

Herdsure[®] protocol for neosporosis in cattle

Herdsure[®] Chapter 6



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Introduction

This protocol describes the process for establishing the neosporosis disease status of cattle herds and for controlling and monitoring the disease where it is found to be present. It is suitable for both dairy and beef suckler herds.

The protocol does not describe procedures for the diagnosis of abortions that may be due to *Neospora caninum*, but it does provide the means to measure and reduce the amount of *N. caninum* infection in the herd.

The neosporosis protocol comprises a sampling, testing and management regime to establish herd health status at three different levels:

Level 1	Provides an indication of the herd's neosporosis status based on a snapshot bleed or historical data.
Level 2	Aims to improve the health status of the herd for neosporosis.
Level 3	Monitors the herd's improved neosporosis health status.

The disease

Introduction

Neosporosis is the disease in cattle caused by *Neospora caninum*, an apicomplexan protozoal parasite with worldwide distribution. The definitive host is the dog. Cattle are an intermediate host of the parasite and infection can result in abortion.

Impact of the disease

Although rare cases of neurological disease in congenitally affected calves have been described, by far the most common sign of neosporosis in cattle is abortion. Neosporosis is the most frequently diagnosed cause of abortion in cattle in the UK. The cost of abortion, which involves not just the loss of the calf but also reduced milk production and additional breeding costs, is well documented. Therefore, the cost of neosporosis to the UK cattle industry is significant.

The organism causes infection of cattle by one of two routes:

- endogenous ('vertical') infection in which the organism passes transplacentally from an infected dam to its calf, or
- exogenous ('horizontal') infection in which the pregnant cow ingests oocysts from dog faeces contaminating the environment, e.g. feed or pasture grass.

Cattle are most commonly infected via the endogenous route.

Both these routes give rise to infection of the foetus. Infection may, in turn, lead to abortion, usually between 3 and 8 months gestation (median 6 months). However, not all cases of intra-uterine infection lead to abortion; some infected calves are clinically normal. It is not currently possible to distinguish between the two routes using available diagnostic tests.

Infection can enter a naïve herd either via infective dog faeces contaminating feed or pastures or by the purchase of congenitally infected animals. It is perpetuated by vertical transmission and dogs may be infected by the ingestion of dead calves or placentae. Therefore, control of the disease is by a combination of biosecurity measures and the identification of infected animals and their removal from the breeding herd.

Testing for the disease

An ELISA (TC0175), which detects antibodies to *N. caninum*, is used for screening in the Herdsure[®] protocol. Sero-positive animals are approximately six to seven times more likely to abort compared to sero-negative animals. The sensitivity of this test is highest in animals that are in the late stage of pregnancy or that have recently aborted. All blood samples taken during the course of this protocol should be collected in plain (red top) vacutainer tubes without anticoagulant.

It is important for the success of the neosporosis protocol that all incidents of abortion are appropriately investigated. For further advice please contact your local AHVLA Regional Laboratory. Relevant samples should be submitted to your local AHVLA Regional Laboratory for diagnostic investigation; all such investigations should be carried out as AHVLA diagnostic submissions outside Herdsure[®]. (See the section entitled 'Additional testing of cattle from Herdsure[®] herds' on page 21 of this handbook.)

Antibody to *N. caninum* fluctuates throughout the life of an infected animal. Confirmation that an animal is not infected requires sampling at a specific time:

- during an animal's pregnancy (10 to 4 weeks before the due calving date), **or**
- at the time of abortion, provided this occurs within the period of 10 to 4 weeks before the due calving date.

Interpretation of results

N. caninum ELISA test results are expressed as 'percentage positivity'. These results will be interpreted by the AHVLA's laboratory management system as negative or positive. Interpretation may change if AHVLA changes the ELISA, but it is currently:

- sero-negative – samples showing a positive inhibition of <30%
- sero-positive – samples showing a positive inhibition of ≥30%.

Keeping track of progress in Herdsure[®]

Each registered holding will be issued an **annual herd progress report**. 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 additional progress report can be produced. This 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.

