INVITED REVIEW

Recent advances in the diagnosis, impact on production and prediction of *Fasciola hepatica* in cattle

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SUMMARY

Fasciola hepatica is a pathogenic trematode parasite of ruminants with a global distribution. Here, we briefly review the current epidemiology of bovine fasciolosis in Europe and discuss the progress made over the last decade in the diagnosis, impact on production and prediction of *F. hepatica* in cattle. Advances in diagnosis have led to significantly improved coprological and serological methods to detect presence of infection. Diagnostic test results have been correlated with intensity of infection and associated production losses, unravelling the impact on carcass weight and milk yield in modern cattle production systems. The economic impact of fasciolosis may, however, go beyond the direct impacts on production as evidence shows that *F. hepatica* can modulate the immune response to some co-infections. Control of bovine fasciolosis remains hampered by the limitations of the currently available flukicidal drugs: few drugs are available to treat dairy cows, many have low efficacies against juvenile stages of *F. hepatica* and there is evidence for the development of drug resistance. This makes research into the prediction of risk periods, and thus the optimum application of available drugs more pertinent. In this field, the recent research focus has been on understanding spatial risk and delivering region-specific spatial distribution maps. Further advances in epidemiological and economic research on bovine fasciolosis are expected to deliver farm-specific economic assessments of disease impact, to leverage non-chemotherapeutic management options and to enhance a more targeted use of anthelmintics.

Key words: *Fasciola hepatica*, ruminant, immunity, global change, anthelmintics, control.

INTRODUCTION

Animal health management has been defined as the promotion of health, improvement of productivity and prevention of disease within the economic framework of farm owner and industry, while recognizing animal welfare, food safety and security, public health and environmental sustainability (Leblanc *et al*. 2006). Prevention and vaccination campaigns are resulting in successful local and regional elimination of several infectious diseases of cattle such as foot and mouth disease (Sutmoller *et al*. 2003), infectious bovine rhinothracheitis (Nardelli *et al*. 2008) and bovine viral diarrhoea (Lindberg *et al*. 2006). In contrast, elimination of endemic parasitic diseases by vaccination has not occurred and is probably unrealistic. In fact, increases in helminth-associated disease frequency and intensity have been reported within the European ruminant sector in recent years (Morgan *et al*. 2013). This includes a substantial increase in laboratory diagnoses of ovine and bovine fasciolosis in the UK, where central collation of such data allows fair temporal comparisons (van Dijk *et al*. 2010). Given this apparent trend, and the significant economic and welfare burden of fasciolosis in cattle (Schweizer *et al*. 2005; Charlier *et al*. 2009*a*), it is timely to re-visit understanding of this disease. Advances in the last decade in diagnosis underpin new knowledge and understanding of impacts on production, including the effect of the host immune response to co-infections and the prediction of disease. In each section, we address these areas and identify remaining knowledge gaps. We conclude with a discussion of research priorities to promote better management of bovine fasciolosis within an economic framework.

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Table 1. Comparable surveys of *F. hepatica* in cattle in three different parts of Europe. Only those surveys in which the sampled population and/or the test used were very similar were included. Prev. = prevalence; Ab-det. = antibody detection

Region	Year	Source	Method	*N*	Prev. (%)	Source
NW Spain	2000	Random sample of dairy cows	Ab-det. ELISA on serum	2238	85	(Sánchez-Andrade, 2000)
	2010	Structured random sample of dairy cows	(Same test)	1034	65	(Arias *et al.* 2010)
Switzerland	1975–2003	Abattoir survey, all ages	Liver inspection	Various	8–15	(Rapsch *et al.* 2006)
	2006	Abattoir survey, all ages	Bayesian approach based on different diagnostic tests	1331	18	(Rapsch *et al.* 2006)
Flanders, Belgium	2006–08	Stratified random sample, dairy herds	Ab-det. ELISA on bulk milk	1762 herds	37–40	(Bennema *et al.* 2011)
	2009–11	Voluntary monitoring campaign	(Same test)	805 to 1116 herds	30–34	(Charlier *et al.* 2013)

BOVINE FASCIOLOSIS: A RESURGENT DISEASE IN EUROPE?

