

# ***Neospora* - a major problem for the British dairy industry**

The farmer's guide to tackling the disease



# Acknowledgements

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## 1. Importance of *Neospora caninum* infection and Bovine Neosporosis

### Q1

#### What is *Neospora*?

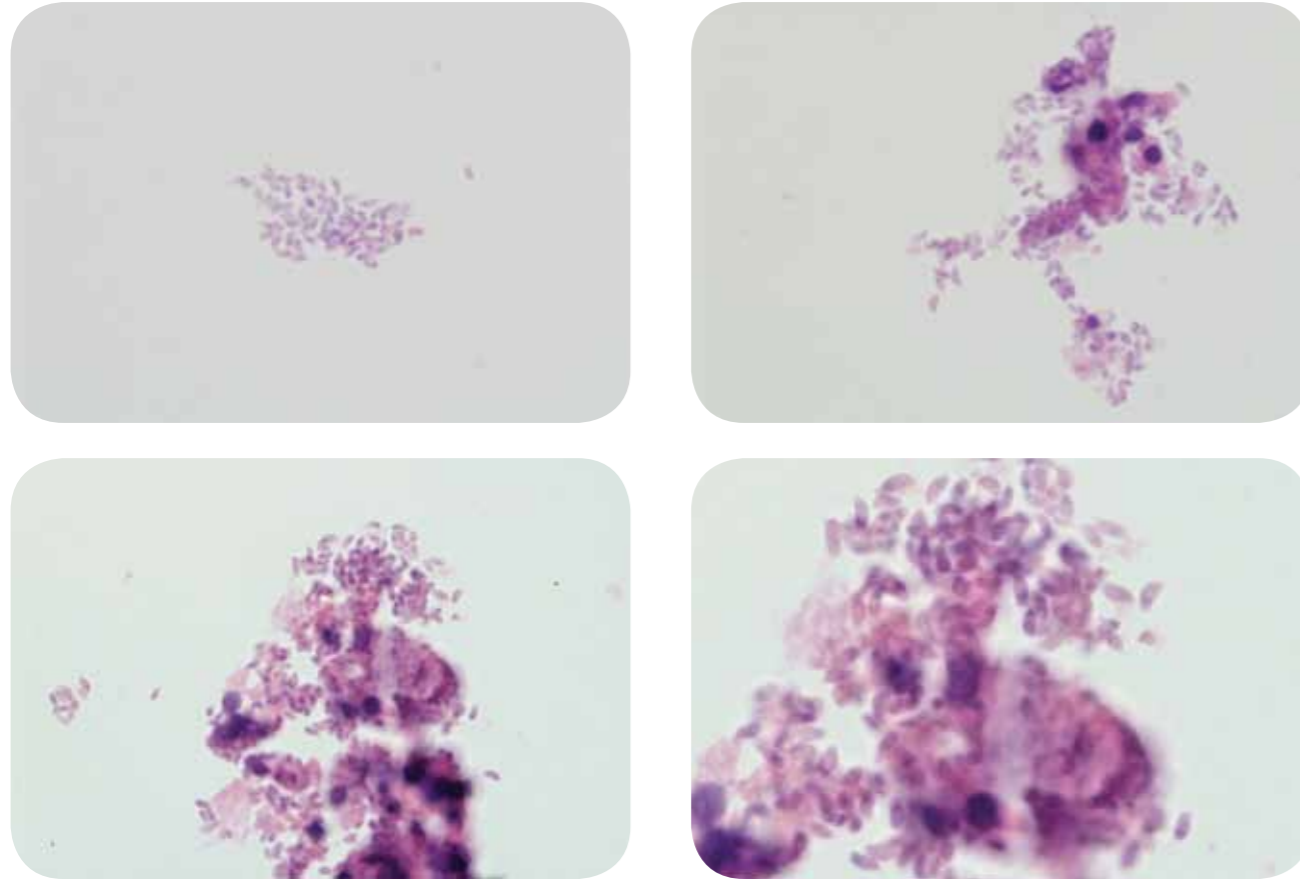
##### Key points:

- *Neospora* is a parasite type organism that can infect cattle
- Infection in cattle can cause serious disease in pregnant cows and result in abortion
- It's a problem worldwide and there is evidence it's on the increase in the UK
- **THERE IS NO EVIDENCE TO SHOW NEOSPOORA CAN INFECT HUMANS**

*Neospora* is a tiny single celled organism (protozoan), about half the size of a red blood cell, that can invade and live inside animal cells. Infection of cattle with *Neospora* can result in serious disease in pregnant animals and the disease neosporosis is recognised as one of the major causes of bovine abortion worldwide. The organism is a parasite that can only live and multiply within cells of an animal and it is often known as *Neospora caninum*, or *N. caninum* for short, as it was first discovered in dogs. In cattle, the parasite can exist in two different forms: the tachyzoite (Figure 1) and the bradyzoite (Figure 2). The tachyzoite stage represents the actively multiplying stage of the parasite that migrates through the body of an infected animal causing infection in

different parts of the body. This stage occurs predominantly during acute infections of the parasite. The bradyzoite stage of the parasite is associated with chronic infection, where it stays hidden within infected cells in structures called tissue cysts (Figure 2). This allows the parasite to persist for a long time within an infected animal. However, these tissue cysts can become reactivated, where the bradyzoites transform back into tachyzoites, and spread throughout the body of the infected animal. If this re-activation, also called "recrudescence", occurs during pregnancy of an infected cow, then the parasite can be transmitted to the foetus, which can lead to the death of the foetus, abortion, or the birth of a persistently infected calf.

Figure 1: Tachyzoites



## Q2

Where does *Neospora* come from?

## Key points:

- *Neospora* parasite was first detected in dogs over 30 years ago
- The link to cattle emerged relatively recently
- It's similar to the *Toxoplasma* parasite transmitted by cats and affecting humans and sheep

The parasite was first detected in dogs in the mid 1980s and that's where it got its *N. caninum* name from. More recently it has been linked to bovine abortions and in the early 1990s it has been confirmed that *Neospora* is a major cause of abortion in cattle. *Neospora* is closely related to *Toxoplasma gondii*, another protozoan parasite, but, unlike *Neospora*, *Toxoplasma* is transmitted by cats and causes abortion in sheep and humans. There is no good evidence that *Neospora* can establish an infection in humans.

It is thought that *Neospora* has existed for a very long time although it was only diagnosed as a significant cause of abortion relatively recently. Evolutionary analyses, based on variations between *Neospora* and *Toxoplasma*, suggest that these parasites have separated from a common ancestor several million years ago. This separation into separate species is most likely linked to the parasites developing different host species specificities.

## B1

Discovery of *Neospora*

A Norwegian scientist, Inge Berkas working at the Veterinary School in Oslo in the mid 1980s, was interested in a fatal disease of dogs which caused a severe inflammation in the brain and sometimes other muscles. Affected animals often also presented with hind limb paralysis. Looking more closely at the lesions found in these animals, he found organisms that looked very similar to a protozoan parasite called *Toxoplasma gondii*. However, when he examined blood samples from these affected dogs he could find no evidence of antibodies to *T. gondii*. Specific antibodies are produced by the immune system of the animal in response to infection and are useful bio-indicators of infection so are often used to try and help diagnose the cause of disease. Inge Bjerkas then did further studies to investigate this new organism and found by examining microscopic sections of infected tissue, that the cyst stage of the new parasite appeared structurally different from that of the closely related parasite *T. gondii*. He also observed that cysts of the new parasite were mainly found in neural tissue. Berkas then travelled to the USA to work with scientists there who were also interested in this new parasite in dogs which they subsequently named *Neospora* (new organism) *caninum* (from dogs). The next important breakthrough came when J.P. Dubey and colleagues, working in the USA, isolated and grew the new organism in the laboratory using tissue culture techniques.

The organism, *Neospora caninum* had been isolated using tissues from puppies that had been congenitally infected and were suffering very similar clinical symptoms as those dogs described by Inge Bjerkas in Norway. Being able to isolate and grow the organism, *N. caninum*, responsible for the disease in the laboratory allowed scientists to develop specific diagnostic tests which included serology based diagnostic assays and immuno-histochemistry techniques which allow the direct staining of infected tissues to highlight the infectious organism. The development of these diagnostic reagents and tests were very significant as they allowed scientists to examine for evidence of the disease in other species. A retrospective analysis of archived tissues from cases of bovine abortion in USA, with a suspected protozoan involvement, showed that they were very likely to have been caused by *Neospora caninum*. Since the early 1990s with the new diagnostic tests available, several reports from other countries around the world showed that *Neospora* was emerging as a major cause of bovine abortion and scientists renewed their efforts to discover more about how the parasite is transmitted, how it causes disease and how it might be prevented and controlled. *Neospora* was isolated from an aborted bovine foetus in USA and then used to experimentally infect naïve cattle where it was possible to reproduce the disease neosporosis, thus the causative agent had been identified.



## How does *Neospora* cause disease?

### Key points:

- Naive\* cows grazing on pastures contaminated by faeces from dogs are susceptible to the parasite
- The pregnant cow may show no outward signs but when the parasite passes to the unborn calf it can cause abortion
- Some calves survive the gestation period and are born with neurological signs or still born, others may look healthy but are infected with the parasite
- An 'abortion storm' can happen when a farm is affected for the first time and has lots of susceptible animals

Disease is mainly seen in dogs and cattle. In dogs the most severe clinical cases are seen in congenitally infected pups where they often present with progressive paralysis of the hind legs and may have difficulty swallowing. Clinically infected bitches can transmit *Neospora* to their foetuses and successive litters from the same bitch may also be born infected.

*Neospora* may cause cattle to abort or to produce still-born calves. Cows of any age may abort from 3 months of gestation to term. The disease neosporosis may present on the farm as outbreaks of abortion or abortion storms where a significant number of cows may abort at one time. This tends to happen when infection is first encountered on a farm and there are a large number of susceptible animals. In other cases the disease may present with lower numbers of sporadic abortions which occur year on year. This is likely to be due to cows that are persistently infected with *Neospora*, that pass the parasite on to the foetus during pregnancy. Some congenitally infected calves may also be born live but show neurological abnormalities and have difficulty, walking and feeding. Other calves may be born infected with the parasite but show no outward clinical signs.

Disease tends to manifest during pregnancy and *Neospora* parasites travel in the blood circulation and invade and grow within the cells of the placenta. The placenta is a critical organ that develops during pregnancy to protect the growing foetus within a sac of fluid in the cow's womb and to transfer oxygen and nutrients from the mother's bloodstream to the developing foetus. The invading parasite develops and multiplies within the cells of the placenta causing tissue damage and lesions, thus damaging the placenta which may be very dangerous for the developing foetus. The parasite may then transfer to the foetal cells and

start growing and multiplying in the foetus causing disease and sometimes death. The *Neospora* parasites locate to the foetal brain where they can cause severe damage of vital tissues and they can also be found in the heart, lungs and liver of the unborn calf.

The timing of infection during pregnancy will affect disease outcome:

- *Neospora* infection early in pregnancy is usually fatal for the developing foetus
- *Neospora* infection at mid-pregnancy may cause foetal death or result in the birth of a persistently infected calf that may show abnormal neurological signs at birth
- *Neospora* infection at late gestation is less likely to result in disease of the foetus and the calf will be born infected with the parasite but with no obvious clinical signs.

In cattle herds where a high level of infection already exists the parasite is very likely to be transmitted to the foetus by vertical transmission. This does not usually result in foetal death as the timing of transmission to the foetus usually happens in mid to late gestation and the calf will be born with no clinical signs but infected with *Neospora*. In a minority of cases the parasite may become active earlier in pregnancy resulting in foetal death. We are still not clear what the trigger is for *Neospora* to become active but it is very likely to involve changes in the immune response of the cow. Once infected with the parasite, cows will remain persistently infected for many years.

\* an animal that has had no prior *Neospora caninum* infection and therefore has no immunity to the parasite.

## The immune system and the parasite

When *Neospora* parasites infect and start multiplying inside the cow, the immune or defence system of the cow becomes activated and tries to limit the spread of infection and contain the parasite. This dynamic relationship between the parasite and the immune system of the cow will determine whether the infection will result in disease or not. In a non-pregnant animal the immune system is able to fight the infection and limits the activity of the fast replicating tachyzoite stage of *Neospora*. As a result the parasite hides within cells and develops into the bradyzoite (slow multiplying) stage. The bradyzoite stage of the parasite is kept under control by the immune system and the parasite lives inside the cow contained within tissue cysts usually in the brain tissue.

