

**DIAGNOSTIC TESTS FOR STRANGLES**

- For detection of chronic carriers
  - Guttural pouch lavage is the only dependable test
- For detection of acute cases/infected in-contacts
  - Nasopharyngeal washes are the most sensitive test
- Nasal or Nasopharyngeal swabs are highly unreliable for detecting chronic or acute cases
- Serologic tests must be interpreted with caution, especially when screening for chronic carriers

Diagnostic samples might be used in 2 main scenarios. Firstly, to help ascertain whether or not a horse is a chronic strangles carrier; and secondly, whether or not it has been infected with strangles recently. The best choice of appropriate tests for strangles differs between these 2 circumstances.

Understanding the role of the guttural pouches in strangles infection is crucial to selection of the right tests. In some (but not all) cases of acute strangles infection, the retropharyngeal lymph nodes (situated beneath the guttural pouches) may abscessate and rupture into the guttural pouches through the floor of the medial compartment(s). In some of these cases the infection may clear, whereas in others it may persist for weeks to years resulting in chronic carriage. Thus essentially *all chronic carriers*, but only *some acute cases*, will have guttural pouch infection.

*Guttural pouch empyema*
IS THE HORSE A CHRONIC CARRIER?

Cases of strangles should generally become free from infection between 6-10 weeks following initial exposure to strangles. Infection persisting longer than this (i.e. 6 weeks to lifelong) are regarded as chronic carriers, many of which show no clinical signs of such.

For selection of appropriate tests, it is important to remember that when *Strep equi* persists in a horse for the medium to long term (>6 weeks), it does so almost invariably within the guttural pouches. Therefore, any test which does not directly sample from the guttural pouches carries a significant risk that infected horses will not be detected (even when repeated several times).

**Guttural Pouch Lavage**

Unlike all alternative tests, when PCR, culture and cytology are performed on bilateral guttural pouch lavages, it is highly unlikely that a strangles carrier will be missed. The technique requires an endoscope but is remarkably easy to perform as long as the details of the procedure are followed exactly as below.

**Equipment needed:**
- Endoscope
- Endoscopic guidewire
- 2 x endoscopic catheters
- 2 x 30 mL syringes of sterile saline

**Technique**
- Sedate horse
- Insert endoscope via *ventral meatus* (push scope ventrally during insertion)
- Approach ipsilateral guttural pouch ostium and insert the guidewire through the dorsal aspect of the ostium and into the guttural pouch
- Orientate the endoscope so that the guidewire is on the pharyngeal luminal side of the endoscope, thus opening the ostium
- Pass the endoscope forwards into the guttural pouch as the wire is slowly withdrawn
- Examine the pouch carefully paying particular attention to the floor of the medial compartment (which overlies the retropharyngeal lymph nodes)
- Remove the guidewire and pass a plastic endoscopic catheter
- Hold the horse’s head reasonably elevated and inject 20-30 mL sterile saline into the pouch
- Aspirate the fluid from the pool that collects in the medial compartment
- Divide the sample between a plain universal container (PCR & culture) and EDTA tube (cytology)
DIAGNOSTIC TESTS FOR STRANGLES

Nasal/nasopharyngeal Swab

Swabs collected from the nasopharynx are fundamentally compromised by the fact that they do not sample from the infected site (the guttural pouches). Numerous instances are seen where repeated negative nasopharyngeal swabs are collected from horses with guttural pouch infections. Therefore although successful isolation of Strep equi from nasopharyngeal swabs is very meaningful, negative nasopharyngeal swabs, even when repeated several times, do not rule out the possibility of Strep equi infection and should not be relied upon to establish freedom from infection.

Serologic testing (antigens A/C)

There are three S. equi antigens that have been targeted in serologic tests comprising the full S. equi M protein (SeM), the N-terminal of SEQ2190 (“Antigen A”) and the N-terminal of SeM (Antigen C). A recent study demonstrated a high rate of false positive results with the SeM ELISA and indicated far better diagnostic usefulness of the combined Antigen A and C assay.

