



# DEFRA / AHT / BEVA EQUINE QUARTERLY DISEASE SURVEILLANCE REPORT Volume 6, No.4: October – December 2010



## Highlights in this issue:

- **EIA situation in Europe**
- **EVA in the United Kingdom**
- **Update on Atypical Myopathy**

### 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 fourth quarterly equine disease surveillance report for 2010 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 Infectious Anaemia (EIA)

As of 16th December 2010, Department for Environment, Food and Rural Affairs (Defra) confirmed that all the remaining horses on the premises in Northumberland, England on which EIA was detected in a horse on 7th September 2010, had tested negative for EIA in their final 90 day blood test. Restrictions were lifted as of 15th December 2010. For more information about this outbreak, [click here](#) and [here](#).

With regards to the outbreak of EIA reported by Defra in Devon on 11th September 2010, as of 23rd December 2010 Defra confirmed that all the remaining horses on the premises had tested negative for EIA in their final 90 day blood test; subsequently restrictions were lifted as of 22nd December 2010. For more information about this outbreak, [click here](#) and [here](#).

**Animal Health** provides registered users with the latest news specific to exotic notifiable farm animal and/or equine disease outbreaks in Great Britain by means of **alerts** that can be sent to the users by a pre-recorded voice message, mobile text, fax and email. If you wish to subscribe to this service, please [click here](#).

#### Equine Viral Arteritis (EVA)

As of 8th December 2010, Defra confirmed EVA in a Warmblood stallion imported from Holland and stabled in West Sussex, England. There was no evidence of infection in any in-contact horses and the stallion was gelded on 22nd December 2010. As of 2nd February 2011 the official restrictions on the EVA positive stallion have been lifted. According to Defra, epidemiological investigations have detected no credible source of infection within the UK and it is presumed that the stallion was infected prior to being moved to the UK from mainland Europe. There is no evidence of onward spread of infection from this case. For more information about this outbreak, [click here](#) and [here](#).

#### Equine Influenza (EI)

**Equine influenza** continues to be of importance within the United Kingdom. In this issue we report on a single small outbreak in Leicestershire.

Equine Influenza outbreaks in the UK are being reported by the new text alert service sponsored by Merial Animal Health, **Tell-Tail**. This service alerts practitioners to outbreaks of equine influenza in the UK by a text message to the practitioner's mobile phone. If you are an equine veterinary practitioner and would like to sign up for this scheme, please register [here](#). This service has also been offered to the members of the National Trainers Federation (NTF). If you would like to contact us regarding outbreaks of equine influenza



virus or would like to sign up for our sentinel practice scheme, please send a message to: [equiflunet@aht.org.uk](mailto:equiflunet@aht.org.uk) or follow the link to [www.equiflunet.org.uk](http://www.equiflunet.org.uk) for more information on equine influenza.

### Equine Herpesvirus-1

Several outbreaks of EHV-1 causing abortion or paralytic disease have been reported in the UK this quarter. In addition to these outbreaks, which are outlined in the Virological Diagnoses for the Fourth Quarter 2010 of this report, in January 2011 an outbreak of EHV-1 causing neurological disease and multiple abortions has been reported on a stud in Oxfordshire. As of 17th January 2011 a Thoroughbred barren mare presented with neurological disease and was euthanased. Following the post-mortem examination and histopathology revealing focal haemorrhages in sections from the spinal cord, EHV-1 was suspected to be the cause of disease in this mare. On 2nd February 2011 virus isolation on spinal cord tissue from this mare was positive and was subsequently confirmed as EHV-1 by PCR. There were 9 mares in total on the premises, and 3 of them (pregnant mares) had shared airspace with the affected mare. These three mares started showing pyrexia, and eventually all three aborted. Only one of these abortions was investigated and as of 28th January 2011 EHV-1 was confirmed to be the cause of abortion on the basis of positive PCR for EHV-1 in mixed fetal tissues. The four mares were all vaccinated against EHV-1/4, as are the remaining mares on the premises which are due to foal and which are being kept in isolation. All necessary precautions have been taken, the Thoroughbred Breeders' Association (TBA) has been informed and the HBLB Codes of Practice is being followed.

### International disease occurrence

#### Contagious Equine Metritis (CEM)

No additional carrier stallions or mares were detected as a sequel to the 2008/09 CEM event in the USA. A total of 292 stallions in 28 states have been screened by bacteriological culture since the USDA, APHIS, VS instituted a stallion testing program in early 2010, none of which have turned up positive for *T. equigenitalis*. The majority of the stallions tested were Quarter horses (114), with an additional 28 different breeds represented among the remaining 178 animals included in the survey. Some 268 of the overall total tested were active breeding stallions with a further 24 being stallions that had been imported into the USA since 2000. All epidemiologically linked horses have been identified and all testing and treatment protocols have been conducted in accordance with Federal, International, and expert guidance and requirements; subsequently the comprehensive epidemiological investigations of the Contagious Equine Metritis (CEM) events in the United States are closed and the whole event has been declared resolved as of 27th December 2010.

One case of CEM in a Lipizzaner on one premises in Seine-et-Marne, France, was confirmed on 24th December using agent isolation.

As previously reported, on 12th July 2010 CEM was reported in Evora, Portugal. This outbreak is ongoing as of 3rd February 2011. For more information about this outbreak, [click here](#).