Biosecurity

It is the responsibility of the herd owner or manager in consultation with their veterinary practitioner to ensure good biosecurity in Herdsure® herds. Below is a list of biosecurity measures that are specific to the control of neosporosis. It is important that herds participating in Herdsure® Levels 2 and 3 conform to these.

- All dogs must be prevented from having access to the calving areas and any carcase material or placentae. This includes farm dogs, visitors' dogs, dogs belonging to members of the public and fox hounds.
- Placentae, stillborn calves and carcasses should be removed from the calving accommodation/paddocks as soon as possible to a secure location ready for removal by fallen stock contractors. This location should be inaccessible to vermin such as foxes, badgers, rats and mice. Dogs must also be prevented from gaining access to potentially infected material.
- The public and dogs must not have access to paddocks used for calving.
- Feed storage facilities, both 'straights' and forage, must be dog-proof to prevent contamination with dog faeces. They should also be vermin-proof to prevent contamination by foxes, badgers, rats and mice.
- Suppliers of feed should provide assurance that measures are in place to prevent contamination of feed by faecal material from dogs.
- Access of dogs to pasture used for cattle grazing or for the production of cattle forage should be kept to a minimum because it could increase the risk of cattle becoming infected with *N. caninum*.
- Added cattle: see the section on added cattle at the end of the protocol.

The protocol

Level 1

Level 1: Objective of Herdsure[®] testing

- The objective of Level 1 is to determine the neosporosis status of the herd. This is done by carrying out a **snapshot bleed**.

The snapshot bleed involves testing a specified proportion of the female breeding herd (using TC0175) 10 to 4 weeks before their expected calving date.

When determining the size of the breeding herd, all the cattle on the farm that are over 2 years of age and are or will be part of the breeding herd should be counted (see Table 5 below). If both milking and suckler cows are present on the same farm they should be considered as one group.

It is recommended that the snapshot bleed is carried out at a time when as many cattle as possible are between 10 and 4 weeks before calving. It is preferable that these are tested at one visit.


The snapshot bleed will provide an early indication of whether neosporosis is on the farm. However, it is not possible to conclusively prove the absence of infection by a snapshot bleed. Where tested cattle give negative ELISA results, these will count as one of the two negative tests results needed to classify them as neosporosis-free in Level 2.

Level 1: Sampling, testing and interpretation protocol

Choosing the animals to bleed for the snapshot bleed

1.1	<p>If the AHVLA laboratory reports for the previous year show a clear indication of sero-positive cows or confirmation of <i>N. caninum</i> in aborted fetuses by foetal serology or foetal histology, it is unnecessary to carry out the Level 1 snapshot bleed; the herd should enter the neosporosis protocol at Level 2.</p> <p>For any herd to complete a valid snapshot bleed, there must be enough cattle within the period 10 to 4 weeks before calving to make up a valid sample size as calculated from Table 5. The veterinary practitioner and farmer should decide if a valid snapshot bleed can be performed. If so, blood samples should be collected as instructed below and submitted to AHVLA Luddington for ELISA testing.</p>
1.2	<p>What to do if there are insufficient suitable animals for the snapshot bleed</p> <p>Herds with protracted calving periods may not have an adequate number of cows in the 10- to 4-week window to satisfy the required sample size. When review of the expected calving dates indicates that a valid snapshot bleed cannot be performed at a single visit, it is advised that herds should enter the protocol directly at Level 2.</p>

Results interpretation

1.3	The ELISA test results will be reported as either negative or positive.
1.4 	It is important that all incidents of abortion are investigated. Blood samples from aborted cows should be submitted to your local AHVLA Regional Laboratory for ELISA antibody testing outside Herdsure®. (See section on 'Additional testing of cattle from Herdsure® herds' on page 21.) All other relevant samples of aborted material should also be submitted to your local AHVLA Regional Laboratory for diagnostic and, if appropriate, statutory investigation with testing outside Herdsure®. Where blood samples prove positive or where <i>N. caninum</i> is confirmed as the cause of abortion in diagnostic samples the Herdsure® consultant must be informed by telephoning the Herdsure® helpline.
1.5	The presence of any infected animals is evidence of 'infected herd' status. The absence of any sero-positive animals does not confirm absence of infection but it is certainly a result that should be viewed optimistically before progressing to Level 2.

