Herdsure® protocol for liver fluke infection in cattle herds

Herdsure® Chapter 3

AHVLA
Animal Health and Veterinary Laboratories Agency

Herdsure®

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Contents

Introduction

This protocol describes the processes used to determine the liver fluke status of a cattle herd and for control and subsequent monitoring of liver fluke status in the herd.

This protocol is suitable for both dairy and beef herds.

The three principal elements of this Herdsure® protocol for liver fluke are:

- sampling and testing to determine status
- advice on appropriate treatments, if any, following test results
- advice on appropriate management measures, if any, to reduce the risk of liver fluke infection following test results.

The protocol addresses only the measurement and control of *sub-clinical* liver fluke infection in cattle herds. It does ***not*** address any aspect of *clinical* liver fluke infection (fasciolosis). Fasciolosis is a significant disease of cattle and other animals which requires veterinary intervention, not provided by Herdsure®, for diagnosis and treatment. Effective control of *sub-clinical* liver fluke infection in cattle herds is likely to reduce the risk of clinical fasciolosis.

This protocol refers only to the control of liver fluke infection in cattle. If other susceptible animals are present on the farm, especially if they share grazing with cattle, liver fluke monitoring and, if necessary, control measures should also be applied to these animals. Your AHVLA Regional Laboratory is able to provide appropriate testing and advisory services for this purpose.

The liver fluke protocol comprises three levels of 'health status':

Level 1	Establishes the liver fluke infection status of the herd.
Level 2	Aims to reduce the burden of liver fluke in the herd to a level at which it does not significantly affect cattle production or welfare.
Level 3	Monitors and aims to maintain the improved (or established as satisfactory at Level 1) health status of the herd for liver fluke infection.

The disease

Introduction

The liver fluke *Fasciola hepatica* is a trematode parasite that infects grazing animals including cattle, sheep, deer, camelid species, rabbits and, occasionally, humans. The parasite causes liver damage by migration of immature larvae and blood loss by the feeding activities of the adult parasite. The term for diseases caused by the liver fluke is 'fasciolosis'.

The parasite has been endemic in the wetter western half of the UK for centuries. Over the past 15 years there has been significant spread to other parts of the UK, including the east of Scotland and East Anglia (Mitchell, 2002; Pritchard *et al*, 2005). This spread has been made possible by:

- the greater movement of infected animals, particularly sheep, to farms that operate poor biosecurity
- changing weather patterns, with increased rainfall and milder winters throughout all of the UK, which benefit the lifecycle of the parasite and its intermediate snail host.

The liver fluke is now considered to be endemic in all regions of the UK.

Eradication of the liver fluke is difficult because wildlife reservoirs can maintain infection on many farms. The objective, therefore, is to reduce infection to a manageable level in order that:

- the economic benefits of control significantly outweigh the cost of control measures
- animal welfare is safeguarded.

Impact of the disease

The parasite can cause significant economic loss, with one estimate in 2001 for the economic loss worldwide put at $200 million (Mulcahy and Dalton, 2001). Another study of the financial loss in Switzerland in 2005 was estimated as €299 per infected animal (Schweitzer *et al*, 2005). A more recent study involving bulk tank milk samples estimated the loss of production in dairy cows as a decrease in annual average milk yield of 0.7 kg per cow per day and an increase in the mean inter-calving interval of 4.7 days (Charlier *et al*, 2007).

Pathogenesis and clinical signs

Lesions in the definitive host are seen in the liver parenchyma caused by migration of immature fluke and in the bile ducts caused by adult flukes. The parasite releases cathepsin protease enzymes, which cause tissue destruction that facilitates migration and feeding.

In addition, damage to the host's liver and bile ducts is caused by the spines attached to the outer layer of the parasite – the tegument.

