DEFRA / AHT / BEVA

EQUINE QUARTERLY DISEASE SURVEILLANCE REPORT

Pilot Issue: October-December 2004

Highlights in this issue:

- **Equine herpes virus-1 (EHV-1) neurological disease outbreaks continue.**
- **Influenza virus-related Encephalopathy update.**
- **Equine viral arteritis (EVA) confirmed in a stallion.**
- **Focus on equine liver disease.**

Important note:
The data presented in this report must be interpreted with caution, as there is likely to be some bias in the way that samples are submitted for laboratory testing. For example they are influenced by factors such as owner attitude or financial constraints or are being conducted for routine screening as well as clinical investigation purposes. Consequently these data do not necessarily reflect true disease frequency within the equine population of Great Britain.

Introducing a new concept

As part of the UK Government's commitment to enhance veterinary surveillance, described in its strategy document published in 2003, the Department for Environment, Food and Rural Affairs (Defra) has now extended the set of species-specific quarterly surveillance reports to include horses and donkeys. However, unlike the other species reports, and in the spirit of partnership promoted by the Animal Health and Welfare Strategy, the Animal Health Trust (AHT), based at its headquarters at Lanwades Park, Newmarket, is coordinating the initiative for the horse, in conjunction with Defra, the British Equine Veterinary Association (BEVA) and a broad network of different laboratories and specialist equine practices.

It is intended when the reporting system is fully up and running that each participating centre will provide anonymised quarterly information on numbers of diagnostic tests performed and discussion of positive results obtained for specified equine infections and disease syndromes. The reports will also include more in-depth discussions of unusual cases and outbreaks, current issues of concern for equine health and welfare and related research findings of relevance. These data will be collated by the AHT, and reports issued two months after the end of the period being reported. Quality assurance standards achieved by reporting centres will be applied in the selection and presentation of data. The information will be made widely available through the Defra, AHT and BEVA websites, In time it is anticipated that, with the co-operation of BEVA members, information on non-infectious diseases and syndromes will be included in reports.

In order to get the ball rolling and establish a reporting framework on which to build, this first report represents a retrospective collation of data arising from the AHT's existing equine surveillance activities. Summary data for diagnostic virology for the whole of 2004 is presented. The remainder of the report concentrates on specific infectious diseases that occurred in Great Britain during the last quarter (October-December) of 2004. These data are all based on ISO 9002 accredited procedures conducted in the AHT's diagnostic laboratories.

AHT Diagnostic Virology 2004

A summary of the virology samples submitted to the AHT for testing during the whole of 2004 is shown in table 1. A total of 30,927 diagnostic tests were conducted in 2004. This represents a decrease on the 35,434 carried out in 2003, however this figure reflects the numerous disease outbreaks that occurred in that year.

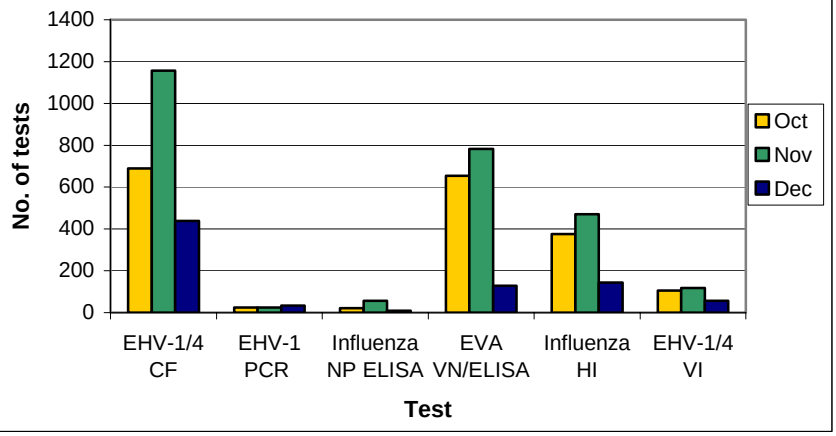
Table 1. AHT diagnostic virology sample throughput and positive results for 2004