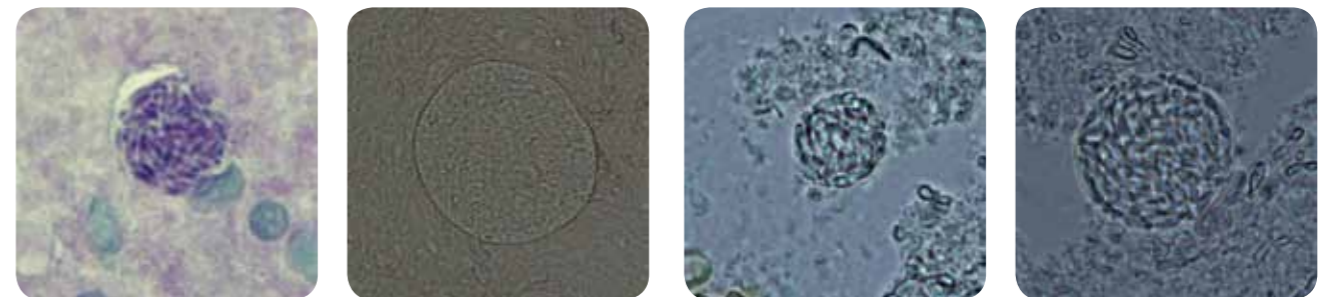
When the cow becomes pregnant the immune system changes naturally to allow the mother to carry the foetus without rejecting it. The immune system is designed to recognise foreign pathogens or tissues and will work to eliminate them from the body. This is why it can be difficult to transplant organs into non-compatible recipients as they are likely to be immunologically rejected. Pregnancy is a very special situation where the immune system of the mother changes to prevent immunological rejection of the foetus.

The parasite takes advantage of the natural changes in the immune system of pregnant cows and becomes active again, escaping from the tissue cysts where it has been contained and travelling via the circulation to the placenta, here it can infect and multiply in the tissues causing damage, going on to infect cells of the developing foetus.

If *Neospora* parasites invade the placenta within the first 3-4 months of pregnancy the cow will make a strong immune inflammatory response in the placenta to try and kill the invading parasite. As result of this inflammatory immune response the placental tissue can become damaged and its function becomes impaired which may have fatal consequences for the foetus.

The maturity of the foetal immune response also has important consequences for disease outcome. For the first 100 days of gestation the foetal immune system is very immature and if the parasite infects the foetus at this stage it will invariably be fatal. However, as gestation progresses the immune system of the foetus begins to mature and the foetus can make its own immune responses and start to defend itself against attack by *Neospora* parasites. Therefore, infections occurring later in gestation, in the last third of the pregnancy, are unlikely to result in foetal death although the calf is very likely to be born infected with *Neospora* parasites.

Figure 2: Tissue cysts with bradyzoites



## How common is *Neospora* infection?

### Key points:

- Statistics on prevalence are sketchy but it's a global problem and one that's on the increase in the UK
- Bulk testing of around 500 GB dairy herds in 2012 has suggested levels of 51% - much higher than previously thought
- Farmers whose cows test free of infection should be aware of the high risk of false negative results, mainly due to the timing of the testing
- More UK herd testing is urgently needed to support the findings of existing sampling

The parasite occurs worldwide where there are cattle, dogs or other suitable hosts. There is only limited data available relating to the *Neospora caninum* prevalence in cattle within the UK. Based on abortion cases that were submitted to veterinary investigation centres, it can be concluded that about 20% of abortions are due to the parasite. Published serology based prevalence studies are only regional and a fairly comprehensive study was conducted in the south-west of England. This study followed 114 dairy farms over a four year period and involved 15,736 cattle. The results of this study show that 12.9% of adult cattle tested positive and that during the four year period about 90% of herds tested positive, with at least a single infected animal at one of the time points. This study confirmed that *Neospora caninum* infection was common in the tested farms. In order to generate more comprehensive prevalence data for

*Neospora caninum* infection in dairy herds, a bulk milk tank testing approach was adopted where 536 bulk milk samples from separate milk tanks were tested in April 2012. In October 2012 a further 477 bulk milk samples were tested, which included 395 milk tanks that were tested previously and 82 newly tested tanks. The milk samples were screened using an ELISA test that detects *Neospora caninum* specific antibodies that are present in the milk of *Neospora* infected cattle. In April 2012, 19.4% of milk samples tested positive for *Neospora* specific antibodies, while in October 2012, 47.6% samples tested positive. When the two data sets are combined, using matched up milk tank results, then 42.1% of the 618 milk tanks tested were positive (Table 1). These results confirm that *Neospora caninum* infection is very common within British dairy herds and farmers are probably not even aware they have the infection within the herd.

Table 1: Summary of Complete Bulk Milk Tank Test Results

	Apr-12	Oct-12	Number of Tanks	Overall Total
<b>Number of negative bulk milk samples:</b>			193	358
		nd	117	
	nd		48	
<b>Number of positive bulk milk samples:</b>			71	260
			122	
			9	
		nd	24	
	nd		34	
<b>Percentage of bulk milk tanks with positive results:</b>	19.4	47.6		42.1

■ = Negative result ■ = Positive result nd = not done

Table 1: A total of 618 (358+260) milk tanks were sampled in April and October 2012. For 223 (117+48+24+34) tanks only a single sample was available, either in April or in October and the missing results are shown as nd (not done). For 395 (193+71+122+9) tanks, milk samples were tested both in April and in October. A total of 358 (193+117+48) milk tanks are considered negative (yellow) because all their milk samples tested negative, while 260 (71+122+9+24+34) tested positive (green) at least once and therefore this indicates that their farms of origin have *Neospora* infected cows. This table shows that 42.1% (260/612) of tanks contained antibodies to *Neospora*.

The difference between the two sampling results is surprising and is likely due to limitations of bulk milk testing by ELISA, rather than an indication of many new infections. The antibody level in the milk of infected cows can fluctuate. As a result, herds that tested positive at one time point can test negative in a subsequent test and this was seen for 9 milk tank samples. The *Neospora* specific antibody levels in individual infected animals can be influenced by pregnancy and in a synchronised herd this can result in seasonal positive/negative results for the bulk milk sample of a whole herd. Since most British dairy farms have similar calving periods this can lead to similar synchronisation of their bulk milk test results, if they have *Neospora* infections within their cattle. This seasonality may explain why herds that tested negative in April could test positive in October and this change from negative to positive was seen for 122 bulk milk tank samples.

Seasonality of the bulk milk test results may influence the overall detected *Neospora caninum* prevalence in the samples tested because more samples were collected in April than in October. Therefore data should only be analysed for the 395 milk tanks for which two milk sample results are available, which gave an overall *Neospora caninum* prevalence of 51.1% (Table 2).

This bulk milk prevalence study confirms that *Neospora caninum* infection is widespread and common within UK dairy herds. It also suggests that single bulk milk test results for identifying *Neospora* infected herds have to be interpreted very carefully due to the high risk of false negative results.

Table 2: Bulk Milk Tank Data with 2 Results Only:

	Apr-12	Oct-12	Number of Tanks	Overall Total
<b>Number of negative bulk milk samples:</b>			193	193
<b>Number of positive bulk milk samples:</b>			122	202
			9	
			71	
<b>Percentage of bulk milk tanks with positive results:</b>	20.3	48.9		51.1

■ = Negative result ■ = Positive result

Table 2 uses only data from bulk milk tank tests, where the same milk tank was tested twice, both in April 2012 and in October 2012. This excludes the data from Table 1, where only single test results were available. A total of 193 milk tanks are considered negative (yellow) because they tested negative twice, while 202 (71+122+9) tested positive (green) at least once and therefore this indicates that their farms of origin have *Neospora* infected cows. This table shows that 51.1% (202/395) of tanks contained antibodies to *Neospora*.

## B3

UK Prevalence of *Neospora*

*Neospora* can infect a wide range of different host animals including dogs, cattle, buffalo, horses, sheep, goats, deer, foxes, coyotes, ferrets, polecats, mink, badgers and rodents. There is no good evidence that *Neospora* can infect and cause disease in people. The two most commonly affected species are dogs and cattle.

Retrospective analysis of post-mortem material from a still-born calf in New South Wales, Australia in the mid 1970s, is the earliest recorded case of bovine neosporosis. Since diagnostic reagents for *Neospora* became available in the early 1990s, bovine neosporosis was reported in many countries including the UK, Ireland, USA, South America, Australia, New Zealand, Sweden, The Netherlands, Germany, Japan, China, South Africa and is very likely to have a worldwide distribution.

Diagnosis of *Neospora* in aborting cattle in the UK is carried out by the Animal Health - Veterinary Laboratory Agency (AHVLA) in England and Wales, the Scottish Agricultural College and Moredun Research Institute in Scotland

and the Veterinary Sciences Division, Department of Agriculture in Northern Ireland. The yearly statistics for the UK are shown in the veterinary surveillance report VIDA [http://vla.defra.gov.uk/reports/rep\\_vida11.htm](http://vla.defra.gov.uk/reports/rep_vida11.htm)

*Neospora* has been the most commonly detected and attributed cause of infectious bovine abortion in UK from 2004 until 2010. A recent survey in Scotland conducted between 2007 and 2010 tested 611 bovine abortion samples by PCR (a molecular based diagnostic test) and found that nearly 20% were positive. A three year longitudinal serology study in the south west of England showed that at least 90% of the 114 farms tested had at least a single *Neospora* infected animal and that 12.9% of the 15,736 cattle tested were positive for *Neospora* using a serology based diagnostic test.

Both dairy and beef cattle are at risk from *Neospora* infection and there is no good evidence that different breeds may have differing susceptibilities.

## B4

Worldwide Prevalence of *Neospora*

Seroprevalence studies have been conducted within dairy and beef cattle worldwide looking for the presence of *Neospora* specific antibodies which are produced by the immune system of the animal following an infection with *Neospora* parasites. These specific antibodies can be detected by a variety of different diagnostic tests using a blood sample from animals and are therefore a useful method to obtain information about the prevalence of *Neospora* infection in live animals. Most of the data available from different countries worldwide is drawn from studies conducted on a regional basis. Very few studies are available giving data on national seroprevalence of *Neospora*.

Data from 131 different studies across the world looking at seroprevalences in dairy cattle was recently reviewed by J.P. Dubey and colleagues in 2007 and showed seroprevalences ranging from 3% to over 60%. The same review also compared seroprevalence studies in beef

cattle around the world and data from 35 different studies showed seroprevalence rates ranging from 2% to 30%.

It is difficult to make direct comparisons between these studies as they were obtained using different serological tests and standards. They do, however, illustrate the global distribution of *Neospora* and the significant economic impact of this disease on cattle production worldwide.

A European study was recently conducted involving both dairy and beef cattle from Germany, Sweden, Spain and The Netherlands using randomised sampling procedures and diagnostic tests that had been standardised among the different participating laboratories. Dairy herd seroprevalences were estimated to be 16% in Sweden; 49% in Germany; 63% in Spain and 76% in The Netherlands. Beef herd seroprevalences were 41% in Germany; 46% in Spain and 61% in the Netherlands.

## Q5

## How big a problem is the parasite?

## Key points:

- It's a significant problem for the UK dairy industry - *Neospora* is the most commonly diagnosed cause of abortion and there are no licensed drugs or vaccine available.
- The impact of new infection can be catastrophic on a dairy herd causing large numbers of cows to abort, leading to inevitable financial losses

In the dairy industry, *Neospora* has been confirmed as the most commonly diagnosed cause of bovine abortion. The recorded incidences in the beef industry in the UK are lower and this is likely to reflect different management styles between these farm enterprises that either result in lower infection rates or in fewer cases of abortions being detected and investigated.

*Neospora* infection of the herd can lead to abortion storms, where many cows lose their calves in a relatively short period of time. This is usually the first indication a farmer has of the parasite. The uncertainty of how long this is likely to go on for and how many abortions are likely to occur makes

this particularly devastating for affected farmers. Abortion storms may be explained by the infection of a group of pregnant cattle in a short time window either through *Neospora* oocyst contaminated pasture, feed or water.