Acute fasciolosis occurs when migration of large numbers of immature fluke cause tissue destruction, haemorrhage and inflammation to the liver that can lead to the death of the animal. It is estimated that 10,000 metacercariae ingested over a short period of time are required to cause acute fasciolosis. The symptoms of acute fasciolosis are seen more commonly in sheep and only rarely in cattle because the bovine liver is larger and more able to withstand this challenge.

Chronic fasciolosis is more common in cattle and occurs when small numbers of metacercariae are ingested over several weeks (trickle infection). The lesions are of hepatic and bile duct inflammation and necrosis and, later, liver and bile duct fibrosis. The fibrotic reaction is more pronounced in cattle compared to sheep. The adult fluke's ability to feed can be limited by this fibrosis.

Clinical signs in cattle are associated with blood feeding of the adult parasite and associated hypoalbuminaemia and manifest as weight loss and reduction in milk yield. In severe cases there is anaemia, subcutaneous oedema ('bottle jaw') and recumbency. Chronic fasciolosis is commonly associated with diarrhoea in cattle.

Metabolic disease associated with chronic fasciolosis has also been described (Mason, 2004).

Subclinical liver fluke infection results in poorer growth rates in growing cattle and lowered milk yields in dairy cows, without any other obvious clinical signs.

Testing for the disease

The gold standard for diagnosis of liver fluke infection is necropsy examination and finding the parasite in the liver.

(1) The detection of liver fluke eggs in faeces

A positive result indicates *current* liver fluke infection. The test has a sensitivity of 60%. There are a number of reasons for this. Faecal egg detection cannot confirm immature fluke infection where no eggs are produced. Also, in chronic infections due to non-specific immunity, it is possible that very small numbers of eggs are produced. Finally, liver fluke eggs can accumulate in the gall bladder and may be excreted intermittently.

The composite fluke egg detection test (TC0689) is used in the Herdsure® liver fluke protocol. It is suitable for screening groups of animals for liver fluke infection and requires the collection and subsequent laboratory pooling and testing of 10 faeces samples.

Testing of four such composite samples, requiring the collection of 40 individual faeces samples, is specified in this Herdsure® protocol. This provides 95% confidence of detecting liver fluke eggs in at least one pool if 20% or more of the cattle in the herd are infected and excreting fluke eggs, assuming a test sensitivity of 60%.

Interpretation

The detection of liver fluke eggs in one or more of the four composites is interpreted as indicating significant liver fluke infection in the group.

(2) The detection of antibody to liver fluke in blood

An ELISA (TC0678) is used to detect antibody to liver fluke in blood. This is a means of detecting past exposure to liver fluke infection.

Measurable antibody develops 3 weeks after ingestion of metacercariae and persists for ***at least*** 3 months following infection.

This test is offered within Herdsure® as an alternative to faecal egg detection, as collection of clotted blood samples by a veterinary practitioner may, under certain circumstances, be more convenient than collection of floor faeces samples by the farmer.

Sampling is by collection of blood samples from a random selection of a calculated number of cattle that will provide 95% confidence of a positive result if 20% or more of the cattle in the herd are infected and possess antibody (see Table 2 following the Level 1 protocol).

Interpretation

Results will be presented as positive, negative or inconclusive.

- A result is positive when there is greater than 20% positivity (when compared to a positive control).
- A result is negative when there is less than 15% positivity (when compared to a positive control).
- A result is inconclusive when there is a percentage positivity between 15% and 20% inclusive (when compared to a positive control).

The presence of one or more positive or inconclusive ELISA results is interpreted as indicating significant liver fluke infection in the group.

(3) The detection of antibody to liver fluke in bulk tank milk (dairy herds only)

An ELISA (TC0692) is used to detect antibody to liver fluke in bulk tank milk.

The test result provides an estimate of the likely percentage of cows (that contributed milk to the bulk tank) that possess antibody to liver fluke. For this to be an accurate reflection of the status of the entire milking cow herd, at least 75% of the cows that are currently milking should have contributed to the bulk tank from which the sample was taken.