Regional increases in laboratory diagnoses of fasciolosis in cattle and sheep (van Dijk *et al.* 2010; Fairweather, 2011), and anecdotal reports from veterinary practitioners of ever-increasing challenges from this disease, raise concerns that risks of infection are indeed increasing. Altered disease patterns could be detected by changes in the prevalence, intensity or distribution of *Fasciola hepatica* (Mas-Coma *et al.* 2008). The distribution can be affected (1) temporally, causing shifts in seasonality or (2) spatially, causing shifts in the geographic range of occurrence. For fasciolosis, increases in prevalence and shifts in the geographic range have been reported in the last decade, primarily in the UK. Fasciolosis was reported in previously unaffected parts of Scotland and East Anglia (Pritchard *et al.* 2005; Kenyon *et al.* 2009). In England, prevalence of infection in dairy herds was 48% in 2003 compared with 72% in 2006 (McCann *et al.* 2010*a*). In these cases, the authors linked increases in prevalence to milder winter temperatures and increased rainfall.

The use of different diagnostic methods or sampling in different areas hampers comparison of the results from prevalence surveys across space and time. Nonetheless, in some enzootic regions, repeated surveys with comparable methodology were carried out over the last decade (Table 1). In north-western Spain, Arias *et al.* (2010) found a seroprevalence of 65% and concluded that despite regular fasciolicide treatment, the control of fasciolosis had not improved in this area compared with a survey carried out 10 years earlier (Sánchez-Andrade, 2000). In Switzerland, Rapsch *et al.* (2006) reported a higher prevalence (18%) than those previously reported (8–15%) in slaughtered cattle. However, the authors concluded that this was mainly due to the higher sensitivity of the detection techniques in their survey compared with the previous ones. In Belgium, no decreases in prevalence were observed between 2006–2008 (Bennema *et al.* 2011) and 2009–2011 (Charlier *et al.* 2013) during which an active monitoring campaign increased awareness of the disease in this region.

Studies in other regions do not allow trends to be assessed over time but show an overall moderate to high prevalence of *F. hepatica* in different regions from southern to sub-Scandinavian Europe (Cringoli *et al.* 2002; Conceição *et al.* 2004; Kuerpick *et al.* 2013*a*). For example, studies in Germany (Pfister and Koch, 2004; Kuerpick *et al.* 2013*a*) and Austria (more specifically Tirol/Carinthia) (Matt *et al.* 2007; Duscher *et al.* 2011) report overall herd-level prevalences of 24 and >70%, respectively and show that herd-level prevalence reaches very high values (up to 97%) in alpine upland farms. In northern Europe, the parasite seems not to be widely

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distributed, with a reported herd-level prevalence of 7% in south-central Sweden (Höglund *et al*. 2010).

The role of climatic factors (milder winter temperatures and higher amounts of rainfall) in increased prevalence of *F. hepatica* seems obvious. However, other, more complex environmental changes could very plausibly affect observed prevalence and disease risk. In England, local increases in the prevalence of fasciolosis in cattle in East Anglia were associated with increased use of sheep to graze environmentally sensitive areas (Pritchard *et al*. 2005). These areas were generally on wet land, resulting from the reversal of previous land drainage schemes in support of conservation and watershed management, while the sheep were imported from areas of the UK where *F. hepatica* was enzootic. In contrast, reversal of pasture drainage measures for nature conservation in northern Germany did not result in detectable changes in fasciolosis in cattle over a 3-year period (Kemper and Henze, 2009). This suggests that the local drivers of transmission and disease risk are complex and to some extent site-specific, and could be important modifiers of predicted macro-scale trends driven by global climate change (Fox *et al*. 2011). This is borne out by statistical models using bulk milk tank antibody levels as a measure of exposure to *F. hepatica*, which are able to explain around 20% of variance when only geographic or climatic factors are considered (Kuerpick *et al*. 2013*a*), rising to 85% when more detailed local factors are also included (Charlier *et al*. 2011).

Considering the published prevalence surveys of *F. hepatica* in European cattle herds over the last decade, it is clear that *F. hepatica* probably affects cattle in every EU member state. Evidence of increasing prevalence of *F. hepatica* is only reported in the UK. Despite the lack of recent published information in some countries with important cattle industries (for example France and countries in Eastern Europe), it is reasonable to say that in other regions throughout Europe, the prevalence is not decreasing despite the greater attention given to control in recent years. In addition, there are cases in which local environmental change appears to have driven disease emergence, while macro-climatic change is predicted to favour rather than hinder transmission. Certainly, there is little basis on which to allay the significant concerns over this disease among animal health professionals in Europe.

