DEFRA / AHT / BEVA

EQUINE QUARTERLY DISEASE SURVEILLANCE REPORT

Volume 1, No. 2: April-June 2005

Highlights in this issue:

- **Methicillin resistant *Staphylococcus aureus* (MRSA) update**
- **Equine grass sickness – evidence for *C. botulinum* toxico-infection as the cause**
- **Pathology focus: options for diagnosis of equine grass sickness**

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 produced by DEFRA, BEVA and the Animal Health Trust. This report collates equine disease data arising from multiple diagnostic laboratories and veterinary practices throughout Great Britain giving a unique insight into equine disease occurrence on a national scale.
This is the second quarterly equine surveillance report produced by Defra, BEVA and the Animal Health Trust. The launch of the first report earlier this year, was accompanied by a flurry of welcome publicity in the veterinary press. This was further enhanced by presentation to a large meeting of interested stakeholders in veterinary surveillance held on 4th July at London Zoo. Presentations from this meeting, including one outlining the equine disease surveillance report initiative may be accessed
online via the Defra website at:

http://www.defra.gov.uk/animalh/diseases/vetsurveillance/bag/july.htm.

A key feature of these reports is the inclusion of focus articles where recognized authorities in their fields discuss in more detail novel diagnostic and research findings arising from the reports. The views expressed in these focus articles are the authors' own and should not be interpreted as official statements of Defra, BEVA or the AHT. Focus articles in this issue have been contributed by Dr Andrew Waller and Katherine Whitwell.

Dr Andrew Waller is the Head of Bacteriology at the AHT, with research interests in respiratory infection with streptococci and, more recently, canine and equine infections with methicillin-resistant staphylococcus aureus (MRSA). The article on MRSA is timely in view of the increasing public health concern over the role of this organism in hospital-acquired infections.

Katherine Whitwell is an internationally recognized expert in equine pathology and consultant in pathology for the AHT and Beaufort Cottage Laboratories. Katherine has a long record of interest in equine grass sickness and her focus article summarises recent exciting developments in the diagnosis of this disease.

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://www.defra.gov.uk/animalh/diseases/vetsurveillance/species/horses/index.htm

A new feature that has now been added to the AHT surveillance report page is a form for registration to receive reports regularly via e-mail as they are produced. The link for this registration form is available via http://www.aht.org.uk/equine_disease_registration.html.

Health And Welfare Strategy For The Horse, Pony And Donkey

BEVA has lead an industry based working party to the production of a draft Health and Welfare Strategy for the Horse, Pony and Donkey, supported by DEFRA. The draft document is now out for public consultation until the 30th of November 2005 and can be viewed by clicking here.

The Health and Welfare Strategy for the Horse, Pony and Donkey aims to identify the real equine welfare concerns in each of the different sectors, from the beach donkey through to the racing thoroughbred, with issues such as disease surveillance, medicines availability and horse identification cutting across each of the sectors. It is an important opportunity for the industry to identify the priority areas that it feels require attention in order to make a significant difference to the overall health and welfare status of our horse population.

The working party would like to encourage a wide participation in the consultation process in order to produce the final strategy document in Spring 2006 as a true representation of the horse industries views.

Virology Disease Report for the Second Quarter of 2005

Table 1: Diagnostic virology sample throughput and positive results for second quarter 2005

	Number of Samples Tested	Number Positive	% Positive	Number of Contributing Laboratories
Serological Tests				
EVA VN/ELISA	3207	133#	4.1%	2
EHV-1/-4 CF test	1003	53*	5.3%	1
EHV-3 VN test	8	4	50.0%	1
ERV-1/-2 CF test	555	0	0%	1
Influenza HI test	587	0	0%	1
Virus Detection				
EHV-1/-4 PCR	83	4	4.8%	1
EVA PCR	1	0	0%	1
Influenza NP ELISA	76	0	0%	1
Influenza VI in eggs	0	0	0%	1
EHV VI	176	3	1.7%	1
Rotavirus	86	40	46.5%	4

VN = virus neutralisation, ELISA = enzyme-linked immunosorbent assay, CF = complement fixation, HI = haemagglutination inhibition, PCR = polymerase chain reaction, NP ELISA = nasopharyngeal swab VI = virus isolation, ERV = equine rhinovirus, EHV = equine herpesvirus, EVA = equine viral arteritis
# = Seropositives include vaccinated stallions
* = Diagnosed positive on basis of seroconversion between paired sera

Equine Herpes Virus (EHV)

EHV Abortion

A single confirmed EHV-1 abortion occurred in an EHV vaccinated Thoroughbred mare on a stud in East Anglia in April. No other animals were affected.

A stud in Shropshire suffered an outbreak of EHV-1 abortion during April and May. Two fetuses were examined by the AHT and the diagnosis confirmed on the basis of positive virus isolation and PCR results. The HBLB Codes of Practice were applied and there were no further abortions. In hindsight it is probable that two abortions and two live but weak foals that subsequently died on the same premises earlier in the season although not investigated at the time, were also the result of EHV infection.

