



**DEFRA / AHT / BEVA
EQUINE QUARTERLY DISEASE
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Highlights in this issue:

- **Strangles - Serological ELISA test for *Streptococcus equi***
- **Dourine – an emerging venereal threat to European horses**

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.



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Introduction

Welcome to the second quarterly equine disease surveillance report for 2011 produced by Department of Environment, Food and Rural Affairs (Defra), British Equine Veterinary Association (BEVA) and the Animal Health Trust (AHT). Regular readers will be aware that this report collates equine disease data arising from multiple diagnostic laboratories and veterinary practices throughout the United Kingdom giving a unique insight into equine disease occurrence on a national scale.

National disease occurrence

Equine influenza

Equine influenza activity has been lower than normally expected for the summer months when horses increase the extent to which they move and mix at shows and events, carrying the virus with them. However, there have been two confirmations of equine influenza recently in Great Britain. On 25th July 2011 Animal Health Trust confirmed equine influenza in a horse in Wiltshire. Diagnosis was made on the basis of seroconversion to H3N8 influenza virus in a haemagglutination inhibition (HI) in a non-vaccinated, cross-bred horse that showed typical clinical signs of frequent coughing at rest and nasal discharge. On 11th August 2011 the Animal Health Trust confirmed equine influenza in a pony in North Lanarkshire, Scotland. Diagnosis was made on the basis of a positive result on nucleoprotein antigen ELISA on a nasopharyngeal swab taken from a non-vaccinated 9-year-old pony mare that had had signs of pyrexia, coughing and mucopurulent nasal discharge for 2 days before being sampled. The affected pony is a single animal on a private premise that had been very recently acquired from a dealer's yard.

International disease occurrence

Contagious Equine Metritis (CEM)

CEM was confirmed recently in France and the United States of America (USA). On 25th July 2011 the *Réseau d'Epidémiologie-Surveillance en Pathologie Equine* (RESPE) reported a single case of CEM in a French Trotter mare in the Department of Calvados, Normandy, northern France. The mare was reported to have signs of metritis with laboratory diagnosis made on 13th July 2011 by the Frank Duncombe Laboratory based on positive bacterial culture of *Taylorella equigenitalis* (*T. equigenitalis*) from a uterine swab. On 27th July 2011 the United States Department of Agriculture (USDA), Washington, USA notified the OIE of the confirmation of CEM through subclinical infection with *T. equigenitalis* in a four-year-old Arabian stallion in Arizona, USA. Diagnosis was made by the National Veterinary Services Laboratories (NVSL) on 21st July by isolation of CEM organism by culture conducted on semen as a routine pre-export test. The source of the infection was not known and there was no known relationship between this positive stallion and any horses associated with the previous USA cases of CEM. A comprehensive epidemiological investigation was initiated by Arizona Department of Agriculture and USDA officials. Controls implemented include quarantine and treatment as necessary of animals in three states that had been bred this season with semen from the infected stallion. All testing and treatment protocols were being conducted in accordance with federal, international, and expert guidance and requirements.



Dourine in Italy

Between 27th May and 22nd June 2011 five epidemiologically linked outbreaks of Dourine involving 10 diagnosed cases was confirmed on five different premises on the island of Sicily and in the Napoli region of mainland Italy. In all outbreaks horses with infection with *Trypanosoma equiperdum* were confirmed by complement fixation test (CFT) performed by the Istituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise (IZS), Teramo, Italy (national laboratory).

The index case was a Friesian stallion at a farm in Catania, Sicily which had been tested negative for Dourine in September 2010. Tracing identified a CFT positive, clinically affected mare on another Catania premises that had attended the stallion farm since March 2011 and had been mated by the stallion in July 2011. Further tracing identified two CFT positive Friesian stallions on a premise in the area of Frattamaggiore, Napoli, mainland Italy with six further cases on premises where the two stallions had originated in Casoria (one case) and Nola (five cases), both in the Napoli region.



A subsequent retrospective survey of 1231 blood samples collected in 2010 for the purposes of West Nile virus surveillance demonstrated 61 (5%) samples positive for Dourine by CFT from 10/15 (67%) regions where samples were available (see map). Further controls have been implemented with additional serological testing of all legally registered breeding horses in nine central and southern regions of Italy. In the region of Campania preliminary results from these control investigations indicated 15/236 (6.4%) animals residing in 11/77 (14.3%) premises were serologically positive for Dourine by CFT. However, the specificity of the dourine CFT is not well characterised and so the results of retrospective serosurveillance need to be interpreted cautiously, particularly in the absence of clinical disease information.

Investigations are continuing into all retrospective serological diagnoses of Dourine in order to better understand the origin and epidemiology of *Trypanosoma equiperdum* infection among horses in Italy. Equine reproduction regulations have been further enforced by veterinary and agricultural authorities throughout Italy and consideration is being given to extending the additional controls of blood testing all registered breeding animals to the whole of the country.



Equine Influenza

On 19th July 2011 the Chief Veterinary Officer for Mongolia notified OIE of the occurrence of clinical H3N8 equine influenza in seven horses among 418 animals in Songinokhairhan district, Ulaanbaatar City, Mongolia. Diagnoses were confirmed by positive results in haemagglutination inhibition (HI) tests and virus isolation conducted by the State Central Veterinary Laboratory (national laboratory). On 10th August 2011 an update to the OIE reported the more widespread occurrence of clinical H3N8 equine influenza in Mongolia. The report detailed 103 separate outbreaks occurring mainly in central and eastern parts of Mongolia (see map) and involving 74,608 cases with 40 deaths from the disease reported.