IMPROVING DIAGNOSIS

The pursuit of the perfect diagnostic test

The classical cornerstone for the diagnosis of fasciolosis in cattle is the microscopic detection of *F. hepatica* eggs in feces. More recently important advances have been made in alternative methods based on detection of *F. hepatica*-specific antibodies in serum or milk and the detection of *F. hepatica*-specific antigens or DNA in the feces.

Detection of eggs is mostly based on sedimentation of eggs, which may be followed by their flotation. Numerous methods have been described in literature and in practice each laboratory has adopted its own variant. In general, the trained eye can easily recognize *F. hepatica* eggs using low magnification conventional microscopy and discriminate between them and others such as *Paramphistomum* spp. eggs. Although the specificity of these methods is high and the detection of eggs indicates current infection (instead of exposure as with antibody detection), a disadvantage of detection of eggs in feces, particularly in cattle, is the low sensitivity (30–70%, depending on the method and study area). However, recent studies have demonstrated that the sensitivity of egg detection methods is largely driven by the volume of feces that is analysed (Conceição *et al*. 2002; Rapsch *et al*. 2006; Charlier *et al*. 2008) and repeated testing or analysing $\geqslant 30$ g of feces can increase the sensitivity up to 90% (Rapsch *et al*. 2006).

Detection of *F. hepatica*-specific antibodies, mostly by ELISA, has been proposed as a more sensitive method. There are several ELISAs that have been well described in the literature. The first, referred to as ES ELISA, uses the complete excretory secretory (ES) products of *F. hepatica*. ES products are relatively easy to produce and as a consequence many laboratories apply their own in-house version of the ELISA. Recently, a more standardized ELISA kit has become available (Svanovir®*F. hepatica*-Ab, Svanova, Uppsala, Sweden). Although cross-reactions of the *F. hepatica* ES products with antibodies against other helminths such as *Dictyocaulus viviparus* (Cornelissen *et al*. 1999) and *Dicrocoelium dendriticum* (Mezo *et al*. 2007) are reported, reasonable sensitivities (86–100%) and specificities (83–96%) of ES ELISAs have been observed under different epidemiological settings (Anderson *et al*. 1999; Cornelissen *et al*. 1999; Salimi-Bejestani *et al*. 2005; Charlier *et al*. 2008; Kuerpick *et al*. 2013*b*). A second ELISA uses a sub-fraction of the ES products as diagnostic antigen, the so-called 'f2' antigen, for which the purification steps are described by Tailliez and Korach (1970). A commercial format (Fasciolosis Verification Test, IDEXX, Hoofddorp, the Netherlands; previous manufacturer: Institut Pourquier) and several evaluations of this kit are available, reporting high sensitivity (88–98%) and specificity (84–98%) (Reichel, 2002; Molloy *et al*. 2005; Rapsch *et al*. 2006; Charlier *et al*. 2008; Kuerpick *et al*. 2013*b*). The MM3-ELISA developed by Mezo *et al*. (2010) uses monoclonal antibodies to capture proteins from a 7–40 kDa fraction (mainly cathepsins L1 and L2) from the ES products (Muino *et al*. 2011). A field evaluation reported a sensitivity of 99% and specificity of 100%. A commercial format is

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available (BIO K 211, Bio-X Diagnostics, Jemelle, Belgium) and further evaluations of this commercial kit are expected in the future. Finally, an ELISA based on recombinant cathepsin L1 is also described, with the major advantage that it does not depend on the availability of living flukes. However, in a direct comparison the sensitivity and specificity were lower than for f2 and ES ELISA, respectively (Kuerpick *et al*. 2013*b*). A recombinant mutant cathepsin L1 ELISA is commercially developed by Ildana biotech (Dublin, Ireland).

Besides increased sensitivity, the major advantage of immunodiagnosis is that it is suitable for high-throughput formats and can be applied on different matrices besides serum. Testing bulk-tank and individual milk samples offers a great advantage in dairy farming as milk samples are collected for other monitoring programmes on a regular basis and there is no need for invasive sampling of the animals (Reichel *et al*. 2005; Salimi-Bejestani *et al*. 2007; Duscher *et al*. 2011). A similar approach has been proposed for beef cattle, using muscle transudate ('meat juice') collected in the abattoir as matrix for *F. hepatica* ELISA (Charlier *et al*. 2009*b*).