A single case of EHV-1 abortion was also confirmed in Oxfordshire in May.

In April, a three-year-old Suffolk Punch mare at stud in Worcestershire aborted at approximately 10 months' gestation. Fetal tissues were positive for EHV-4 by PCR. No other animals were affected.

EHV-1 Neurological Disease

A presumptive case of paralytic EHV-1 was investigated in April in a 12-year-old Thoroughbred cross gelding in Staffordshire. The unvaccinated gelding presented with acute onset severe ataxia. Paired sera showed a marked seroconversion to EHV,

although no virus was isolated from nasopharyngeal swabs. The animal responded rapidly to corticosteroids and has since recovered. In contact animals remained unaffected.

EHV-1 Respiratory Disease

A 9-week-old foal that had been suffering from pneumonia was found dead in June at a stud in Oxfordshire. *Post mortem* examination showed the lungs to be positive for EHV-1 by PCR.

EHV-3 Venereal Disease

Four cases of coital exanthema due to infection with EHV-3 were confirmed during June. A single case was diagnosed in a mare in Warwickshire on the basis of characteristic vulval lesions and a high VN antibody titre. EHV-3 was isolated from infected penile lesions from an Andalusian stallion imported from Holland at stud in West Yorkshire. The stallion also had a high VN antibody titre. Two mares that had been recently covered by this same stallion also developed clinical signs. A Welsh pony mare in Aberdeenshire developed characteristic vulval lesions soon after covering and was diagnosed on the basis of clinical signs and high VN antibody titres. Six in-contact animals remained unaffected.

Other viruses

There were no confirmed laboratory diagnoses of infection with equine influenza or equine viral arteritis during this quarter although 133 blood samples, including from vaccinated horses, were found to possess antibodies to equine arteritris virus.

Bacteriology Disease Report for the Second Quarter of 2005

A summary of the diagnostic bacteriology testing undertaken by different contributing laboratories is presented in Table 2. For contagious equine metritis (CEM) 10 of 28 HBLB approved laboratories contributed data. None of the 10 contributing laboratories isolated the organism and no infection was confirmed during the quarter. Among 9 laboratories testing for *S. equi*, the causative agent of strangles, there was an 19.4% isolation rate from 959 samples tested, again highlighting the relatively high prevalence of this compared with other infections tested for during the same period.

Table 2: Diagnostic bacteriology sample throughput and positive results for second quarter 2005

	Number of Samples Tested	Number Positive	% Positive	Number of Contributing Laboratories
CEMO	10781	0	0%	11
Strangles (*S. equi*)	959	186	19.4%	9
Salmonellosis	209	1	0.5%	6
MRSA	3	0	0%	1

CEMO = contagious equine metritis organism (*Taylorella equigenitalis*)
MRSA = methicillin resistant *Staphylococcus aureus*

Antibiotic resistance

Table 3 summarises data related to antibiotic resistance for a broad range of sample types and/or sites spanning many different bacterial species as judged by antibiotic sensitivity discs.

It should again be noted that the table entries represent summary data from 5 different reporting laboratories rather than resistance patterns for individual bacterial isolates. As such the data may be of limited clinical usefulness and should be interpreted with extreme caution. Proposed changes in future data collection from laboratories contributing to these reports should allow developing trends to be monitored in subsequent reports.

None of the submitting laboratories this quarter reported isolation of methicillin resistant *Staphylococcus aureus* (MRSA) from equine samples.

Table 3: Antibiotic resistance patterns observed among 5 contributing laboratories during the second quarter 2005 (R denotes antibiotic resistance recorded this quarter)

Sample Type/Site	**Antibiotic**													
Bacterial spp.	**Amik**	**Amp**	**Ceft**	**Enro**	**Eryth**	**Fucid**	**Gent**	**Marbo**	**Neom**	**Oxyt**	**Pen**	**Strep**	**TMPS**	**Tica**
Wound/Abscess														
Staphylococcus	**R**	**R**	**R**		**R**		**R**	**R**	**R**		**R**		**R**	
Pseudomonas		**R**			**R**							**R**		
E. coli		**R**			**R**		**R**		**R**	**R**			**R**	
Burkholderia			**R**							**R**			**R**	
Tracheal Wash														
Staphylococcus		**R**								**R**	**R**			
Streptococcus				**R**					**R**			**R**		
Sinus Swab														
Burkholderia													**R**	
Pseudomonas										**R**			**R**	**R**
Nasal Swab														
Staphylococcus												**R**		
Streptococcus							**R**							
Proteus					**R**									
Synovial Fluid/Membrane														
Acinetobacter							**R**			**R**			**R**	**R**
Burkholderia							**R**			**R**			**R**	**R**
Chryseomonas							**R**			**R**			**R**	**R**
Staphylococcus											**R**			
Corneal Scrape/Conjunctival Swab														
Streptococcus						**R**			**R**					
Clitoral/Penile Sheath Swab														
Streptococcus		**R**			**R**		**R**					**R**		
Klebsiella										**R**	**R**		**R**	
Pseudomonas			**R**								**R**		**R**	
Endometrial Swab														
E.coli													**R**	
Streptococcus		**R**												
Vaginal Swab														
Proteus		**R**												