Hendra virus in Australia

In Queensland between 30th June and 27th July ten horses and one dog on eight premises (Beaudesert [1 horse], Mt Alford [3 horses, 1 dog], Park Ridge [1], Kuranda [1], Hervey Bay [1], Boondall [1], Logan [1] and Chinchilla [1]) were confirmed with Hendra virus infection. These infections are rare spillover events from the natural hosts for Hendra virus, flying foxes (also called fruit bats). On 27th July 2011 the Chief Veterinary Officer for Australia, notified the OIE of the confirmation of serological evidence of Hendra virus infection in one of three dogs on a premises in Mount Alford, Queensland that was already under restriction for three recent equine cases. The diagnosis was made at the Australian Animal Health Laboratory (AAHL), Geelong, Victoria by serological ELISA and virus neutralisation test (VNT) applied to serum. PCR tests applied to samples on 4th July 2011 and 25th July 2011 were though negative for Hendra virus. The dog has not had a reported recent illness. It is most likely that the dog contracted the infection from one of three horses on this property that have died from Hendra between mid-June and early July 2011. All dogs are tested where they have had possible exposure to infected horses. National policy is that domesticated animals that have evidence of infection with Hendra virus, even if they are not ill, are euthanased because of the potential risk they may pose to people.

In New South Wales between 30th June and 24th July five horses on four premises (Wollongbar [2 cases], Macksville [1], Lismore [1] and Mullumbimby [1]) were confirmed with Hendra virus infection. Samples from the dead horses were sent to the Elizabeth Macarthur Agricultural Institute for laboratory analysis and results confirmed Hendra virus. All properties were placed in quarantine and the dead horses buried. Horse and companion animal movements on and off quarantined properties are not permitted. On all four properties it is likely that contact with flying foxes was the likely source of infection, although the second Wollongbar case may have become infected by close contact with the first infected horse. All horses that have had close contact with an infected horse are



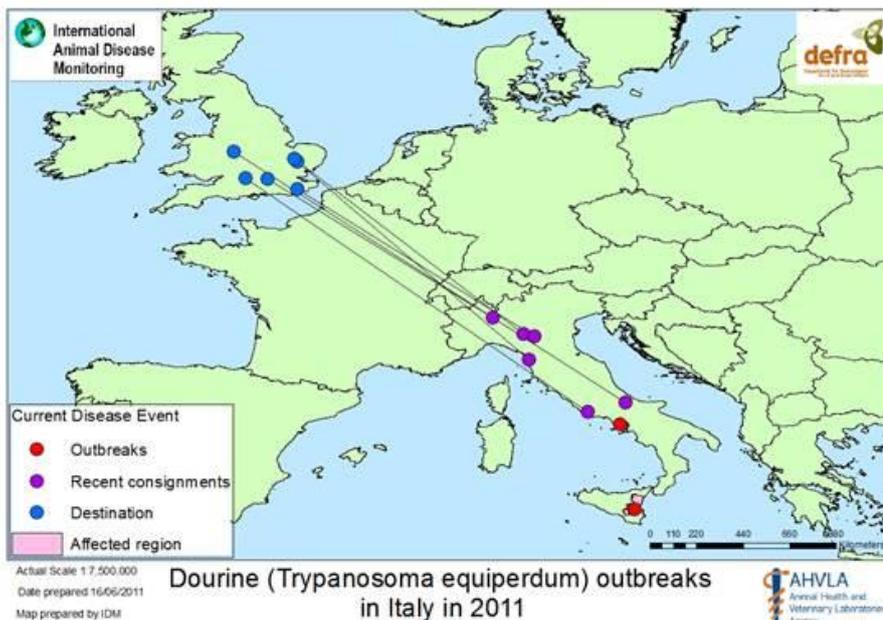
sampled and tested three times, at approximately 1, 16 and 32 days after contact as part of the control measures after infection is confirmed. The Hendra cases seen in 2011 represent a marked increase in numbers and the first real incursion into NSW.

West Nile Virus (WNV)

WNV was confirmed recently in Greece and the United States of America (USA). On 1st August 2011 the Ministry of Rural Development and Food in Athens, Greece notified OIE of the occurrence of clinical WNV infection in two horses among 35 animals on a premises in Attiki. Initial clinical diagnoses were confirmed by positive results in real time PCR, competitive ELISA and IgM capture ELISA conducted by the Virology Department of the Institute of Infectious and Parasitic Diseases at the Centre of Athens Veterinary Institutes (national laboratory). In addition in the framework of active sero-surveillance being undertaken a single subclinical infection was confirmed on 3rd August 2011 in a horse on a premise with 11 horses in Kentriki-Macedonia. Diagnosis was by competitive ELISA and IgM capture ELISA conducted by the national laboratory. In the USA there have been six cases of WNV encephalitis diagnosed for the year to date with cases occurring in Georgia (1 case), North Dakota (3), Pennsylvania (1) and California (1).

Defra news

On 8th August 2011 Defra announced that Mr Michael Seals, MBE, a livestock and arable farmer from Derbyshire, has been appointed as the Chair of the new Animal Health and Welfare Board for England (AHWBE). The Board will bring experts including farmers, veterinarians, welfare experts and others from outside Government together with the Chief Veterinary Officer and civil servants to make direct policy recommendations on policy affecting the health and welfare of all kept animals such as farm animals, horses and pets. The AHWBE will hold its first meeting towards the end of 2011. Mr Seals will form part of the selection panel for other external board members who are expected to be appointed in October. The Terms of Reference for the Board can be found at <http://www.defra.gov.uk/food-farm/animals/ahwbe/>.



Following the confirmation of Dourine, a parasitic venereal disease of horses caused by infection with the protozoa *Trypanosoma equiperdum*, in Italy in May 2011, Defra published two preliminary outbreak assessment reports, which are available on Defra's website ([Click here](#)). In recognition of the significance of clinical dourine being confirmed in mainland Europe we include a Focus article on the disease.

The situation assessment provided by Defra considered Dourine to be slow moving as it is only transmitted by sexual contact, not vector borne unlike other Trypanosome infections.