Another reason why this disease is so devastating is that there is no treatment to help infected animals and as yet there is no effective vaccine that will prevent infection or that protects from abortion or transmission of the parasite from dam to foetus. This means the only current option to help prevent or control the disease is the adoption of farm management practises that assure bio-security and limit the risk of transmission of the parasite.



## Q6

## How will the parasite affect me economically?

### Key points:

- Direct costs are associated with abortions and loss of the calf
- Indirect costs include increased calving interval and failing to get the cow back in milk
- Infected animals have a reduced life expectancy
- Financial modelling completed as part of this project predicts the cost of *Neospora* in an average 121\* cow herd to be in the order of £3,000 per year

\* Figures for 2011 from DairyCo

The economic effect of *Neospora* infection is associated with the cost of abortion, either directly as the loss of a calf but more importantly, in the case of the dairy industry, failing to get a cow back into milk may result in her experiencing prolonged dry periods. Losses of foetuses during the early stages of pregnancy will result in cows coming back into oestrus and the consequences of this are increased calving intervals. This in turn leads to reduced total daily milk yields for the calving interval.

Farm based studies have shown that *Neospora* infected animals have a reduced life expectancy as they are often culled earlier than uninfected animals, probably due to

reduced milk yields or fertility issues. This means that these animals often have a lower total milk yield over their life than uninfected cows. The costs of rearing and keeping them are higher than the income they have generated during their life making them often a net loss for the affected farmer.

Another financial impact is that farmers who try to breed out *Neospora* infected animals may not be able to rear enough uninfected replacement stock to maintain their herd and may have to buy in replacement animals losing their closed herd status.

## Q7

## How long will the infection last for?

### Key points:

- Infected cattle stay infected for life
- Parasites in a persistently infected cow can become active again in subsequent pregnancies and transmit the disease to her foetus
- In these cases this can lead to abortion, or the birth of a persistently infected calf
- This is the primary reason why the disease is very hard to eradicate

Individual animals that become infected are expected to stay persistently infected for life. More importantly, the parasite in these persistently infected cows may become active again in subsequent pregnancies and be transmitted to their foetuses. This leads either to abortions or the birth of persistently infected calves, which in turn can pass

on this parasite to their offspring. This is a very effective transmission mechanism for the parasite that allows *Neospora* to be maintained within a herd for many cattle generations, without the need of a definitive host spreading the disease to other cows.

## Q8

## What proportion of my cattle will abort?

### Key points:

- Not all infected cattle will abort
- Infected cows are 3-7 times more likely to abort
- Abortions are more common in heifers and recently infected cows



This question is more difficult to answer because it will depend on the proportion of cattle that are infected within a herd. Not all infected animals will abort but infected cows are 3 - 7 times more likely to have an abortion than non-infected cows. Abortions are more common in *Neospora* infected heifers or recently infected pregnant cows. In subsequent pregnancies the risk of abortion becomes less and significantly fewer cows that aborted due to *Neospora*

infection will abort again due to the parasite in following pregnancies.

After an abortion storm the frequency of abortions within the herd drops and a lower number of abortions are usually seen over time. This may be explained by the fact that abortion storms are due to a synchronised infection of a large number of naïve animals.

## Q9

### Will my cows abort again?

#### Key points:

- Little information is available in this area. However, it is thought that around 5% will abort again due to *Neospora* infection



There are very few comprehensive studies providing this information and the available data is based on working farms that had abortion storms. From these studies it may be expected that about 5% of cows that aborted once due to *Neospora* infection will abort again due to it in a subsequent pregnancy. However, this percentage is likely to be an

underestimate because working farms are likely to have removed cows that did not get pregnant and these figures are also likely to exclude cows that lost their calf very early during pregnancy. It is also very important to get an accurate diagnosis of the cause of abortion as many other infectious agents may be involved.



## B5

### Impact of *Neospora* infection

The main economic losses associated with *Neospora* infection are the loss of the calf due to abortion, early foetal death and resorption which may manifest as return to service, infertility and increased time to conception, stillbirth or neonatal mortality. The cost associated with abortion will vary depending on the age and genetic value of the dam. As *Neospora* may cause disease as a result of a primary infection or due to a previous persistent infection, then the developing foetus in a persistently infected cow is potentially exposed to the effects of *Neospora* from conception to birth. Therefore effects on reproductive performance should be considered alongside abortion. Associated

losses also include those involved in establishing a diagnosis; increased calving interval; re-breeding those cows that aborted; costs of replacement cows that are culled and sometimes loss of milk yield.

Diagnostic disease surveillance data shows that *Neospora* is the most frequently diagnosed cause of infectious bovine abortion in the UK. It is clear infection with *Neospora* will have a significant economic impact on individual affected farms and a serious impact on cattle production.

**Abortion risk and early foetal death**

Several independent studies in USA, The Netherlands and the UK have estimated that cattle infected with *Neospora* are 3 - 7 times more likely to abort than uninfected cattle, with congenitally infected heifers at most risk in their first pregnancy. In England and Wales it was estimated that 12.5% of abortions (n=6000) may be due to *Neospora*.

Early foetal death is likely to present as a return to service, increased time to conception and therefore increased calving interval. There is very little documented evidence to quantify the extent of early foetal losses due to *Neospora*. However, data from experimental infections has shown that challenges with the parasite in early gestation are highly likely to result in foetal death.

**Premature culling**

Reduced fertility is one of the most common reasons for culling cattle and a study of a 2000 cow dairy herd in the USA showed that cows seropositive for *Neospora* were 1.6 times more likely to be culled than sero-negative animals. A study of dairy cattle from the Maritime provinces in Canada (New Brunswick; Nova Scotia and Prince Edward Island) showed that *Neospora* seropositive cows were more likely to be culled prematurely than seronegative animals and that the total annual economic losses associated with an average 50 cow dairy herd due to neosporosis was \$2304 Canadian dollars. A seroprevalence study examining 134 dairy herds in Canada showed that cows that were

seropositive for *Neospora* had a 1.43 times greater chance of being culled than seronegative animals. In the Netherlands a large scale study of 83 herds randomly selected from the Dutch dairy herd population showed that *Neospora* seropositive cows were 1.73 times more likely to be culled than seronegative animals. In the same study, 17 herds were also examined where there had been well documented abortion epidemics due to neosporosis; in these cases, seropositive cows were 1.88 times more likely to abort than seronegative cows.

**Milk production**

Persistent infections with *Neospora* have not been shown to have a direct effect on milk yield of the infected cow. However, indirect effects of *Neospora* infection on milk yields have been demonstrated and these are all linked to abortions and fertility issues caused by parasite. The most obvious costs are associated with the abortion event, especially during late gestation, which can result in extended dry periods of a cow.

Very early calf-losses due to *Neospora* infection are often not seen because the foetus, when aborted, is either too small to be detected or was absorbed by the dam. Both cases will result in the dam going back into service and leading to increased calving intervals and reduced fertility, requiring mating several times or multiple artificial insemination attempts. Increased calving intervals means that cows will spend more time in their less productive stages of the milk production cycle, which can lead to reduced efficiency of the cow as calculated by dividing the total milk yield of the animal by its age when the animal was culled.



## 2. Diagnosis of *Neospora* infection and Bovine Neosporosis

### Q10

#### How do I know if I have the parasite in my herd?

##### Key points:

- Antibodies to *Neospora* can be detected in milk and blood
- *Neospora* can be detected by submitting abortions for laboratory analysis

Diagnosis of *Neospora* infection can be reached by detecting parasite specific antibodies in the blood of infected animals or by submitting abortion cases, including parts of the placenta, to your vet for diagnosis.

Most farmers will not know that they have *Neospora* within their herd because the parasite can stay dormant within infected animals for many years. Often the only signs of infection at herd level are sporadic abortions or possibly some fertility issues resulting in increased calving intervals or reduced milk yields. In these circumstances it is rare that

this parasite is detected. In most cases a diagnosis is only reached after a detailed investigation into herd fertility issues or increased numbers of abortions on a farm. For example, after an abortion storm, when a significant number of pregnancies are lost within a short period of time. Another way of increasing farmer awareness of the infection is through the increased requirement for *Neospora* testing by some cattle health schemes. Routine testing of the whole herd will inform the farmer if his/her animals are likely to be infected with the parasite.

## Q11

### How is Neosporosis diagnosed?

#### Key points:

- From abortions, *Neospora* can be diagnosed by a veterinary laboratory
- For living animals, a bulk milk test can give an indication of your herd status while blood tests can show individually infected animals

A diagnosis of neosporosis may be reached as the cause of an abortion by pathological examination of abortion cases if foetus and placenta are submitted to a vet. However, in live animals the only available tests to determine if a cow is infected are based on detection of parasite specific antibodies in the blood or milk.

**For abortions:** The aborted foetus and any parts of the placenta should be submitted to your vet or a veterinary investigation centre. They will perform a post mortem examination and take relevant samples to reach a diagnosis. To confirm that *Neospora* is a cause of an abortion, a diagnosis is usually based on a combination of information: 1) finding evidence of the parasite and parasite induced damage by pathological examination of the foetal brain and the placenta 2) detecting *Neospora* specific antibodies in blood samples taken from dam and/or foetus 3) DNA based technologies can be used to detect the DNA of the parasite in the foetus or placenta 4) absence of other pathogens

in the abortion case and 5) farm based history, if evidence exists that previous abortions on the farm were caused by *Neospora*.

**For living animals:** Detection of *Neospora* specific antibodies in blood or milk using serological tests is the only method that can be used to determine if an animal is infected. A positive serology based test result is usually accurate and can be trusted as an indicator that an animal is infected with *Neospora*. Unfortunately, a positive serological test result is not a reliable indicator that an abortion was caused by *Neospora* because not all seropositive dams will lose their calves during pregnancy. As a result the dam could have passed on the infection to the foetus but the abortion could have been due to a different cause. Single negative serology based test results are not that reliable in determining that an animal is free of infection and should be treated with caution when buying in or choosing replacement stock.

## Q12

### What do I need to submit to get a diagnosis for abortion cases?

#### Key points:

- Submit foetus and placenta
- In some cases *Neospora* can be difficult to detect so a negative result does not rule out *Neospora* as the cause

It is important to submit foetus and placenta for diagnosis to your veterinary practice or a veterinary investigation centre. If more than one aborted foetus / placenta is available then it is better to submit multiple fetuses with their placentas to increase your chance of getting a diagnosis. The submitted samples will be processed and some tissues will be collected and sent for specialised tests

in order to determine if a pathogen can be detected and if the cause of the abortion can be attributed to this pathogen. Routine tests will be looking for evidence of bacterial, viral and parasitic infections. Veterinarians may follow up submissions of an abortion case by taking blood samples from the dams that aborted, to determine if these animals are infected with *Neospora*.

## B6

### Diagnosis of *Neospora* in bovine abortions

One of the main limitations for the diagnosis of this parasite, either from abortion material or from live infected animals, is that the parasite cannot be cultured easily, unlike some bacteria or viruses. As a result the diagnostic tests depend on either detecting the parasite directly or by detecting parasite induced damage within infected tissues, using pathology based diagnostics. Alternatively, evidence of infection can be obtained by detecting antibodies that are specific for *Neospora* and are produced by infected animals.

Pathology based diagnosis depends on the submission of an aborted foetus and the associated placenta. The pathologists will collect tissue sections from these and process them for microscopy to look for evidence of parasite specific lesions and cysts. These signs will provide evidence that the tissues were infected

by the parasite, especially if the parasite itself can be seen in sections that were stained with *Neospora* specific antibodies. Further evidence that an abortion was due to *Neospora* can be obtained if these sections show evidence of an active infection (infiltration of inflammatory cells, associated with the parasite).

Often the final cause of an abortion is based on a differential diagnosis, where *Neospora* was detected, ideally with signs of an active infection, possibly with a positive serology result, but also the absence of other pathogens or other possible causative explanations for the abortion.

Molecular biology based techniques have recently been adopted by some laboratories as part of their differential diagnostic tools. These techniques involve

the detection of *Neospora* specific DNA fragments within samples. This test is very specific for the parasite and also allows the detection and discrimination of other closely related parasites. A further advantage of this test is that it can utilise autolysed samples that would not normally be suitable for pathological examination. However, the disadvantage of this test is that it does not, by itself, determine if the parasites were the actual cause of the abortion. It can only confirm that the parasite was present. However, it is

a valuable additional tool that supports a differential diagnosis.