Another technique is the detection of *F. hepatica* antigens in feces through ELISA (Espino and Finlay, 1994; Abdel-Rahman *et al*. 1998; Mezo *et al*. 2004). The test described by Mezo *et al*. (2004), using the MM3-antibody directed towards *F. hepatica* cathepsins is available commercially (BIO K 201, Bio-X Diagnostics, Jemelle, Belgium). This copro-antigen test is described as an ultra-sensitive and specific test capable of detecting prepatent infections from 4 weeks post-infection onwards and animals infected with as low as 1–2 flukes (Mezo *et al*. 2004). Despite recent field evaluations of the commercial test reporting rather low sensitivity (Duscher *et al*. 2011; Salem *et al*. 2011), the test has been successfully evaluated to detect triclabendazole resistance in sheep by assessing copro-antigen reductions after flukicide treatment (Flanagan *et al*. 2011; Gordon *et al*. 2012).

Finally, DNA-based techniques have so far received relatively little attention in the context of liver fluke diagnosis. This can be justified as the most obvious source of DNA is *F. hepatica* eggs in the feces and the techniques would thus not offer great advantage above classical microscopical quantification of eggs. However, a recent study in sheep shows that PCR-technology could be useful to detect infections as early as 2 weeks post infection, before serum antibody develops in many infected animals and before eggs appear in feces and this with great sensitivity and specificity (Martinez-Perez *et al*. 2012). Another promising application is the use of a LAMP (loop mediated isothermal amplification) assay on fecal samples. Ai *et al*. (2010) describe this assay that is even more sensitive than conventional PCR technology. Moreover, DNA amplification is possible under isothermal conditions, quick and visible by the naked eye, thus has potential as a pen-side diagnostic test.

The current paradigm change from diagnosing the infection to its impact

The previous cited work on diagnosis is characterized by a clear progression: the reported tests show, in chronological order, increased sensitivity, specificity and precision compared with previous tests. Yet, this has not (yet) resulted in a major reduction in disease prevalence.

A possible explanation for this is that the tests focus on detecting presence/absence of infection while no clear message on control is provided (Vercruysse and Claerebout, 2001). In cattle, fasciolosis is a chronic disease, therefore, there is limited value in knowing the status of presence/absence of infection in a cow or herd or diagnosing infections at 2 weeks *vs* 8 weeks post-infection; it is more relevant to have some information on intensity of infection and associated production losses to convince farmers that further diagnosis, and treatment, are worth considering.

A study by Charlier *et al*. (2008) showed that while the majority of naturally infected animals had a low fluke burden, signs of liver damage (visible liver lesions or increases in the bile duct enzyme γ-glutamyltransferase) only appeared when >10 flukes were present. Coprology applied on 4 g of feces identified only these highly infected animals, while coprology applied on 10 g of feces also identified animals with lower worm burdens (Charlier *et al*. 2008). The copro-antigen ELISA showed good correlation between test results and fluke burden and could also be a valuable aid to control (Mezo *et al*. 2004; Charlier *et al*. 2008). Antibody detection ELISAs also discriminate to some extent between no, low, moderate and high fluke burdens (Charlier *et al*. 2008; Salimi-Bejestani *et al*. 2008).

Diagnostic test results have been directly correlated with production parameters and with production responses after flukicide treatment (Charlier *et al*. 2007, 2009*b*, 2012*a*; Mezo *et al*. 2011; Kuerpick *et al*. 2012). Establishing these relationships adds value to the diagnostic test because not only can information be given on the likelihood of the presence of infection, but also on the likely impact it has on animal performance. Moreover, this information can then be used to calculate herd-specific, albeit crude estimates of the direct costs due to *F. hepatica* (Charlier *et al*. 2012*b*) and provide support for decisions on liver fluke control.

It is clear that this new approach of moving from purely detecting infection to an economic diagnosis (assessing the economic impact) is still in its infancy. Production impacts will vary in different epidemiological and farm management settings.