The EU electronic trade notification system (TRACES) which holds certificates for horses consigned for breeding purposes, or registered horses moving to the UK for longer than a temporary movement, reported that 8 such horses (including a foal) were consigned to the UK since May 1st 2011 (see map). Due to the EIA situation in Italy, the UK currently has in place extra checks on horses originating there. All horses which have spent time in Italy and are entering the UK for reasons other than temporary movements are restricted and sampled for EIA. This would be expanded to include testing for Dourine for the foreseeable future, and back tracing to cover the last few weeks in Italy.

According to Defra the Dourine outbreaks in Italy continue to be an emerging situation until more information about the source of infection is known. There are no legislative requirements for horses destined for EU trade to be pre-export tested for Dourine, but EU legislation (90/425/EEC) allows post import checks for compliance purposes, which may include testing samples. On this basis and taking into account the current low level of trade from Italy, Defra still considers the risk of introduction of Dourine into the UK in horses originating in Italy “very low” (very rare but cannot be excluded occur) or “low” (rare but does occur). The level of checks on consignments of horses for breeding and reproduction originating in Italy will remain in place to ensure compliance with EU trade, and additional tests will be carried out due to the presence of clinical and subclinical disease in Italy. The situation will be continuously monitored and the risk level might be revised in the future.

Focus articles

In this report we are pleased to include two focus articles, one on the parasitic equine venereal disease Dourine, which has been recently confirmed in horses in Italy as reported above and the other on use of serology in preventing, investigating and controlling outbreaks of strangles. The first focus article provides an overview of Dourine and is adapted from the recently adopted Horserace Betting Levy Board (HBLB) Code of Practice prepared by Sidney Ricketts and Andrew McGladdery of Rossdale and Partners, James Crowhurst of Newmarket equine Hospital and Richard Newton of the Animal Health Trust. The second article is provided by Mr Edd Knowles of Bell Equine Clinic, near Maidstone Kent and provides a short guide to use of the relatively new serological blood test for infection by *Streptococcus equi*, in the investigation and control of strangles in horses.

We reiterate that the views expressed in this focus article are the authors' own and should not be interpreted as official statements of Defra, BEVA or the AHT.

Access to all of the equine disease surveillance reports can be made on a dedicated page on the Animal Health Trust website at http://www.aht.org.uk/equine_disease.html or via the BEVA and Defra websites:

<http://www.beva.org.uk/>
<http://archive.defra.gov.uk/foodfarm/farmanimal/diseases/vetsurveillance/reports/listing.htm>

We would remind readers and their colleagues that a form is available on the AHT website for registration to receive reports free of charge, via e-mail, on a quarterly basis. The link for this registration form is available via

http://www.aht.org.uk/equine_disease_registration.html



Virology Disease Report for the Second Quarter of 2011

The results of virological testing for April to June 2011 are summarised in Table 1 and include data relating to Equine Viral Arteritis (EVA), Equine Infectious Anaemia (EIA) and West Nile Virus (WNV) from the Veterinary Laboratories Agency (VLA), Weybridge. The sample population for the VLA is different from that for the other contributing laboratories, as the VLA's tests are principally in relation to international trade (EVA and EIA). VLA now provides testing for WNV as part of clinical work up of neurological cases on specific request and provided the appropriate Regional Veterinary Lead of Animal Health Veterinary Laboratories Agency (AHVLA) has been informed.

Table 1: Diagnostic virology sample throughput and positive results for the second quarter 2011

	Number of Samples Tested	Number Positive	Number of Contributing Laboratories
Serological Tests			
EVA ELISA	2438	75 [#]	6
EVA VN	932	103 [#]	3
VLA EVA VN	377	22 [#]	1
EHV-1/-4 CF test	825	7 [*]	2
EHV-3 VN test	23	7	1
ERV-A/-B CF test	259	0 [*]	1
Influenza HI test	272	0 [*]	1
EIA (Coggins)	460	0	3
EIA ELISA	1521	0	6
VLA EIA (Coggins)	753	0	1
VLA WNV (PRNT)	0	0	1
Virus Detection			
EHV-1/-4 PCR	62	12	2
EHV-2/-5 PCR	16	10	1
Influenza NP ELISA ^{**}	92	2	2
Influenza Directigen	55	0	1
Influenza VI in eggs	2	0	0
EHV VI	98	14	1
EVA VI/PCR	1	0	1
VLA EVA VI/PCR	11	0	1
Rotavirus ^{##}	94	28	11

ELISA = enzyme-linked immunosorbent assay, VN = virus neutralisation, VLA = Veterinary Laboratories Agency, CF = complement fixation, HI = haemagglutination inhibition, Coggins = agar gel immuno diffusion test, PCR = polymerase chain reaction, NP = nucleoprotein, VI = virus isolation, EVA = equine viral arteritis, EHV = equine herpes virus, ERV = equine rhinitis virus, EIA = equine infectious anaemia
[#] = Seropositives include vaccinated stallions, ^{*} = Diagnosed positive on basis of seroconversion between paired sera
^{##} = Testing methods for Rota virus detection included an ELISA and a Latex Agglutination test, both performed on faecal samples
^{**} = The relatively high number of NP ELISA tests performed is largely due to requirements for international equine movement. All horses travelling to Australia must now have 2 NP ELISA tests performed prior to travel. The figures above include tests performed for international trade purposes.



Of the 22 EVA VN positives detected by the VLA, 1 was an import sample, 13 were export samples and 8 samples were submitted for diagnosis.

The 753 agar gel immuno diffusion tests for EIA (AGID; Coggins) were conducted for international trade purposes and they were all negative.