Test, or group	Number of Samples Tested	Number Positive	% Positive
Serological Tests			
EVA VN/ELISA	9461	361[#]	3.8%
EHV -1/-4 CF test	7761	81*	1.0%
EHV-3 VN test	22	4	18.2%
ERV-1 CF test	3940	10*	0.3%
ERV-2 CF test	3940	6*	0.2%
Influenza HI test	4129	36*	0.9%
Influenza SRH test	119	31*	26.1%
Virus Detection			
EHV-1/-4 PCR	341	32	9.4%
EVA PCR	34	1	2.9%
Influenza NP ELISA	291	13	4.5%
Influenza VI in eggs	13	8	61.5%
EHV VI	877	23 EHV-1, 3 EHV-4	3.0%

VN- virus neutralization, ELISA- enzyme-linked immunosorbent assay, CF- complement fixation, HI- haemagglutination inhibition, SRH- single radial haemolysis, PCR- polymerase chain reaction, NP ELISA- VI- virus isolation,
ERV- equine rhinovirus
[#] Seropositives include vaccinated stallions
* Diagnosed positive on basis of seroconversion between paired sera

A graphical summary of the virological tests conducted during the final quarter of 2004 is shown in figure 1. A seasonal peak in respiratory disease is illustrated by the high number of submissions for influenza and equine herpes virus (EHV) screening. Submissions of fetal and placental tissue for polymerase chain reaction (PCR) testing for the presence of EHV-1 as a cause of abortion showed a slight increase in this quarter. Equine viral arteritis (EVA) screening maintained the moderate level of testing typically seen outside the pre-breeding season.

Figure 1. Diagnostic tests conducted in the last quarter of 2004 by month



Disease Report for the Final Quarter of 2004

Equine Herpes Virus

During the final quarter of 2004 PCR was used to detect the presence of EHV-1 in 84 samples of fetal and placental tissues. Virus isolation was attempted on 278 submissions of blood, nasal swabs, and tissue. A complement fixation test was performed on 2285 serological samples to test for the presence of antibody to EHV-1 and 4. A summary of the positive diagnoses are presented below.

EHV-1 Abortion

A single EHV-1 abortion in a 5-year old thoroughbred mare on a stud in Hertfordshire was confirmed based on histopathology and PCR.

EHV-1 Neurological Disease

Paralytic EHV-1 outbreaks have continued to be diagnosed during 2004, including the last quarter, following an increase in number of cases during 2003. These generally present as single or small numbers of neurological cases seen alongside more widespread respiratory or subclinical infection. Several outbreaks have occurred among vaccinated animals. Molecular characterisation of isolates from these outbreaks has shown a strong association between neurological disease and a specific mutation within the viral genome[1].

EHV-1 neurological disease was confirmed on a livery yard in Essex in October in an unvaccinated 14-year-old Arab-cross mare that developed urinary retention, hind limb paresis and recumbency. Serological and virological screening demonstrated no evidence of viral activity among 8 in-contact animals. The affected mare was supported in a sling and made a good recovery within a month.

Two cases of EHV-1 neurological disease occurred on a small Thoroughbred stud in Leicestershire at the end of November. The first case, confirmed by virus isolation from heparinised blood, was a non-vaccinated mare that presented with sudden onset recumbency. The mare was subsequently euthanased. A direct contact of the first case then presented with hind-limb ataxia and urinary overflow. Control measures to limit spread by movement restrictions, and serological and virological screening of all 24 animals on the premises was performed successfully in accordance with Horserace Betting Levy Board Codes of practice. Screening showed that the infection was restricted to a group of 8 animals, including the two clinical cases, housed together in an American barn. This type of housing is recognised as a risk factor for the transmission of EHV-1.

EHV-4 Respiratory Disease

EHV-4 was confirmed as the cause of mild respiratory disease including pyrexia, nasal discharge, and coughing on two Thoroughbred training yards this quarter. Virus was isolated from nasopharyngeal swabs and there was seroconversion in paired sera.

Equine Influenza Virus

During the final quarter of 2004 a haemagglutination inhibition test was used to screen 990 serum samples for the presence of antibody to influenza virus, and 87 submissions were tested for the presence of virus using a nucleoprotein enzyme linked immunosorbent assay (NP ELISA). A summary of the positive diagnoses is presented below.

Influenza infection was confirmed in a vaccinated 12-year-old Cob gelding, on a premises in Oxfordshire during September. The pony died following clinically severe respiratory disease, and at *post mortem* examination influenza virus was detected by immunostaining of lung tissue. No signs were observed in two vaccinated in contact animals. Disease among vaccinates has increased in recent years and research to establish the reasons for this is ongoing.