Antibiotic Abbreviations: Amik = Amikacin, Amp = Ampicillin, Ceft = Ceftiofur, Enro = Enrofloxacin, Eryth = Erythromycin, Fucid = Fucidin, Gent = Gentamicin, Marbo = Marbocyl, Neom = Neomycin, Oxyt = Oxytetracycline, Pen = Penicillin, Strep = Streptomycin, TMPS = Trimethoprim Potentiated Sulphonamide, Tica = Ticarcillin.

Note: The frequency of resistance to different antibiotics represented here may simply reflect differences in numbers of tests performed and not be a true reflection of resistance in a single bacterial isolate.

Focus article - Methicillin resistant *Staphylococcus aureus* (MRSA) update
Courtesy of Andrew Waller, PhD, Head of Bacteriology, Animal Health Trust

There is increasing evidence that inter-species transmission of methicillin resistant *Staphylococcus aureus* (MRSA) occurs, with recent publications identifying MRSA in many species other than humans, including the horse (Lee 2003, Middleton et al. 2005, O'Mahony et al. 2005, Weese et al. 2005).

A recent paper documented the recovery of MRSA in Ireland from 25 animals comprising 14 dogs, 8 horses, one cat, one rabbit and a seal (O'Mahony et al., 2005). Analysis of the strains by pulsed field gel electrophoresis (PFGE) showed that most isolates from non-equine animals were indistinguishable from each other and from the predominant pattern obtained from the most prevalent MRSA strain in the human population in Ireland. However, isolates from the 8 horses were unlike the patterns obtained from the other isolates or any patterns previously reported in Irish studies of human isolates. Unfortunately, the authors were unable to assign specific MRSA subtypes to any of the strains analysed. Another report analysed 31 MRSA strains isolated from companion animals (mainly cats and dogs) by PFGE (Rich et al. 2005). Of these, 25 (81%) were found to be human epidemic MRSA-15 (EMRSA-15) and 4 were human EMRSA-16, demonstrating the risk posed to animals by this now widespread human infection.

We recently began analysis of both methicillin resistant and sensitive *Staphylococcus aureus* (MRSA and MSSA) isolates passing through the Animal Health Trust and Royal Veterinary College veterinary diagnostic laboratories by multilocus sequence typing (MLST). MLST is a highly discriminatory method of characterising bacterial strains on the basis of the sequence of internal fragments of seven housekeeping genes (Enright et al. 2000). Sequence data generated can not only provide useful information regarding the prevalence and geographical spread of particular strain types, but also enables questions regarding the evolution of resistant strains to be addressed. Using this technique, 3 of 5 MRSA isolates from dogs suffering clinical disease analysed to date were found to be identical to the human EMRSA-15 (sequence type 22 (ST22)), 1 of 5 represented a new strain of MRSA, with only a single nucleotide change in the *pta* gene compared with EMRSA-15 and one strain was identified as EMRSA-16 (ST36). A case of equine MRSA in a scirrous cord wound was found to be ST8 (EMRSA-2, -6, -7, 12 or -14) (Enright et al. 2002). ST22, ST36 and ST8 each represent distinct arms of a *Staphylococcus* dendrogram (Figure 1).

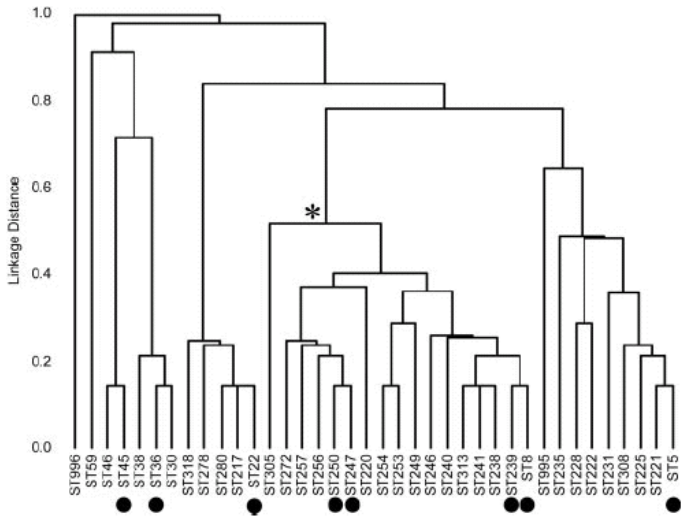


Figure 1. Relatedness of MRSA isolates from a recent human study (Enright et al. 2002).
*** identifies a major cluster of related subtypes (ST)**
• denotes the 7 major international EMRSA STs and another ST (ST36), which is frequently identified in the UK

Further MLST analysis of many more isolates will enable us to determine if the equine host environment selects for a different population of MRSA and whether equines are at risk of infection by other MRSA subtypes.