Submissions of multiple foetuses and their placentas will increase the chances of obtaining a reliable diagnosis for the cause of an abortion. The placenta is of particular importance in order to determine cause as this is often the site of the active infection and including it, or parts of it, in the submitted material will increase the chances of reaching an accurate diagnosis.

## Q13

### Can I test living animals and how does the test work?

#### Key points:

- Blood based tests detect antibodies to *Neospora* in the blood serum
- Milk based tests work in a similar way, antibodies can be detected in milk

**Blood based tests:** In living animals detection of infection with *Neospora* can only be made indirectly by use of serology because detection of the parasite directly in the blood is very unreliable, even in experimentally infected animals. As a result, the only way to confirm that individual animals or a herd is infected with *Neospora* is by a veterinarian taking blood samples. These blood samples will be sent for *Neospora* specific serology tests. Testing all animals within a herd will give an indication of the prevalence of infection within a herd. The currently available serology tests may give false negative results for a proportion of cases and therefore a single test result will never confirm that an animal/herd is free of *Neospora*.

**Milk based tests:** Bulk milk testing for *Neospora* specific antibodies have also been developed. This is usually used as an epidemiological tool to determine the proportion of dairy herds that are infected with *Neospora* within a country. It can be used by individual farms as a quick and easy early indication if they have *Neospora* within their herd. The advantage of this test is that it is non-invasive and that one test will give a result for the whole dairy herd. The drawback is the potential lack of sensitivity of the test. Therefore, the test is better utilised to confirm that there is a problem on a farm rather than providing evidence that the herd is not infected. This is because the test relies on detecting significant levels of antibodies in the bulk milk sample but the contribution of a single infected animal can be diluted out.

## Q14

### What does a positive test result mean?

#### Key points:

- A positive result in submitted abortion samples means *Neospora* specific lesions have been found. These positive results are fairly conclusive
- A positive serum antibody test means the animal has had some exposure to the organism

**Positive pathology results for foetus or placenta:** If a positive diagnosis was reached for an abortion case by pathological examination of a foetus or its placenta, then this will mean that the pathologist investigating the abortion case will have seen at least one *Neospora* specific lesion in the foetal brain or in the placenta. Often this observation is further supported by detection of the actual parasite associated with the lesions. In these cases the diagnosis is fairly conclusive that the abortion was caused by *Neospora* infection.

**Positive serological results for foetus or dam:** A positive antibody test result will mean that the animal had exposure to the parasite and has built up a specific immune response to *Neospora*. These results and interpretations are reliable

but it does only confirm that the animal is infected. A positive serology result for a dam post abortion does not confirm that the cause of the abortion was *Neospora*. Many dams that are infected in the late stages of pregnancy will be antibody positive but produce an apparently healthy calf that is congenitally infected with *Neospora*. A positive result for a foetal sample will show that the foetus had been exposed the parasite during pregnancy and was able to mount an immune response. Experimental data has shown that the foetus is capable of producing *Neospora* specific antibody responses from day 100 of gestation onwards. Therefore a negative result may occur with a younger foetus <100 days of gestation, as a result of the immaturity of its immune system.



## Q15

### What does a negative test result mean?

#### Key points:

- A negative pathology result on a foetus or placenta is not conclusive as detection of lesions can be difficult. This is why all abortion samples should be submitted to a laboratory
- A negative serum antibody result means that no antibodies were detected by the test. This can occur in infected cows as antibody levels fluctuate considerably over time
- Testing cows during pregnancy and calving will give more accurate results

#### Negative pathology results for foetus or placenta:

A negative pathology result means that the investigative pathologist did not see any specific evidence that the abortion was caused by *Neospora*. If only the foetus was submitted, without placenta, then it may be easy to miss the parasite and obtain a negative pathology result. The most distinctive and conclusive evidence that an abortion is due to *Neospora* is usually found in the cotyledons of the placenta. Another contributing factor for not reaching a diagnosis may also have been the condition of the foetus and placenta when it was submitted for diagnosis.

#### Negative serological results for foetus or dam:

A negative serology for *Neospora* means that the tests used

did not detect sufficient parasite specific antibodies to give a positive result. However, this does not mean that an animal is not infected because *Neospora* specific antibody levels fluctuate throughout the life of an infected animal and as a result a negative serology test result could be a false negative result. Testing an animal at different stages during its life, especially during pregnancy or around parturition, will increase confidence levels of negative test results. Experimental data has shown that the foetus is capable of producing *Neospora* specific antibody responses from day 100 of gestation onwards. Therefore a negative result may occur with a younger foetus <100 days of gestation, as a result of the immaturity of its immune system.

## Q16

### When is the best time to test animals?

#### Key points:

- Because antibodies fluctuate, the best time to blood test cows is during pregnancy and after calving
- And testing cows after abortions

The best time for testing animals is usually during or following an active infection of the animal by *Neospora* because at this stage the animal will have sufficient circulating antibody in its blood stream against the active stage of the parasite to give a positive test result. However, later, when the parasite has been encysted and hidden from the immune system, the antibody titres, specific for the active form of the parasite,

drop, potentially leading to negative test results. Based on our understanding of the parasite, the best time to test to reduce the risk of getting a false negative result is during pregnancy, directly after an abortion or after suspected new infection. Testing an animal several times will reduce the risk of falsely identifying an animal as uninfected.



## Can I test my calves when they are born?

### Key points:

- Testing calves at birth is not very reliable
- Cows transfer their antibodies to calf through colostrum so the antibodies detected may be from the mother
- Having said that, if the cow has antibodies it is likely that the disease would have been transferred during pregnancy
- Calves take time to build their own antibodies so a negative result would not be conclusive

Calves can be tested by serology but unfortunately the results require careful interpretation. If a calf registers positive for *Neospora* when it is tested shortly after it is born, then the *Neospora* specific antibodies detected could have originated from its mother and were absorbed from her colostrum. However, if the dam had high levels of *Neospora* specific antibodies in her colostrum then it is also very likely that the calf is infected. In order to avoid colostrum antibodies, calves would need to be blood sampled before

the first colostrum feed, which is not very practical. A negative test result for a calf is also not necessarily a true reflection of its infection status because if a calf got infected at an early stage *in utero* (<100 days of gestation) it may not be capable of immunological recognition of the parasite and it may not develop *Neospora* specific antibodies. As a result these calves are born antibody negative but are infected. It should be noted, however, that this is a rare occurrence.

## B7

### Diagnosis using Serology

Many of the serological tests for detecting *Neospora* infection in cattle were generated in the 1990s and there are now many commercial serology based diagnostic test kits available. However, the majority, if not all of these tests, involve the detection of *Neospora* specific antibodies that recognise the tachyzoite stage of the parasite. The tachyzoite stage is the actively growing stage of the parasite that is found during acute *Neospora* infections but as the infection progresses the parasite differentiates into the bradyzoite stage, contained within

tissue cysts which is how the parasite may persist in the host animal. During this transformation, the parasite changes its antigens (markers that are recognised by the immune system) and thereby its appearance with regard to the host's immune responses. This disguising strategy allows the parasite to hide from the immune response raised against the tachyzoite. Unfortunately, as a consequence, the antibody levels, detected by the diagnostic tests, drop and this can result in negative test results although the animals are still infected.

Positive results are normally easily interpretable and are a reliable indicator that an animal is infected. However, negative results are more difficult to interpret because they could either represent an uninfected animal or an infected animal in which the infection is dormant. This would not be a significant problem if the parasite would not become reactivated but, unfortunately, such reactivation occurs frequently in *Neospora* infected animals during pregnancy. Around 90% of persistently infected cows will transmit the parasite to their offspring. When the parasite becomes activated it is in the tachyzoite stage, again resulting in an increase in specific and detectable antibody levels to the tachyzoite leading to positive serology results. This means that an infected animal can test positive and negative, depending whether it has an active infection or a quiescent, persistent infection.

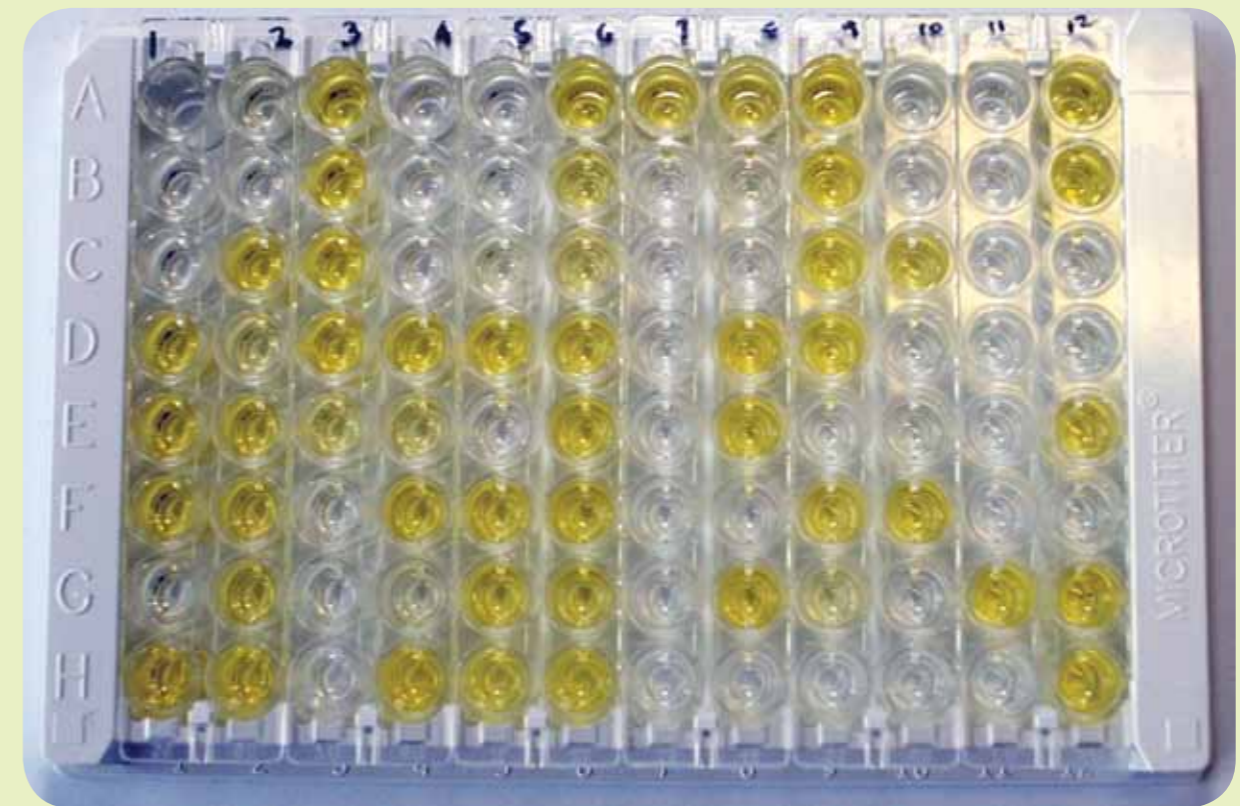
This may explain why a dam that is antibody negative may produce an infected calf. The dam had an active

infection earlier in pregnancy during which the parasite was transmitted to the foetus. By the time the dam gives birth the antibody levels within the blood of the dam have dropped below the "cut-off" threshold giving a negative result, while the antibody levels in the infected calf are still elevated, giving a positive result.

The fluctuations in antibody levels detected in these tachyzoite based diagnostic serology tests require that animals may need to be tested several times in order to reach a reliable diagnosis. This is a particular concern when buying in replacement stock and also for identifying all infected animals within a farm.

Scientists at the Moredun Research Institute along with international collaborators are currently investigating the use of antigens from the bradyzoite stage of the parasite as complementary targets for the development of improved serology based diagnostics that may help to accurately identify persistently infected animals.

### ELISA



This image depicts an ELISA plate, which is used to measure antibody levels in either serum or milk. Each well on the plate represents a sample, which was tested. A colour reaction is used to visualise the levels of specific antibodies in a sample. The intensity of the colour reaction is proportional to the levels of antibodies present. The colour intensity can be measured giving a numerical value, often referred to as OD (optical density) reading. This OD value is compared to standards to determine if the colour reaction is strong enough to confirm that the sample contains enough antibodies to verify that the donor is infected. If the value is below this threshold then the sample is considered negative.