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Moreover, van der Voort *et al*. (2013) show that the economic impact of production losses will depend on the farm's efficiency of input-output transformation. Previous studies have measured the impact on key production indicators such as milk yield or carcass weight. A much closer collaboration between agricultural economists and parasitologists will be needed to manage parasitic infections within the whole farm economic context (van der Voort *et al*. 2013).

IMPACT ON PRODUCTION

Fasciola hepatica infections are reported to affect weight gain, milk production, milk solids content and fertility of cattle (Black and Froyd, 1972; Lopez-Diaz *et al*. 1998; Torgerson and Claxton, 1999). Although the general negative impact of *F. hepatica* on production parameters is well accepted, Dargie (1987) expressed his concern on the lack of appropriately designed studies to quantify these impacts. These (older) studies are well summarized by Schweizer *et al*. (2005), who estimated mean losses of a 9% reduction in weight gain in growing cattle, 10% reduction in milk yield, an extension of the service period by 13 days and an increment of 0·75 services per conception.

More recently, a number of additional studies have provided new figures that may provide more representative estimates for modern cattle production systems. Loyacano *et al*. (2002) observed a 6% increase in body weight of beef heifers ($N = 372$) that were raised under repeated flukicide treatment compared with untreated controls. In abattoir studies Charlier *et al*. (2009*b*) and Sanchez-Vazques and Lewis (2013) related *F. hepatica* infection status (based on *F. hepatica* meat juice ELISA and liver condemnation) to carcass parameters, while adjusting for potential confounding factors such as age and herd-clustering effects. Charlier *et al*. (2009*b*) observed a marginal significant mean reduction in warm carcass weight of 0·7% (3·4 kg) in Belgian Blue suckler cows ($N = 1450$) and no significant effects on conformation score or fat coverage. Sanchez-Vazques and Lewis (2013), using records ($N = 328\,137$) from a 6-year period and different beef breeds assessed a 0·5% reduction in cold carcass weight and a reduction in the price of the carcass by 0·3%. The odds for liver fluke-infected animals receiving a higher conformation or fat score compared with uninfected animals was 0·89 and 0·97, respectively.

Correlating bulk tank milk *F. hepatica* ELISA results with herd average annual milk production, while controlling for potential confounding factors resulted in an average reduction of milk yield of 0·7 kg cow^{-1} day^{-1} ($\approx 3\%$) (Charlier *et al*. 2007). Mezo *et al*. (2011) report a milk yield reduction in cows categorized as 'highly positive' based on *F. hepatica* (bulk-tank) milk ELISA of 1·5 kg cow^{-1} day^{-1} ($\approx 5\%$) and 2 kg cow^{-1} day^{-1} ($\approx 6\%$) on the herd and animal level, respectively. Finally, a randomized placebo-controlled trial administering closantel at dry-off indicated that these losses are largely recoverable as treatment resulted in a 1·1 kg increase in peak production and a longer persistence of lactation (9%) resulting in a 305-day milk production increase of 303 kg (Charlier *et al*. 2012*a*). There was no effect of treatment on the fat or protein concentration of the milk.

A less clear picture is obtained for the effect of *F. hepatica* on reproductive performance. Using a high experimental infection dose of 600 metacarcariae, Lopez-Diaz *et al*. (1998) found that first oestrus was delayed by 39 days in infected heifers compared with uninfected controls. This effect was linked to significantly higher oestradiol and lower progesterone concentrations in the serum of the infected animals. Loyacano *et al*. (2002) report non-significant but higher pregnancy rates (67 *vs* 54%) in beef heifers under repeated flukicide control compared with untreated controls. Charlier *et al*. (2007) report that an increase in anti-*F. hepatica* antibody level in bulk tank milk was associated with an increase in the mean intercalving interval of 4·7 days in dairy herds. In contrast, Simsek *et al*. (2007) and Mezo *et al*. (2011) found no association between the *F. hepatica* serological status and repeat breeding or calving to conception interval.

In conclusion, new studies, underpinned by improved diagnostic tools, are generating insights into the production impact of fasciolosis in modern cattle systems. There is sound evidence that milk production in dairy cattle or carcass weight of slaughtered beef cattle in *F. hepatica*-infected herds will decrease on average by 3–5% or 0·5–0·7%, respectively. Also muscle conformation and carcass fat composition may be affected. However, studies quantifying the effect of *F. hepatica* on growing heifers or on reproductive performance remain limited. Current studies support deleterious effects on reproductive performance, but mainly under high infection challenge and in heifers. There is a need for randomized controlled clinical trials to assess the effects under field circumstances.