In a more worrying development the *mecA* gene has been identified in canine isolates of *Staphylococcus intermedius* (MRSI) by ourselves and others (Kania et al. 2004, Guardabassi et al. 2004). MRSI has the potential to cause widespread disease in animals including equines and new schemes, including MLST, are urgently required to improve understanding of the epidemiology of this infection and limit its dissemination.

Toxic and Parasitic Disease Report for the Second Quarter of 2005

A summary of the diagnostic toxicosis and parasitology testing undertaken by a small number of the contributing laboratories is presented in Tables 4 and 5, respectively.

The data presented for diagnostic toxicosis testing are based on information from a combination of *post mortem* examinations and allied laboratory tests, including histopathology.

Table 4: Diagnostic toxicosis sample throughput and positive results for second quarter 2005

	Number of Samples Tested	Number Positive	% Positive	Number of Contributing Laboratories
Grass Sickness	23	16	69.6%	3
Tetanus	1	1	100%	1
Botulism	1	1	100%	1
Ragwort	5	4	80.0%	2
Hepatic toxicoses	62	17	27.4%	2

Equine grass sickness (EGS) was the main toxicosis that was investigated during the quarter, with approximately 65% of clinically presumptive cases being confirmed by laboratory testing. As the second quarter is the predominant time of the year for occurrence of EGS it was considered pertinent to provide two focus articles on the disease in this report. The first, presented below reviews the evidence in support of EGS being caused by a toxicoinfectious form of botulism. The second article, provided courtesy of Katherine Whitwell FRCVS, is presented as a pathology focus and summarises the current state of play with respect to diagnostic options for the disease.

Focus article - Equine grass sickness – evidence for *C. botulinum* toxico-infection as the cause

Equine grass sickness (EGS or equine dysautonomia) is a debilitating and frequently fatal neurodegenerative disease of horses, which as its name suggests predominantly affects grazing animals. Although the cause of EGS has still not been definitively identified, there is both strong historical and modern evidence to show that it is caused by local neurotoxin production by the bacterium *Clostridium botulinum* within the equine gastrointestinal tract.

The botulinum hypothesis was initially proposed in late 1919, when *post mortem* examinations on EGS cases suggested acute toxaemia of bacterial origin (Tocher *et al.,* 1923; Tocher, 1924). A 'large anaerobic bacillus' was isolated from cases and found to have the morphological characteristics and toxigenic properties of *C. botulinum* and similarities between the signs of EGS and those of botulism were recognised.
In 1922 and 1923 Tocher and co-workers performed controlled vaccine trials involving more than 2000 horses, with half the horses on each premises receiving an antitoxin neutralised botulinum toxin vaccine and the other remaining as unvaccinated controls (Tocher *et al.,* 1923; Tocher, 1924, Wood *et al.,* 1999; Collier *et al.,* 2001). Results showed a highly statistically significant reduction in EGS mortality rate among the vaccinated group in both years of the study. In 1922, EGS mortality among non-vaccinated horses was 9.3% compared with 3.2% among those that had been inoculated once and 2.3% for those that received two doses of vaccine. In the 1923 trial, in which a more potent vaccine based on *C. botulinum* isolated from chronic EGS cases was used rather than the human vaccine used in the 1922 trial, mortality among controls and vaccinated horses was 8.2% and 1.5%, respectively. Furthermore, no horses that received two doses in 1923 succumbed to the disease. However, the botulism theory for EGS was eventually abandoned after Professor Gaiger from the University of Liverpool, who had his own streptococcal theory as to the cause of EGS, publicly rejected the results and criticised the trial.

In the 1990s, the hypothesis that EGS was indeed a toxico-infectious form of botulism involving a type C strain botulinum toxin (BoNT/C) was re-visited by Keith Miller. Subsequently, Ian Poxton and co-workers have demonstrated a significant association between presence of both the botulism organism and BoNT/C in histologically confirmed EGS cases compared with apparently healthy controls (Figure 1) (Hunter *et al.,* 1999).