### 3. Transmission of *Neospora* infection

#### Q18

#### How does the parasite get onto a farm?

##### Key points:

- Infection can be transmitted on farm via a number of routes
- A persistently infected cow can be purchased which can potentially pass the disease onto her offspring
- A recently infected dog that is on the farm can transmit via its faeces
- Recently infected dogs can contaminate drinking water and cattle feed through faecal contamination
- Wildlife may be a source of infection

There are several potential routes by which *Neospora* can be brought onto a farm: restocking with infected cattle; dogs; wildlife or via contaminated drinking water and cattle feed.

- The most common route to import *Neospora* onto a farm is by inadvertently bringing in persistently **infected cattle** that appear clinically healthy. These animals may not have had any abortions themselves but if they do abort their foetus or if they give birth, then placentas and dead foetuses may contain the parasite. If any of these tissues get eaten by dogs then they can become infected and produce oocysts which are the infective stages of the parasite for cattle. These infected animals have a very high likelihood of transmitting *Neospora* vertically (for explanation see diagram on P27) and this may occur over several generations.
- If recently **infected dogs** come onto the farm and shed parasite eggs (oocysts) in their faeces, which can survive more than 6 months, then this can be a major risk to

uninfected cattle. Especially if the parasite oocysts within the faeces contaminate cattle feed or water.

- Wildlife** is potentially another source of infection, either by acting as definitive hosts (although there is no good evidence of this to date) or by acting as intermediate hosts eg: rodents, rabbits or birds. If infected wildlife is eaten by a naïve dog, then this could lead to the dog shedding oocysts.
- Drinking water** for cattle may become contaminated by *Neospora* eggs depending on the source of the water. Stream or river water can be contaminated with dog faeces upstream if there are areas where dogs are kept, walked or exercised.
- Cattle feed** may also be a source of infection if it is contaminated with dog faeces containing oocysts. Therefore dogs should be kept away from areas where cattle feed is stored.

## B8

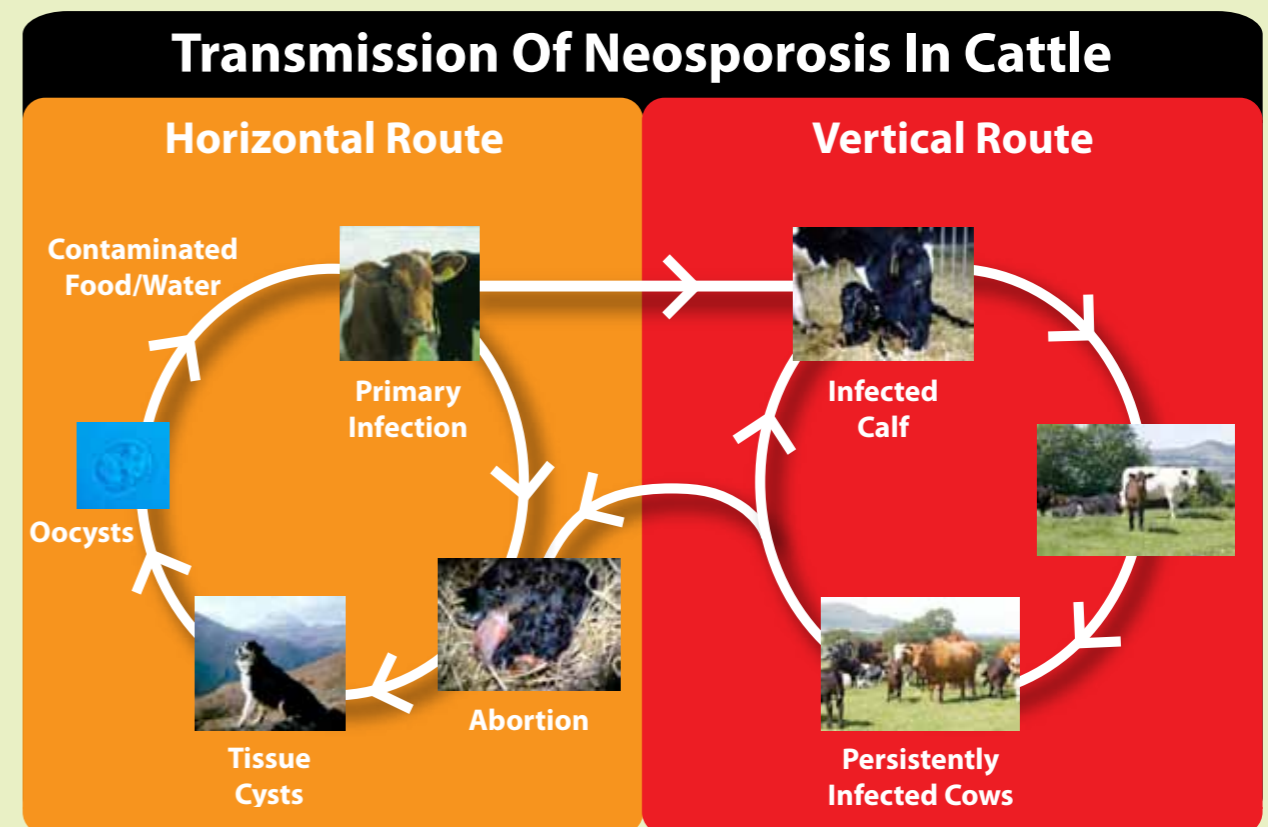
### Life Cycle and Transmission Routes:

*Neospora* has a complex life cycle and transmission routes (Figure 3). Definitive hosts, e.g. dogs, will produce parasite oocysts (eggs) in their faeces, which leads to environmental contamination. These oocysts have a tough outer shell, are very stable in the environment and stay infective for 6 months or longer in temperate conditions. If these oocysts end up in drinking water or feed, they can transmit the infection to cattle. Initially the parasite will invade and infect a variety of different cells in the bovine host where they multiply rapidly and disseminate through the bloodstream to other tissues in the animal. This active rapidly multiplying stage of the parasite is called “tachyzoite”. Once the immune system starts to recognize the tachyzoite, then the parasite changes tactics and tries to hide from the immune system by staying dormant inside tissue cysts, which may contain several hundred parasites. This slow multiplying stage of the parasite is called “bradyzoite”. If a definitive host eats meat containing *Neospora* bradyzoites within tissue cysts then this part of the life cycle is completed. However, the parasite has developed a back up strategy; during pregnancy it becomes activated again, breaks out of the tissue cysts and migrates to the placenta. Infection of the placenta

may lead to abortion, which would provide a source of contaminated meat for definitive hosts. In addition, the parasite may transmit to the foetus, which can lead to the birth of congenitally and persistently infected calves. As adults these animals may have abortions due to the parasite or they may transmit it to their offspring resulting in birth of infected although clinically normal calves. Congenital transmission of the parasite from dam to offspring allows *Neospora* to survive in herds for many generations and infection may stay undetected because the only clinical sign would be sporadic cases of abortions. However, contamination of animal feed and drinking water with oocysts produced by the definitive host may lead to abortion storms in naïve cattle herds.

There is no good evidence of horizontal transmission (see diagram on P29) between cattle. Experimental work has shown that it is possible to infect very young neonatal calves, 6 hours old, with colostrum samples spiked with live tachyzoites. However, this is not thought to be a significant natural route of transmission. Similarly, *Neospora* contaminated semen is not thought to be a major issue in the transmission of the parasite to cattle.

Figure 3: Lifecycle



## Q19

### How does the parasite infect livestock?

#### Key points:

- This can be by two main routes
- Direct dog to cattle via faecal contamination
- From infected cow to calf

There are two main routes for the transmission of *Neospora* to livestock; these involve either dog to cattle or dam to foetus.

**Dog to cattle** transmission occurs when a naïve dog eats *Neospora* infected meat (abortion cases, placentas or wildlife). The parasite multiplies inside the gut cells of the dog and this leads to the production of parasite eggs that are shed in the faeces of the dog. These oocysts are infectious to cattle and therefore dogs should be kept away from cattle pasture, feed and water. Dog to cattle transmission can lead to abortion storms where many naïve cows are infected in a short period and as a result abort at about the same time.

The second route is **dam to foetus** and this occurs during pregnancy. There are two ways by which this can happen: a) a cow gets infected during pregnancy or b) she was infected previously and reactivation of the persistent

parasite infection occurs during pregnancy due to natural changes in the mother's immune system. Both of these scenarios may result in the transmission of the parasite to the foetus. This can either lead to abortion of the foetus or to the birth of a persistently infected calf that can appear clinically healthy. This calf (if female) and its mother are both very likely to transmit the parasite to their offspring in subsequent pregnancies. Research at Moredun has shown that the timing of the infection during pregnancy will have a significant effect on the likelihood of transmission and the clinical consequences. Infection occurring in the first three months of gestation has a reduced likelihood of transmission to the foetus compared to infection occurring later in pregnancy. However, transmission of the parasite from the mother to the foetus will have more severe clinical consequences if it occurs in early gestation compared to later in pregnancy. Thereby, the parasite can be maintained within a herd for many generations without the need for new infections by oocysts from dogs.

## Q20

### How long will dogs shed oocysts for?

#### Key points:

- A dog that has not been exposed to *Neospora* and picks up infection will shed oocysts in their faeces around 3-9 days after infection
- This shedding of oocysts will last around 2-3 weeks
- After this period no further oocysts are produced and the dog is no longer a risk to the herd

If a naïve dog becomes infected, then the parasite may establish infection in the gut cells of the dog. There it will multiply and re-infect more gut cells. The parasite will undergo changes and sexual development which will lead to the production of parasite oocysts that are shed in the faeces of the dog. From infection of the dog until the emergence of oocysts in the faeces, can take 3 to 9 days depending on the level of the initial infection. Shedding of oocysts by dogs

lasts for about a couple of weeks. By then the dog's immune system has managed to control the infection in the gut and no further oocysts are produced. Once immune, the dog is not thought to shed significant numbers of oocysts again in subsequent infections. *Neospora* oocysts are usually only detectable in dog faeces after the first exposure of the dog to the parasite and therefore it is usually only observed in faeces from young dogs, often from puppies.





## Q21

### Should I get rid of my dog?

#### Key points:

- No
- If you test your dog and it is positive then it is unlikely to be a risk as it has probably stopped shedding oocysts
- If your dog has tested negative then there is a risk if it goes on to become infected while on the farm. Instigating simple bio-security procedures\* will prevent the dog getting infected and therefore no risk to the cattle herd

If your dog tested positive for *Neospora* specific antibodies then it is very likely that it will have stopped shedding oocysts as the dog has developed a detectable immune response to the parasite. As specific antibodies may persist in an animal, then detection of these antibodies does not necessarily correlate with an acute and active infection. It will also mean that the dog is not very likely to shed significant numbers of oocysts again and, as a result, it is not a continuing risk to your cattle. If your dog is antibody

negative for *Neospora* then it could be a risk to your cattle if it subsequently becomes infected. Therefore, it is advisable to instigate good bio-security measures on the farm that prevent dogs having access to calving areas or places where pregnant cattle are kept. This will prevent dogs becoming infected with *Neospora* in the first place. It is also advisable to keep dogs away from cattle feed and areas that could result in the contamination of drinking water for cattle. \* For more information please see Q40

## B9

### Transmission from dog to cow

Dogs and other canid species are the definitive hosts for *Neospora* infection, meaning that the parasite completes the sexual stages of its lifecycle in the gut epithelial cells of an infected dog. When a naïve dog ingests *Neospora* infected meat, containing tissue cysts, then it will start to shed parasite oocysts in its faeces a few days later. Oocysts are the infective stages of the parasite, which are environmentally stable and can stay infective for many months in cool and moist conditions. The oocysts can get killed by heat, freezing or drying out.

Dogs only shed significant numbers of oocysts during their first infection and the shedding is relatively short, only for a couple of weeks. During this time the

immune system of the dog starts to control and resolve the infection. In subsequent infections, the protective immune responses are re-activated and infection is controlled before significant numbers of oocysts are produced. As a result oocyst shedding is usually only seen in very young dogs and these are a particular risk factor for cattle farms.

The infection status of dogs may be determined by serology and it is thought to be safer to have an immune dog than a naïve dog because they represent a lower risk when it comes to shedding oocysts after a new exposure to the parasite.