Seroconversion was used to diagnose influenza in a 9-year-old Welsh Cob mare on a livery yard in Hampshire in November. Eight of the 30 animals kept on the premises were affected. The horse had last been vaccinated in July, and showed typical signs of pyrexia, mucopurulent nasal discharge and coughing at rest.

In late November/early December influenza was diagnosed on 2 adjacent non-Thoroughbred premises in Wales by NP ELISA on nasopharyngeal swabs. Non-vaccinated animals were mainly affected on both premises. Characterisation of viruses isolated in eggs has shown them to be America-like lineage H3N8. The phylogenetic tree shown in figure 2 demonstrates the relationship of this strain to other isolates. Recent UK strains are highlighted.

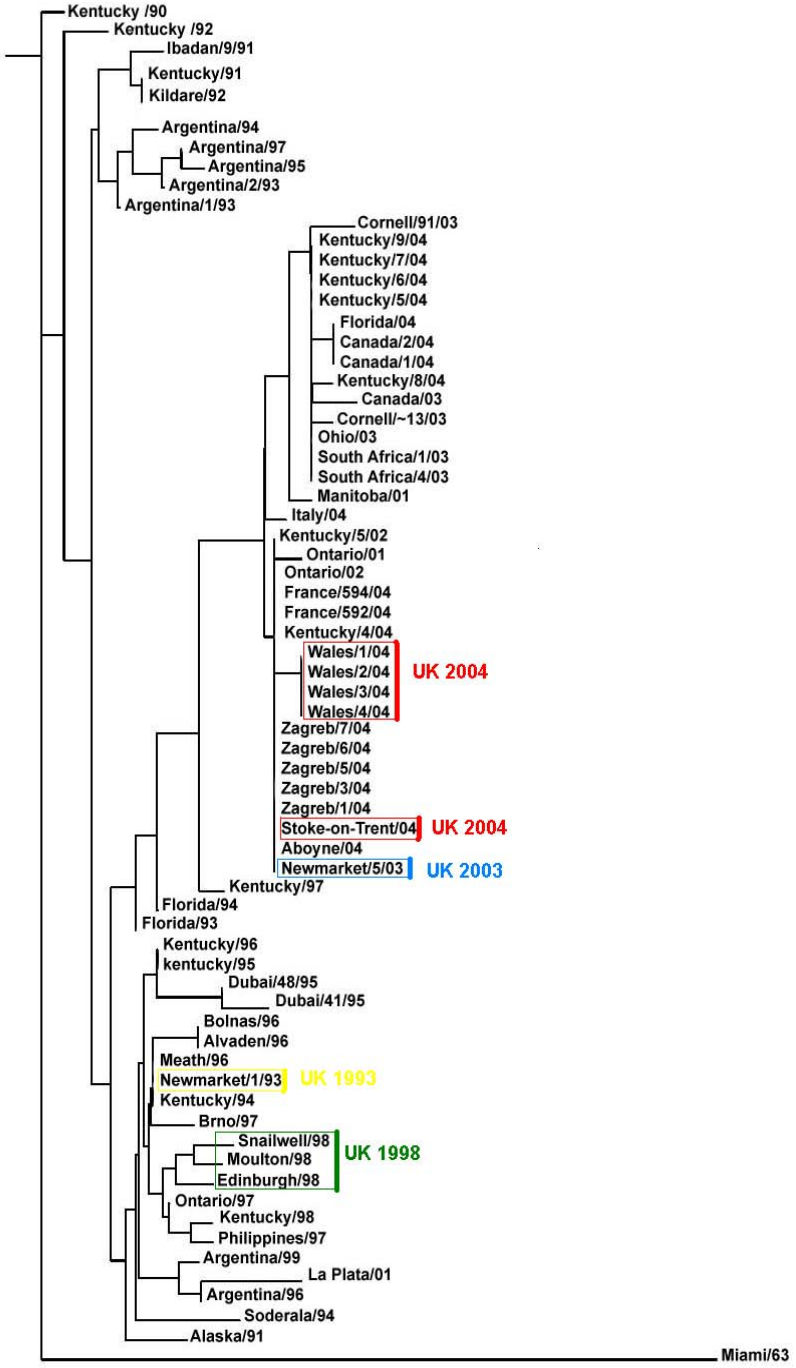
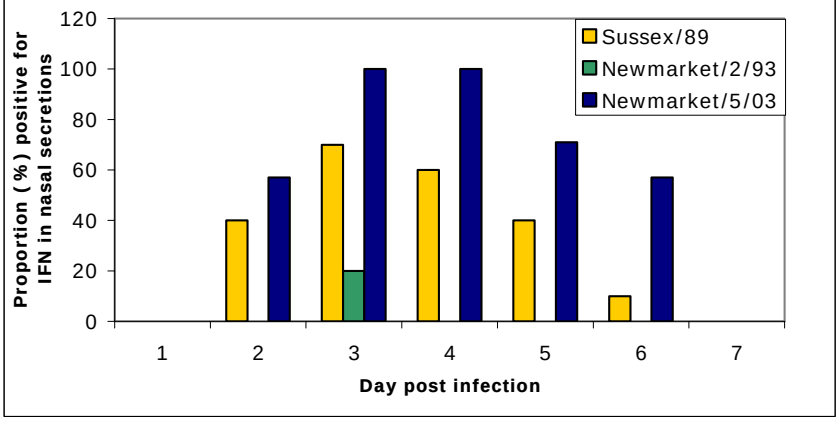


