Trematodes in farm and companion animals

The comparative aspects of parasitic trematodes of companion animals, ruminants and humans is presented by Maggie Fisher BVetMed CBiol MRCVS FRSB, managing director and Peter Holdsworth AO Bsc (Hon) PhD FRSB FAICD, senior manager, Ridgeway Research Ltd, Park Farm Building, Gloucestershire, UK

Trematodes are almost all hermaphrodite (schistosomes being the exception) flat worms (flukes) which have a two or more host life cycle, with snails featuring consistently as an intermediate host.

Dogs and cats residing in Europe, including the UK and Ireland, are far less likely to acquire trematode or fluke infections, which means that veterinary surgeons are likely to be unconfident when they are presented with clinical cases of fluke in dogs or cats. Such infections are likely to be associated with a history of overseas travel.

In contrast, the importance of the liver fluke, *Fasciola hepatica* to grazing ruminants is evident from the range and significance of this parasite in sheep and cattle. This importance has been reflected historically, in the research and development investment for chemotherapy, vaccinology, lifecycle intervention and animal welfare in livestock. For over half a century this investment has delivered a battery of treatments for fluke in cattle and sheep.

This article aims to provide an update on fluke in ruminants whilst utilising the depth of knowledge about fluke in ruminants to provide a context for fluke in companion animals. The public health significance of fluke will also be reviewed briefly.

Figure 1: Rumen fluke attached to the interior surface of the rumen.

KEY SPECIES

A number of trematode species are potential parasites of dogs and cats. The whole list of potential infections is long and so some representative examples are shown in Table 1. A more extensive list of species found globally in dogs and cats has been compiled by Muller (2000). Dogs and cats are relatively resistant to *F hepatica*, so despite increased abundance of infection in ruminants, there has not been a noticeable increase of infection in cats or dogs.

In ruminants, the most important species in Europe are the liver fluke, *F hepatica* and the rumen fluke, *Calicophoron daubneyi* (see Figure 1).

The rumen fluke has been recognised relatively recently as a common species, exceeding the prevalence of liver fluke in Ireland in one survey where approximately 31% of sheep and 44% of cattle were infected (Anon, 2011). A third species in ruminants, *Dicrocoelium dendriticum*, occurs less commonly (see Table 1). *Fasciola gigantica* is another species exotic to Europe but a significant cause of disease in cattle and humans elsewhere.

Examples of human infections are shown in Table 1.

DISTRIBUTION

Trematode distribution is determined by the availability of suitable hosts for all stages of the life cycle and environmental conditions that permit survival and transmission of immature stages outside a host.

Presence alone does not result in infection in potential hosts. For example, *Alaria alata* can infect dogs and cats and has been identified in a fox in Ireland (Wolfe et al, 2001), but evidence is lacking of it having entered the dog or cat populations in Ireland. Fluke infections of dogs and cats in Europe are relatively rare with examples of *Opisthorchis* spp and *Metorchis* spp infection of dogs that had been fed on raw fish (Schuster et al, 2007). *Opisthorchis felineus* infection has been identified as an emerging zoonosis in Europe, with infection reported in humans and companion animals in a number of European counties including Italy (Pozio et al, 2013). Fluke infections in dogs and cats are more common in parts of the American continent, with *Nanophyetus salmincola* found in dogs in north-west North America. *Alaria* spp are seen in northern North America. Infection with the lung fluke, *Paragonimus kellicotti*, is seen in both dogs and cats in the US. *Heterobilharzia americanum*, the canine schistosome, occurs in dogs in swamp lands of Louisiana along the coast and in the Mississippi delta. In more tropical regions, *Platynosomum fastosum*, the cause of 'lizard poisoning' in cats occurs, with cats infected by eating the final