Virological Diagnoses for the Second Quarter of 2011

Equine Herpes Virus-1 (EHV-1) Abortion

Four single cases of EHV-1 abortion were reported in this quarter. Three cases were in Thoroughbred mares of which two were vaccinated and the other was in a vaccinated Polo pony mare. A further outbreak of EHV-1 abortion in two non-vaccinated Warmblood mares on one premises was reported on 28th April. In all cases EHV-1 infection was confirmed at the Animal Health Trust on the basis of positive PCR and/or virus isolation for EHV-1 in mixed fetal tissues. All necessary precautions were taken and the HBLB Codes of Practice were followed.

Equine Influenza

A single case of equine influenza was confirmed in a five-year-old non-vaccinated riding pony in Cardiff, South Wales, United Kingdom. The affected pony was new to the premise, and presented with clinical signs of cough, nasal discharge and pyrexia on arrival. The diagnosis was confirmed at the Animal Health Trust by nucleoprotein antigen ELISA applied to a nasopharyngeal swab. The affected pony was isolated and no further cases were reported.

Equine Herpes Virus-3 (coital exanthema)

In this quarter, one case of EHV-3 infection was confirmed in a Clydesdale at the Animal Health Trust by virus isolation from a swab.



Bacteriology Disease Report for the Second Quarter 2011

A summary of the diagnostic bacteriology testing undertaken by different contributing laboratories is presented in Table 2. For contagious equine metritis (CEM) 29/30 HBLB approved laboratories in the UK contributed data.

VLA CEMO Data for the period April to June 2011

We are again pleased to include data relating to CEM testing from the Veterinary Laboratories Agency (VLA), in this quarterly report. The sample population for the VLA is different from that for the other contributing laboratories as the VLA tests are principally in relation to international trade and/or outbreak investigations.

Strangles

Strangles remains endemic in the UK, especially among parts of the non-Thoroughbred horse population. Diagnoses are confirmed in the UK based on traditional culture of *S. equi* and qPCR on respiratory samples and/or seroconversion using a serological ELISA.

Table 2: Diagnostic bacteriology sample throughput and positive results for the second quarter 2011

	Number of Samples Tested	Number Positive	Number of Contributing Laboratories
CEMO (HBLB)	7084	0	29
CEMO (VLA)	340	0	1
<i>Klebsiella pneumoniae</i>[#]	7086	3	29
<i>Pseudomonas aeruginosa</i>	7086	39	29
Strangles*culture	2353	166	20
Strangles PCR	1352	92	2
Strangles ELISA	2004	407	1
Salmonellosis	476	12	18
MRSA	595	0	13
<i>Clostridium perfringens</i>	106	10	7
<i>Clostridium difficile</i> (toxin by ELISA or immunochromatography)	102	18	9
Borrelia (by ELISA)	32	12	1
<i>Rhodococcus equi</i>	339	10	6
<i>Lawsonia intracellularis</i>^{**}	18	2	2

CEMO = contagious equine metritis organism (*Taylorella equigenitalis*); HBLB = HBLB accredited laboratories; [#] =capsule type 1,2,5; VLA = VLA reference laboratory; **Streptococcus equi* subsp. *equi*; MRSA = methicillin resistant *Staphylococcus aureus*. ** *Lawsonia intracellularis* identified using PCR applied to faeces; ¹ reproductive tract samples only ^{###} = an overseas sample from Italy

VLA Salmonella results

From 18 strains typed by the VLA the serotypes reported were *S. Typhimurium* (9 isolates), *S. Newport* (5 isolates), *S. 4,5,12:l:-193*, *S. Kottbus*, *S. Montevideo* and *S. Oslo* (1 isolate respectively). These 18 positive samples represent 11 incidents. The following definition of an incident applies: “An incident comprises the first isolation and all subsequent isolations of the same serovar or serovar and phage/definitive type combination of a particular *Salmonella* from an animal, group of animals or their environment on a single premises, within a defined time period (usually 30 days).”



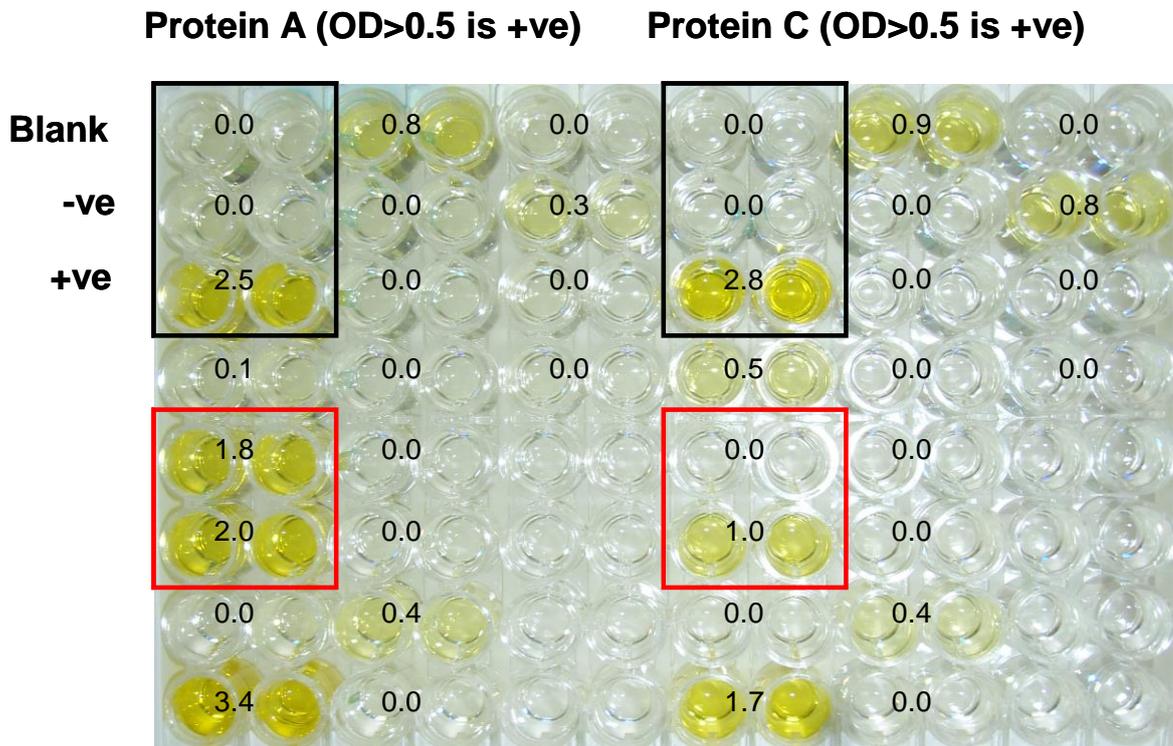
Focus Article: Serological ELISA test for *Streptococcus equi* (Strangles)