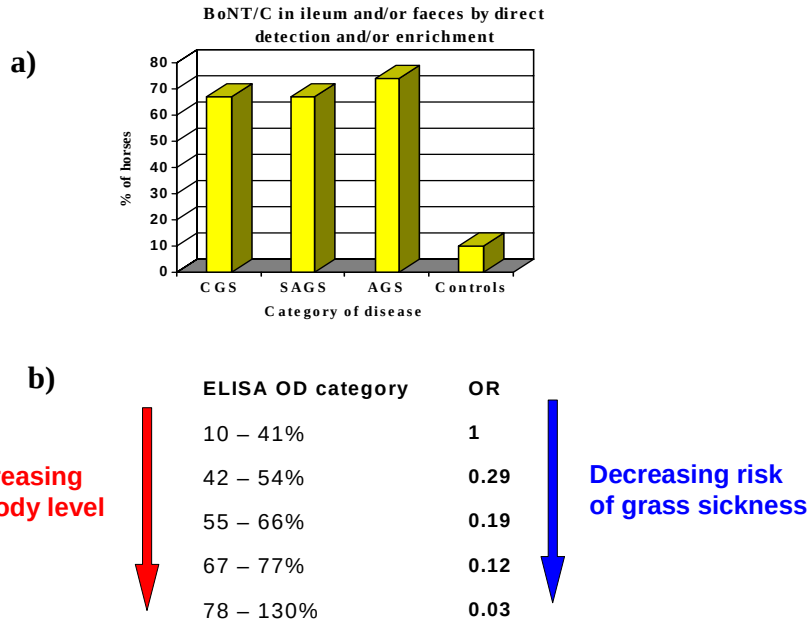


Figure 1: a) presence of *C. botulinum* and/or its toxin is associated with all forms of EGS (Hunter et al. 1999), b) increasing levels of anti-botulinum antibody, having adjusted for age, are associated with a biological gradient of decreasing risk (OR = odds ratio) of developing EGS (McCarthy 2002)

It has been further shown that EGS cases have significantly lower antibodies to *C. botulinum* and its type C toxin than horses that have either been in contact with EGS or have grazed frequently affected land (Figure 2) (Hunter and Poxton, 2001; McCarthy et al. 2004). Findings are strongly supportive of EGS being a toxico-infection with *C. botulinum* type C and it is now proposed that toxin production and absorption occurred mainly in the ileum due to overgrowth from normal large intestinal flora and/or to spore germination in association with nutritional triggers.

Table 5: Diagnostic parasitology sample throughput and positive results for second quarter 2005

	Number of Samples Tested	Number Positive	% Positive	Number of Contributing Laboratories
Endoparasites				
Strongyles	20	8	40.0%	1
Tapeworms	255	4	1.6%	2
Cyathostomes	626	109	17.4%	4
Dictyocaulus	2	0	0%	1
Ascarids	188	2	1.1%	1
Trichostrongyles	22	1	4.5%	1
Ectoparasites				
Lice	352	2	0.6%	5
Ringworm	63	4	6.3%	13
Mites	329	4	1.2%	5

Relatively small proportions (<7%) of samples tested were positive for tapeworms, lice, ringworm and/or mites whereas larger proportions (>15%) of faecal samples were positive for strongyles and/or cyathastomes.

Equine *Post Mortem* Examinations (PME)

Reports on gross PME were received from 4 laboratories. Reports are presented regionally below:

East Anglia

Ten full PME were performed in Newmarket on foals and adult horses over the quarter April to June 2005. In addition forty-one abortions and neonatal foal deaths were investigated and these results will form part of a report covering the 2004-5 breeding season in a future issue.

The findings of interest from these PME are briefly described below:

A three month-old Thoroughbred foal was examined with severe pyogranulomatous pneumonia due to infection with Rhodococcus equi. A focal abscess in one lung lobe cultured R.equi.

Welfare investigation of an adult male, mixed breed horse found emaciation due to combined cyathostomiasis and Salmonellosis causing typhlocolitis with concurrent dermatophilosis and severe louse infestation.

PME was performed on an adult thoroughbred mare that had undergone successful surgery to correct a colonic torsion but subsequently suffered severe ischaemic compromise throughout the digestive tract, marked peritonitis and adhesion formation necessitating euthanasia. The cause of the original torsion was not apparent.

An imported, five year old American Quarter Horse stallion that became acutely ill post transport was examined and found to have massive internal fluid exudation, haemorrhage and oedema. At first it was unclear whether there was a primary vasculitis responsible for the changes and tests were performed to rule out EVA and EHV (serology, PCR and virus isolation were negative). Instead a diagnosis of purpura haemorrhagica secondary to S.equi guttural pouch empyema was made. The vasculitis was caused by deposition of antibody/antigen complexes within the endothelium.

A five year-old, in-foal, Thoroughbred brood mare was diagnosed with EGS on gross pathology (linear oesophageal ulceration and distended fluid filled stomach – a colon impaction had already been surgically corrected – no obstructive cause was found for this at surgery). Histological examination showed degenerate neurones and nuclear pyknosis affecting the submucosa and myenteric plexi and ganglia confirming the diagnosis.