Published evidence indicates that 93% of horses “involved in outbreaks of strangles” will have values ≥0.5 for antigens A and/or C. However, questions have arisen regarding the sensitivity of the test in chronic carriers. Thus a positive result strongly suggests prior exposure to Strep equi and the need for follow up guttural pouch lavage to establish whether infection is historical or current. However, a negative result should not be used to rule out the possibility of chronic guttural pouch carriage. Additionally, the use of a 0.5 cutoff is questionable with evidence suggesting 0.3 would be more appropriate figure.

IS THE HORSE AN ACUTE STRANGLES CASE/AN INFECTED IN-CONTACT?

By no means all acutely infected strangles cases have guttural pouch infection (caused by rupture of retropharyngeal lymph nodes into the guttural pouches). Therefore guttural pouch lavage is not the best test for these.

Nasopharyngeal Lavage

The advantage of this technique is that it collects bacteria from a large surface area of the nasopharyngeal mucosa than other methods such as nasal or nasopharyngeal swabbing, and therefore has a significantly higher diagnostic rate.

Equipment needed:
- Either an endoscope or a 50 cm sterile tube or dog urinary catheter
- 50-60 mL warm sterile saline
- Sterile universal container

Technique:
- Sedate horse
- Insert tube/catheter/endoscope into nasopharynx (typically about 40 cm)
- Ensure head hangs down and squirt in sterile saline
- Collect fluid as it exits nostrils into the sterile universal container
**DIAGNOSTIC TESTS FOR STRANGLERS**

**Direct swabbing/aspiration from abscesses**

Although one would imagine that direct sampling from an abscess should be a highly reliable sample, evidence indicates as few as 20% of samples collected from strangles abscesses are culture positive and perhaps only 80% PCR positive.

**Serologic testing (antigens A/C)**

There are three *S. equi* antigens that have been targeted in serologic tests comprising the full *S. equi* M protein (SeM), the N-terminal of SEQ2190 (“Antigen A”) and the N-terminal of SeM (Antigen C). A recent study demonstrated a high rate of false positive results with the SeM ELISA and indicated far better diagnostic usefulness of the combined Antigen A and C assay.

Published evidence indicates that 93% of horses “involved in outbreaks of strangles” will have values ≥0.5 for antigens A and/or C. However, exactly how long it takes for horses to seroconvert post infection is not known but it is advisable that at least 2-3 weeks have passed since the last time point of possible exposure before judging a horse not to have been exposed in an outbreak. Additionally, the use of a 0.5 cutoff is questionable with evidence suggesting 0.3 would be more appropriate figure. Where positive results are obtained these indicate exposure and not necessarily active infection. Further bacteriologic tests (as above) are then required to determine current versus historic infection.

<table>
<thead>
<tr>
<th>Test result</th>
<th>Suggested advice/response</th>
</tr>
</thead>
<tbody>
<tr>
<td>BLOOD TEST (ANTIGEN A OR C SEROLOGY)</td>
<td></td>
</tr>
<tr>
<td>Positive blood test (A or C &gt; 0.3)</td>
<td>Current infection is possible but requires guttural pouch lavage (chronic cases) or nasopharyngeal washes (acute cases) to rule out or confirm</td>
</tr>
<tr>
<td>Negative blood test in acute cases</td>
<td>Probably not become infected in last 2 weeks but a further test in 2-3 weeks is required.</td>
</tr>
<tr>
<td>Negative blood test in possible chronic case</td>
<td>Interpret very cautiously as chronic carriers are seen without seroconversion. Consider guttural pouch lavage.</td>
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</table>
# DIAGNOSTIC TESTS FOR STRANGLES