Species	Intermediate host(s)*	Final host(s)	Location in final host	Appearance of egg in faeces
Family *Diplostomatidae*				
Alaria alata	Snail, frog	Fox, dog, cat, human	Small intestine	98-134µm x 62-68µm operculate
Family *Dicrocoeliidae*				
Dicrocoelium dendriticum	Snail, ant	Ruminants (dog, human)	Bile ducts	35-40µm x 29-30µm dark-brown operculate contains miracidium
Family *Fasciolidae*				
Fasciola hepatica	Snail	Ruminants (human)	Bile ducts	130-150µm x 90-100µm thin-shelled, operculate, yellow-brown
Fasciola gigantica	Snail	Ruminants (human)	Bile ducts	Similar to but larger than *F hepatica* 170-190µm x 90-100µm
Family *Heterophyidae*				
Apophallus donicus	Snail, fresh water fish	Heron, fox, dog, cat	Small intestine	35-40µm x 26-32µm
Cryptocotyle lingua	Snail, marine fish	Fish-eating birds and mammals, dogs and cats	Small intestine	34-38µm x 16-20µm
Heterophyes heterophyes	Snail, brackish water fish	Fish-eating mammals, fox, dog, cat, human	Small intestine	
Family *Opisthorchidae*				
Opisthorchis felineus	Snail Fresh water fish	Cat, dog, pig, fox, human	Bile ducts	Approximately 30µm contains miracidium
Family *Paragonimidae*				
Paragonimus kellicoti	Snail, crayfish	Wild carnivores, cat, dog	Lungs	90 x 50µm unipolar cap
Family *Paramphistomidae*				
Calicophoron daubneyi	Snail	Ruminant	Rumen	130-150µm x 90-100µm thin shelled, operculate, colourless
Family *Schistosomidae*				
Heterobilharzia Americana	Snail	Dogs, wild carnivores, humans	Mesenteric blood vessels	60-80µm x 74-113µm non-operculate contains miracidium
Schistosoma japonicum	Snail	Human, cattle, dog	Mesenteric blood vessels	
Schistosoma mansoni	Snail	Human, primates, rodent, dog	Mesenteric blood vessels	
Family *Troglotrematidae*				
Nanophytus salmincola	Snail, salmon	Fish-eating mammals and birds, dog, cat, human	Small intestine	64-80µm x 34-50µm indistinct operculum with small adjacent knob

Table 1: Summary of parasitic trematode species in dogs, cats, ruminants and humans. *Snail is consistently the first intermediate host, where a second intermediate host is named, this occurs in the lifecycle after the snail stage.

intermediate host, a lizard.

F hepatica occurs globally in ruminants while other species tend to be less widespread (see Table 2).

LIFECYCLES

The typical lifecycle of a fluke is illustrated by that of the liver fluke (see Figure 2). Miracidia hatch from eggs passed by the final host and infect snails. The species of snail is relatively specific for the fluke species, with *Galba truncatula* associated with both *F hepatica* and *C daubneyi*, for example. The two species are able to parasitise the same intermediate host, with examples of the two species co-existing in the same snail (Jones et al, 2015). The association of *C daubneyi* with *G truncatula* was reconfirmed in a recent survey conducted in Wales, whilst in the same survey, *F hepatica* stages were identified in two other species of snail, although the epidemiological significance of this has still to be determined (Jones et al, 2015). Asexual reproduction occurs in the snail, which results in the release of cercariae. These in turn, may encyst on grass to form metacercariae (see Figure 2) as in the case of liver fluke or may invade a second intermediate (or paratenic) host such as fish (see Table 1) or, in the case of *D dendriticum*, an ant.

The lifecycle is completed when the metacercarial stage or its equivalent is eaten by the final host. The exception to trematode infection occurring by ingestion is seen in the schistosomes where the infective stage penetrates the skin of the final host when the host is in water.

Dogs and cats may feature as final hosts within what is normally, a typical lifecycle, such as that of *A alata* where the second intermediate host is an amphibian, with companion animals infected by eating an infected frog (see Figure 3). Alternatively, for some fluke species dogs and/or cats are normal final hosts, as exemplified by the canine schistosome *H americana*, or, in the case of other species, they may form a reservoir for human infection.

Canada/US and South America	Europe	Africa	Russia	East Asia
Alaria alata ***Dicrocoelium dendriticum*** ***Fasciola hepatica*** ***Heterobilharzia americana*** ***Nanophyetus salmincola*** ***Paragonimus kellicotti*** ***Schistosoma mansoni***	***Alaria alata*** **(in wildlife)** ***Apophallus domicus*** **(Eastern Europe)** ***Dicrocoelium dendriticum*** ***Cryptotyle lingua*** **(Greenland)** ***Fasciola hepatica*** ***Heterophyes heterophyes*** **(France)** ***Opisthorchis felineus*** **(Central Europe including Italy)**	***Schistostoma mansoni*** ***Dicrocoelium dendriticum*** ***Fasciola hepatica***	***Fasciola hepatica*** ***Opisthorchis felineus***	***Dicrocoelium dendriticum*** ***Fasciola hepatica*** ***Heterophyes heterophyes*** ***Schistosoma japonicum***

Table 2: Global distribution of fluke species.