## Equine Infectious Anaemia (EIA)

As previously reported, following the investigation launched on 20th January 2011 after the UK reported having confirmed the disease in two horses of a consignment from Romania via Belgium, EIA was confirmed in seven single cases in Assebroek and Brugge (West Flanders), Warsage, Fumal, Charneux and La Reid (Liège), and Knesselaere (East Flanders). These seven outbreaks in Belgium were resolved as of 14th December 2010. For more information, [click here](#).

Regarding the EIA situation in Germany, currently there are 22 continuing outbreaks of EIA in Germany. Restrictions have been placed on the affected premises and epidemiological investigations are still ongoing. In 2010 a total number of 27 outbreaks occurred, of which five outbreaks have been reported as being resolved and restrictions have been lifted. During 2010 19 EIA outbreaks have been reported on premises in the Federal State of Bayern, five outbreaks in the Federal State of Hessen and one outbreak each in Nordrhein-Westfalen and Rheinland-Pfalz. As far as the regional distribution is concerned, all outbreaks have occurred in the southern half of Germany. For more information about these outbreaks, [click here](#).

On 13th October 2010, one case of EIA was reported in a French Trotter in Gironde, France. The affected horse had not shown clinical signs and was tested as part of the epidemiological investigation involving horses related to the index outbreak confirmed on 3rd March 2010, in Dordogne. Restrictions have been placed on the affected premise; all the horses have been isolated and are due to be screened as part of the investigation. The affected horse was euthanased. In addition to this outbreak, on 16th November 2010, one case of EIA was confirmed in Ille-et-Vilaine. The affected horse was imported from Romania in 2008 via Belgium. Restrictions have been placed on the affected premises and epidemiological investigations are ongoing. For more information about the EIA outbreaks in France, [click here](#) and [here](#).

As previously reported, on the 2<sup>nd</sup> July 2010 EIA was confirmed in a horse in Western Macedonia and Thrace, Greece. As of 3<sup>rd</sup> February 2011, this outbreak is continuing and investigations are ongoing. For more information about this outbreak, [click here](#).

As reported by RESPE on 20th January 2011 following a report from the *Ministère de l'Alimentation, de l'Agriculture et de la Pêche* (France), EIA has been confirmed in Hadju-Bihar, Hungary. Following the screening of 700 horses under an epidemiological investigation for EIA, one horse tested positive and has subsequently been euthanased. Also in Hungary and as reported by RESPE on 3<sup>rd</sup> February 2011, EIA has been confirmed in a horse in Győr-Moson-Sopron. Following the screening of 400 horses under an epidemiological investigation for EIA, all horses except for one horse tested negative. The positive horse, which was standing on a premises with other 10 horses, has subsequently been euthanased. Restrictions have been placed on the affected premises. For more information about these outbreaks, please [click here](#).

In January 2011 two horses were confirmed positive for EIA on a premises in northeast Missouri, USA. The first case was a Belgian horse that was quarantined upon confirmation of infection and subsequently euthanased. Testing of the remaining horses on the premises turned up a second seropositive animal, which was also euthanized. State animal health officials tested 696 other horses in the vicinity of the affected farm of which a number had contact with the 2 infected animals but found no additional cases of infection.



The source of virus for the two cases of EIA has not been determined. For more information, [click here](#).

### Equine Piroplasmosis

Regarding the **Equine Piroplasmosis** (EP) outbreaks in the US, extensive testing and follow-up investigation for evidence of EP caused by *Theileria equi* or *Babesia caballi* has continued throughout the fourth quarter of 2010 following the discovery of EP on a ranch in south eastern Texas in 2009. Of 2,500 horses tested since that event, 413 *T. equi* seropositive horses were determined to be epidemiologically linked to the index premises in Texas. A considerable number of the positive horses were relocated back to the index ranch to be managed under long-term quarantine on that facility. Many of these animals have been included in a USDA approved EP treatment research program or in other research studies conducted by USDA, ARS.

A not insignificant number of seropositive horses on premises other than the index ranch have been euthanased by their owners. Some others are currently being kept under state quarantine on individual premises in Alabama, Indiana, North Carolina, Tennessee and Texas. Aside from the aforementioned findings, an additional 143 horses (137 positive for *T. equi* and 6 positive for *B. caballi*) unrelated to the index premises in Texas, have been identified in a total of 16 states. While many of the positive horses are racehorses belonging to the Quarter horse breed, other breeds including Thoroughbreds are also represented. A not insignificant number of these horses were imported in the USA at some time in the past, many from known EP-endemic countries. Currently, a total of 9 states (Arkansas, Colorado, Florida, Kentucky, Iowa, Louisiana, New Mexico, Oklahoma and Texas), require EP testing for horses competing in sanctioned horse racing events. Further testing for EP is continuing with respect to interstate movement of horses, movement to equine events and trace-back investigations. [Click here](#) for more information about the EP situation in the US.

### West Nile Virus (WNV)

The WNV outbreak reported on 4th October 2010 in Dobric, Bulgaria was declared resolved as of 30th December 2010. In addition, the outbreak reported on 2nd November in Varna, Bulgaria has also been declared resolved. For more information about these outbreaks, [click here](#).

As previously reported, on 27th August 2010 WNV was reported for the first time in Greece ([click here](#) for more information). As of 3rd February 2011 the situation regarding the outbreaks of WNV in Greece has not changed with respect to the last report; 27 outbreaks involving 559 susceptible horses, 30 cases and 3 deaths are still ongoing.