A seven-year-old Warmblood gelding with a history of acute colic, hindlimb stiffness, rapidly progressive ataxia and eventual recumbency was examined. Findings were limited to a diffuse myodegeneration that did not appear to be infective and a CK of over 66000 suggesting an acute, severe rhabdomyolysis. The animal had been feeding on silage and although no characteristic gross lesions of botulism infection were apparent it could not be completely ruled. Testing for EHV1 and EHV4 by PCR, serology and virus isolation suggested a recrudescence of EHV-4 infection (detected by PCR on mixed CNS tissues) secondary to the underlying muscular disease but no evidence of recent EHV infection.

A two month-old Thoroughbred foal was euthanased as a result of progressive hindlimb lameness. A diagnosis was made of severe, osteochondrosis affecting both the left and right femoro-tibial joints with extensive cartilage defects. Incidentally the foal had a row of healed rib fractures along one side presumed to be parturient damage.

A nine week-old Thoroughbred foal was examined after being found dead. The foal was suffering from an acute, severe interstitial pneumonia and a diagnosis of Pneumocystis carinii, based on monoclonal antibody tests, and co-infection with EHV-1, identified in lung tissue by PCR, was made. It is unclear whether the EHV-1 was the primary pathogen that enabled the Pneumocystis infection to take hold or whether there was recrudescence of latent EHV-1 infection as a result of intercurrent disease. Immunostaining was performed to determine the relative significance of the two pathogens in the disease process.

Post mortem examination was performed on a three month-old Thoroughbred foal with severe pyogranulomatous pneumonia due to infection with Rhodococcus equi. A focal abscess in one lung lobe cultured R.equi and it is thought that this provided a source of infection for the foal each time antibiotic therapy ceased eventually causing an insurmountable septicaemia. Multiple valvular changes were noted in the heart and great vessels thought to be the result of cardio-pulmonary infection and subsequent endocarditis. The cause of death is likely to have been circulatory failure.

Additionally one case of neurological disease and one case of renal failure were examined at PME.

South West

Ten full PME were performed at Bristol University Veterinary School during the second quarter of 2005. These included one case of neonatal death (no diagnosis), three cases of arthritis/ tendonitis, one small intestinal infarction of unknown cause, three cases of

trauma: one laceration, two fractures (unspecified), one case of emphysema, two colic surgery recovery complications.

Midlands

One case of sudden death was examined at PME and found to have a caecal rupture.

Scotland

Thirty six PME were performed during the last quarter. These comprised a single case of neurological disease, diagnosed as cauda equine syndrome, fifteen tumours, ten fractures and ten cases of gastro-intestinal disease.

Pathology focus: options for diagnosis of equine grass sickness
Courtesy of Katherine Whitwell FRCVS, Consultant Equine Pathologist

When clinical illness suggests grass sickness in horses, accuracy of diagnosis is essential for optimal therapy and management of affected and in-contact horses. Since Obel (1955) discovered that the underlying defect in affected horses was degenerative changes in autonomic neurones, the most reliable confirmatory tests have been based on demonstrating pathognomonic neuronal damage.

Other tests provide supportive evidence but none are specific enough to be 100% reliable. Some tests used in practice and diagnostic labs include

Assessments based on clinical evaluations

a) Clinical appraisal of the nature, severity and duration of clinical signs, which may present as peracute, acute, subacute or chronic is fundamental to diagnosis (Milne 1996). *Rhinitis sicca* (drying of the nasal mucous membranes; Figure 1) is one of the more specific signs in chronic cases.

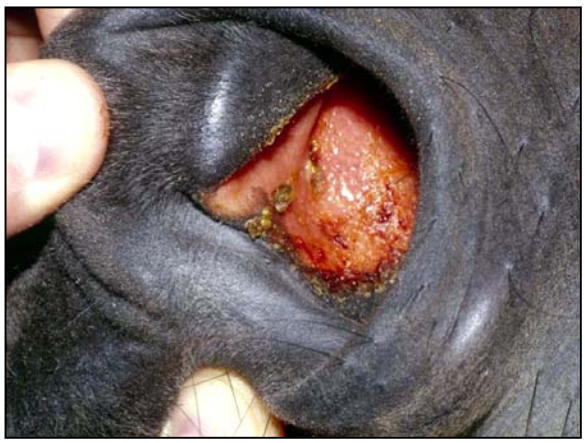


Figure 1: Rhinitis sicca within the left nostril of a horse with chronic grass sickness

b) Surgical appraisal of the appearance, and texture of the alimentary tract and its contents at laparotomy with exclusion of other conditions.
c) Physiology-based clinical tests
i) The use of barium swallow in conjunction with oesophageal radiography in the standing horse to evaluate motility of the oesophagus (Greet and Whitwell 1986).
ii) The effect of phenylephrine eye drops on the size of the palpebral fissure and angle of the eyelashes of horses with ptosis (Hahn and Mayhew 2000).