If a herd has more than one bulk milk tank, each should be sampled and each sample should be submitted for separate testing.

Interpretation

Results will be presented as positive, negative or inconclusive.

- A result is positive when there is greater than 40% positivity (when compared to a positive control).
- A result is negative when there is less than 27% positivity (when compared to a positive control).
- A result is inconclusive when there is a percentage positivity between 27% and 40% inclusive (when compared to a positive control).

A positive or inconclusive result is interpreted as indicating that 25% or more of the cows contributing to the bulk tank possess antibody to liver fluke.

A negative result is interpreted as indicating that less than 25% of the cows contributing to the bulk tank possess antibody to liver fluke.

Biosecurity

Effective control of liver fluke infection requires appropriate biosecurity measures to prevent the introduction, or re-introduction, of fluke infection onto the farm and into the herd.

Ideally, animals that are to be introduced onto the farm from elsewhere should be **either**:

- sampled and tested for antibody to liver fluke, with a 'negative' result required before entry to the farm of destination or release from quarantine (note that a false negative result could arise from ingestion of liver fluke metacercariae by a naïve animal in the 2 weeks before sampling)

or

- treated with a flukicide (that kills both adults and immature liver fluke) 4 weeks before they are moved onto the pastures of the farm of destination. (This allows time for any fluke eggs in the intestinal tract to be expelled before arrival.)

If this is not possible, then animals should be quarantined on arrival on the farm of destination. They should not have access to pastures for 4 weeks after being treated with a flukicide (that kills both adults and immature liver fluke). Faeces should not be put directly onto grazing land because of the risk of seeding pasture with fluke eggs.

Both pre-movement and quarantine treatments may need to be repeated if bought-in animals have been very recently infected.

The risk of introducing infected animals that have fluke that are resistant to triclabendazole should be considered. If there is evidence of lack of efficacy of triclabendazole on the farm of origin, added animals should be given another flukicide before quarantine. The risk of introducing resistant fluke is reduced by not sourcing animals from farms with a history of non-efficacy of triclabendazole or any other flukicide.

Treatment and control

If liver fluke infection is detected by Herdsure® liver fluke Level 1, Level 2 or Level 3 testing, then treatment is indicated.

Treatment strategies should be reviewed by the veterinary practitioner and modified, if necessary, to address local risks and farm management practices. In particular, rainfall and temperature have a significant effect on the risk of animals acquiring liver fluke infection. The risk of liver fluke infection is likely to increase significantly if there is high rainfall in summer and this may warrant further treatments in addition to those recommended below.

As stated above, fasciolosis is potentially a very severe disease and it is the duty of the person responsible for the care of the cattle on the farm to obtain and implement veterinary advice, beyond the scope of this protocol.

Which animals should be treated?

All animals that are considered to have been at risk of infection from the grazing of metacercariae at pasture should be treated.

When should treatments be given?

For non-lactating cattle

The minimum number and timing of recommended treatments are:

- Treatment 1: in winter, as soon as possible after the receipt of a positive Herdsure® liver fluke result.
- Treatment 2: in the subsequent May, following the receipt of a positive Herdsure® liver fluke result.
- Treatment 3: in the subsequent autumn or early winter (possibly at housing) following the receipt of a positive Herdsure® liver fluke result.

In years in which weather (rainfall and temperature) and possibly other local factors indicate a high risk of infection, additional treatments may have to be given.

For lactating cattle

In most dairy herds it is likely that treatment will be given at the time of ‘drying-off’ in order to comply with manufacturers’ instructions and to avoid lengthy periods of milk withdrawal. A local assessment of risk should be made to determine if more immediate treatment of milking cows is necessary.

What type of flukicide should be used?

- Treatment 1: a flukicide effective against both adult and immature liver fluke.
- Treatment 2: for cattle that are housed over the winter, a flukicide effective against adult liver fluke; for cattle that graze pastures that may be contaminated with liver fluke metacercariae, a flukicide effective against both adult and immature liver fluke.
- Treatment 3: a flukicide effective against both adult and immature liver fluke.