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The disease

Strangles is caused by infection with *Streptococcus equi* (*S. equi*). Clinical signs include pyrexia, mucopurulent nasal discharge and abscess formation in the retropharyngeal and submandibular lymph nodes. Diagnosis is usually based on the characteristic clinical signs and culture (and/or PCR) of nasopharyngeal swabs or pus (reviewed by Sweeney and others (2005)). Many, perhaps even the majority of, cases in the UK do not show classical clinical signs. Instead they show milder atypical signs or may have subclinical infections (Slater 2010).

Two factors underlie strangles importance as a disease. Firstly, around 10% of cases develop more severe clinical signs which occasionally prove fatal. Secondly *S. equi* has evolved to persist in the equine population. After normal infection, a proportion of cases, known as carriers, remain persistently but asymptotically infected. Carriers appear outwardly normal but infection usually persists in the guttural pouches; in-contact horses can become infected through intermittent shedding of *S. equi*. Together with the unrecognised and thus untreated atypical cases, carriers are likely to contribute significantly to the disease persistence. Identification of both carriers and atypical cases is difficult by conventional means.



The test

In 2008 the Strangles ELISA blood test was launched by the Animal Health Trust. The test detects IgG, to two *S. equi* specific antigens (A and C), identified through sequence analysis of *S. equi* and numerous strains of *S. zooepidemicus*. Exposure to *S. equi* within the last 6 months can be detected with a sensitivity of 93.3%, and a specificity of 88.0% (Waller, A.S. personal communication). The test has proved popular and 5129 samples were submitted in 2010 (DEFRA/AHT/BEVA 2010).



Test use and interpretation

The test has 3 main uses:

- Screening horses of unknown history prior to movement onto disease free premises to allow carriers to be identified and treated before movement.
- Follow up testing after an outbreak e.g. at a livery yard. In many cases it can be unclear which horses have been exposed to *S. equi* and thus may be carriers.
- Confirmation of infection in atypical cases or those in which conventional testing has failed to demonstrate the presence of *S. equi*.

A positive ELISA result indicates a serological response to exposure but not necessarily current infection or carrier status. Only a small minority of seropositive horses are likely to be carriers. Whilst a negative result makes carrier status highly unlikely, a positive result is an indication for additional testing (guttural pouch endoscopy or repeated nasopharyngeal swabbing) to confirm or refute carrier status.

Positive results can occur in vaccinated horses. The Equilis Strep E vaccine has only recently been reintroduced and the persistence and magnitude of IgG titres to these antibodies in vaccinated horses has not been well characterised. False positive results due to the assay not being 100% specific can occur in clinically normal horses.

Samples taken early in the course of the disease (within 2 weeks) may give negative results if insufficient time has elapsed for a serological response to exposure. False negative results may also occur in a small minority of cases (around 6.7%) that do not develop a typical immune response to *S. equi* exposure.

Active infection can be demonstrated by a rising IgG titre on paired serum samples taken 10-14 days apart.

Future directions

In some cases the delay in receiving test results can hamper diagnosis or delay the movement of horses. Supported by a grant from Land Rover Burghley Horse Trials, work is currently underway at the Animal Health Trust to develop a patient side ELISA capable of producing a result in 10 minutes.

References

- DEFRA/AHT/BEVA (2010) Equine Quarterly Disease Surveillance Reports. http://www.aht.org.uk/equine_disease.html accessed 1/8/11.
- Slater, J.S. (2007) Bacterial Infections of the Equine Respiratory Tract. *In* Equine Respiratory Medicine and Surgery. Eds McGorum, B.C., Dixon, P.M., Robinson, N.E. and Schumacher, J. Saunders Elsevier, Philadelphia pp327-353
- Sweeney, C.R., Timoney, J.F., Newton, J.R. and Hines, M.T. (2005). *Streptococcus equi* Infections in Horses: Guidelines for Treatment, Control and Prevention of Strangles. *J. Vet. Intern Med* **19**: 123-134



Toxic and Parasitic Disease Report for the Second Quarter 2011

A summary of diagnostic toxicosis and parasitology testing undertaken by contributing laboratories is presented in Tables 3 and 4 respectively. Results for toxicosis are based on histopathologically confirmed evidence of disease only (where applicable).

Table 3: Diagnostic toxicosis sample throughput and positive results for the second quarter 2011

	Number of Samples Tested	Number Positive	Number of Contributing Laboratories
Grass Sickness	28	22	2
Hepatic toxicoses	18	0	2
Atypical myopathy	1	1	1
Tetanus	-	-	-

Grass sickness surveillance data (www.equinegrasssickness.co.uk):

A total of 44 equine grass sickness (EGS) cases have been reported for the second quarter (April to June 2011), of which 30 cases occurred in England, eight cases occurred in Scotland, and the location of six cases was not reported. The median age of affected horses was 5 years (range 1.9 – 32 years). A greater proportion of cases occurred in males: 59% of cases were geldings (n=26), 4.5% (n=2) were stallions/colts, 31.8% (n=14) were mares/fillies and sex was not recorded for 2 cases.