<table>
<thead>
<tr>
<th>Test result</th>
<th>Suggested advice/response</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Culture</strong></td>
<td></td>
</tr>
<tr>
<td>Positive nasal swab (suspect acute cases)</td>
<td>Confirms active <em>S. equi</em> infection</td>
</tr>
<tr>
<td>Negative nasal swab (suspect acute cases)</td>
<td><em>S. equi</em> infection still perfectly possible (many acute cases test negative)</td>
</tr>
<tr>
<td>Positive abscess swab (suspect acute cases)</td>
<td>Confirms active <em>S. equi</em> infection</td>
</tr>
<tr>
<td>Negative abscess swab (suspect acute cases)</td>
<td><em>S. equi</em> infection still perfectly possible (many acute cases test negative)</td>
</tr>
<tr>
<td>Positive nasopharyngeal swab (suspect carriers)</td>
<td>Confirms active <em>S. equi</em> infection</td>
</tr>
<tr>
<td>Negative nasopharyngeal swab (suspect carriers)</td>
<td><em>S. equi</em> infection still perfectly possible (many confirmed carriers test negative repeatedly)</td>
</tr>
<tr>
<td>Positive guttural pouch lavage (suspect carriers)</td>
<td>Confirms active <em>S. equi</em> infection</td>
</tr>
<tr>
<td>Negative guttural pouch lavage (suspect carriers)</td>
<td><em>S. equi</em> infection still possible (approx. 40% of confirmed carriers test negative)</td>
</tr>
<tr>
<td><strong>PCR</strong></td>
<td></td>
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<tr>
<td>Positive nasopharyngeal swab</td>
<td>Confirms presence of <em>S. equi</em> and probable active infection</td>
</tr>
<tr>
<td>Negative nasopharyngeal swab</td>
<td><em>S. equi</em> infection still perfectly possible (many confirmed carriers test negative repeatedly)</td>
</tr>
<tr>
<td>Positive guttural pouch lavage</td>
<td>Confirms presence of <em>S. equi</em> and probable active infection</td>
</tr>
<tr>
<td>Negative guttural pouch lavage</td>
<td><em>S. equi</em> infection unlikely</td>
</tr>
<tr>
<td><strong>Cytology</strong></td>
<td></td>
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<tr>
<td>Inflammatory guttural pouch lavage</td>
<td>Infection of pouch likely which may be caused by <em>S. equi</em></td>
</tr>
<tr>
<td>Non-inflammatory guttural pouch lavage</td>
<td><em>S. equi</em> infection unlikely but possible</td>
</tr>
</tbody>
</table>
EQUINE HERPESVIRUSES

Of the currently recognised herpesviruses in horses. EHV1, EHV3 and EHV4 are alpha-herpesviruses and are of the greatest clinical importance. The gamma-herpesviruses EHV2 and EHV5 may be associated with ocular and respiratory infections in horses although their importance is relatively minor.

ALPHA HERPESVIRUSES

EHV 1 and 4

EHV-1 infection is widespread and it should be expected that all horses will be at risk of infection resulting in subclinical infection or respiratory disease, and less frequently abortion, neonatal mortality and myeloencephalopathy (risk dependent on specific strains). Most horses are inevitably exposed to EHV1 early in life and long-term latent infection is then established with the possibility of recrudescence and shedding at times of stress (e.g. foaling, sales, mixing, training, transport...). The prevalence of latent EHV1 infection in horse populations has been estimated at between 60 and 90% but may well be higher as more sensitive detection techniques are applied.

EHV4 is a common cause of relatively mild respiratory disease and also a rare cause of abortion in mares. Neurologic disease associated with EHV4, if it exists, is apparently very rare indeed. EHV4 is endemic worldwide and antibodies can be detected in almost 100% of horses.

Diagnosis

- **Serology** (red top tubes)
  - Paired samples 2-3 weeks apart looking for around a 4 x increase in titre (complement fixation test). Serologic tests don’t generally distinguish between antibodies to EHV1 and EHV4 although an ELISA targeting the C-terminal portion of glycoprotein G of both viruses is available for distinguishing serologic response to each virus. Both the CF and ELISA tests only reliably detect recent infections (<2-3 months previously). Antibody titres in CSF are not helpful due to leakage of serum from damaged blood vessels into the CSF.