Rationale and sample size calculations for the snapshot bleed

Sampling should be carried out when as many cows as possible are in the 10 to 4 weeks pre-calving period.

It is assumed that, if *N. caninum* infection is present, at least 5% of adult cows will have measurable antibody between 10 and 4 weeks before calving. The test sensitivity in this period is 90%. To be 95% confident that at least one positive animal is detected, **at least** the number of in-calf cows (between 10 and 4 weeks before calving) shown in Table 5 should be sampled. Animals for sampling should be selected randomly.

If more than the minimum number of qualifying cows are present, all of these may be tested as the results will be of use during Level 2.

Table 5: Statistical-based sampling according to group size

Group size	Cows to sample
<23	all
23–25	22
26–30	26
31–35	31
36–40	35
41–50	36
51–55	39
56–60	43
61–80	47
81–100	51
101–150	53
>151	61

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Level 2

Level 2: Objective of Herdsure® testing

- The aim of Level 2 is to improve the neosporosis status of the herd by identifying and removing sero-positive cows. Successful completion of Level 2 will result in all the cows in the herd achieving neosporosis-clear status. The Level 2 testing is known as the progressive bleed.





An animal will be confirmed free of *N. caninum* infection when it has achieved two clear ELISA antibody tests on samples collected as described in the section 'Testing for the disease'. Any negative ELISA results gained in a Level 1 snapshot bleed will be credited to the individuals now entering Level 2.

Animals with positive ELISA test results should be removed from the breeding herd.

Once an animal is confirmed free of *N. caninum* infection, all the calves born to her are considered free of *N. caninum* and will not require antibody testing. Thus the amount of required testing of cows will reduce over time until the entire breeding herd is free. At that point all home-bred animals can be considered free of *N. caninum* infection.

Only when all the breeding animals have been confirmed free of *N. caninum* can the herd be classified as neosporosis-free and move to Level 3.

Level 2: Sampling, testing and interpretation protocol

2.1	Identification of breeding females
 	<p>The veterinary practitioner will be sent a list of all adult females on the farm. Those tested during Level 1 will be annotated with their snapshot bleed test result. This list should be photocopied and a copy returned to the Herdsure® consultant. A column is included to indicate those animals that are not in the breeding herd by annotating them 'NB'. This information is needed to produce the 'Neospora Status Reports'. (See the section below on 'Tracking the Level 2 progressive bleed.)</p>
2.2	Sampling of breeding females
 	<p>In Level 2, each breeding cow should be tested between 10 and 4 weeks before calving. This is a progressive bleed with sampling and testing repeated until each cow has two negative ELISA results. All the samples taken for the progressive bleed should be submitted to AHVLA Luddington. The eligible animals should be batched in groups of those fulfilling the 10- to 4-week pre-calving window to help economy of veterinary visits.</p>
<p>This table shows possible combinations of sampling times which will confirm</p>	

freedom from neosporosis in an individual animal (born to a dam of unknown status).

Period for sampling	Option 1		Option 2	
	First sample*	Second sample	First sample*	Second sample
10–4 weeks pre-calving	X		X	
10–4 weeks pre next calving		X		
Abortion, if 10–4 weeks pre-calving				X

*The ELISA result of the first sample must be negative in order to proceed to the second.

To assist with management of this process, the HMS will provide a list of all female cattle known to be in the breeding herd, showing the ELISA results of the individuals where appropriate. This list will be updated regularly (see section on ‘Tracking the Level 2 progressive bleed’ below).

2.3 Dealing with sero-positive cattle

Sero-positive animals should be removed from the herd.