Figure 2. Phylogenetic tree of America-like strains of influenza H3N8 virus.

Influenza virus-related Encephalopathy

Equine influenza is usually a transient and self-limiting disease. However, during the large outbreak of equine influenza in the UK during 2003, there were reports of unusually severe clinical signs among unvaccinated animals[2]. Two influenza-infected horses developed neurological signs, and one was euthanased. *Post mortem* examination of the brain of that horse revealed viral-type, non-suppurative encephalitis, and influenza virus antigen was demonstrated by immunostaining of nasal mucosa.

Figure 3. The proportion of horses positive for IFN in nasal secretions following experimental infection with different strains of influenza virus[2]



Experimental infection of ponies not previously exposed to equine influenza virus with a strain representative of the 2003 outbreak resulted in more severe clinical signs and higher levels of cytokine in nasal secretions than observed those observed following challenge with other strains of equine influenza virus. A graphical summary of these findings is shown in figure 3. In the absence of an alternative explanation, the possibility that infection with a highly pathogenic strain of influenza virus had given rise to neurological complications was suspected. Since virus was not identified in central nervous system tissue, the lesions are thought to arise as a result of an aberrant host immune response.

Equine Viral Arteritis

During the final quarter of 2004 a total of 1564 samples were screened for seroconversion. Eight semen samples were tested for virus using PCR and virus isolation techniques. Details of the diagnosis of EVA in an imported stallion and a change in test procedure conducted at the AHT are presented below.

At the end of October 2004 EVA was confirmed in a recently imported Dutch stallion on a premises in Suffolk[3]. Prior to importation the stallion had been blood tested for equine arteritis virus (EAV) and been found negative. However, whilst the horse was in quarantine awaiting onward transport to a non-EU country, the EVA virus neutralisation test was performed in accordance with the OIE regulations for international trade and a positive result for antibody was obtained. Since there was no evidence that the animal had been vaccinated, it was placed under statutory restrictions in accordance with the Equine Viral Arteritis Order 1995, whereupon isolation in of EAV in cell culture identified the stallion as a venereal shedder. Sequence data was used to provisionally identify the virus as a European strain, and the State Veterinary Service conducted investigations to establish when the animal became infected, and trace in contact animals.

The stallion was returned to Holland in January 2005, and restrictions on the quarantine premises were lifted. It is fortunate that the horse remained in quarantine throughout its time in the UK, and had not been used for breeding purposes. Investigations revealed that

it had come into contact with three mares still in the UK, all of which were tested negative for EAV. This incident serves to highlight the need for vigilance, and the importance of observing guidelines laid out in the Horserace Betting Levy Board Codes of Practice.

November 2004 saw a change in the way that serological screening of EVA is conducted at the AHT. Several laboratories have recently reported difficulties whilst carrying out the OIE reference laboratory approved virus neutralisation (VN) test. These problems relate to serum cytotoxicity, which produces a cytopathic effect on the rabbit kidney indicator cell cultures. This can be difficult to distinguish from a positive test result. Investigation of this phenomenon has indicated that serum cytotoxicity is associated with the use of a tissue-culture-derived equine herpesvirus vaccine[4]. It occurs due an anti-cellular antibody response directed against the indicator cells. This link between vaccination and cytotoxicity is illustrated by the graphs in figure 4, which show a) increasing toxic VN titre with increase number of vaccine doses, and b) non-linear decay in antibody with increasing time since last vaccination. To avoid the implications of ambiguous test results on both disease surveillance and pre-breeding programmes, other methods of screening for EAV antibody have been investigated, and an ELISA test is now routinely used at the AHT.

