of the distribution suggests that the disease is poised to spread northwards along the coast. At the moment liver fluke affects mainly dairy herds (and sheep in parts of southern Queensland) but the impact on the beef industry is likely to increase as the parasite spreads northwards and as producers in traditional dairying areas switch to beef.

7 Recommendations

The Institut Pourquier ELISA should be adopted in place of faecal egg count as the standard test for diagnostic and disease monitoring applications and for certification of animals being sent to Western Australia.

Provided suitable aquatic environments are available along the Queensland coast, there seems to be little chance of halting the northward spread of *P. columella* (and probably *A. viridis*). Likewise, it would be very difficult to control the movement of infected cattle. Therefore, an ongoing surveillance programme, possibly using liver condemnation data from abattoirs, should be implemented to monitor the spread of liver fluke and to detect new pockets of infection. A programme to promote awareness of liver fluke disease and its economic impact, and management strategies among producers, stock inspectors and veterinarians should also be considered.

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9 Appendices

9.1 Appendix 1 - Extension material and media articles

1. Information brochure distributed to producers
2. Fact sheet with information on treatment options for liver fluke
3. Fact sheet with information on snail identification
4. Information on liver fluke and liver fluke information sessions appearing on the DPI&F web site
5. DPI&F media release
6. Articles in regional newspapers

9.2 Appendix 2 - Papers submitted to refereed journals

1. Final draft of a paper on validation of the ELISA that has been accepted by Veterinary Parasitology

2. Draft of a paper on the distribution of liver fluke and its intermediate snail host in Queensland that will be submitted to the Australian Veterinary Journal

9.3 Appendix 3 - Conference papers

1. Paper presented at the Australian Parasitology Association conference in Perth in September 2004.

2. Presentation to Queensland beef producers exporting cattle to Western Australia in December 2004.