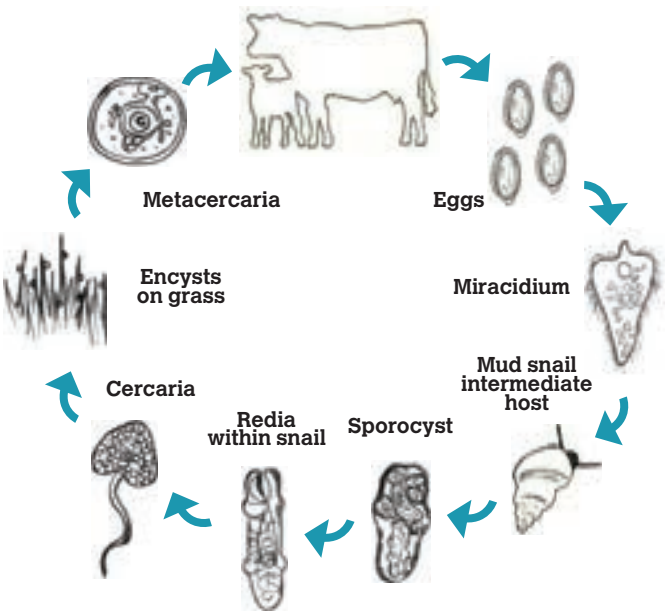

Figure 2: Lifecycle of the liver fluke, *F hepatica*.

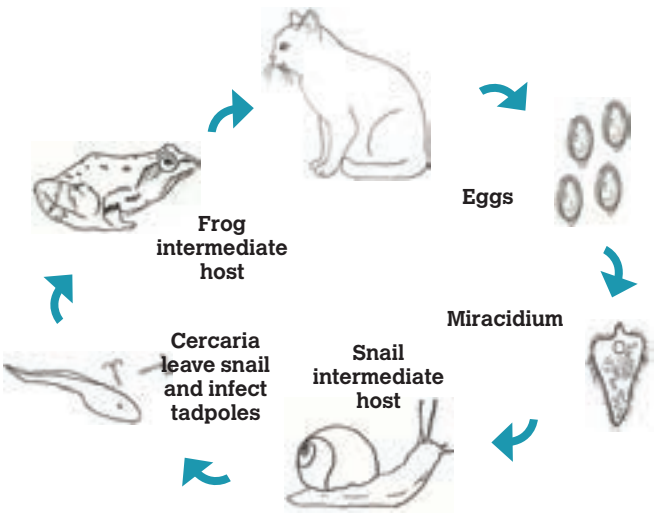

Figure 3: Lifecycle of the fluke, *A alata*.

Untreated, adult fluke are often long-lived, producing eggs that are shed intermittently in the faeces of the final host.

CLINICAL SIGNS

Depending on the fluke species, clinical signs may be associated with migration of immature stages or with the presence of adult fluke. Clinical signs associated with juvenile stages is most likely after a large infection over a short period of time. For example, migration of immature *F hepatica* through the liver can be associated with inappetance, weight loss and even death (Mulligan, 2011). A recent report of deaths within a flock of sheep associated with many immature rumen fluke migrating from the small intestine to the rumen, associated with poor condition and profuse scour, illustrates the potential for rumen fluke to be pathogenic (SAC, 2016). Migration of immature *Alaria spp* in dogs may be associated with pulmonary signs in affected dogs or cats (Bowman, 1999).

One exception to low levels of infection being well tolerated is seen in 'salmon poisoning' in dogs. The acute disease is associated with ingestion of *Nanophyetus salmincola cercariae* within raw fish and is caused by co-infection with *Neorickettsia helminthoeca*. Clinical signs include haemorrhagic enteritis, with fever, anorexia, vomiting and lymphadenopathy (Muller, 2000).

Various clinical signs are associated with adult fluke infections, largely dependent on the size of the infection and the affected organs. For example, canine schistosomosis is associated with watery diarrhoea, weight loss, vomiting and lethargy. Lizard poisoning in cats as a result of *P fastosum* infection can result in hair loss, poor condition, diarrhoea, vomiting, icterus, dehydration, slight fever and harsh respiratory signs (Basu and Charles, 2014).