Sero-positive animals should not be used for breeding. If herds have a high prevalence of positive animals they can be removed from the herd over a period of time to reduce the financial impact of their loss to the herd, provided they are not then used for further breeding. If these animals are inseminated, served or used as recipients for embryos they are at risk of either aborting or producing an infected calf. They may be retained and used for embryo donation or for fattening before they are removed from the herd or culled.

2.4 Progress through Level 2

The progressive blood sampling of female breeding cattle in the herd should continue until they have all reached an *N. caninum* infection-free status with two negative blood tests.

Calves born to cows with *N. caninum* infection-free status will be deemed to have inherited that free status and will not require any further testing.

2.5 Investigation of abortions

Any abortions that occur within the herd must be investigated to determine whether or not *N. caninum* has played a role.

The minimum requirement for herds that are in Level 2 of the Herdsure[®] protocol for neosporosis is the submission of a blood sample from the cow that has aborted for the *N. caninum* ELISA test. However, a definitive diagnosis of neosporosis as the cause of abortion can be made only by the submission of an aborted foetus. The relevant samples should be submitted to your local AHVLA Regional Laboratory for diagnostic investigation. Advice on these can be obtained from the local AHVLA Regional Laboratory. All tests on maternal blood samples and aborted foetuses will be carried out according to procedures for diagnostic submissions to AHVLA and not as part of Herdsure[®]. (See section on 'Additional testing of cattle from Herdsure[®] herds' on page 21.)

All positive *N. caninum* ELISA results must be reported to the Herdsure[®] consultant by telephoning the Herdsure[®] helpline. All such cattle should be dealt with as in paragraph 2.3.

Tracking the Level 2 progressive bleed: *Neospora* Status Reports

At Level 2, the HMS helps to manage the progressive bleed by storing results from each Herdsure[®] neosporosis submission. *Neospora* Status Reports (see example below) are used to summarise the status of individual animals. The *Neospora* Status Report has four main functions:

- It gives a picture of progress of the herd.
- It defines the status of all the breeding animals in the herd.
- It provides a means of recording calving dates for planning tests of in-calf animals.
- It can be used to identify which animals are to be used for breeding. When a *Neospora* Status Report is received for the herd, any cattle included in it that will **not** enter the female breeding herd should be annotated 'NB'. A photocopy of the amended report should be sent to the Herdsure[®] consultant who will ensure that these cattle are removed from the database list of female breeding cattle.

Neospora Status Reports are available at 1-, 2- or 6-monthly intervals but will be sent to the veterinary practitioner 6-monthly unless otherwise requested.

The figure below shows the layout for *Neospora* Status Reports. In this example the farm is halfway through the second year of a progressive bleed; most animals have been tested once and around half have been tested twice. Each of the target animals is listed by ear tag and date of birth (from CTS). Customers can use the first column to note expected calving dates in order to help with planning of sampling visits.

The status column indicates the current status of each animal. The status codes are explained in the key. The remaining columns show first and second test results for each animal where these have been carried out.

Neospora Status Report

Date 01 January 2012
Practice Gridlington Vets
Farm Home Farm, Gridlington
CPH 12/123/1234

Key	
NN	<u>Two negative results – confirmed sero-negative</u>
N	<u>One negative result – potentially sero-negative</u>
P	<u>POSITIVE (should be removed from herd)</u>
DN	<u>Dam Negative (NN so no test needed for offspring)</u>
U	<u>As yet Untested</u>