See the following websites for further information about flukicide treatments:
www.noahcompendium.co.uk
www.norbrook.co.uk

Mixed grazing

On mixed cattle and sheep farms where fluke infection has been diagnosed, treatment of sheep is advised. Please contact your AHVLA Regional Laboratory if you require advice on the treatment or control of liver fluke infection in sheep. Other susceptible animals on the farm, such as goats and camelids, should also be treated.

Other control measures

It is advisable to keep lactating cattle on the drier, less infective pastures, particularly in the autumn because of difficulties in treating the dairy herd. Wetter areas of the farm, or around ponds and streams, can be fenced off. Drainage of land is largely discouraged now, particularly in regions that are designated environmentally sensitive areas. For the same environmental considerations, molluscicides are not a realistic means of control.

Resistance to triclabendazole

Resistance to triclabendazole has been reported in liver fluke in sheep in the UK, the Netherlands and Australia. This has implications for the treatment of cattle.

Forecasting fasciolosis

The following factors increase the risk of infection:

- warmer and wetter weather
- encouragement of wetland habitats and other environmentally sensitive schemes
- increased movement of sheep across the UK that introduce infection to farms, if biosecurity measures are not implemented on arrival
- greater survival of metacercariae on pasture from the previous year because of milder winters, and so the metacercariae would be present to infect grazing animals newly turned out to grass
- greater survival of fluke eggs over the winter so that they are present to infect snails in the spring, thus beginning the lifecycle
- preferential grazing of cattle in wet habitats favoured by the intermediate snail hosts.

The most important of these factors are increased rainfall and warmer temperatures (Ollerenshaw, 1974).

Monthly and quarterly disease surveillance reports produced by AHVLA will highlight periods of high risk of liver fluke infection. See http://www.defra.gov.uk/vla/reports/rep_surv.htm.

Keeping track of your herd's progress in Herdsure®

An **annual herd progress report** will be issued to Herdsure® members. The progress report will detail the level achieved for each protocol for which the herd is enrolled on the date of issue.

For a small fee, an updated progress report can be produced. The updated progress report, like the annual report, will detail the level achieved for each protocol for which the herd is enrolled on the date of issue.

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The protocol

Level 1

Level 1: Objective of Herdsure® testing

- Level 1 establishes the liver fluke infection status of the herd.

Level 1: Sampling protocol

Also refer to the flowchart summary of the protocol at the end of Level 3.

1.1	**When to sample:** ▪ in November, December or January **and, for dairy herds:** ▪ when at least 75% of the milking cows are contributing milk to the bulk tank. (If fewer than 75% of cows are contributing milk to the bulk tank in November, December or January, contact the Herdsure® helpline for guidance.)
1.2	**What to sample:** ▪ the main cow herd: the lactating cows in a dairy herd and the main group of suckler cows in a beef herd **and** ▪ the other 'at risk' cattle (see paragraph 1.3).
1.3	**Definition of 'at risk' cattle group** **The 'at risk' groups for sampling should *include*:** ▪ cattle that have grazed pastures of the subscribed farm **and** experienced an interval of at least 12 weeks between the earliest exposure to pasture (in the preceding grazing season) and sampling. **The 'at risk' group for sampling should *exclude*:** ▪ the main cow herd (sampled separately) ▪ cattle that have been treated for liver fluke infection within the 3 months before sampling ▪ bought-in cattle, wherever possible (contact the Herdsure® helpline if further guidance is necessary) cattle with a significantly low risk of having acquired liver fluke infection for any other reason (for example, animals that have grazed pastures of likely low risk of harbouring fluke-infected snails)