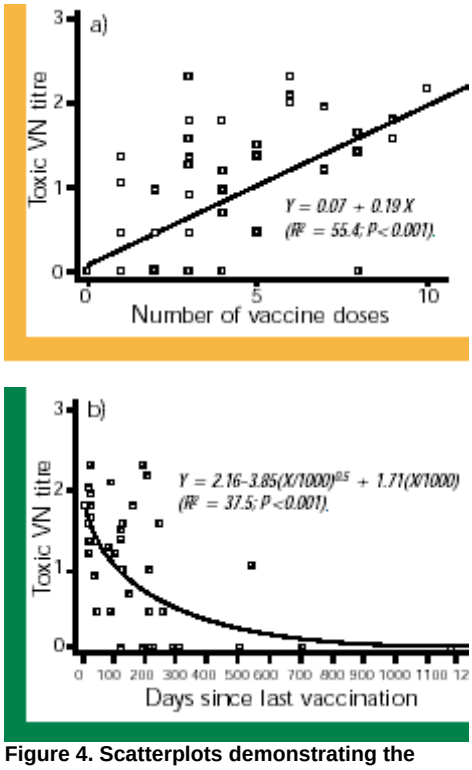


Figure 4. Scatterplots demonstrating the relationship between vaccine administration and cytotoxicity. Best-fitting linear regression lines are shown[4].

Equine Liver Disease

In October 2004, a polo pony was presented with signs of acute liver failure that failed to respond to intensive medical therapy. Elective euthanasia was undertaken, and *post mortem* examination of the first pony revealed diffuse severe subacute necrotising hepatitis. Two further ponies from the same premises developed similar clinical signs of liver disease and were hospitalised. Liver biopsy revealed similar changes to those present in the first pony, and intensive supportive therapy was given, resulting in recovery. A fourth pony then developed similar signs, and was also treated successfully. All four ponies had been in France in August, and their dietary history included high-level copper supplementation. Immunoperoxidase testing for EHV-1 and EHV-4 proved negative in the first pony, and copper levels in the liver of this animal were normal. The aetiology of the hepatopathy remains undetermined.

Many of the biochemical and serological tests used routinely in the evaluation of suspected equine hepatopathy cases have been shown to be of poor diagnostic value[5]. Biopsy is recognised as the 'gold standard', and is able to establish the presence of liver disease, provide a specific diagnosis, guide therapeutic choice and also help determine prognosis in cases of suspected liver disease.

Table 2. Summary of scores assigned to the severity of 5 histopathological criteria contributing to a total liver biopsy (minimum score = 0, maximum score = 14)[6]

	Severity			
Histopathological	**absent**	**mild**	**moderate**	**severe**
Fibrosis	0	0	2	4
Irreversible cytopathology	0	1	2	2
Inflammatory infiltrate	0	0	1	2
Haemosiderin	0	0	0	2
Biliary hyperplasia	0	0	2	4

A biopsy scoring system has recently been developed that is based upon rating five pathological processes within the liver and weighting the score towards those processes that have been shown through survival analysis to be the most detrimental to long-term liver function[6]. The histopathological criteria and scoring scheme are shown in table 2. The biopsy shown in figure 5 illustrates a case of severe hepatopathy due to ragwort poisoning.