As previously reported, on 27th September 2010 the OIE confirmed the first occurrence of West Nile Virus (WNV) in equids in Portugal. An outbreak involving a positive horse on a premises with only one susceptible horse was reported in Lisboa e Vale do Tejo, near Lisbon. The affected horse was been humanely destroyed on welfare grounds and the outbreak was declared resolved as of 4th October 2010. However, on 29th October 2010 a second outbreak involving a positive horse and 70 susceptible horses was reported in the same area. As of 3rd February 2011 this outbreak is still ongoing. For more information about this outbreak, [click here](#).



On 29th December 2010 three outbreaks were reported in Romania (two in Braila and one in Constanta). These three outbreaks have involved 6 cases and 9 susceptible horses. The three outbreaks are located nearby mosquito breeding areas. As of 3rd February 2011 all three outbreaks are ongoing. Please [click here](#) for further information about these outbreaks.

As reported in last quarter's issue, on 10th September 2010 the first occurrence of West Nile Virus (WNV) in equids was confirmed in Spain. As of 3rd February 2011 the situation regarding the outbreaks of WNV in Spain has not changed with respect to the last report. The 31 outbreaks reported are ongoing, there are 845 susceptible horses and 39 cases of which two have died. For more information, [click here](#).

A total of 86 cases of WNE were recorded over the approximate three month period since late September 2010. The greatest number of cases during the period were recorded in Florida (18), California (15), Texas (8), Colorado (5) and Pennsylvania (5), Missouri (4), Kentucky (3), Tennessee (3) and Utah (3) and 1/2 cases in many other states. The vast majority of cases of WNE either had not been vaccinated, had no history of vaccination or had not been vaccinated within the previous 12 months.

### **Focus articles**

In this report we are pleased to include two focus articles. Following the several cases of Atypical Myopathy in the south-west of England, Jo Ireland and Fatima Cruz from the AHT give an overview on the most relevant up to date information of this disease.

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](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/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](http://www.aht.org.uk/equine_disease_registration.html).



## Virology Disease Report for the Fourth Quarter of 2010

The results of virological testing for October to December 2010 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 local DVM has been informed.

**Table 1: Diagnostic virology sample throughput and positive results for the fourth quarter 2010**

	Number of Samples Tested	Number Positive	Number of Contributing Laboratories
<b><u>Serological Tests</u></b>			
EVA ELISA	1752	10 <sup>#</sup>	5
EVA VN	355	1 <sup>#</sup>	3
VLA EVA VN	1217	24 <sup>#</sup>	1
EHV-1/-4 CF test	601	8 <sup>*</sup>	1
EHV-3 VN test	0	0	1
ERV-A/-B CF test	259	2 <sup>*</sup>	1
Influenza HI test	248	7 <sup>*</sup>	1
EIA (Coggins)	304	0	3
EIA ELISA	683	0	3
VLA EIA (Coggins)	1934	0	1
VLA WNV (PRNT)	9	0	1
<b><u>Virus Detection</u></b>			
EHV-1/-4 PCR	94	4	2
EHV-2/-5 PCR	12	7	1
Influenza NP ELISA**	189	1	1
Influenza Directigen	235	0	1
Influenza VI in eggs	0	0	1
EHV VI	152	8	1
EVA VI/PCR	0	0	1
VLA EVA VI/PCR	10	1	1
Rotavirus	18	2	5

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  
<sup>#</sup> = Seropositives include vaccinated stallions, <sup>\*</sup> = Diagnosed positive on basis of seroconversion between paired sera  
<sup>\*\*</sup> = 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 24 EVA VN positives detected by the VLA, 14 were export samples, 3 were serum samples from stallions for artificial insemination (AI) certification, 5 samples were submitted privately for testing and 2 samples were submitted for diagnosis from overseas. Of the 10 semen samples received for EVA testing one tested positive on virus isolation and RT-PCR; this positive relates to the EVA outbreak in West Sussex, England.



The 1934 agar gel immuno diffusion tests for EIA (AGID; Coggins) were conducted for international trade purposes and they were all negative.

### **Virological Diagnoses for the Fourth Quarter of 2010**

#### **EHV-1 Abortion**

Two single cases of EHV-1 abortions in two Thoroughbred mares have been reported in this quarter. In both cases EHV-1 infection confirmed was on the basis of positive PCR for EHV-1 in mixed fetal tissues consistent with characteristic histopathology evident in formalin fixed tissue sections and also virus isolation from placenta and/or fetal tissues. No further cases were reported on either of the affected studs. All necessary precautions were taken and the HBLB Codes of Practice were followed.

#### **EHV-1 paralytic and respiratory disease**

On 8th December 2010 an outbreak of EHV-1 causing multiple cases of fever and paralytic disease was reported in a livery yard in Warwickshire, England. There were at least 10 horses out of a total of 40 on the premises reported with clinical signs which varied from fever and mild inco-ordination to moderate/severe neurological signs without obvious respiratory signs. As of 9th December one severely affected horse was euthanased; the remaining affected horses were treated symptomatically. All the horses on the yard were current for equine influenza vaccinations but were not vaccinated against EHV-1/-4. Initial clinical suspicion of EHV-1 infection was supported with evidence of high titres by complement fixation (CF) test in some affected horses in the absence of recent vaccination. Submission of post mortem material from the euthanased horse provided the opportunity to confirm the presumptive diagnosis of paralytic EHV-1 on the basis of spinal cord tissues testing positive for presence of EHV-1 by PCR. In addition, virus isolation from nasopharyngeal swabs and heparinised bloods in the in-contact horses gave rise to three other horses testing positive for EHV-1. Restrictions were placed until further notice and an epidemiological investigation was undertaken including laboratory screening by means of virological and paired serological testing.