Chronic infection can result in clinical signs, for example, chronic liver fluke infections in cattle result in long-term changes to the liver including thickening and calcification of the bile ducts. In humans, chronic infections are invariably associated with severe morbidity, with symptoms mainly organ-specific related to the location of the adult worms. In fascioliosis, the adult worms lodge in the larger bile ducts and the gall bladder, where they cause inflammation, fibrosis, blockage, colic pain and jaundice. Liver fibrosis and anaemia are also frequent with clonorchiosis and opisthorchiosis, where the adult worms lodge in the smaller bile ducts of the liver, local inflammation and fibrosis is caused eventually resulting in cholangiocarcinoma, a severe and fatal form

of bile duct cancer, hence two species of fluke, including *Opisthorchis viverrini*, (but not O *felineus*), are classified as carcinogenic agents. Paragonimiosis, where the final location of the worms is the lung tissue, can result in symptoms similar to those associated with tuberculosis including a chronic cough with blood-stained sputum, chest pain, dyspnoea and fever.

DIAGNOSIS

Diagnosis of fluke infection depends on history (normally involving overseas travel in the case of cats and dogs) and relevant clinical signs. Clinical signs are related to, in the case of immature fluke, the organ(s) that the fluke migrate through and, in the case of adult fluke, their location. Elimination of differential diagnoses, haematology and biochemistry parameters may also assist in reaching a diagnosis. For example, fluke infection of the liver in dogs can be associated with elevated liver enzyme levels. Radiography and ultrasound imaging may also be helpful.

Microscopical detection of *Fasciola* eggs, from faeces in faecal egg counts (FEC), while widely and routinely used is relatively unreliable and time consuming to perform (Martinez-Perez et al, 2012). Detection of fluke eggs requires modification of the normal flotation techniques used for nematode egg detection. For example with *F hepatica* detection a sedimentation technique is commonly used. The use of water in FEC causes some fluke eggs to hatch and so dilution fluids have to be modified. Trematode eggs from different species have common features such as an operculum, but there are a wide variety of egg appearances, making identification a specialist skill. Egg appearance for a range of trematode species are shown in Table 1. It may be possible to confirm diagnosis in the research setting by deoxyribonucleic acid identification of species.

Factors including host age, faecal water content and the number of aliquots tested per sample (Honer, 1965) and maturity of fluke can contribute to the variability in detecting *Fasciola* eggs in faeces. All these factors can result in recurring false-negative results. However, the more faecal samples tested the increase in sensitivity of detection (Rapsch et al, 2006). As is the case with nematode infections, the number of eggs detected per gram of faeces does not necessarily relate to the intensity of the worm infection present in an infected host (Alvarez Rojas et al, 2014). *C daubneyi* eggs are similar in size and appearance to those of *F hepatica*, so may add another layer of complexity if mis-identified. They can be distinguished by their colour as they are almost colourless whereas *F hepatica* eggs are yellowish-brown.

Post-mortem examination and identification of immature or adult fluke remains a gold standard for confirmation of infection, although this may not be possible for companion animals. Liver fluke diagnosis can be confirmed at post-mortem examination of animals that have succumbed or been selected from a flock/herd and euthanased. This also permits intensity of *Fasciola* infections to be established (Urquhart et al, 1996). Livers are examined for juvenile and bile ducts for adult worms and associated pathological changes. Examination of bile ducts for adult worms is relatively easy, with locating immature worms within the liver tissue more difficult as they may be as small as a pinhead. Pseudoparasitism may be seen when a carnivore, including humans, ingests raw liver containing fluke eggs which may, in turn, be passed in faeces.

Research into tests identifying either antigens or antibodies has attracted a lot of investment for liver fluke. For example,various antigens from *F hepatica*, including excretory/secretory products, tegumental components, crude extracts from adult worms and recombinant proteins have been used in assays, with several commercialised to detect antibodies, for example:

- Bio-X Diagnostics (La Jemelle, Belgium): an indirect antibody-detection test was commercially established using specific serum antigen MM3 (Mezo et al, 2003). This test purportedly was able to detect serum antibodies in sheep infected with small numbers (5-40) of metacercariae (Alvarez Rojas et al, 2014);
- Pourquier Kit (Montpellier, France): a fraction 2 (F2) antigen-based commercial test kit for use only with cattle. Rapsch et al, 2006, used the kit to estimate the prevalence of fascioliasis in cattle at an abattoir with a resulting sensitivity of 91.7% compared to 69% for coprology and 63.2% for classical meat inspection.