	▪ cattle that have co-grazed with the main cow herd and that are thought to have the same or lower risk of having acquired liver fluke as the adult cows (for example, suckler calves). **In a *dairy* herd,** the 'at risk' group could, therefore, include dry cows and other cows not contributing to the bulk tank, replacement heifers, grazing youngstock and calves and bulls that have had access to pasture. **In a *beef* herd,** the 'at risk' group could, therefore, include cows managed separately from the main suckler herd, heifer replacements, beef youngstock and weaned calves and bulls that have had access to pasture.
1.4	**Farms with a dairy component** **(1)** A milk sample should be collected from the bulk tank (see Appendix 2). **(2)** In addition, the 'at risk' group (see paragraph 1.3) should be sampled by **either**: ▪ collection of 40 floor faeces samples (see Appendix 2) **or** ▪ collection of clotted blood samples (red top) from a statistically valid number of cattle (see Table 2 below). Additionally, if the cattle on a single subscribed farm are subdivided so that they exclusively graze two or more distinct and separate areas of land then they should be sampled as if they are two separate herds. For example, if a farm keeps a group of cattle on one set of pastures and an entirely different group of cattle on an entirely different set of pastures, each group should be sampled separately.
1.5	**Farms with no dairy component** **(1)** If there is no dairy component in the herd, then the main herd of suckler cows should be sampled by **either**: ▪ collection of 40 floor faeces samples (see Appendix 2) **or** ▪ collection of clotted blood samples (red top) from a statistically valid number of cattle (see Table 2 below). **(2)** In addition, if there is also an 'at risk' group (see paragraph 1.3), it should be sampled by **either**: ▪ collection of 40 floor faeces samples (see Appendix 2) **or** ▪ collection of clotted blood samples (red top) from a representative number of cattle (see Table 2 below). Additionally, if the cattle on a single subscribed farm are subdivided so that they exclusively graze two or more distinct and separate areas of land then they should be sampled as if they are two separate herds. For example, if a farm keeps a group of cattle on one set of pastures and an entirely different group of cattle on an entirely different set of pastures, each

	group should be sampled separately.

1.6	All samples should be submitted in the same parcel with the paperwork provided (in advance) to AHVLA using the Herdsure® Business Reply labels.

Table 2: Statistical-based sampling according to group size

Number of cows in group	Number of cows to test from group
1–10	All
11–15	10
16–30	12
31–50	13
51–190	14
>190	15

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Assumptions

- It is expected that, if liver fluke is present in the herd, at least 20% of cattle in a group will possess antibody.
- Cattle that are at a significantly lower risk of having acquired liver fluke on the farm (e.g. animals that have not grazed or that have been treated) are excluded from the group to be sampled.
- All animals for testing should be selected at random.
- Sero-positivity is relatively independent of age, stage in lactation cycle and seasonality.

Level 1: Testing protocol and interpretation of results

1.7	Clotted blood samples will be tested by ELISA (TC0678) for antibody to liver fluke. Results will be presented as positive, negative or inconclusive. ***Interpretation*** ▪ A serum sample with percentage positivity of greater than 20% (of a positive control) is regarded as positive. ▪ A serum sample with percentage positivity of between 15% and 20% (of a positive control) is regarded as inconclusive. ▪ If one or more of the samples from the group are recorded as positive or inconclusive for antibody, the herd will be classed as significantly infected.
1.8	Faeces samples will be pooled into four composites of 10 samples each. The composite faeces fluke egg detection test (TC0689) will be used to determine the presence of fluke eggs in each of four composite samples. Results will be presented as positive or negative. ***Interpretation*** ▪ Detection of one or more fluke eggs in a composite sample (of 10 individual samples) is regarded as positive. ▪ If one or more of the four composites are found to be positive for fluke eggs, the herd will be classed as significantly infected.
1.9	Bulk milk will be tested by ELISA (TC0692) for antibody to liver fluke. Results will be presented as positive, negative or inconclusive. ***Interpretation*** ▪ A bulk milk sample with percentage positivity greater than 40% (of a positive control) is regarded as positive. ▪ A bulk milk sample with percentage positivity between 27% and 40% (of a positive control) is regarded as inconclusive. ▪ A positive or inconclusive result indicates that 25% or more of the cows contributing to the bulk tank sample possess antibody to liver fluke.