FASCIOLA HEPATICA AND CO-INFECTIONS

Fasciola hepatica has a well-defined, direct effect on the health and productivity of cattle but there is a growing awareness of the impact of infection on an animal's susceptibility to other pathogens. Infection with *F. hepatica* is known to be associated with a powerful anti-inflammatory immune response, dominated by an antibody response of the IgG_1 isotype and a cellular response associated with the cytokines interleukin (IL)4, IL10 and Transforming Growth Factor-β (Brady *et al*. 1999; Flynn *et al*. 2007). Dalton *et al*. (2013) describe three

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well-defined molecules, secreted by *F. hepatica* with immunomodulatory properties. This strongly polarized immune response appears to have consequences for the host and particularly its ability to control intracellular pathogens. For example it has been known for many years that *Salmonella dublin* infection in cattle is associated with the presence of *F. hepatica* (Aitken *et al*. 1978; Vaessen *et al*. 1998).

In mice, infection with *F. hepatica* increases susceptibility to the bacterial infection *Bordetella pertussis* and decreases the efficacy of the *B. pertussis* vaccine (Brady *et al*. 1999). This is associated with induction of a potent T helper (Th) cell type 2 response by *F. hepatica* and corresponding suppression of Th1 and interferon (IFN)-γ responses required to control the *B. pertussis* infection. These findings led researchers to investigate the interactions between fluke and other pathogens normally controlled by Th1 responses. One important pathogen affecting cattle in many countries of the world is *Mycobacterium bovis*, the causative agent of bovine tuberculosis (BTB). Although the question, 'does *F. hepatica* infection lead to a change in the susceptibility of cattle to BTB?' has not yet been addressed effectively, recent work suggests that at the very least, the diagnosis of BTB may be compromised in fluke-infected cattle. Flynn *et al*. (2007) showed that cattle co-infected with the avirulent *M. bovis* strain, Bacille Calmette Guerin (BCG), had a significantly reduced diagnostic response to the bacterium. Diagnosis of BTB is based on the single intradermal comparative cervical tuberculin (SICCT) test, in which purified protein derivative (PPD), an antigen of *M. bovis*, is inoculated into the skin of a cow. If the animal is infected with *M. bovis*, there is a specific delayed-type hypersensitivity response, involving activation of antigen-specific T cells and secretion of IFN-γ. The magnitude of the response and associated thickening of the skin is used to diagnose infection. Suppression of this response has been demonstrated in calves co-infected with *F. hepatica* under experimental conditions (Flynn *et al* 2007, 2009; Claridge *et al*. 2012). Perhaps more significantly, these findings have been extended to cattle in the field, again enabled by improved diagnostic tests. Comparison of the spatial distribution of prevalence of *F. hepatica* infection and incidence of BTB in over 3000 dairy herds in England and Wales, showed almost no overlap and logistic regression analysis found presence of *F. hepatica* was significantly negatively associated with diagnosis of BTB (Claridge *et al*. 2012). These data, together with results from experimental co-infections, suggest that fluke infection in dairy cattle suppresses the diagnostic SICCT test for BTB. This finding has important consequences for the control of BTB. It is also important to investigate the interaction between fluke and other intracellular infections that cause significant disease in cattle. Johnes disease caused by *Mycobacterium avium paratuberculosis*, bovine viral diarrhoea virus and *Clostridium* spp. are all significant pathogens of cattle whose control may be affected in animals exposed to *F. hepatica*. The effect of *F. hepatica* on vaccine efficacy is also not well understood.

Interaction between pathogens is complex and the outcome in co-infected animals may well depend on which pathogens are present. For example, whilst the immune response to *B. pertussis* is significantly compromised in mice co-infected with *F. hepatica*, the immune response to the potent Th1 inducing parasite, *Toxoplasma gondii*, is unaffected by the presence of a fluke infection (Miller *et al*. 2009). Much more research is required to fully understand the interaction between different pathogens and the overall outcome of such co-infections in cattle naturally exposed to a plethora of pathogens.