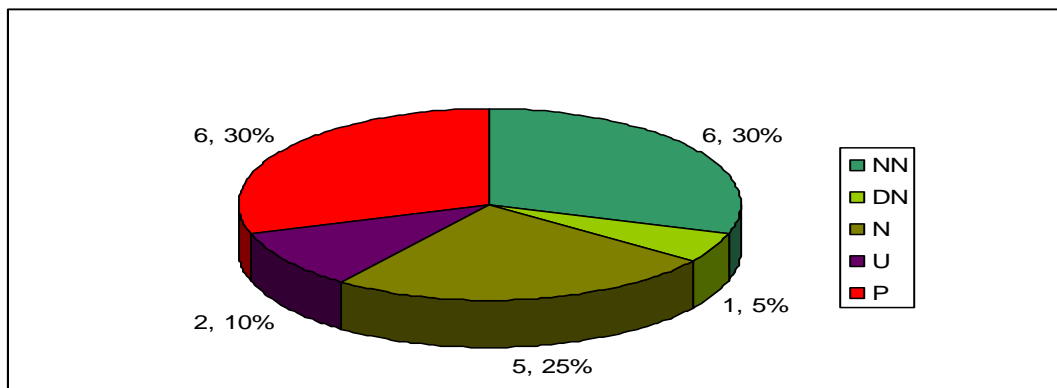
				Neospora test results			
				First test		Second test	
Due to calve?	Ear tag	Born	Status	Date	Result	Date	Result
	UK281526700065	01/02/08	NN	01/11/10	Negative	20/11/11	Negative
	UK602440100006	02/02/08	NN	30/11/10	Negative	20/11/11	Negative
	UK602440100024	06/02/08	P	30/11/10	Negative	20/11/11	POSITIVE
	UK602440100061	07/02/08	NN	01/11/10	Negative	20/11/11	Negative
	UK602440100066	08/02/08	NN	01/11/10	Negative	20/11/11	Negative
	UK602440100076	09/02/08	P	01/11/10	POSITIVE		-
	UK602440100122	10/02/08	P	30/11/10	POSITIVE		-
	UK602440100146	12/02/08	NN	12/12/10	Negative	20/11/11	Negative
	UK602440100150	13/02/08	U				
	UK602440100157	14/02/08	NN	01/11/10	Negative	20/11/11	Negative
	UK602440100164	15/02/08	P	30/11/10	Negative	20/11/11	POSITIVE
	UK602440100178	16/02/08	N	12/12/10	Negative		
	UK602440200011	17/02/08	N	20/12/10	Negative		
	UK602440200018	18/02/08	N	01/11/10	Negative		
	UK602440200019	14/02/10	DN				
	UK602440200025	20/02/08	P	01/11/10	POSITIVE		-

Current herd status

Veterinary practitioners can choose the frequency of these reports for each of their registered farms.

Pie chart as visual guide to the herd’s neosporosis progress

This pie chart gives a visual impression of the herd’s current status with respect to neosporosis. (See above for key.)




Level 3

Level 3: Objective of Herdsure[®] testing

- Level 3 testing aims to monitor and maintain the improved neosporosis health status of the herd.

Monitoring the continued freedom from infection is carried out by ELISA testing of all aborting cows.

Level 3: Sampling and testing protocol

3.1 	Strict biosecurity and adherence to the added animal policies must be maintained. (See the section on biosecurity at the end of this protocol.)
3.2	<p>Investigation of abortions</p> <p>Any abortions that occur must be investigated to confirm whether <i>N. caninum</i> has played a role.</p> <p>The minimum requirement for herds that are in Level 3 of the Herdsure[®] neosporosis protocol is the submission of a blood sample from each aborting cow for the <i>N. caninum</i> ELISA test. However, a definitive diagnosis of neosporosis as the cause of abortion can be made only by the submission of an aborted foetus. The relevant samples should be submitted to your AHVLA Regional Laboratory for diagnostic investigation. Advice on these can be obtained from your AHVLA Regional Laboratory. All tests carried out on maternal blood samples and aborted foetuses will be carried out according to procedures for diagnostic submissions to AHVLA and not as part of Herdsure[®]. (See section on ‘Additional testing of cattle from Herdsure[®] herds’ on page 15.)</p> <p>All positive <i>N. caninum</i> ELISA results and all results confirming <i>N. caninum</i> as the cause of abortion following examination and testing of abortion material must be reported to the Herdsure[®] consultant by telephoning the Herdsure[®] helpline. All such cattle should be dealt with as in paragraph 2.3.</p>
3.3	<p>Action if neosporosis is diagnosed as a cause of abortion</p> <p>If <i>N. caninum</i> infection is found to have played a part in the abortion, then the herd will revert to Level 2 while a herd investigation is carried out in the following way.</p> <p>Immediate blood sampling for ELISA testing of all adult animals in the same cohort as the aborting cow should be carried out. This cohort is defined as all animals which are due to calve or have calved from 1 month before to 1 month after the calving date of the aborted cow.</p> <p>If all of the cows and heifers in the cohort are sero-negative, the herd may return to Level 3.</p> <p style="text-align: right;">Sero-positive results in one or more animals in the cohort suggest an external</p>