Ingesting oocysts is the only way by which cattle can become infected with *Neospora*, other than by transmission from an infected dam to its foetus during pregnancy. Infection of cattle through oocysts is often referred to as horizontal transmission. It is linked to oocyst contaminated pasture, feed or water resulting in the infection of several cattle at the same time. This kind of "point source" infection has been determined as the usual cause of abortion storms where significant numbers of pregnancies are lost during a relatively short time period.

Although devastating when it happens, horizontal transmission is considered to be a minor route of transmission for the parasite because it is not frequently confirmed. It is also not the most efficient route by which the parasite is maintained within cattle populations. It is, however, essential for parasite survival because it is a mechanism by which it can be transmitted to previously uninfected cattle populations.

## Q22

### Can foxes spread the infection?

#### Key points:

- Foxes have been implicated in spreading infection but there is no evidence as yet to confirm this

There is some debate whether foxes are another definitive host that could produce oocysts following exposure to the parasite. *Neospora* like oocysts have been detected in faeces from foxes by separate investigators but the only attempt at an experimental infection of foxes with *Neospora* did not lead to the production of oocysts. As a result the definitive evidence that foxes can shed oocysts is still lacking. Based on

the experience with dogs and with closely related parasites it is very likely that shedding of oocysts would only be seen in very young animals e.g. fox cubs. Seroprevalence studies of foxes has shown that 0.9% of 549 tested foxes from Great Britain were positive for *Neospora* and a further study detected *Neospora* DNA in 4.8% of 83 foxes tested (Table 3).

Table 3: *N. caninum* infection in wild carnivores

Species	Number of Animals		Mean % Positive
	Tested (n=)	Positive (n=)	
Mink ( <i>Neovison vison</i> )	65	3	4.6
Fox ( <i>Vulpes vulpes</i> )	83	4	4.8
Polecat ( <i>Mustela putorius</i> )	70	13	18.6
Ferret ( <i>Mustela furo</i> )	99	10	10.1
Badger ( <i>Meles meles</i> )	64	7	10.9
Stoat ( <i>Mustela erminea</i> )	9	0	0.0

## Q23

### What about other wildlife?

#### Key points:

- There is no conclusive evidence that any wildlife in the UK can shed *Neospora* oocysts however further research is required in this area

This is an area which requires further research. In other countries of the world it was shown that wolves and coyotes can shed *Neospora* oocysts but there is no conclusive evidence of this in any wildlife species in the UK. Faecal samples of many very different host species have been tested but the only UK species which has conclusively been shown to shed oocysts are dogs. Further studies have shown

that most species investigated can act as intermediate host for *Neospora* and they will become persistently infected but do not shed oocysts. Relevant UK species are rodents, rabbits, different bird species, including chicken, as well as ferrets, polecats, mink and badgers (Table 3).

## B10

### Transmission routes involving wildlife

All hosts for *Neospora*, that have been shown to produce parasite oocysts in their faeces, belong to the canid family. These include wild dog species like dingoes and African wild dogs but also wolves and coyotes.

In the United Kingdom, dogs are the only confirmed definitive hosts that can spread *Neospora* infection via oocysts. However, there is still considerable discussion about the role of the fox. Some researchers state that they are definitive hosts while other scientists are more cautious. The reason for this difference in opinion is based on conflicting observations. It is known that foxes can be infected with *Neospora* and this is widely accepted. Data from research carried out at the Moredun Research Institute has shown that 5 out of 549 foxes (0.9%) tested had antibodies to the parasite. Another Moredun study found that 4 out of 83 foxes (4.8%) had detectable levels of parasite DNA in their brain. These data show that foxes can be infected but they may just act as intermediate host, like cattle, without the ability to produce oocysts.

In Germany, an experimental challenge of young foxes and dogs was conducted by feeding them *Neospora* infected tissue. The faecal samples produced were screened for the presence of oocysts. The puppies produced oocysts while none of the fox cubs produced oocysts, which would argue against foxes being definitive hosts. However, *Neospora* like oocysts were detected in 2 faecal samples collected from wild foxes in separate studies. In both surveys only a single sample tested positive and with a very small number of oocysts. In one study, DNA was extracted from the faeces and *Neospora* was confirmed to be present. Unfortunately, these studies could not conclusively show that the oocysts were *Neospora* oocysts and that they were actually produced in the gut of the fox. They could have passed through the digestive tract of the fox, after ingesting *Neospora* oocysts in contaminated water or food. As a result of these conflicting observations it is still unclear if the parasite can complete its lifecycle in foxes, which results in the production of oocysts.

Further studies conducted at the Moredun Research Institute have shown that many wild carnivorous species in the UK can be infected with *Neospora* (Table 3) but no data exists to show that any of these species actually shed *Neospora* oocysts following an active infection. There is now also substantial evidence that many other

animal species may be naturally infected with *Neospora* including mice, rats, rabbits, hares, deer and chicken. These species are more likely to act as intermediate hosts and may act as reservoirs for the infection for carnivores, including dogs.

## Q24

### How long will the parasite oocysts survive for?

#### Key points:

- Little information is available in this area. However, knowledge from similar organisms suggest that the oocysts survive for long periods and stay infective for many months
- The presence of ammonia and heat (i.e. in a muck midden) can help destroy oocysts

Experimental data for *Neospora* oocyst survival has not been done or published because very few groups have produced oocysts from experimental infections. As a result oocyst survival is based on data from a very closely related parasite called *Toxoplasma gondii*. *Toxoplasma* oocysts can survive for more than a year in favourable environmental

conditions, which are cool and moist. These oocysts survive well in water. Because these parasites are closely related and their oocysts are morphologically very similar, it is probably fair to assume that *Neospora* oocysts will also persist in the environment for a long period and that they will stay infective for many months.

## Q25

### How does the parasite survive within the herd?

#### Key points:

- As there is no treatment or vaccine the parasite is difficult to eliminate from a herd
- Infected herds remain persistently infected because the parasite can be transmitted from infected dams to their offspring

Once a herd is infected with *Neospora* it is very difficult to eliminate the parasite from the herd because there is no treatment or vaccine that can clear the parasite from infected animals. A further complication is that the parasite transmits very effectively from infected dams to their foetuses which will either lead to abortions or to the birth of infected calves that can appear clinically healthy at birth. The apparently healthy calves, in turn, can transmit the infection to their offspring in subsequent pregnancies. As a result the

parasite can persist within a herd over many generations without the need for dogs to infect more cattle. *Neospora* persisting in a herd will manifest itself by sporadic abortion cases. Abortion material together with infected placentas may be a source of infections in naïve farm dogs that have not had prior contact with the parasite, leading to the production of oocysts and possibly infection of cattle and contamination of the immediate farm environment.

## Q26

### Is my infected cow a risk to my other uninfected adult cows?

#### Key points:

- There is no evidence of adult cow to adult cow transmission of *Neospora*

No, there is no evidence of adult cow to adult cattle transmission of *Neospora*. The only way by which adult cattle can become infected with *Neospora* is through ingestion of oocysts. Therefore, if the definitive host, i.e. dogs, can be taken out of the transmission cycle then all naïve adult cattle are safe. This will allow farmers the opportunity to breed out *Neospora* from their herd by choosing replacement heifers from *Neospora* uninfected cows and over time eliminate the infected animals from the herd. There is no good evidence

of horizontal transmission between cattle. Experimental work has shown that it is possible to infect very young neonatal calves, 6 hours old, with colostrum samples spiked with live tachyzoites. However, this is not thought to be a significant natural route of transmission. Similarly, *Neospora* contaminated semen is not thought to be a major issue in the transmission of the parasite to cattle.

## B11

### Transmission between dam and calf

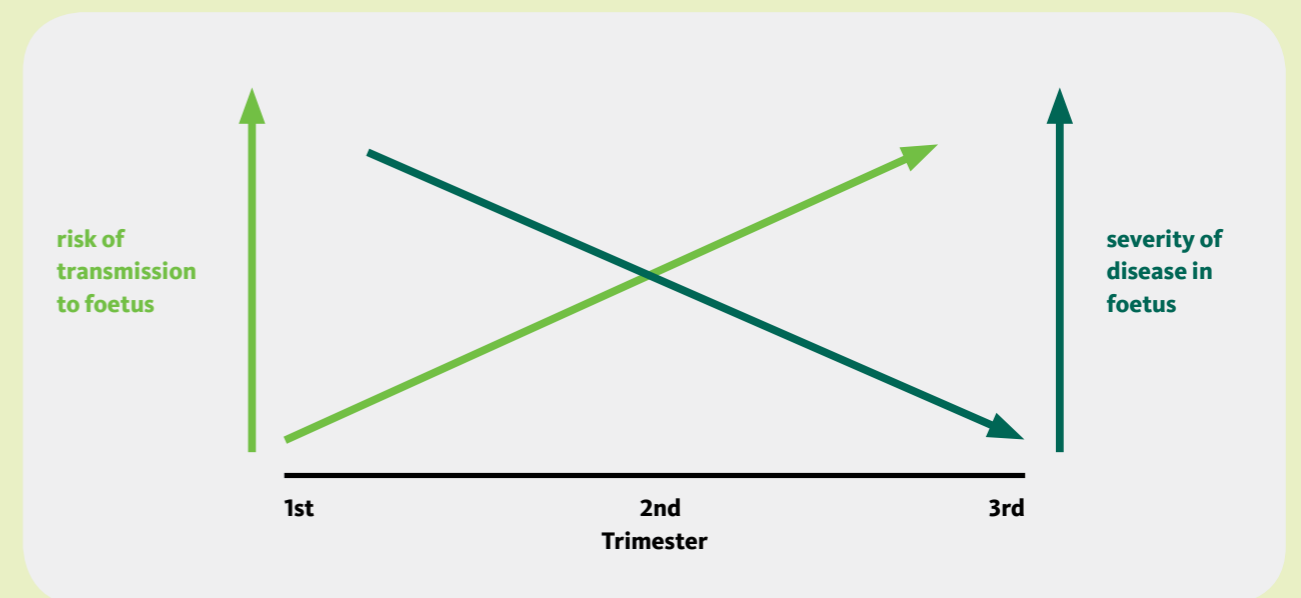
Transmission of *Neospora* from an infected dam to its foetus is considered to be the most important transmission route for maintaining the parasite within the cattle population. It is estimated that about 90% of infected dams pass the parasite on to their offspring during pregnancy. This means that once the parasite is within a herd, it may be maintained there for many generations, without the need of a definitive host such as a dog.

With each birth, the infected cow produces a placenta that may contain parasites that are infective to dogs, giving the opportunity for oocysts to be produced that could infect other cattle. Even if no transmission of the parasite occurs via the placenta to dogs, then there are still future chances that the parasite will be transmitted to dogs in subsequent pregnancies. Also, infected female calves have the opportunity to pass on the parasite to their offspring and to produce infected tissue samples that could act as a source of infection for dogs and other carnivores.

It is known a cow will consume her own placenta after giving birth and also on occasion placental material left by another cow in the herd. It has been speculated this might provide another route of transmission for *Neospora*. However, there is no good evidence that this is an important natural transmission route.

The timing of *Neospora* infection or reactivation of a persistent infection of the parasite during pregnancy is very important in determining the clinical outcome of the infection, as demonstrated in Figure 4. If the infection occurs during the first trimester of pregnancy then (although there is a relatively lower chance of transmission compared to later in pregnancy) then there is a high risk of the foetus dying as it does not have the ability to produce a protective immune response that can control the parasite. As the pregnancy progresses the foetus matures and so does its immune system. The immune responses of the dam naturally change during pregnancy to accommodate the safe development of the foetus and protect it against immunological rejection (as the foetus may resemble a foreign tissue graft within the mother). The immune responses induced as a result of pregnancy are less able to control the multiplication of the *Neospora* parasites. If the dam becomes infected later in pregnancy she is less able to prevent the transmission of *Neospora* parasites to the placenta. However, at the later stages of gestation the foetus is much better equipped to control the infection should transmission occur. This means that *Neospora* infections later in pregnancy usually result in the birth of persistently infected calves rather than abortions. Female calves, infected during pregnancy, are very likely to transmit the parasite to their subsequent offspring and a proportion of these animals will have abortions during their first pregnancy.