The majority of affected horses (72.7%, n=32) were purebreds, predominantly native pony breeds (n=13) and Cob breeds (n=10) and 25% (n=11) were crossbred.

Fifty percent of cases (n=22) were reported to have acute EGS, 27.2% (n=10) had subacute EGS and 11.4% (n=5) were diagnosed with chronic EGS. Of the five horses with chronic EGS, only one was reported to have been euthanased due to the disease.

Diagnostic information was provided for 75% of cases (n=33); of these, 45.5% (n=15) were diagnosed based on clinical signs alone. Thirteen horses underwent surgery and diagnostic confirmation obtained by biopsy examination in the majority of cases (69.2%, n=9); seven horses that underwent surgery also had a subsequent post-mortem examination. In total, post-mortem examination was performed in twelve cases, of which eight had biopsy samples obtained for confirmation of diagnosis by histopathology.

It should be noted that the grass sickness surveillance scheme receives data from a wider population in comparison to the data presented in Table 3 and different diagnostic criteria were used. For more information about the grass sickness surveillance please refer to previous reports published in Vol.2 No.4 and Vol.4 No.2.



Table 4: Diagnostic parasitology sample throughput and positive results for the second quarter 2011

	Number of Samples Tested	Number Positive	Number of Contributing Laboratories
<u>Endoparasites</u>			
Ascarids	2796	28	16
Cyathostomes	1957	357	15
Dictyocaulus	552	12	10
Strongyles	3748	1039	22
Tapeworms (ELISA based testing)	13	12	3
Tapeworms (Faecal exam)	2207	27	13
Trichostrongylus	30	0	2
Strongyloides	2072	218	15
<i>Oxyuris equi</i>	176	1	7
Fasciola	139	8	4
Coccidia	438	2	8
Cryptosporidia	0	0	0
VLA <i>Theileria equi</i> (CFT)*	136	4	1
VLA <i>Theileria equi</i> (IFAT)**	480	24	1
VLA <i>Theileria equi</i> (cELISA)***	157	10	1
VLA <i>Babesia caballi</i> (CFT)*	141	6	1
VLA <i>Babesia caballi</i> (IFAT)**	482	9	1
VLA <i>Babesia caballi</i> (cELISA)***	162	6	1
VLA <i>Trypanosoma equiperdum</i> (CFT)*	241	3	1
VLA <i>Trypanosoma equiperdum</i> (IFAT)**	2	0	1
<u>Ectoparasites</u>			
Mites	611	7	16
Lice	719	17	16
Ringworm	671	175	21
Dermatophilus	536	16	15
Candida	47	1	2

*Complement Fixation Test; CFT suspect/positive samples are tested in IFAT test

Indirect Fluorescent Antibody Test; *competitive Enzyme-linked immunosorbent assay; positive cELISA results are not undergoing confirmatory testing



Focus article: Dourine – an emerging venereal threat to European horses

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Introduction

Following the disclosure of an outbreak of Dourine, due to infection with the protozoan parasite *Trypanosoma equiperdum*, in Italy earlier in 2011, the Codes of Practice Sub-Committee of the Horserace Betting Levy Board's (HBLB) Veterinary Advisory Committee, undertook its annual review of the Codes of Practice in September 2011. The review concluded that it was timely to include for the first time a specific Code of Practice for Dourine to provide European breeders with guidance as to how to deal with this emerging venereal disease threat. Dourine is a serious and often chronic venereally transmitted disease of horses, which is notifiable by law. There is no proven long term cure for the disease and no vaccine is available, so establishing freedom from disease is the basis of prevention. The following article is based around the HBLB Code of Practice which was drafted by the authors prior to approval as the basis of the HBLB's Code of Practice.

The Disease

Dourine is caused by the protozoan parasite, *Trypanosoma equiperdum* and is a serious, often chronic, venereally transmitted disease of horses and other equids. Once widespread, dourine has been eradicated from many countries but is still seen in horses in Asia, Africa, South America, southern and eastern Europe, Mexico and Russia and was reported in June 2011 in Sicily and then just north of Naples, on the Italian mainland.

Notification

European Council Directive 90/426 of 26th June 1990 makes dourine compulsorily notifiable in the EU. In the UK, dourine is also **notifiable by law** under the Infectious Diseases of Horses Order 1987. Under the Order, anyone who owns, manages, inspects or examines a horse, which is affected or is suspected of being affected by the disease must notify Defra via the appropriate Regional Veterinary Lead of Animal Health Veterinary Laboratories Agency (AHVLA). See Defra's website ([Click here](#)) for AHVLA contact information.

Under the Order, Defra may declare the premises where disease is suspected to be as an infected place and impose restrictions on horses at those premises. A veterinary inquiry will be carried out under the direction of Defra to determine if dourine is present. The Order provides Defra with powers to enforce measures for vector control and disinfection.

There is currently no proven, effective long-term cure for dourine. Any horse testing positive will be required to remain under official breeding and movement restrictions indefinitely. There are no powers for compulsory humane destruction of infected horses, as a result there is no provision for compensation. Any decision as to the necessity for humane destruction will have to be taken by owners based on economic and welfare considerations.



Clinical Signs

Clinical signs of dourine are highly variable in manifestation and severity. The disease is characterized mainly by swelling of the genitalia, cutaneous plaques and neurological signs but severity varies with the virulence of the strain, the nutritional status of the horse, and stress factors. Clinical signs often develop over weeks or months, frequently waxing and waning with relapses, probably precipitated by stress. This can occur several times before the animal either dies or experiences an apparent recovery. The mortality rate is believed to be in excess of 50%.