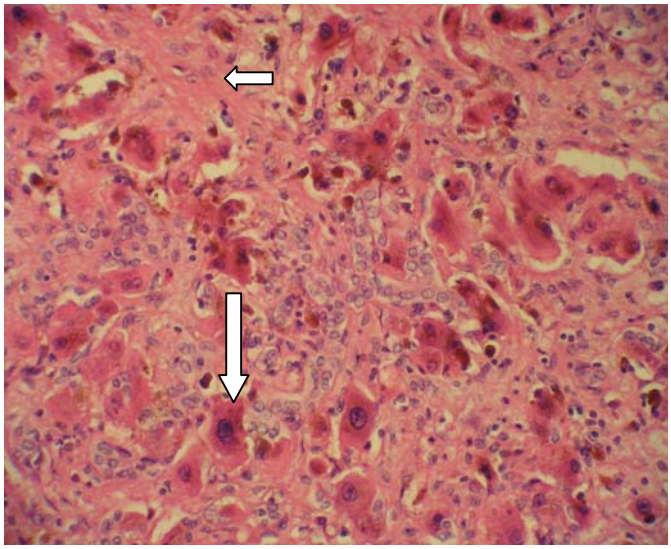
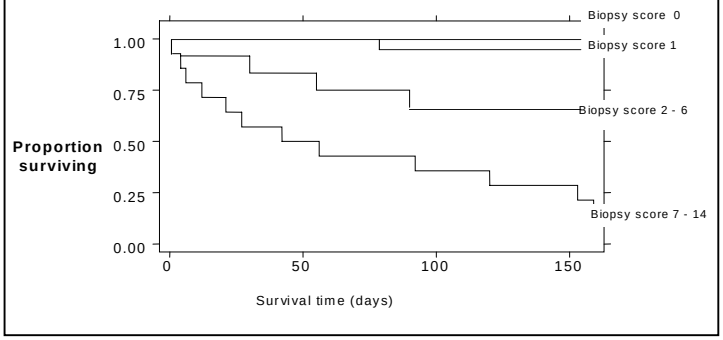


Figure 5. Severe hepatopathy typical of ragwort poisoning. Note megalocytosis (large arrow) and fibrosis (small arrow).

This case would obtain a high biopsy score and thus carry a poor prognosis. The Kaplan-Meier plot in figure 6 shows the markedly reduced proportion of horses with the highest biopsy scores that survive to 150 days post-biopsy compared to those with lower ratings. This biopsy scoring system has been shown to provide a more refined method of determining prognosis for horses suffering from diffuse hepatopathies, and could prove useful in the clinical assessment of such cases.

Figure 6. Kaplan-Meier plot stratified by biopsy score[6]



Equine *Post Mortem* Examinations

Nine full *post mortem* examinations were performed on adult horses at the AHT during the last quarter of 2004. These included two welfare investigations in relation to suspected neglect, a polo pony in acute liver failure (discussed above), a horse that had suffered from acute severe hyperaesthesia and nasal dermatitis, and a pony with a pedunculated lipoma and volvulus of the small intestine.

A twelve-year-old Welsh mountain pony with a history of sudden weight loss and ventral oedema was examined following elective euthanasia. Gross examination revealed watery fluid in the small intestine, and abscessation of the lungs. Histological examination of the small intestine revealed extensive infiltration of the mucosa by atypical and mitotically active lymphoid cells, which expressed CD3 on immunostaining. *Rhodococcus equi* was cultured from the lung lesions. The final diagnosis was of alimentary T-cell lymphoma, and it was suspected that the *Rhodococcus* infection of the lungs was the consequence of immunosuppression.

Three of the horses that were examined had died suddenly at exercise. One exhibited aortic arch pathology and gastric tympany, whilst cardiac dysrhythmia was considered to be the likely cause of death in the others. The presumptive nature of these diagnoses was due to the absence of gross lesions. This finding has been observed in the majority of animals that are examined having succumbed to sudden cardiac death. Histological or electrophysiological factors are thought to be critical in determining whether cardiac failure occurs in a previously normal horse. Further research to elucidate the changes that take place prior to a catastrophic cardiac event, and to establish the epidemiology of cardiac sudden death, would be of particular relevance to the racing industry.

A number of fetal and foal submissions were also examined. This type of investigation will be discussed in detail in a future communication.

Strangles (*Streptococcus equi* Infection)

During the last quarter of 2004 the bacteria *Streptococcus equi* was isolated on 69 occasions. Forty-six of these isolations confirmed strangles in a horse for the first time. The remainder were follow-up samples, submitted to monitor disease progression

Europe's first strangles vaccine was launched at the British Equine Veterinary Association congress in September 2004. Produced by Intervet UK Ltd, Equilis Strep E is a live vaccine containing a modified strain of *S. equi* that does not grow in mammalian cells. If there is good uptake of this vaccine, which is recommended for use in horses deemed to be at medium to high risk of exposures to the pathogen, a change in the epidemiology of *S. equi* infection in the UK may soon be observed.

References for further reading

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