Figure 4: Frequency and severity of *Neospora caninum* infection of foetuses during pregnancy



## Q27

### How can I be sure that bought replacement heifers are no risk to my other animals?

#### Key points:

- There is no risk to other cattle in the herd via direct transmission from bought in heifers
- There is a threat though, if recently purchased heifers abort and the foetal material is eaten by a dog that has not been exposed to *Neospora*
- Testing heifers coming onto the farm would be helpful but remember, a negative test is not always definitive.

Quarantine of newly purchased cattle is good practice when buying in animals but for *Neospora* this is not necessary because there is no evidence of transmission between adult livestock. The only risk from these newly purchased heifers would be if dogs had access to and ate placental material from these animals which could result in the dogs becoming infected with *Neospora* and subsequently shedding oocysts.

This would contaminate the immediate farm environment, posing an infection risk to other cattle.

If possible, testing replacement cattle for antibodies to *Neospora* in blood samples before they are brought onto the farm would be helpful. Although as discussed previously in the diagnostic section, the results from the serology testing would have to be interpreted carefully.

## Q28

### Why do some cows abort but not others?

#### Key points:

- It depends on when infection occurs
- In general, cows infected during early to mid pregnancy abort. Cows infected in late pregnancy will give birth to a persistently infected calf.
- The same applies for persistently infected cows. If the parasite becomes active in early to mid pregnancy then abortion can occur. If activation is in late pregnancy, a persistently infected calf is likely

Experimental infection studies with *Neospora* at Moredun have shown that timing of the initial infection plays a crucial role in the clinical outcome of the pregnancy. If infections occur early in pregnancy then the maternal immune system can mount a strong and protective response. This can prevent the parasite from invading the placenta and infecting the foetus. However, if the parasite manages to reach the foetus during the first trimester of pregnancy then it can multiply unhindered because the immune system of the foetus is not sufficiently developed to control the parasite. Multiplication of the parasite within the placenta also provokes an inflammatory immune response in the placenta as the mother's immune system is alerted to try and fight the infection. This inflammatory response in placental tissue, and the immunological vulnerability of the foetus, may lead to abortion.

If the infection occurs later during pregnancy then the immune system of the foetus is more mature and may manage to contain but not clear the infection. This may lead to the birth of persistently infected calves. As pregnancy progresses, the maternal immune responses change as well to allow the pregnancy to continue without the threat of rejecting the foetus as a foreign entity. However, this means that the dam is also less able to prevent the parasite

reaching the placenta, causing infection of the foetus. Many abortions are caused by infections during early to mid-pregnancy. Infections later in gestation can cause the birth of live infected calves that may show some neurological clinical signs at birth or are born apparently healthy with no clinical signs. It is also thought that the same is true for the timing of reactivation of the parasite in infected dams. Studies looking at changing patterns of specific antibody responses of infected cattle over pregnancy showed that increases in specific antibody production occurring in early to mid-gestation may indicate that these cattle were more likely to abort their foetus. A rise in specific antibody titres in late gestation was more likely to result in the birth of a clinically normal but persistently infected animal. Changes in specific antibody levels will reflect parasite activity in the animal i.e. when the parasite becomes active and starts multiplying this will induce a specific antibody response. Therefore monitoring changes in the specific antibody levels in *Neospora* infected cattle can give an indication of whether an animal is likely to abort or not.

There is no good evidence that the breed of cattle will have much effect on the susceptibility or resistance to *Neospora* infection. Studies with buffalo have shown them to be more resistant than cattle to infection with *Neospora* parasites.

## Q29

### Are heifers more like to abort?

#### Key points:

- Generally, yes. This is because older cows have more time to develop immunity to the parasite

There is evidence that *Neospora* infected heifers are more likely to abort than infected cows in later pregnancies. This is probably due to the fact that infected cattle are more likely to abort in the first pregnancy after becoming infected, while older cows are better able to control the infection due to priming of their immune systems and fewer calves are lost.

About 5% of cattle that have aborted once due to *Neospora* infection may abort again due to neosporosis. However, most infected cows, regardless of age, will transmit the parasite to their foetuses *in utero* and these animals are more likely to abort their calves in their first pregnancy, in comparison to older, infected animals.

## Q30

### How can I avoid getting the parasite onto my farm?

#### Key points:

- It's a difficult process but it is possible
- Having a closed herd that is free from *Neospora* obviously prevents the infection coming in via purchased cows
- An effective bio security plan will be essential in putting a stop to infection from dogs

If you do not have *Neospora* already, then having a closed herd will eliminate one of the main transmission routes of bringing *Neospora* onto your farm via persistently infected cattle. As there is no reliable diagnostic test that will identify all *Neospora* carrier animals with a single test, it is impossible to be sure a bought in animal is not infected. If replacement stock need to be bought, then buying from a herd that is part of a cattle health scheme already testing for *Neospora*, or a herd that is known to be *Neospora* free,

will help. It is also advisable to screen replacement cattle, when they are on your farm, remembering that they are no risk to your herd unless they abort or give birth to infected calves. Another important strategy is good rodent control to avoid an infected rodent population establishing on the farm because they will have access to the calving area and may be a source of *Neospora* infection for a dog. It is also important to keep dogs away from calving areas and from access to cattle feed.



## Q31

### How can my animals become infected if I have a closed herd?

#### Key points:

- Infection can come in to the herd from dogs so effective bio security is essential

Having a closed herd will prevent the risk of importing the problem onto the farm with infected cattle. However, there are other ways that the parasite can come onto your farm. The main alternative sources would be contamination from other dogs that have access to your fields, and wildlife hosts

that could shed oocysts (although there are no confirmed definitive hosts in the UK other than dogs). Animal feed and water contaminated with oocysts, and water run-off, containing oocysts, from surrounding areas onto your farm are also potential routes for infection.

## Q32

### Can *Neospora* infect me?

#### Key points:

- There is no evidence that *Neospora* infects humans

Researchers have looked very extensively, worldwide, for evidence that *Neospora* may infect and cause disease in humans. The simplest tests to conduct are serology based because serum is easy to obtain. In the UK, no evidence was found that humans can get infected with *Neospora*. However, studies in South America have claimed that there was

some evidence in immuno compromised people who were exposed to *Neospora* but, even in these samples, antibody levels were very low and probably borderline. Although it is possible to experimentally infect Rhesus Macaque monkeys, there is currently no good clinical evidence that *Neospora* naturally infects or causes disease in humans.

## 4. Control of *Neospora caninum* infection and Neosporosis

### Q33

#### Is there a vaccine to protect against *Neospora* infection?

##### Key points:

- Currently there is no licensed vaccine in the UK
- Vaccines are, however, being used in non EU countries including the USA. Vaccination efficacy trials are ongoing in a number of UK research institutions including Moredun.

Currently, there is no licensed vaccine to protect cattle against Neosporosis in the UK and Europe. There is a vaccine to aid against *Neospora* associated abortion that is licensed and marketed in the USA and is also available for purchase in South and Central America and New Zealand. This vaccine is made from killed tachyzoites. Field trials on *Neospora* affected farms have given variable results, where in some cases fewer abortions were seen, while on other farms the vaccine had little or no effect.

Research at Moredun has shown that a vaccination approach is a feasible option to protect cattle against *Neospora*-

associated abortion. Results from the research show it is much easier to induce protective immunity using a live vaccine, although a major drawback of using this approach is that it would not be desirable for it to exist in the animal due to the risk of vertical transmission to the foetus. The vaccine should be targeted at naïve animals and should be administered prior to mating. Ideally, the vaccine should be designed in such a way that it is possible to diagnostically distinguish between vaccinated and infected animals as this would have important implications for cattle health schemes based on animals being seronegative to the parasite

### Q34

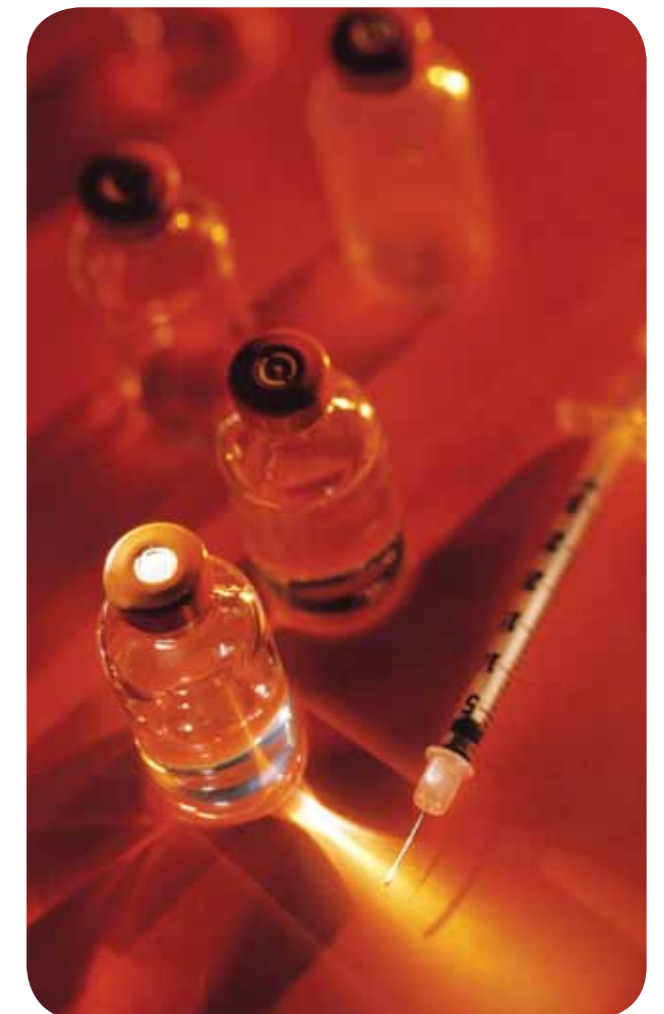
#### Why is the vaccine not available in the UK?

##### Key points:

- Some non EU countries market *Neospora* vaccines. However, evidence suggests that they are not very effective in controlling the disease and therefore aren't licensed in the UK
- Many believe urgent research funding should be made available to develop an effective vaccine

The vaccine has not been licensed for use in the UK or any other country within the European Union due to more stringent legislative requirements here than in the USA. To licence a product in Europe there would have to be evidence of efficacy.

Various research groups, including scientists at the Moredun Research Institute, are working on the development of a vaccine that will reduce and prevent *Neospora* abortion in cattle. Some of the research approaches tested have proved to be effective using experimental bovine models of neosporosis. It is hoped that new vaccines may be available soon to help protect cattle against Neosporosis.



## Vaccines for Neosporosis

Currently there is no vaccine against Neosporosis that is licensed for use in cattle in the UK or any other European country. In the USA there is a vaccine based on killed *Neospora* tachyzoites that is administered twice to animals during pregnancy. However, published work on the efficacy of this vaccine in field conditions has given conflicting results. In one comprehensive study a wide spectrum of results were obtained where at best the vaccine did show a reduction of disease, while on other farms it had either no effect or more disease was seen after the vaccine was used. The manufacturers of the product suggest that this vaccine may help prevent abortion if naïve animals are vaccinated prior to challenge.

The licensing standards for vaccines are very different in the USA to those in Europe. In the USA no efficacy data is required to obtain a licence, the manufacturers just need to show that the vaccine is not harmful. In Europe, however, manufacturers have to provide evidence that the vaccine is effective and that it is not harmful. Attempts to obtain a licence for the USA vaccine in Europe have failed so far.

### Current vaccine research:

Live vaccines are very effective in inducing protective immune responses because they more closely mimic the natural infection in the host animal. Several research groups are currently exploring the use of live attenuated vaccines as a suitable approach to protect against bovine neosporosis.

Attenuated *Neospora* parasites are able to infect animals without causing disease. It is important that the attenuated strains used as vaccines do not result in persistent infection as the parasite may become reactivated during subsequent pregnancies resulting in disease. Attenuated parasite strains can be obtained from natural infections in the field, by prolonged parasite culture *in vitro* or by serial passage in animals. This approach has resulted in several effective vaccines against other protozoan parasites, for example to protect against congenital *Toxoplasma* infection in

sheep and tropical theileriosis in cattle. Attempts have failed, so far, to produce a suitable attenuated *Neospora* strain that does not cause persistent infection in the host animal. A different approach has been selected by a Spanish research group that has attempted to identify a naturally occurring attenuated *Neospora* isolate that does not cause clinical disease. They have managed to identify different isolates with different degrees of pathogenicity and they are currently assessing the potential of these strains to act as vaccines against bovine neosporosis.