source of infection and means the herd restarts Level 2 and all the breeding animals must requalify for Level 3. This will mean that the neosporosis-free status of all the breeding female cattle in the herd will be lost and the herd will begin sampling and testing for Level 2 again.

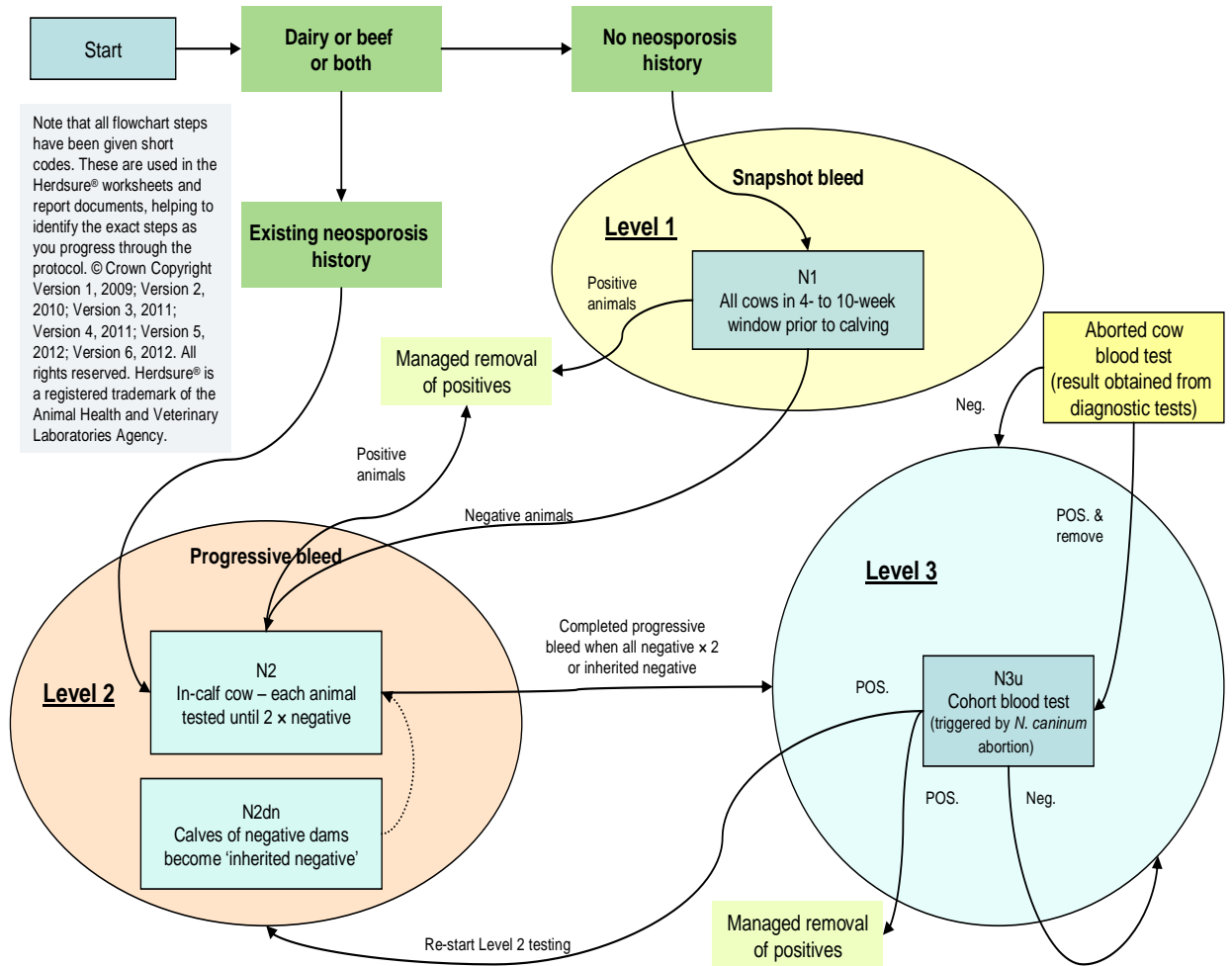
Farm biosecurity should be reviewed and, if necessary, improved following the breakdown in order to identify potential risks and routes of entry of *N. caninum* infection.