What happens when Herdsure® Level 1 testing has been completed?

This depends on the results of testing.

- If one or more of the test results for the farm are positive or inconclusive, the herd enters Level 2 for a 12-month period.
- If all of the test results for the farm are negative, the herd enters Level 3.

Level 2

Level 2: Objective of Herdsure® testing

- Level 2 aims to reduce the burden of liver fluke in the herd to a level at which it does not significantly affect cattle production or welfare.

Level 2: Treatment and continued monitoring

Entry to Level 2 is by:

- detection of liver fluke infection in the herd by the Herdsure® Level 1 protocol

or

- demonstration of fasciolosis by another means (such as clinical diagnostic testing, necropsy or by meat inspection).

Level 2 of the Herdsure® liver fluke protocol spans a period of 12 months.

The veterinary practitioner should use the advice in this handbook and relevant information elsewhere to devise an appropriate 12-month treatment and control programme for liver fluke infection on the farm.

A herd progress report is issued after 12 months and the herd either remains at Level 2 or proceeds to Level 3.

Liver fluke infection in the herd should be treated according to the guidance given above in the section on 'Treatment and control'.

Management actions to reduce the risk of acquisition of liver fluke infection should be implemented according to the guidance given above in the section on 'Treatment and control – other control measures'.

Biosecurity actions to reduce the risk of the introduction of liver fluke infection should be implemented according to guidance given above in the section on 'Biosecurity'.

Level 2: Sampling protocol

Also refer to the flowchart summary of the protocol at the end of Level 3.

2.1	**When to sample** Continued monitoring for liver fluke infection is achieved by sampling: ▪ in November, December or January **and, for dairy herds:** ▪ when at least 75% of the milking cows are contributing milk to the bulk tank. (If fewer than 75% of cows are contributing milk to the bulk tank in November, December and January, contact the Herdsure® helpline for guidance.)

2.2 **What to sample**

Sample collection is the same as in Level 1, namely:

Farms with a dairy component

(1) A milk sample should be collected from the bulk tank (see Appendix 2).

(2) In addition, the 'at risk' group (some or all of the remainder of the herd: dry cows, suckler cows, bulls and young-stock (see paragraph 1.3)) should also be sampled by **either**:

- collection of 40 floor faeces samples (see Appendix 2)

  or

- collection of clotted blood samples (red top) from a representative number of cattle (see Table 2 given in the Level 1 protocol).

Farms with no dairy component

(1) If there is no dairy component in the herd, then the main herd of suckler cows should be sampled by **either**:

- collection of 40 floor faeces samples (see Appendix 2)

  or

- collection of clotted blood samples (red top) from a representative number of cattle (see Table 2 given in the Level 1 protocol).

(2) In addition, the 'at risk' group (some or all of the remainder of the herd: dry cows, suckler cows, bulls and young-stock (see paragraph 1.3)) should also be sampled by **either**:

- collection of 40 floor faeces samples (see Appendix 2)

  or

- collection of clotted blood samples (red top) from a representative number of cattle (see Table 2 given in the Level 1 protocol).

Additionally, if the cattle on a single subscribed farm are subdivided so that they exclusively graze two or more distinct and separate areas of land then they should be sampled as if they are two separate herds. For example, if a farm keeps a group of cattle on one set of pastures and an entirely different group of cattle on an entirely different set of pastures, each group should be sampled separately.

2.3 Sampling reminders will be sent by AHVLA each November.

What happens after the herd has been within the Herdsure® Level 2 testing, treatment and management protocol for 12 months?