#### **EHV-4 Respiratory infection**

EHV-4 was isolated from a nasopharyngeal swab in a 3 year-old stallion which showed respiratory signs.

#### **Equine Influenza**

On 4th November 2010 equine influenza was diagnosed in a four year-old horse in Leicestershire, UK. Diagnosis was confirmed by the Animal Health Trust on the basis of positive nucleoprotein (NP) ELISA on a nasopharyngeal swab. The affected horse, which had a primary vaccination for EI in the past that lapsed, showed respiratory signs and at the time of reporting was the only affected animal among a group of 20 horses (most of them vaccinated for EI) on a livery yard. Genetic characterisation of the isolate obtained from this outbreak could not be carried out due to failure to isolate virus from the primary sample.



## **Bacteriology Disease Report for the Fourth Quarter 2010**

A summary of the diagnostic bacteriology testing undertaken by different contributing laboratories is presented in Table 2. For contagious equine metritis (CEM) 27 out of 28 HBLB approved laboratories in the UK contributed data.

### VLA CEMO Data for the period October to December 2010

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 fourth quarter 2010**

	Number of Samples Tested	Number Positive	Number of Contributing Laboratories
<b>CEMO (HBLB)</b>	1163	0	27
<b>CEMO (VLA)</b>	999	0	1
<b><i>Klebsiella pneumoniae</i><sup>#</sup></b>	1156 <sup>1</sup>	2	27
<b><i>Pseudomonas aeruginosa</i></b>	1153 <sup>1</sup>	13	27
<b>Strangles*culture</b>	1435	101	18
<b>Strangles PCR</b>	872	77	2
<b>Strangles ELISA</b>	992	183	1
<b>Salmonellosis</b>	628	17	18
<b>MRSA</b>	291	6	11
<b><i>Clostridium perfringens</i></b>	146	8	6
<b><i>Clostridium difficile</i> (toxin by ELISA or immunochromatography)</b>	155	18	9
<b><i>Borrelia</i> (by ELISA)</b>	27	13	1
<b><i>Rhodococcus equi</i></b>	568	1	7
<b><i>Lawsonia intracellularis</i><sup>**</sup></b>	76	22	4

CEMO = contagious equine metritis organism (*Taylorella equigenitalis*); HBLB = HBLB accredited laboratories; <sup>#</sup> =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; <sup>1</sup> reproductive tract samples only

### VLA *Salmonella* results

From the 16 strains typed by the VLA the serotypes reported were *S. Enteritidis* (five isolates), *S. Newport* (three isolates), *S. Typhimurium* (two isolates), serotype 4,5,12:1:- (two isolates), *S. Agama*, *S. Anatum*, *S. Nagoya* and *S. Berta* (one isolate respectively). These 16 positive samples represent 13 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)."

For more information from Defra about *Salmonella* in the UK, please [click here](#).



## **Focus Article: Recent advances in *Rhodococcus equi***

**Professor Jose A. Vazquez-Boland, DVM, PhD. Microbial Pathogenesis Unit, Centre for Infectious Diseases, College of Medicine and Veterinary Medicine, University of Edinburgh.**

The genus *Rhodococcus* belongs to one of the largest microbial groups on earth, the *Actinobacteria*. The rhodococci include "friendly" organisms that produce antibiotics and other clinically useful secondary metabolites, but also major pathogens, such as the causative agents of tuberculosis (TB), leprosy or diphtheria. They are widely distributed in the environment and are important in biotechnology due to their extraordinary metabolic versatility and biodegradative properties. The genus also includes an animal pathogen, *Rhodococcus equi*, a soil inhabitant that uses herbivore manure as growth substrate and is ubiquitous in the farm environment.

### **Horse pathogen**

*R. equi* is the causative agent of a severe respiratory disease of horses that is a leading cause of mortality in foals. Rhodococcal pneumonia typically affects foals between one and six months of age, follows an insidious course with sudden onset of overt symptoms, and is generally fatal if antibiotic treatment is not rapidly administered. Secondary enterocolitis and mesenteric lymphadenitis are other common manifestations. Less frequent forms of presentation include synovitis, uveitis, osteomyelitis and septic arthritis. The lung infection is contracted through inhalation of contaminated soil dust or the breath of infected animals. *R. equi* can also multiply in the intestine, contributing to its dissemination via an oral-faecal cycle that enriches the farm environment with virulent (plasmid-bearing) strains. Transmission is more likely to occur in conditions that favour aerosolisation of contaminated soil particles, i.e. dry weather and crowded paddocks. The organism becomes endemic on stud farms, where it represents a real challenge as there is no effective vaccine available. This is compounded by the intrinsic resistance of *R. equi* to many antibiotics (e.g. penicillins, cephalosporins, sulfamides, quinolones, tetracyclines, clindamycin, and chloramphenicol) and the recent emergence of acquired resistance to currently used drugs. The intracellular localization of the pathogen complicates its therapeutic management, making it necessary to administer lengthy treatments, of up to three months or more, with no guaranteed success [1,2,3].

Due to its high fatality rate, the lack of effective early diagnosis and preventative measures, and the costs of the prolonged antibiotic treatments (often administered prophylactically in endemic studs), *R. equi* has a major economic impact and is recognized as one of the most important infectious problems that afflict the equine industry worldwide.