The main drawback of live vaccines is there are safety concerns that these vaccine strains could revert back to virulence and cause disease themselves. A further limitation of these vaccines is that it may be difficult to distinguish between vaccinated and naturally infected animals. This raises concerns if testing for these pathogens is included in Cattle Health Schemes to get disease free accreditation for farms. However, a major advantage of live vaccination approaches is that they do work very effectively in protecting against disease.

Other options that are being investigated are subunit vaccines, where only parts of the parasite are included in the vaccine formulations. The challenges for these types of vaccines are mainly that it is more difficult to induce protective immune responses as the vaccine is made using killed antigens and different delivery strategies have to be developed to help induce the required immune responses.

Research at Moredun has identified the critical immune responses that would have to be induced by an effective vaccine and scientists are working on a live and a killed vaccination strategy. A bovine model of neosporosis has also been developed to allow the efficacy testing of vaccine candidates. Live immunisation of naïve cows prior to mating is effective in protecting against a live challenge administered to pregnant cattle at mid-gestation. These studies and others across the world give optimism that new, more effective vaccines will soon be available to protect against bovine neosporosis.

## Can I treat my infected animals?

### Key points:

- There are no licensed drugs for the treatment of *Neospora* infection in cattle

Unfortunately, there are no licensed drugs for the treatment of *Neospora* infection in cattle. There are some drugs that are used to limit the effect of infection in dogs. These drugs are only effective in suppressing multiplication of intracellular tachyzoites but they have no effect on the parasite cysts, the persistent stage of the parasite in the animal. There are no drugs that can cure infected animals.

There is experimental data on the efficacy of certain compounds to reduce tachyzoite multiplication *in vitro*. There have also been reports of treatment of cattle with the drug Toltrazuril having reduced likelihood of *Neospora*-associated abortions. For the dairy sector a further complication may be milk withdrawal periods associated with these drugs, especially if the animals would need to be treated for a prolonged period during pregnancy to avoid abortion.

## If you had an abortion storm, what can you expect in subsequent years?

### Key points:

- Most herds that experience an abortion storm will see reduced numbers of sporadic abortions in subsequent years

The abortion storm is the most devastating manifestation of *Neospora* infection in cattle herds and usually occurs after a primary point source infection of the herd due to oocyst contamination of pasture, feed or water. Usually there are more infected animals than just those that aborted, as other cows that were not pregnant or were at a different stage of pregnancy when the infection occurred may not have shown clinical signs of disease. This means that some of these

animals may abort at a later stage or that their off spring may abort in subsequent pregnancies. Most herds that experienced an abortion storm will see reduced numbers of sporadic abortions in subsequent years. However, care should be taken to avoid oocysts shed by infected dogs entering food or water supplies of cattle to avoid further risk of abortion.

## Q37

### What can I do to prevent my uninfected animals becoming infected with *Neospora*?

#### Key points:

- Uninfected cows will not get the parasite from infected cattle
- The only known route of infection is from dogs
- Adopt good biosecurity measures to prevent dogs accessing cattle areas

There is no good evidence for direct transmission of the parasite between cattle, other than from a dam to its calf during pregnancy. This means that the major route by which cattle can become infected with *Neospora* postnatally is by the ingestion of oocysts that were excreted in the faeces of an infected dog. The advantage of this transmission route is that there is no direct danger of keeping infected cows with uninfected cattle. This gives farmers more time to develop control strategies, without the need to cull out all infected animals immediately. However, good biosecurity

measures should be implemented that will stop dogs accessing cattle areas, especially places where pregnant cattle are kept and designated calving areas. This will help prevent dogs becoming infected with the parasite. Further, dogs should not be allowed in areas where cattle feed is kept and it is also important to make sure that dog faeces cannot contaminate feed or drinking water. Another measure that has been suggested is good rodent control, as rodents can become infected with *Neospora* and may act as a reservoir for the infection for dogs.

## Q38

### Can I get rid of the parasite once I have got it in my herd?

#### Key points:

- Once in a herd, *Neospora* will be very difficult but not impossible to eradicate
- See answer to Q39 on how to manage the infection out of your herd. Also a good idea to speak to your vet

There are no drug treatments as yet that can eliminate the parasite from an infected animal, which means that the only way to eradicate the parasite from the herd is by removing all infected animals. Culling out all infected animals from an infected herd is rarely an economically viable option and is only likely to be used when a very small proportion of the animals within the herd are infected. However, because there is no transmission between adult cattle, a slower approach can be chosen. This is called "breeding out". For this control strategy, the infection status of all animals within the herd

needs to be determined and only replacement stock bred from uninfected dams will be kept whilst infected cows are culled out over time based on their productivity.

However, it is important to note that it is extremely difficult to detect and eliminate *Neospora* oocysts from the environment and therefore there is always the danger that naïve cattle can become infected via consumption of oocysts contaminating the farm area.

## Q39

### Should I get rid of my positive cows?

#### Key points:

- Culling out infected cows would not normally be a practical or commercial option
- Once the level of herd infection is known it is recommended the farmer seeks veterinary advice to determine the best course of action

Culling out all infected animals from a herd is not usually a practical option and it will depend on the proportion of infected animals within a herd (i.e. will it significantly affect the productivity of the herd?) It also depends on the type of farm (i.e. does the farm rely on sales of animals?) For some owners of pedigree herds it may be acceptable to lose a higher proportion of their animals to be able to claim a *Neospora* infection free status when they sell their livestock. For a dairy farmer the decision should focus on productivity of animals. Especially since there is no transmission between adult cattle (the route is from dam to calf during pregnancy and dog to cattle). This allows other options to minimise the

impact of the disease like breeding out the infection from the herd. It is a much slower approach based on selecting breeding stock from uninfected animals and using their offspring as replacement stock for infected, less productive animals.

The other consideration is that having a herd of naïve, *Neospora* free cattle may also be a risk as these animals would be very vulnerable if they picked up infection from oocysts in the environment and would have no immunity or resistance to the parasite.



## Q40

### What does 'adopting good biosecurity measures to prevent *Neospora* infection or transmission' mean?

#### Key points:

- Biosecurity measures will reduce the risk of introducing and transmission of *Neospora* onto and within the farm by blocking potential transmission routes of the parasite
- A list of biosecurity best practice measures are shown below
- By operating these on farm you can also help prevent livestock picking up infections from other pathogens

Biosecurity measures that prevent *Neospora* infection or transmission refers to farm management practices that can be adopted on a farm which will either reduce the risk of the parasite getting onto the farm or reduce/prevent the parasite being transmitted to uninfected animals. Listed below are a range of measures that are designed to reduce the risk of *Neospora* infection and transmission. They are also good general security methods that will help mitigate the risk from other pathogens on the farm.

#### *Neospora* specific biosecurity measures are:

- Having a closed herd and not buying in any replacement stock will reduce the risk of introducing *Neospora* onto the farm (current diagnostic tests are not able to detect all infected animals).
- Breed replacement stock from cows that are known to be uninfected with *Neospora* as this will help to breed out the parasite from the herd.
- Dispose of cattle tissues left over from a calving, or aborted fetuses, in a safe and timely manner. If infected with *Neospora* these pose a high risk of transmitting the parasite to dogs and other hosts, such as rodents / wildlife.
- Prevent dogs having access to calving areas or parts of the farm where pregnant cattle are kept in order to stop dogs becoming infected from eating placenta or abortion material.
- Do not feed your dog raw meat and this will prevent exposure to the parasite.
- Make sure dogs cannot get access to areas where cattle feed is kept or fields that are used for grazing to prevent dog faeces, which could be contaminated with *Neospora* oocysts, being ingested by cattle.
- Dogs should not be allowed to access cattle water troughs or fields where the rain run-off will be washed into the livestock drinking water. This will reduce the risk of potentially contaminated dog faeces ending up in drinking water where the *Neospora* oocysts can survive for many months.
- Dog faeces should be disposed of safely in the household waste or in the midden, where the heat is sufficient to kill *Neospora* oocysts.
- Operate good rodent control because they can become infected with *Neospora*. Infected rodents are not a direct risk to the cattle on the farm but dogs eating infected rodents can become infected with *Neospora* and potentially pass the parasite to cattle.
- If public footpaths cross the farm or fields used by cattle, then it is advisable to put up notices reminding dog walkers to pick up their dog's faeces, to avoid passing on any infection to livestock.

## Q41

### How can I minimise the economic effect of Neosporosis on my milking herd?

#### Key points:

- Again, once the level of infection is known the producer should seek veterinary advice to determine the best course of action

In order to work out what can be done to minimise the economic impact of the disease on production it is essential to know what proportion of cattle within the herd are infected with *Neospora*. This will give a quick indication if culling out infected animals is a viable option but it also identifies which animals are either infected or are free of infection.

A good biosecurity strategy should be adopted that will minimise the risk of new infections due to oocysts shed by dogs. This approach will also lessen the urgency of having to remove infected animals from within the herd because there is no direct transmission between adult cattle.

Having removed/reduced the risk of new infections within the herd, other than dam to calf transmission during pregnancy, then a slow process of breeding out the infection from within the herd can begin by choosing replacement stock from *Neospora* uninfected animals and by culling out the least productive infected livestock over time.

In order to minimise the losses of not being able to get replacement stock from *Neospora* infected cows there is the option to mate *Neospora* infected cows with beef bulls or use beef bull semen in insemination programmes. This means that in successful pregnancies the dam is in milk and the calves can be sold for meat, which will compensate for the loss of breeding stock.



## Farm management practices to reduce the impact of *Neospora* infection

Good farm management practices should be adopted by farmers regardless if you have *Neospora* on a farm or not because they will help to prevent introduction of the parasite onto the farm. It will also reduce the risk of spreading the infection further once it is there. These practices initially focus on dogs: stopping dogs becoming infected and also preventing infected dog faeces contaminating pasture and animal feed or water.

### Prevention of dogs becoming infected:

One of the most important ways to stop the spread of *Neospora* on the farm is to make sure that the farm dogs do not become infected. This can be achieved by restricting their access to where cattle are kept, especially areas for pregnant cattle and calving. This will limit one of the main sources of infection for the dog. The dogs also should not be fed potentially infected meat, which could be any raw meat. Another important strategy is good rodent control on the farm as these could be infected with *Neospora* and thus may act as a reservoir of infection for dogs.

### Prevention of dog faeces contaminating cattle feed or water:

A second layer of control that should be implemented is preventing dog faeces from contaminating cattle feed and water. Access of dogs should be controlled in areas where cattle feed is kept, where cattle graze and areas where water run-off could wash dog faeces into drinking water.

Public footpaths and right of access over which the farmer has no control cause particular difficulties when they go across fields used for grazing. This, potentially, could lead to introduction of *Neospora* oocysts onto the farm. The only option is to educate dog owners to pick up the dog faeces while walking on farm land.

### Prevention of introducing *Neospora* onto a farm:

Control of dog faeces is important for reducing the risk of introducing *Neospora* infection onto a farm but so is keeping a closed herd (if that is an option.) However, if purchasing replacement stock is required then buy them where possible from reputable farms with good reproductive health history. Some Cattle Health Schemes have started to include *Neospora* testing which may help in the identification of suitable farms for the purchase of replacement cattle.

*Neospora* infected animals, brought into a *Neospora* free herd, are normally no risk to the other members of the herd, except if they abort. Then they produce material that is infective to dogs. Also, if the infected cattle produce infected but apparently healthy calves, then this will increase the number of infected stock. Having animals of unknown infection status within the herd increases the risk of spreading the disease. Therefore, bought in replacement stock should be tested to confirm if they are infected or not. To be sure of an animal's infection status, they should be tested on more than one occasion, ideally before and during the first pregnancy, in order to increase chances of avoiding false negative test results.

### Web Links:

**Moredun Research Institute:**  
<http://www.moredun.org.uk/>

**Neospora at the Moredun Research Institute:**  
<http://www.moredun.org.uk/research/research-%40moredun/reproductive-diseases/neospora>

**Prof Elisabeth Innes:**  
<http://www.moredun.org.uk/staff/prof-elisabeth-innes>

**Dr Frank Katzer:**  
<http://www.moredun.org.uk/staff/dr-frank-katzer>

### Suggested Further Reading:

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