There is also a commercialised assay available to detect excretory/secretory antigen in faeces:

- Bio-X Diagnostics direct faecal antigen test in an enzyme-linked immunosorbent assay (ELISA) format. Alvarez Rojas et al (2014) summarised the advantages and disadvantages of currently available diagnostic tests for *F hepatica*. Detection of *Fasciola* antigens in host body fluid has taken primary focus as these tests have an advantage over antibody detection because antigenemia implies recent and active infection (Tak et al, 2014).

Similar tests exist for some trematode infections in dogs and cats but because each test is specific for a particular infection, such tests are best used as a confirmation and are unsuitable for a 'fishing expedition'. The presence of *Opisthorchis spp* and *Metorchis* spp antibodies were detected in sledge dogs using ELISA tests adapted for canine serum (Schuster et al, 2007), and levels fell in repeat samples taken 10 weeks after treatment.

PUBLIC HEALTH

Liver fluke infection in the UK and Ireland is estimated to result in around 30% of livers being rejected at slaughter, due to the pathology associated with infection (Skuce and Zadoks, 2013). In 1994, the annual cost associated with *Fasciola* species was estimated at ~$3bn worldwide (FAO, 1994).

Humans can be infected with liver fluke by accidentally ingesting metacercariae on vegetation. This results in liver fluke establishing in the liver in a similar way to fluke in sheep or cattle. Early and light infections of the various fluke infections in humans often pass unnoticed, as they are asymptomatic or only scarcely symptomatic. However, as with

Age of fluke (weeks)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Flukicide														
Albendazole										Some activity		Effective		
Oxyclozanide														
Nitroxynil							Variable efficacy			Effective				
Closantel														
Triclabendazole	Effective			Effective										

Table 3: Effectiveness of *F hepatica* treatments against immature and mature fluke (adapted from Fairweather and Boray, 1999).

other host species, if the worm load is high, general malaise is common and severe pain can occur, especially in the abdominal region, and this occurs most frequently in the case of fascioliosis.

Normally, adult fluke in the final host do not constitute a direct infection risk to other animals or humans as infection is acquired through ingestion of the metacercarial or cercarial stage. However, dogs or cats may be reservoir hosts for some zoonotic fluke infections (see Table 1).

CONTROL

Control here is taken to include the treatment of an individual animal or human, mass treatment of a group of individuals or a series of interventions that may or may not include flukicidal use to eliminate, reduce or prevent infection.

Fluke infections in dogs or cats in the UK or Ireland are likely to be individual events, with treatment of the affected individual(s) necessary, together with assessing for any likelihood of the infection having established its lifecycle locally. In fluke infections in dogs and cats, there is a lack of licensed treatments, relying instead on recommendations from published papers. For example, oral treatments of 20 or 50mg/kg bodyweight praziquantel on one occasion or 20mg/kg twice at 24-hour intervals were successful in eliminating *Metorchis* spp and *Opisthorchis spp* infection from a kennel of sledge dogs as assessed by elimination of eggs and reduction of antibody titres (Schuster et al, 2007). Praziquantel at a dose-rate of 20mg/kg/day for three to five days, is the preferred treatment for infection with *P fastosum* in cats (Basu and Charles, 2014). *Heterobiliharzia* in dogs has been treated with fenbendazole at 40mg/kg for 10 days (Bowman, 1999). Praziquantel at 23mg/kg for three days has been shown to be effective against *P kellicotti* infection in cats. Treatments for other fluke infections in dogs and cats have been reviewed by Bowman (2009).

Treatment of *F hepatica* infection in sheep or cattle can be conducted using one of a range of licensed flukicides (see Table 3), although only triclabendazole (TCBZ) is effective against all stages of fluke.

As treatments do not have residual activity, they remove only those fluke present at the time of treatment and so are combined with management strategies into control programmes for cattle or sheep – more information can be found in control of worms sustainably ([COWs] www.cattleparasites.org.uk/) and SCOPs (www.scops.org.uk/), respectively. Treatment is complicated by a lack of flukicides approved for use in lactating dairy cows, anthelmintic resistance to TCBZ and a lack of suitable diagnostics.

Against a backdrop of increased liver fluke infection in sheep and cattle in the UK and Ireland, much work has gone into designing control programmes for ruminants. However, such programmes are not turnkey to implement, having to consider factors such as the farm history, topography, geographical location and the prevailing weather.