### **A multihost pathogenic actinomycete**

*R. equi* research has been traditionally "equinocentric" and the appreciation of the organism as a multihost bacterial pathogen has been largely neglected. *R. equi* is often isolated from submaxillary pyogranulomatous adenitis in pigs and TB-like abscesses in retropharyngeal and pulmonary lymph nodes in cattle [2]. Data from abattoir surveys in Ireland indicate that up to 4% of suspected bovine TB cases may in fact be *R. equi* infections [4]. Since the emergence of the AIDS pandemic, *R. equi* has also gained prominence as a human opportunistic pathogen. Human cases are generally associated with immunosuppressive conditions, and in HIV patients they usually present as TB-like purulent cavitary pneumonia with a high mortality (50-55%) [5]. *R. equi* infections have been reported in a variety of other animal species, including dogs and cats [6].

The number of reports of non-equine infections is on the rise, probably due to increased awareness about this pathogen and the application of improved detection techniques. However, *R. equi* is still frequently misidentified in the laboratory. A careful differential diagnosis must always be carried out in any suspected case of mycobacterial (TB) infection to exclude *R. equi*.

### **Pathogenesis and molecular virulence determinants - before the genome**

*R. equi* is a facultative intracellular parasite of macrophages that replicates within a modified endosomal compartment, the *R. equi*-containing vacuole (RCV). *R. equi* virulence depends on the

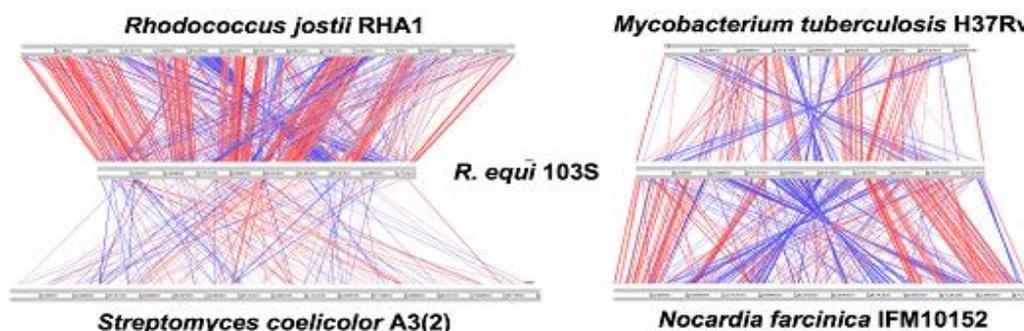


presence of a circular plasmid of 80 to 90 kb. This plasmid confers the property of arresting RCV acidification and maturation and its loss results in an inability to cause disease in foals and to replicate in macrophages *in vitro* and in mouse tissues *in vivo*. A surface lipoprotein antigen, VapA encoded in the plasmid, is a key mediator of these effects [2,3,7]. The *vapA* gene is located in a 21-Kb pathogenicity island (PAI) together with several other *vap* genes [8]. Recent findings from our laboratory indicate that specific virulence plasmid types are associated with specific animal hosts (horse, cattle, pigs). The host-associated plasmids differ in *vap* gene complement within the PAI, suggesting an involvement of the Vap proteins in the determination of *R. equi* host tropism [9]. This unique plasmid-determined host specificity constitutes a new paradigm in bacterial pathogenicity. The precise role and mechanism of the Vap proteins remain unknown.

Chromosomal factors are also likely to participate in *R. equi* pathogenesis. The *R. equi* glyoxylate shunt enzyme isocitrate lyase is required for full virulence in macrophages and mice, consistent with lipids being a major carbon source for the organism *in vivo*. Cell wall mycolic acid-containing glycolipids may promote survival within phagocytes and granuloma formation. Mannose-capped lipoarabinomannans may inhibit phagosome maturation or trigger the release of interferon- $\gamma$  and IL-12 from infected cells, as shown in *Mycobacterium tuberculosis* (Mtb). Other possible chromosomal factors include cholesterol oxidase [45, 46], the capsular polysaccharide, iron uptake systems [43, 44], and homologues of the HtrA chaperone, NarG nitrate reductase and PepD peptidase [47]. However, their roles in virulence, if any, remain largely speculative [2,3].

### The *R. equi* genome

To gain insight into the biology and virulence mechanisms of *R. equi*, in 2004 we determined with the Sanger Institute and an international collaborative consortium the complete genome sequence of a prototypic foal isolate, strain 103S (accessible at NCBI [http://www.ncbi.nlm.nih.gov/nuccore/NC\\_014659](http://www.ncbi.nlm.nih.gov/nuccore/NC_014659)). An overview of the genome was presented at a Havemeyer Foundation-sponsored international workshop we organised in 2008 in Edinburgh [10], and a paper with a detailed analysis has been recently published [11] (<http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1001145>). *R. equi* 103S has a genome of just above 5 millions base pairs, with a circular chromosome of 5,043 Kb and a virulence plasmid of 80.6 Kb. The G+C content is 68.8%. Whole genome comparisons showed it is highly similar to that of the soil-restricted versatile biodegrader *Rhodococcus jostii* RHA1 (9.7 Mb) [50] and of two recently sequenced environmental rhodococci, *Rhodococcus erythropolis* PR4 (6.89 Mb) and *Rhodococcus opacus* B4 (8.17 Mb) (<http://www.nite.go.jp/index-e.html>); it is, however, significantly smaller in size, due to genome expansion in environmental rhodococci, not reductive evolution in *R. equi*. Next in overall genome similarity was *Nocardia farcinica* IFM 10152 followed by Mtb (Fig. 1).