This depends on the results of testing:

- If one or more of the test results for the farm are positive or inconclusive (see paragraphs 1.7, 1.8 and 1.9), the herd re-enters Level 2 for a further 12-month period. The liver fluke treatment and control programme is reviewed by the veterinary practitioner and the farmer and improved, if necessary.
- If all of the test results for the farm are negative, the herd enters Level 3.

Level 3

Level 3: Objective of Herdsure® testing

- Level 3 monitors and maintains the improved, or otherwise satisfactory, liver fluke status of the herd.

Level 3: Continued monitoring

Entry to Level 3 is by:

- all test results being negative following sampling according to the Herdsure® liver fluke Level 1 protocol

or

- all test results being negative following treatment, management and sampling according to the Herdsure® liver fluke Level 2 protocol.

Level 3 of the Herdsure® liver fluke protocol spans a period of 12 months.

A herd progress report is issued after 12 months and the herd either remains at Level 3 or reverts to Level 2.

Management actions to reduce the risk of the introduction of liver fluke infection should be implemented according to the guidance given above in the section on 'Treatment and control – other control measures'.

Level 3: Sampling protocol

Also refer to the flowchart summary of the protocol at the end of Level 3.

3.1	**When to sample** Continued monitoring for liver fluke infection is achieved by sampling: ▪ in November, December or January **and, for dairy herds:** ▪ when at least 75% of the milking cows are contributing milk to the bulk tank. (If fewer than 75% of cows are contributing milk to the bulk tank in November, December and January, contact the Herdsure® helpline for guidance.)

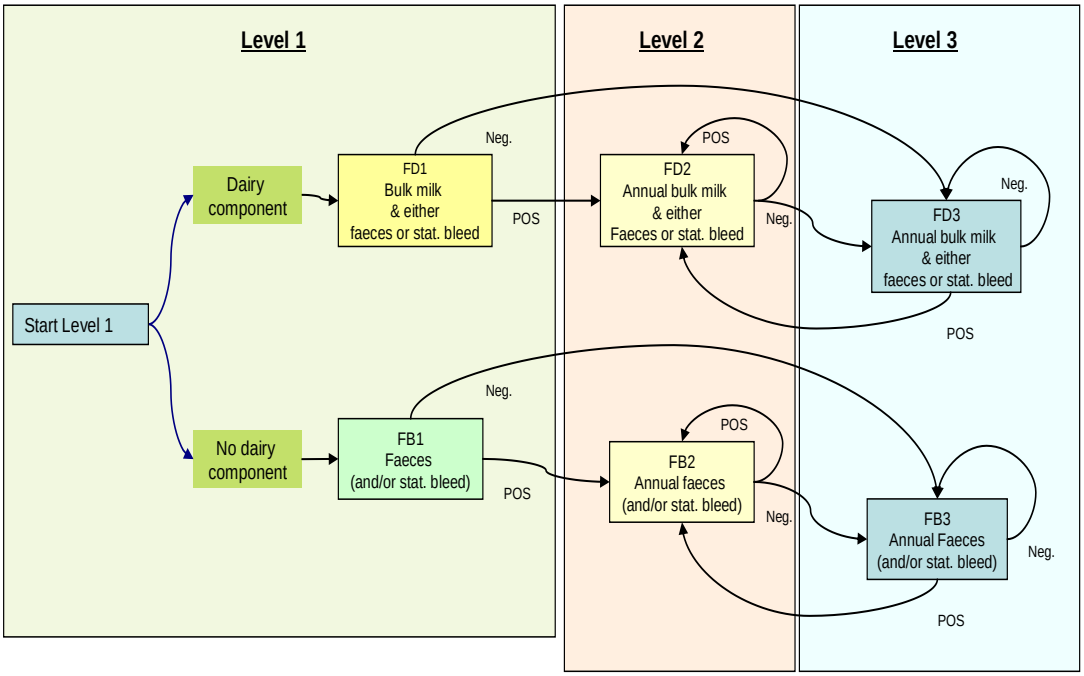
3.2	**What to sample** Sample collection is the same as in Level 1, namely: **Farms with a dairy component** **(1)** A milk sample should be collected from the bulk tank (see Appendix 2). **(2)** In addition, the 'at risk' group (some or all of the remainder of the herd: dry cows, suckler cows, bulls and youngstock (see paragraph 1.3)) should also be sampled by **either**: ■ collection of 40 floor faeces samples (see Appendix 2) **or** ■ collection of clotted blood samples (red top) from a representative number of cattle (see Table 2 given in the Level 1 protocol). **Farms with no dairy component** **(1)** If there is no dairy component in the herd, then the main herd of suckler cows should be sampled by **either**: ■ collection of 40 floor faeces samples (see Appendix 2) **or** ■ collection of clotted blood samples (red top) from a representative number of cattle (see Table 2 given in the Level 1 protocol). **(2)** In addition, the 'at risk' group (some or all of the remainder of the herd: dry cows, suckler cows, bulls and youngstock (see paragraph 1.3)) should also be sampled by **either**: ■ collection of 40 floor faeces samples (see Appendix 2) **or** ■ collection of clotted blood samples (red top) from a representative number of cattle (see Table 2 given in the Level 1 protocol). Additionally, if the cattle on a single subscribed farm are subdivided so that they exclusively graze two or more distinct and separate areas of land then they should be sampled as if they are two separate herds. For example, if a farm keeps a group of cattle on one set of pastures and an entirely different group of cattle on an entirely different set of pastures, each group should be sampled separately.
3.3	Sampling reminders will be sent by AHVLA each November.