IMPROVING DISEASE FORECASTING

The emergence of fasciolosis in some areas, increasingly obvious production effects, and the threat of anthelmintic resistance with unfocused drug use, emphasize the need for prediction and forecasting tools at regional and farm scales. The development and death rates of the free-living stages of *F. hepatica* and the population dynamics of the snail intermediate hosts are strongly influenced by climate, and this underpins seasonal patterns of infection in temperate areas (Dalton, 1999; van Dijk *et al*. 2010), and provides a basis for predictive models. Attempts to forecast autumnal fasciolosis risk from climatic variables such as environmental temperature and, especially, rainfall have been made for a long time and, with climate change, this field is currently attracting renewed attention. The objective of fluke forecasting is first to determine whether a farm is in a fluke risk area, second what the expected within-year fluke prevalence will be on this farm (in terms of above- or below-average risk) and third exactly when pasture contamination becomes dangerous, in order to guide treatment decisions or evasive strategies. So far, no models have been able to make successful predictions at all these levels.

Forecasting systems have been available since the 1950s (Ollerenshaw and Rowlands, 1959; Malone *et al*. 1987; McIlroy *et al*. 1990; Gaasenbeek *et al*. 1992). They are all based on the use of indices of temperature and humidity in the months that most influence *Fasciola* epidemiology to warn farmers about above-average predicted autumnal disease risk. However, the seasonal temperature-driven developmental window for *Fasciola* can vary substantially between similar eco-climatic zones (Yilma and Malone, 1998; Bossaert *et al*. 1999). Therefore forecasting systems should be regarded as region-specific.

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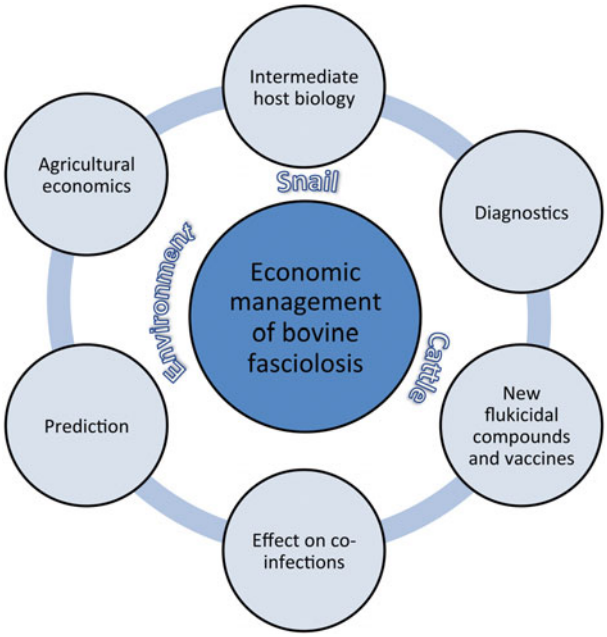

Fig. 1. The key areas for future research to promote economic management of bovine fasciolosis.

Recent research efforts have invariably focused on quantifying and predicting spatial risk. These efforts build further on the work of Malone and colleagues who introduced the use of geographic information systems (GIS) to study the spatial distribution of liver fluke (Malone *et al*. 1992). Recognizing the multiple causation of disease, researchers have explored risk factors other than rainfall and temperature by constructing models providing the best fit for estimates of *Fasciola* abundance. In the UK, McCann *et al*. (2010*b*) identified significant predictors such as soil pH and the slope of land whereas in Australia the variable that best explained spatial fluke distribution was irrigation (Durr *et al*. 2005). In Cambodia, Tum *et al*. (2004) built their maps using factors including inundation risk, proximity to rivers and elevation. However, Bennema *et al*. (2011) showed that, in addition to factors describing the parasite's environment, the inclusion of farm management factors such as the mowing of pastures and the length of the grazing season were also vital for the correct prediction of infection risk in Belgian cattle. The differing variables that correlate with *Fasciola* abundance in different studies show that, like the older forecasting systems, current models predicting spatial risk should be built at the national, or regional, scale and are unlikely to be applicable to other countries. McCann *et al*. (2010*a*) furthermore showed that, even using a plethora of predictors, it is as yet impossible to reliably predict risk at the farm level, with the postcode (roughly NUTS 2 equivalent) level the smallest scale achievable. It appears that GIS-based modellers face the same dilemmas that mechanistic modellers of temporal disease risk, across disciplines, have found hard to deal with (Smith, 2011): (1) to pursue on a road of ever-increasing complexity, ultimately producing models that accurately predict risk at spatially fine scales yet not being widely applicable; or (2) to focus on the simplest model still giving valuable insights on trends, but unsuitable for the prediction of farm-level risk needed for decision support.