**Fig. 1.** Pairwise chromosome alignments of *R. equi* and selected *Actinobacteria*. Red and blue lines connect homologous regions (tBLASTx) in direct and reverse orientation, respectively

### Niche adaptive features

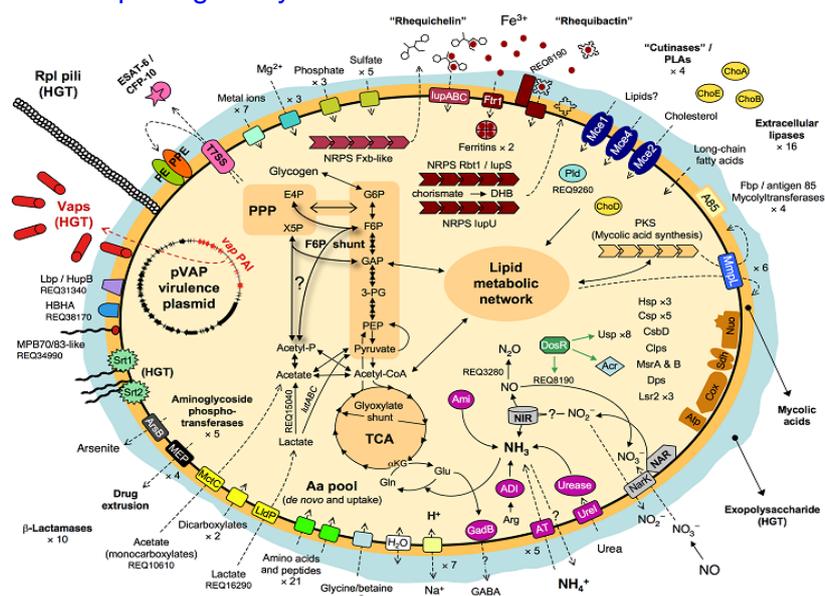
The 103S genome possesses a large complement of genes involved in lipid metabolism but lacks sugar transporters and PTS components, consistent with the inability of *R. equi* to use carbohydrates. *R. equi* is capable of synthesizing all amino acids from inorganic nitrogen and has the potential for anaerobic respiration via denitrification and nitrogen assimilation from nitrate/nitrite (Fig. 2). This metabolic profile probably represents a specialization vis-à-vis two key *R. equi*



habitats: the volatile fatty acid-dominated environment of herbivores' distal intestine and feces; and the intramacrophage and granulome environments, presumably rich in membrane-derived lipids and poor in amino acids, sugars and oxygen. The inability to use sugars is unique among related actinomycetes and possibly confers a competitive advantage against carbohydrate-fermenting intestinal microbiota (which generate large quantities of short-chain fatty acids, a primary carbon source for *R. equi*). It lacks the extensive metabolic network and catabolic abilities of environmental rhodococci, as well as the extensive secondary metabolism found in many other *Actinobacteria*. In contrast, it has a larger than average secretome and many surface proteins (406 or 8.9% of genes) and regulators (464, 10.26% of genes), consistent with its dual lifestyle as soil saprotroph and intracellular parasite. *R. equi* is also well endowed to survive desiccation, important for its dustborne transmission during hot, dry weather. As typical in soil bacteria, the 103S genome is heavily shielded with an array of antibiotic resistance determinants, including 10  $\beta$ -lactamases, five aminoglycoside phosphotransferases and four multidrug efflux pumps. This illustrates how naturally selected resistance traits may have a significant impact on the clinical management of bacterial infections [11].

### Cooptive virulence

The *R. equi* genome has provided important clues to understand how virulence was shaped in this pathogen and in the actinomycetes. Our findings suggest a mode of virulence evolution in which a few decisive niche (host)-adaptive HGT events in a direct ancestor of *R. equi*, such as the acquisition of the plasmid *vap* "intramacrophage survival" PAI, triggered the rapid conversion of a "preparasitic" commensal organism into a pathogen via the appropriation or cooption of pre-existing bacterial functions. Gene cooption is a key evolutionary mechanism by which traits that evolved for one purpose serve new functions in different circumstances, thus allowing rapid adaptive changes. A way in which gene cooption operates is through critical modifications in the expression of the appropriated genes to adapt their function to the new needs. Whole-genome microarray transcription profiling and expression network analysis were used to identify new *R. equi* virulence genes by their coregulation with known pathogenicity determinants from the virulence plasmid, and we found that a number of chromosomal metabolic genes were placed under the regulatory influence of the *vap* PAI. Two of the *vap* PAI-coregulated genes encoded chorismate mutase and anthranilate synthase enzymes involved in aromatic amino-acid biosynthesis. Gene deletion and complementation analysis demonstrated that these two enzymes were required for full intramacrophage proliferation capacity, providing experimental support to our HGT-driven cooptive virulence model [11]. Recognizing the importance of gene cooption in bacterial virulence provides a rational framework for understanding and interpreting the emergence and evolution of microbial pathogenicity.



**Fig. 2.** Schematic overview of relevant metabolic and virulence-related features of *R. equi* 103S. See ref. [11] for details.