Genital oedema and reproductive tract mucopurulent discharges are often the first signs. Mares develop a mucopurulent vaginal discharge, and the vulva becomes oedematous; this swelling may be marked leading to vaginal prolapse and extend along the perineum to the ventral abdomen and mammary gland and may result in depigmentation, similar to that seen in coital exanthema with EHV-3 infection. Abortion can occur with more virulent strains. Stallions develop oedema of the prepuce and glans penis with paraphimosis in some cases, and can develop a mucopurulent urethral discharge. The swelling may spread to the scrotum, perineum, ventral abdomen and thorax and may also become depigmented.



Characteristic raised oedematous patches 2-10 cm in diameter (sometimes called 'silver dollar plaques'; arrowed left) may appear on the skin on the neck, hips, lower parts of the abdomen and particularly over the ribs. These cutaneous plaques usually last for 3 to 7 days and are pathognomonic for the disease, although they do not occur with all infecting strains.

Neurological signs can develop with signs of progressive weakness, incoordination and, eventually, paralysis. Facial paralysis, which is generally unilateral, may be seen in some animals. Conjunctivitis and keratitis are common, and in some outbreaks, ocular disease may be the first sign of dourine and anaemia and intermittent fever may also be found. Dourine also results in a progressive loss of condition and affected animals may become emaciated, although the appetite remains good.

Transmission of Disease

Dourine is caused by the protozoan parasite, *Trypanosoma equiperdum*, which unlike other trypanosomal infections, is sexually transmitted during natural mating or by artificial insemination (AI) with infected semen. Transmission from stallions to mares is more common, but mares can also transmit the disease to stallions. *T. equiperdum* can be found in the vaginal secretions of infected mares and the seminal fluid, mucous exudate of the penis, and sheath of stallions. Periodically, the parasites disappear from the genital tract and the animal becomes non-infectious for weeks to months. Transmission is most likely early in the disease process as non-infectious periods are more common late in the disease. Male donkeys can be asymptomatic carriers and sexually immature animals that become infected can transmit the organism when they mature.

Rarely, infected mares pass the infection to their foals, possibly before birth or through colostrum and milk and infections may also occur through mucous membranes such as



the conjunctivae. There is currently no evidence that arthropod vectors play a significant role in transmission of dourine, but this possibility cannot be ruled out.

Prevention

There is no vaccine available for dourine. As dourine is primarily a venereal disease, prevention of natural mating or AI with infected horses (stallions or mares) is the most important means of control. Prevention of dourine is therefore based on the establishment of freedom from infection and this is done by testing blood for presence of antibodies against *T. equiperdum*, which is more reliable than testing for the presence of the protozoan parasite itself.

Any introductions of horses from endemic areas or areas of incursion should be isolated and blood tested for antibodies by complement fixation test (CFT) or indirect fluorescent antibody test (IFAT). Horses in isolation must not be allowed to mate and semen must not be collected or used for AI until negative dourine test results are confirmed. Any seropositive results, or any horses showing clinical signs of dourine should be reported as required by national law (Defra in UK) and will then be dealt with under official supervision. Dourine should be eradicated from an incursion into a non-endemic area by identification of the source, thorough tracing and testing of all in-contacts and euthanasia of infected and seropositive horses.

Stallions or mares should not leave endemic areas or areas of incursion without veterinary confirmation that:

- The horse(s) has/have not been in contact with cases of Dourine.
- The horse(s) is/are healthy and show(s) no clinical signs of Dourine, prior to leaving
- Negative CFT blood sample result(s) for Dourine, performed by an authorised laboratory, collected within one month of leaving.

On arrival in an area where Dourine does not occur, these stallion(s) or mare(s) should be isolated until repeat negative CFT blood sample result(s) for Dourine, performed by an authorised laboratory, collected 10-14 days after arrival, has been obtained. Under no circumstances should the stallion(s) or mare(s) involved be mated and no semen should be collected and used for AI purposes before this reassurance has been obtained.

Diagnosis

Due to the variability and possible absence of outward signs of dourine, clinical diagnosis is not always possible and laboratory diagnosis is necessary to confirm diagnoses of dourine.

The complement fixation test (CFT) is the prescribed test for international trade, and has been used successfully in eradication programs. Some uninfected animals, particularly donkeys, often have non-specific CFT reactions due to anticomplementary activity of their serum, thereby rendering results difficult to interpret. Indirect fluorescent antibody tests may help to resolve these cases. Enzyme linked immunosorbent assays (ELISAs) and agar gel immunodiffusion (AGID) tests have also been used to diagnose dourine. Although no serological test is specific for dourine as cross-reactions occur with other trypanosomes (especially *T. brucei* and *T. evansi*), this is not a problem where these infections are all considered to be exotic and requiring eradication.

CFT and confirmatory IFAT should always be used to test horses with clinical signs, to test horses that have been in contact with others who have or are at risk of having dourine and



for official export certification. In such cases, serum or clotted blood samples for dourine testing must be sent to the Veterinary Laboratories Agency, Weybridge.

Definitive diagnosis by identification of the parasite is not undertaken for routine screening as the organisms are extremely difficult to find and are usually not detectable in blood smears. *T. equiperdum* cannot be distinguished microscopically from *T. evansi*.

Control of infection

If dourine is suspected in any horse, stop all breeding activities immediately, identify and isolate the horse(s) concerned, notify Defra via the appropriate Regional Veterinary Lead of Animal Health Veterinary Laboratories Agency (AHVLA) and seek veterinary advice about the welfare of the horses and the next steps.

If dourine is confirmed, further action will be controlled by Defra. Mating, teasing, collection/insemination of semen and movement of horses on and off the premises must stop until the disease outbreak is confirmed to be over.

Any venereal contacts with confirmed infected horses must be isolated and will be blood tested to determine if they produce antibodies, i.e. to determine if they have become infected.