Identification and exclusion of snail habitats from livestock offers some measure of control. Drainage eliminates the snail and offers an effective means of control, but environmental schemes to protect wetland areas has reduced the opportunities for this. Keeping stock off the wettest fields in the autumn and the winter, when the incidence of disease is at its highest, can reduce the risk from fluke, together with preventing access to ponds and bogs, where possible.

Most *F hepatica* control programmes utilise flukicidal treatments as a mainstay. The choice of product and frequency of use will depend on the level of fluke challenge (which is assisted by forecasts), the time of year, and the management and husbandry systems on the farm. For example, for treatment in late summer and autumn, when new fluke infections will be establishing in their final hosts, a flukicide with activity against immature stages should be chosen. However, over reliance on TCBZ should be avoided whenever possible.

Control of foodborne trematodioses in humans aims to reduce the risk of infection and at controlling associated morbidity. Humans with confirmed infection can be treated with flukicides (see Table 4), and programmes have been devised for the mass treatment of populations identified as being at risk of infection.

Simplistically, prevention of infection relies on ensuring that final hosts do not have access to infective stages in the environment or in intermediate hosts. For example, rumen fluke and liver fluke prevention could rely on avoidance of introduction of infection and/or preventing access to wet areas or ponds that may provide habitats for the intermediate host snail *G truncatula*, and thus sources of metacercariae

Disease	Recommended drug and dosage
Clonorchiosis and opisthorchiosis	Praziquantel: 5mg/kg three times daily for two to three consecutive days
Fascioliosis	Triclabendazole: 10mg/kg in single administration (a double dose of 20mg/kg can be administered in case of treatment failure)
Paragonimiosis	Triclabendazole: 2 x 10mg/kg in the same day or Praziquantel: 25mg/kg three times daily for three days

Table 4: Summary of recommended fluke treatments for humans. Reference: WHO Foodborne trematodiases. Fact sheet updated March, 2016.

Figure 4: Prevention of fluke infection in domestic mammals depends on consistently preventing access to the infective stage. Here, the fencing around this wet-bottomed pit has fallen into disrepair, permitting grazing stock to access this typical *G truncatula* habitat and thus permit perpetuation of the liver fluke lifecycle.

on local vegetation. As with all such strategies, prevention relies on the management strategies such as fences being maintained (see Figure 4). Prevention of human infection depends on avoiding sources of cercariae, for example in raw or smoked fish or raw shell fish in endemic areas.

CONCLUSION

In contrast to *F hepatica*, fluke infections of dogs and cats are exotic infections to the UK and Ireland and, if encountered, are likely to have been acquired whilst travelling. Fluke infections of dogs and cats are often zoonotic, and so, whilst dogs and cats do not provide a direct threat to humans, they may be reservoir hosts for zoonotic infection. Accurate diagnosis provides some challenges, as do control and prevention. Control of the liver fluke, *F hepatica*, is an ongoing challenge for grazing ruminant production in the UK and Ireland. Research into understanding the emergent *C daubneyi*, its impact and control is ongoing. The lifecycle of *F hepatica* can be used as an example of other fluke life cycles Fluke infections are of considerable public health importance.

REFERENCES ON REQUEST

ACKNOWLEDGEMENTS

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READER QUESTIONS AND ANSWERS

1. SALMON POISONING IN DOGS IS CAUSED BY:

A *Calicophoron daubneyi*
B *Fasciola gigantica*
C *Nanophyetus salmincola*
D *Neorickettsia helminthoeca*

2. WHICH FLUKE INFECTION IS AN EMERGING PARASITE OF DOGS AND HUMANS IN CONTINENTAL EUROPE?

A *Alaria alata*
B *Apophallus donicus*
C *Opisthorchis felineus*
D *Paragonimus kellicoti*

3. WHAT WAS THE COST OF THE LIVER FLUKE ESTIMATED TO BE GLOBALLY EACH YEAR?

A $3m
B $3bn
C $0.3bn
D $ 0.03bn

4. WHAT IS THE PREVALENCE OF RUMEN FLUKE IN IRELAND?

A 44% of sheep and 31% of cattle
B 50% of sheep and 50% of cattle
C 31% of sheep and 44% of cattle
D 13% of sheep and 41% of cattle

5. CHRONIC FLUKE INFECTION IN HUMANS CAN RESULT IN:

A Recovery without clinical signs
B Cholangiocarcinoma
C Loss of memory
D Increased longevity

ANSWERS: 1: D; 2: C; 3: B; 4: C; 5: B