With the sequencing and analysis of the 103S genome, a first key milestone towards achieving a full understanding of *R. equi* biology and pathogenesis has been achieved. New virulence determinants have been identified (**Fig. 2**), among which a cytoadhesive pilus essential for lung colonization in mice (manuscript in preparation). We are now developing the functional analysis of the *R. equi* genome to decipher the intimate mechanisms used by the pathogen to cause infection and identify novel vaccine targets. Work is in progress in our laboratory on a promising vaccine candidate and a serological diagnostic marker identified through our work with the genome.

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## **Toxic and Parasitic Disease Report for the Fourth Quarter 2010**

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 fourth quarter**

	<b>Number of Samples Tested</b>	<b>Number Positive</b>	<b>Number of Contributing Laboratories</b>
Grass Sickness	17	4	2
Hepatic toxicoses	21	6	4
Atypical myopathy	1	0	1
Tetanus	0	0	0

**2010**

**Table 4: Diagnostic parasitology sample throughput and positive results for the fourth quarter 2010**

	<b>Number of Samples Tested</b>	<b>Number Positive</b>	<b>Number of Contributing Laboratories</b>
<b><u>Endoparasites</u></b>			
Ascarids	1537	36	16
Cyathostomes	1297	362	13
Dictyocaulus	347	6	9
Strongyles	2423	717	21
Tapeworms (ELISA based testing)	19	17	4
Tapeworms (Faecal exam)	969	4	10
Trichostrongylus	48	1	1
Strongyloides	1320	279	10
<i>Oxyuris equi</i>	365	8	6
Fasciola	112	10	2
Coccidia	259	1	4
Cryptosporidia	11	0	2
VLA <i>Theileria equi</i> (CFT)*	412	5	1
VLA <i>Theileria equi</i> (IFAT)**	1028	46	1
VLA <i>Theileria equi</i> (cELISA)***	487	8	1
VLA <i>Babesia caballi</i> (CFT)*	412	0	1
VLA <i>Babesia caballi</i> (IFAT)**	1025	29	1
VLA <i>Babesia caballi</i> (cELISA)***	487	1	1
<b><u>Ectoparasites</u></b>			
Mites	21	1	1
Lice	361	1	13
Ringworm	581	138	20
Dermatophilus	301	27	15
Candida	60	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

**Grass sickness surveillance data ([www.equinegrasssickness.co.uk](http://www.equinegrasssickness.co.uk)):**

A total of ten equine grass sickness (EGS) cases have been reported for the fourth quarter (October to December 2010), making a total of eighty-six reports in 2010. Six cases occurred in England, one case occurred in Scotland, and the location of three cases was not reported. Of the affected horses, 70% were geldings (n=7), 20% were mares (n=2), and 10% were stallions (n=1). The median age of affected horses was 5.6 years (range 7 months – 11 years), and mean age was 5.9 years. The majority of horses (n=7, 70%) were purebreds, of which five were cobs, two horses were crossbreds, and for one case breed was not reported.

Of the ten cases reported, seven were acute cases (70%), two were subacute cases (20%) and one was a chronic case (10%). Information regarding outcome was available for seven cases, with no horses reported to survive. Five horses were euthanased (two acute, two subacute and one chronic case) and two cases of acute EGS died naturally. Diagnostic information was not available for two cases, and where reported, four cases (50%) were diagnosed based on clinical signs alone. One case was diagnosed by post-mortem examination and two further cases (20%) underwent surgery, one of which was diagnosed by biopsy examination and the other at post-mortem examination following surgery.

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.4 No.2 and Vol.2 No.4.



## **Report on Post-mortem Examinations for the Fourth Quarter 2010**

### **East Anglia**

*A total of 88 cases were examined including 67 aborted fetuses.*

Of the aborted fetuses examined this quarter, umbilical cord torsion was suspected as the precipitating cause in 32 of 67 cases. A premature placental separation was found to be the cause of two abortions, whereas placental insufficiency was the underlying cause in eight cases of abortion. Post-mortem examination and histology revealed placental atrophy and degeneration in four cases of abortion. Two abortions were found to be due to illness in the mare (septic foot in one case and three surgeries requiring general anaesthesia in the second case). Placentitis was found to be the cause of three abortions whereas EHV-1 was confirmed by virus isolation and PCR in placenta and fetal tissues in two abortions. No definitive cause was determined for fourteen cases of abortion, however infectious agents were excluded.

There were two cases of neonatal death reported in this quarter. The first case was due to septicaemia whereas the second case presented a congenital malformation with associated intestinal herniation.

Following neurological disease a mare was euthanased and EHV-1 was confirmed by PCR and virus isolation in spinal cord samples.

Ten horses were examined following gastrointestinal disease, causes of death were as follows: two cases of gastric rupture (one of them associated to equine dysautonomia), a case of right dorsal colitis and peritonitis, a case of colonic torsion, a rectal tear, septicaemia secondary to intestinal infection in a 6 months-old colt, a case of toxic shock following colic, a case of severe, diffuse, eosinophilic and granulomatous enteritis and finally a case of Equine Grass Sickness in a 2 year-old Thoroughbred filly. The cause of death in the tenth case could not be determined.

Following respiratory signs a horse was euthanased and diagnosed with a maxillary sinus neoplasia.

The two musculoskeletal cases reported in this quarter include a case of skull fracture leading to subdural and submeningeal haemorrhage and a case of severely malformed left hind limb joints.

Other cases reported include three welfare/neglect cases (two of them presented emaciation and cyathostomosis whereas the third presented emaciation and radial paralysis following an orthopaedic surgery), a case of severe haemoabdomen secondary to uterine artery rupture and a case of brain swelling following a general anaesthesia with a possible diagnosis of post anaesthetic cerebral necrosis.