Assessment based on clinical chemistry

a) Serum biochemical tests: Although certain changes do occur regularly, none can be depended on to confirm or exclude a diagnosis.

b) Urinalysis: Horses with acute and subacute grass sickness generally have significantly higher urinary protein, creatinine and specific gravity, and a lower pH, which may support the diagnosis (Fintl et al. 2002).

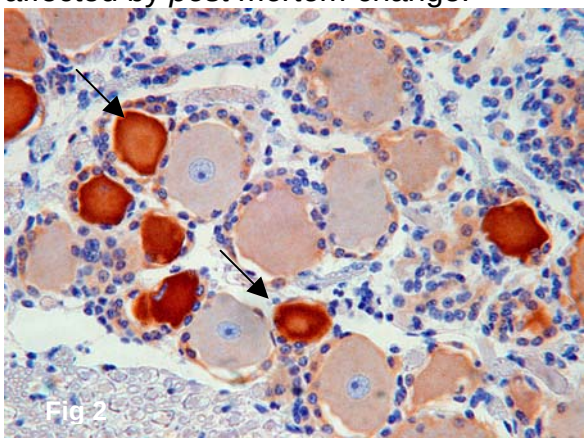
Assessment based on histopathology

a) Samples from the living horse: In most cases a diagnosis can be made from an examination of the integrity of the neurones in an ileal biopsy obtained at laparotomy (Scholes et al.1993).

b) Samples taken at *post mortem* examination: Routine histology on any of five ganglia (coeliaco-mesenteric, cranial cervical, stellate, thoracic chain, caudal mesenteric) can provide a diagnosis. The coeliaco-mesenteric ganglion is the largest and is the one usually examined when a full *post mortem* examination is undertaken. Diagnosis can be achieved without recourse to evisceration by examination of a cranial cervical ganglion (they lie in the caudal wall of the guttural pouches).

Recent developments

a) Immunostain for synaptophysin: Hilbe et al. (2005) have drawn attention to a useful and reliable stain for identifying degenerate neurones in biopsy or *post mortem* material (see figure 2). The marker provides improved assessment of neuronal integrity in ganglia affected by *post mortem* change.



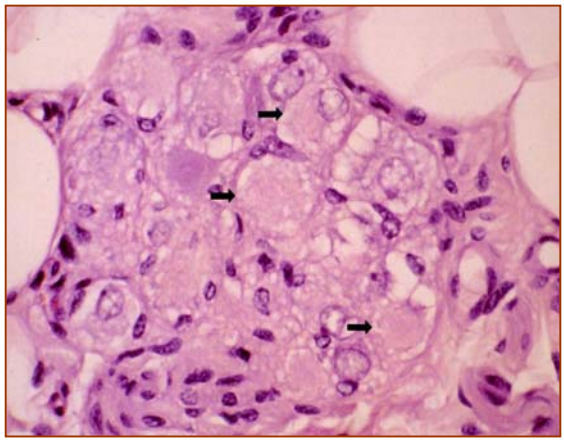


Figure 2: Trigeminal ganglion of a horse with EGS immunostained for synaptophysin. Degenerate neurones are immunostained dark brown (arrows).
Figure 3: Photomicrograph of autonomic nerve plexus in wall of rectum from horse with EGS showing degenerate (chromatolytic) neurones (arrowed). Image courtesy of Dr Andy Wales.

b) Skin biopsy: Assessment of the status of immunoreactive nerve fibres innervating small dermal blood vessels has recently been found to provide a reliable means of identifying the more chronic cases (Begara-McGorum – work in progress)

c) Rectal biopsy: Evidence from *post mortem* studies suggests that degenerate neurones can be reliably identified by examination of two or more rectal biopsies (see figure 3). This may prove clinically useful, but has not yet been validated in live horses (Wales and Whitwell 2005).

References for further reading

Collier, D.St.J., Collier, S.O. and Rossdale, P.D. (2001) Grass sickness – the same old suspects but still no convictions! *Equine veterinary Journal* **33** 540-542.

Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus. *Journal of Clinical Microbiology* **38** 1008-15.

Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. (2002) The evolutionary history of methicillin-resistant Staphylococcus aureus (MRSA). *Proceedings of the National Academy of Science USA* **99** 7687-92.

Fintl, C, Milne, E.M. & McGorum, B.C. (2002) Evaluation of urinalysis as an aid in the diagnosis of equine grass sickness. *Veterinary Record* **151** 721-724.

Greet, T.R. & Whitwell, K. E. (1986) Barium swallow as an aid to the diagnosis of grass sickness. *Equine Veterinary Education* **18** 294-297.

Guardabassi L, Loeber ME, Jacobson A. (2004) Transmission of multiple antimicrobial-resistant Staphylococcus intermedius between dogs affected by deep pyoderma and their owners. *Veterinary Microbiology* **98** 23-7.