Key to flowchart summary of the Herdsure® protocol for liver fluke infection

Step	Step name	
FD1	Initial sampling for herds with a dairy component	Bulk milk sample for liver fluke antibody ELISA (TC0692) for dairy herds together with a representative number of blood samples for fluke ELISA antibody testing (TC0678), or 40 faeces samples for fluke egg identification (TC0689) from 'at risk' cattle.
FB1	Initial sampling for beef herds	Blood samples from representative cattle for fluke ELISA antibody testing (TC0678), or 40 faeces samples for fluke egg identification (TC0689) from suckler cows and 'at risk' cattle. Blood samples from one group and faeces samples from the other, or the same sample type for each group are acceptable.
FD2	Annual sampling for herds with a dairy component	Bulk milk sample for liver fluke antibody ELISA (TC0692) for dairy herds together with a representative number of blood samples for fluke ELISA antibody testing (TC0678), or 40 faeces samples for fluke egg identification (TC0689) from 'at risk' cattle.
FB2	Annual sampling for beef herds	Blood samples from representative cattle for fluke ELISA antibody testing (TC0678), or 40 faeces samples for fluke egg identification (TC0689) from suckler cows and 'at risk' cattle. Blood samples from one group and faeces samples from the other, or the same sample type for each group are acceptable.
FD3	Annual sampling for herds with a dairy component	Bulk milk sample for liver fluke antibody ELISA (TC0692) for dairy herds together with a representative number of blood samples for fluke ELISA antibody testing (TC0678), or 40 faeces samples for fluke egg identification (TC0689) from 'at risk' cattle.
FB3	Annual sampling for beef herds	Blood samples from representative cattle for fluke ELISA antibody testing (TC0678), or 40 faeces samples for fluke egg identification (TC0689) from suckler cows and 'at risk' cattle. Blood samples from one group and faeces samples from the other, or the same sample type for each group are acceptable.

Flowchart summary of the Herdsure® protocol for liver fluke infection

Liver fluke



Note that all flowchart steps have been given short codes. These are used in the Herdsure® worksheets and report documents, helping to identify the exact steps as you progress through the protocol. © Crown Copyright Version 1, 2009; Version 2, 2010; Version 3, 2011; Version 4, 2011; Version5, 2012; Version 6, 2012. All rights reserved. Herdsure® is a registered trademark of the Animal Health and Veterinary Laboratories Agency.