### **Home Counties**

*Twenty-one cases were examined in this quarter.*

One aborted fetus was examined in this quarter, and even though the precipitating cause could not be determined, EHV was excluded.



One neurological case that was euthanased was reported in this quarter; the cause of disease was could not be determined.

The twelve cases of gastrointestinal disease reported include seven cases of colic (within these a case of colic surgery, euthanased due to bad prognosis, a case of recurrent colic, a case of a colic with poor prognosis, a case of colic and strangulation, a case with an associated peritonitis and a case of rupture), a case of fibrino-haemorrhagic enteritis, a case of malabsorption that was diagnosed with a lympho-plasmacytic, eosinophilic colitis, a case of a strangulating lipoma, a case of equine dysautonomia and finally a horse which post-mortem examination revealed a pedunculated lipoma with associated ischaemic necrosis of the distal jejunum and ileum.

Respiratory cases include a horse that presented lung emphysema and was euthanased, and a case of airway surgery complication.

There was only one case of neoplasia reported in this quarter; a horse with a disseminated lymphoid neoplasia.

The only musculoskeletal case reported was a horse that presented a comminuted fracture in the tibia and was subsequently euthanased.

Two neglect cases were reported: the first one presented an intraabdominal haemorrhage whereas the second one presented a severe parasitosis.

The cause of death for the last case reported in this quarter was not determined.

### **South West**

*Eleven cases were examined in this quarter.*

Three cases of abortion was reported in this quarter, the cause for one of them was placental insufficiency whereas the cause for the remaining two abortions could not be determined; however, infectious agents were excluded

A neurological case was reported in this quarter. The cause of death was determined to be acute liver failure, necrosis and hepatic encephalopathy with a likely toxic cause.

Five cases were examined following gastrointestinal disease. In the first case duodenal perforation with associated peritonitis was found to be the cause of death. The second case showed a jejuno-ileocaecal intussusception and cyathostominosis; the third case presented a displacement of large colon with extensive colonic mucosal oedema and cyathostominosis. The fourth case had a gastric impaction followed by gastric rupture and intestinal parasitism. This case also presented diaphragmatic rupture but was probably post-mortem. Finally, the fifth case presented a severe ecto and endoparasitism with extensive subcutaneous oedema.

A case of musculoskeletal disease was reported; post-mortem examination revealed a chronic subluxated left fore fetlock in a donkey.



Following a sudden death in a horse, post mortem examination revealed haemorrhages in the thorax, peripancreatic tissue and ventricular endocardium. A coagulopathy was suggested as a possible underlying cause.

### **Northern England**

*Thirteen cases were examined in this quarter.*

Seven gastrointestinal cases were reported in this quarter; the causes of death were as follows: two caecal ruptures, an ileocaecocolic intussusception, a strangulating lipoma, a large colon rupture and a typhlocolitis caused by *Clostridium difficile* and that was a result of antibiotic treatment due to a wound on upper right forelimb. The cause of death for the seventh case (colic) was not determined.

Following post-mortem examination and histology a horse was diagnosed with a plasma cell myeloma.

A single musculoskeletal case was reported and it was diagnosed of pedal osteitis with distal interphalangeal joint involvement.

Ragwort toxicity was reported to be the cause of death in a case following post-mortem examination and histology.

Three neglect cases were reported in this quarter. These cases presented diarrhoea and they were diagnosed with Cyathostomosis.

### **West Midlands**

*One case was examined in this quarter.*

Post-mortem examination in one case following a history of sudden death overnight revealed a 360° twist of small intestine with displacement of large intestine.

### **Scotland**

*Seven post-mortem examinations were reported in this quarter.*

Three gastrointestinal cases were reported in this quarter, including a case of strangulating lipoma of the terminal colon, a case of ulcerative colitis with concurrent pneumonia and a case of gastric rupture.

Only one neoplasia case (disseminated lymphosarcoma) was reported in this quarter.

Musculoskeletal cases reported include an angular limb deformity in a foal and a suspensory ligament desmitis.

The last case reported was diagnosed with a spinal haemorrhage that was related to trauma.



## **Northern Ireland**

*Eight post-mortem examinations were examined in this quarter.*

An 11-year-old gelding with a history of colic was diagnosed with caecal entrapment and associated oedema and engorgement of the caecal wall.

Four cases of enteritis were examined during this quarter. A recently-wormed six-year-old male donkey had a small number of fluke eggs present in the faeces and mild hepatic portal fibrosis with cholestasis. The intestines were autolytic and unsuitable for further examination. Cyathostomes were associated with enteritis in a three-year-old female donkey, a yearling colt and a two-year-old filly. The filly also had ventral oedema and degenerative myopathy associated with a low liver vitamin E level.

A pregnant adult female donkey was euthanized and submitted for examination. There were multiple intramural nodules containing nematodes (*Strongylus vulgaris*) at the root of the aorta, in the descending aorta and in the mesenteric artery. There were also multiple myocardial and renal infarcts.

A ten-year-old mare was diagnosed with multiple hepatic hydatid cysts, hepatic and pulmonary abscessation and a subcutaneous abscess on the side of the head.

An emaciated seven-year-old male horse was submitted for examination. The contents of the large intestine were coarse and fibrous and while there was a “seven year hook” no other dental or alimentary tract abnormalities were detected. The bladder was distended and there was a large volume of fine gritty material in the urine.



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**We would welcome feedback including contributions on focus articles  
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