Knowledge of parasite ecology at different latitudes could be used to modify established models. Improved high-throughput diagnostic tests, alongside now widely available remotely sensed climatic data, make it ever more feasible to develop statistical models of spatio-temporal risk. However, changes to the underlying epidemiological processes that are implicitly, not explicitly, captured in such models would limit ability to extrapolate conclusions and forecasts between regions or into future climates. Changes in seasonality and the relative importance of rainfall and temperature in driving parasite abundance and availability are core to this problem. Fortunately, improved diagnostics also have the potential to better validate mechanistic models of the ecology of the free-living stages, including the snail host, which could explain regional differences and be robust to future changes. In general terms, since it is likely that rainfall is an important determinant of annual risk whereas especially temperature determines when pasture becomes dangerous (Gettinby *et al*. 1974), the effects of both should be included in such models. A first challenge will be to quantify the effects of temperature on, notably, survival of eggs at pasture, the size of snail populations, as well as individual snails, and metacercaria. A second, major, challenge, shared with models of many other parasites, is to identify parameters which link rainfall to the microclimate experienced by the parasite and snail as well as relate them to their developmental success. A third challenge will be to quantify the spatial distribution of snail habitats on farms. In the longer term, after an assessment of the variance in crucial rates at various spatial levels, these models should also incorporate parasite adaptation. As the life cycle of the parasite is complex, it will be essential to use sensitivity analyses to identify key parameters and strip prediction models down to the key drivers (Smith, 2011). This would enable models to be run with limited farm-level input data, on mobile platforms, and hence enhance their value in decision support.

CONCLUSIONS AND PERSPECTIVES

Our review highlights that fasciolosis should be considered an important production disease of cattle in Europe. In the countries that report results, prevalences remain high, despite ongoing control efforts and renewed attention to the disease.

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In several areas significant scientific progress has been made over the last decade such as the development of diagnostics, understanding and prediction of spatial distribution of disease occurrence and effects of fluke on susceptibility to co-infection and diagnosis of other infections. In other areas such as the development of new flukicidal compounds little progress has been made (Fairweather and Boray, 1999) and therefore has not been included in this review. In Fig. 1, we identify the key areas for future research. As stated above, major progress could come from new anthelmintic medicines. The major bottlenecks for current flukicides are (1) their limited activity against immature stages; (2) the prohibition of their use in animals producing milk for human consumption; and (3) the development of anthelmintic resistance. The development of new medicines that overcome some or all of these issues would be a major advancement. The low discovery and development rate of new medicines may to a large extent be explained by low economic incentives to the pharmaceutical industry. However, with the increasing concerns about fasciolosis the balance is tilting the other way and investment in new drug development is needed. In addition, the European Commission and the pharmaceutical industry are making considerable efforts to promote research into vaccine development (Golden *et al*. 2010) and establish maximum residue limits for all flukicidal drugs (European Commission Implementing Decision C(2012)8604), potentially allowing their use in dairy cattle.

Some topics are receiving renewed attention such as the biology of intermediate host and free-living stages. Better understanding of the climatic and environmental conditions on their propagation/survival is key to improved temporal and spatial prediction of disease occurrence.

Development of diagnostics, which has contributed much already to advances in understanding and application, should further focus on the relationship between morbidity and productivity in different epidemiological settings. The production impacts need to be further defined in growing cattle. Better understanding in this area is also expected from randomized clinical field trials that include assessment of fertility parameters. The evidence that *F. hepatica* modulates the immune response and affects the susceptibility and diagnosis of co-infections is an area that requires further elucidation, particularly where those diseases are part of a national control programme. A better understanding of the overall impact of fluke infection will allow close collaboration with agricultural economists and will place fasciolosis control next to other key farm management and animal health decisions in the whole-farm economic context. Ultimately, this would mean that we would stop controlling the parasite and start managing it.

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CONFLICT OF INTEREST

The SVANOVIR®F.hepatica-Ab ELISA is commercialized by Boehringer Ingelheim Svanova under a licence agreement with Ghent University.

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