Inform:

- Owners (or persons authorised to act on their behalf) of horses at, and due to arrive at, the premises.
- Owners (or persons authorised to act on their behalf) of horses that have left the premises.
- Recipients of semen from the premises.
- The national breeders' association.

T. equiperdum is a parasite, which cannot survive outside a living host. It dies quickly with its host. Various disinfectants, including 1% sodium hypochlorite, 2% glutaraldehyde and formaldehyde, as well as heat of 50-60°C, will kill the parasites in the environment, but their transient life outside the host makes this unnecessary, although good stable hygiene is always recommended.

Treatment

There is currently no effective treatment for dourine although treatment has been attempted with quinapyramine sulphate (3 mg/kg, given subcutaneously). However, *T. equiperdum* may persist in asymptomatic carrier horses after treatment and these horses are considered unsafe for breeding purposes.

Any treatment to alleviate the signs of the disease and otherwise support the horse will be determined by the attending veterinary surgeon, until such time as a positive diagnosis is confirmed by CFT. Compulsory slaughter of infected horses to eradicate the infection is considered the best policy.

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Report on Post-mortem Examinations for the Second Quarter 2011

East Anglia

A total of 50 cases were examined including 30 aborted fetuses/neonatal deaths.

Of the 18 aborted fetuses examined this quarter, umbilical cord torsion was identified in two cases, placentitis in five cases, EHV-1 infection in three cases, terminal peripartum asphyxia/hypoxia in four cases, partial allantochorial separation and premature placental separation in two separate cases, one case of presumed placental insufficiency and one case delivered by caesarean section that had no definitive cause of death identified.

There were 12 cases of neonatal death reported in this quarter. Two cases were associated with dystocia, three had congenital malformations, and two with sepsis. The remaining five cases included a full term uterine death in a mare with pituitary adenoma, a foal with isoerytholysis despite a washed red cell transfusion, a case of severe volvulus, a case of atelectasis and one with a severe congenital cardiac defect resulting in hypoxia.

One animal with neurological disease was confirmed histologically with lymphocytic encephalomyelitis.

Six horses were examined following gastrointestinal disease. These included two cases each of colon torsion and intestinal adhesions and single cases each of cyathostomiasis and typhlocolitis.

Among three animals with respiratory signs, there was: one horse was confirmed with EHV-5 associated pulmonary fibrosis on the basis of histopathology and PCR, one case of *Rhodococcus equi* associated pneumonia in a seven-week old Thoroughbred foal and one case of severe acute exercise induced pulmonary haemorrhage (EIPH) in a three-year-old Thoroughbred that suffered sudden death whilst training.

The two cardiac cases reported in this quarter included a case of histologically confirmed epicardial fibrosis and a case of necrotising endocarditis, consistent with a bacterial cause.

Among four welfare/neglect cases examined during the quarter, one horse euthanased on humane grounds had suffered a lacerated tendon, one case had emaciation and chronic marked laminitis, one case had weight loss presumed to be due to multiple age-associated conditions and one case died from accidental suffocation.

Four other cases reported included one case of necrotising myositis, one case of uterine rupture, one case of hepatic lipidosis and one case of suspected acute and severe anaphylaxis.

Home Counties

No cases were examined in this quarter.

South West

Seven cases were examined in this quarter.

Two cases of gastrointestinal disease were investigated, one case was diagnosed with cyathostomiasis and the other had ruptured small intestine secondary to 360 degree torsion.



Two cases of musculoskeletal disease were reported with one animal having arthritis of the right fore fetlock (with left recurrent laryngeal nerve neuropathy and associated laryngeal muscle neurogenic atrophy also noted) and the other having denuded cartilage of the medial femoropatellar joint.

The other three cases included a case of sudden death due to suspected taxine toxicity (Yew poisoning), a cardiopulmonary accident and a case of peri-rectal neoplasia.

Northern England

Nine cases were examined in this quarter.

Six cases of gastrointestinal disease were reported in this quarter. The causes of death included three cases of colon torsion and single cases each of caecal rupture, grass sickness, and evisceration following incisional infection.

Three cases of musculoskeletal disease were reported including polysynovitis secondary to Lyme's disease, severed carotid artery secondary to occipital bone fracture and a fractured growth plate of left fore fetlock joint in a foal.

West Midlands

No cases were examined in this quarter.

Scotland

Five post-mortem examinations were reported in this quarter.

Two gastrointestinal cases were reported, including a case of suspected clostridiosis which tested positive for *C. chauvei* and *C. novyi* by IFAT and a case of intestinal cicatrisation.

A welfare/neglect case identified trauma to the neck resulting in massive haemorrhage in the neck leading to compromise of the airway and two cases of suspect adverse reaction to penicillin injection were investigated.

Northern Ireland

Ten post-mortem examinations were conducted in this quarter, including four stillborn or aborted fetuses.

Four aborted fetuses were examined including one submission of two stillbirths that cultured *Bacillus licheniformis* from the stomach contents of both fetuses. No definitive cause of abortion was determined in the other two cases.

Five cases were examined for gastrointestinal disease. An adult gelding with a history of acute colic was diagnosed with small intestinal mesenteric torsion with associated venous infarction along with diaphragmatic rupture and small intestinal herniation into the thoracic cavity. A two-year-old gelding with a history of unresponsive weight loss and collapse was examined and extensive transmural lymphohistiocytic colitis and typhlitis with associated cyathostome infection along with gastric ulceration was diagnosed. A five-year old mare with no history of illness was found dead with gastric volvulus and fibrinous peritonitis. A nine year-old donkey was found dead with nephrosplenic colonic entrapment and a dead fetus in utero which was being expelled.

No definitive cause of death was reached in the remaining two cases.



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**We would welcome feedback including contributions on focus articles
and / or case reports to the following address:**

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