Key to flowchart summary of the Herdsure® protocol for neosporosis

Step	Step name	
N1	Snapshot bleed	Where the calving pattern allows for a significant proportion of the breeding herd to be sampled 10 to 4 weeks before calving, all of these cows should be tested using ELISA test TC0175. The herd includes both dairy and suckler cows where present. For the minimum number to be sampled refer to Table 5 in Level 1. A list of ear tags of breeding age cattle will be provided to assist with the sampling process. Positive animals are removed from the herd.
N2	In-calf blood sampling	A sampling worksheet is provided for submission of blood samples from all in-calf cows, 10 to 4 weeks before calving. Samples are submitted for the ELISA test TC0175. Positive animals are removed from the herd; negative animals are repeat tested in the window 10 to 4 weeks before calving until they are classified negative (2 x negative tests). The Herdsure® consultant may be able to assist with planning optimum sampling visits.
N2dn	Inherited negative – no testing	No sampling is needed here. Calves of dams that are classified negative (two negative Herdsure® tests) inherit their negative status. When this occurs the <i>Neospora</i> Status Report will be updated automatically.
	Abortion – ‘diagnostic submission only’	Although not part of the Herdsure® sampling protocol, any abortion must be investigated by submitting samples to your local AHVLA laboratory. As part of this investigation the minimum required is a TC0175 of a maternal blood sample. Positive results must be notified to the Herdsure® consultant.
N3u	Cohort blood sampling	Abortions involving neosporosis trigger immediate blood sampling of all in-calf animals in the same cohort. Samples will be tested by the ELISA for antibody to <i>N. caninum</i> (TC0175). This cohort is defined as all cows due to calve or that have calved from 1 month before to 1 month after the date of the abortion. Samples should be submitted using the unscheduled sample submission form (Form C in the ‘Forms’ section at the back of this handbook).

Flowchart summary of the Herdsure® protocol for neosporosis

Neosporosis



Procedures for added animals

Added animals are one of the most likely ways to introduce infection to a herd. Minimising the number of added animals will keep the risk of introducing infection low. You may wish to consider a closed herd policy.

If possible only buy animals that have been confirmed negative according to the Herdsure® protocol. These animals can be added without testing.

Cattle of 'unknown health status' can be introduced, but they should be subject to testing as described below before they join the remainder of the herd. Blood samples from all added animals should be submitted to AHVLA Herdsure accompanied by Form C (Unscheduled Submissions) for TC0175.

Added animals should preferably be blood sampled at the farm of origin and sero-positive animals should not be purchased.

If this is not possible, added animals should be tested on arrival and, if sero-positive to *N. caninum*, should be removed from the herd as soon as possible. Under no circumstances should any offspring be retained from sero-positive added animals to introduce into the breeding herd.

Animals that give negative results cannot be regarded as free of infection on the basis of one blood test. Sero-negative animals should be regarded as *potentially* infected until they have achieved two negative blood tests taken between 10 and 4 weeks before two calvings. This could include the pre-purchase blood sample if taken in this period of gestation. Although sero-negative cattle can enter the herd, they will be recorded on the HMS as only having attained one clear test and thus Level 3 herds will revert to Level 2 status. Only when all breeding cattle in the herd have achieved two clear blood tests at the appropriate stage of pregnancy can the Level 3 status of the herd be reinstated.

Embryos must only be implanted into sero-negative recipients.