Hahn, C.N. & Mayhew I.G. (2000) Phenylephrine eyedrops as a diagnostic test in equine grass sickness. *Veterinary Record* **147** 603-606.

Hilbe, M., Guscetti, F., Wunderlin, S. & Ehrensperger, F. (2005) Synaptophysin: an immunohistochemical marker for animal dysautonomia. *Journal of Comparative Pathology* **132** 223-227.

Hunter, L.C. and Poxton, I.R. (2001) Systemic antibodies to Clostridium botulinum type C: do they protect horses from grass sickness (dysautonomia)? *Equine Veterinary Journal* **33** 547-553.

Hunter, L.C., Miller, J.K. and Poxton, I.R. (1999) The association of Clostridium botulinum type C with equine grass sickness: a toxicoinfection? *Equine veterinary Journal* **31** 492-499.

Kania S.A., Williamson N.L., Frank L.A., Wilkes R.P., Jones R.D., Bemis D.A. (2004) Methicillin resistance of staphylococci isolated from the skin of dogs with pyoderma. *American Journal of Veterinary Research* **65** 1265-8.

Lee J.H. (2003) Methicillin (Oxacillin)-resistant Staphylococcus aureus strains isolated from major food animals and their potential transmission to humans. *Applied and Environmental Microbiology* 69 6489-94.

McCarthy H.E., French N.P., Edwards G.B., Poxton I.R., Kelly D.F., Payne-Johnson C.E., Miller K., Proudman, C.J. (2004) Equine grass sickness is associated with low antibody levels to Clostridium botulinum: a matched case-control study. *Equine Veterinary Journal* **36** 123-9

Middleton J.R., Fales W.H., Luby C.D., Oaks J.L., Sanchez S., Kinyon J.M., Wu C.C., Maddox C.W., Welsh R.D., Hartmann F. (2005) Surveillance of Staphylococcus aureus in veterinary teaching hospitals. *Journal of Clinical Microbiology* **43** 2916-9.

Milne, E.M. (1996) Clinical diagnosis and management of acute and sub-acute grass sickness; *Equine veterinary Education* **8** 71-73.

Obel, A. (1955) Studies on grass disease. The morphological picture with special reference to the vegetative nervous system. *Journal of Comparative Pathology* **65** 334-346.

O'Mahony R., Abbott Y., Leonard F.C., Markey B.K., Quinn P.J., Pollock P.J., Fanning S., Rossney A.S. (2005) Methicillin-resistant Staphylococcus aureus (MRSA) isolated from animals and veterinary personnel in Ireland. *Veterinary Microbiology* **109** 285-96.

Rich M., Roberts L., Kearns A. (2005) Methicillin-resistant staphylococci isolated from animals. *Veterinary Microbiology* **105** 313-4.

Scholes, S.F.E., Vaillant, C., Peacock, P., Edwards, G.B. and Kelly, D.F. (1993) Diagnosis of grass sickness by ileal biopsy. *Veterinary Record* **133** 7-10.

Tocher, J.F. (1924) Grass sickness in horses. *Transmissions of the Royal Highland Agricultural Society of Scotland* **36** 65-83.

Tocher, J.F., Brown, W., Tocher, J.W. and Buxton, J.B. (1923) 'Grass sickness' investigation report. *Veterinary Record* **3** 37-45, 75-89.

Wales, A.D.and Whitwell, K.E. (2005). Potential role of multiple rectal biopsies in the diagnosis of equine Grass Sickness. *Veterinary Record* (in press).

Weese, J.S. (2005). Methicillin-Resistant Staphylococcus aureus: An Emerging Pathogen in Small Animals. *Journal of the American Animal Hospital Association* **41** 150-157**.**

Wood, J.L.N., McGorum, B.C. and Mayhew, I.G. (1999) Equine dysautonomia: has grass been blamed unfairly all this time? *Equine Veterinary Journal* **31** 451-452.

Acknowledgements

This report was compiled by the Animal Health Trust. We are extremely grateful to the following laboratories for contributing data for this report.

Beaufort Cottage Laboratories
BioBest Laboratories Ltd
Central Diagnostic Services, University of Cambridge
Department of Pathology, University of Bristol
Greenwood, Ellis and Partners
Hampden Veterinary Hospital
J.S.C Equine Laboratory Ltd
Minster Equine Veterinary Clinic
NationWide Laboratories
Oakham Veterinary Hospital
Ratley Lodge Laboratory
The Equine Veterinary Hospital, Arundel
Three Counties Equine Hospital
Veterinary Pathology Unit, R(D)SVS, University of Edinburgh
Vetlab Services Ltd

All laboratories contributing to this report operate Quality Assurance schemes. These schemes differ between laboratories, however, all the contagious equine metritis testing reported was accredited by the Horserace Betting Levy Board.

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