- **Virus identification** (nasal/nasopharyngeal swabs, buffy coat (green or purple top tubes), foetus/placenta)
  - Virus isolation performed on nasal/nasopharyngeal swabs or buffy coat samples in heparinised blood but takes several days. Gauze swabs and viral transport media are required. It is advisable during an outbreak that in-contacts are also sampled (especially with signs of pyrexia) to increase the diagnostic rate.
  - PCR allows for rapid identification of the presence of EHV1 infection. It can be performed on:
    - nasal/nasopharyngeal swabs in viral transport media. If viral transport medium is not available, then a dry swab in a sterile tube can be submitted.
    - buffy coat samples from heparinised or EDTA blood (check with lab).
    - aborted foetuses (or at least the foetal liver, lung, spleen, adrenal gland and thymus) as well as placenta and, where possible, endometrial biopsies.
    - CSF is rarely helpful due to such a low viral content.

- **Histopathology**
  - Post mortem examination of foetuses, placentae, endometrial biopsies, brain and spinal cord helps confirm EHV infection.
EQUINE HERPESVIRUSES

**EHV 3**

This the cause of coital exanthema, a contagious venereal pustular/vesicular dermatitis affecting the penis and vulva occasionally seen in breeding animals. Transmission might also occur from insects and equipment. Signs appear about a week after infection and the clinical disease lasts for 2-3 weeks. Depigmented spots may be left after infection has cleared.

**Diagnosis**

- **Serology** (red top tubes)
  - Paired samples 2-3 weeks apart looking for around a 4 x increase in titre (virus neutralisation test)
- **Virus identification** (swabs from lesions)
  - Virus isolation is the usual method
  - PCR is possible but not currently commercially available

**GAMMA HERPESVIRUSES**

**EHV 2**

Conjunctivitis or keratitis might be seen associated with EHV2 infection. PCR can be performed on conjunctival/corneal scrapes or swabs.

**EHV 5**

Interest has been growing recently in the apparent association between EHV5 and the progressive inflammatory interstitial lung disease known as equine multinodular pulmonary fibrosis (EMPF). Bronchoalveolar lavage samples from many such cases have been found to be positive for EHV5 by PCR.
EQUINE VIRAL ARTERITIS

The disease has been notifiable under certain specific circumstances under the Equine Viral Arteritis Order 1995. Under the Order, anyone who owns, manages, inspects or examines a horse must notify the Animal and Plant Health Agency (Tel. 03000 200 301) when:

- they suspect the disease in a stallion, either on the basis of clinical signs or following blood or semen testing;
- they suspect disease, either on the basis of clinical signs or following blood testing, in a stallion or mare that has been mated or artificially inseminated within the past 14 days.

EVA is a common contagious respiratory disease worldwide especially in Standardbred and Warmblood populations. It is rarely encountered in the UK as a clinical disease although it is not unusual to encounter seropositive horses in the UK that have been exposed previously to the virus during time spent abroad. The virus is spread by respiratory and venereal routes.

Following infection of stallions (but not mares, geldings, sexually immature colts), around 30–50% become carriers for variable periods of time and are a key natural reservoir of the virus. All carrier stallions will be seropositive but not all seropositive stallions will be carriers.

Outbreaks typically arise following transmission during breeding a carrier stallion with a susceptible mare. Further dissemination to in-contacts via respiratory disease in the mare and aerosolised virus follows. Horses may shed virus for approximately two weeks from the respiratory tract and then recover with solid immunity (detected later as seropositivity). Only stallions carry the risk of longer term infection. Importantly, semen remains infectious after chilling or freezing.

**SIGNS**

The majority of EVA infections are subclinical and unrecognised. Clinical EVA infection is associated with a variable incubation period of between 2-14 days. It results in widespread vasculitis and may manifest as any or all of the following:

- pyrexia, anorexia and depression
- respiratory signs
- nasal discharge
- conjunctivitis, epiphora and photophobia ("pink-eye")
- oedema of the legs, scrotal, preputial, mammary and periorbital areas
- general urticarial rash
- abortion in mares (single cases or outbreaks)
- fatal neonatal pneumonia/enteritis
- oral ulcers

Respiratory disease within a week or two of breeding (natural breeding or chilled or frozen AI) is a big alarm for EVA. Abortion accompanied by respiratory signs is also indicative. Conjunctivitis is also a sign not otherwise seen in many respiratory cases.
EQUINE VIRAL ARTERITIS

DIAGNOSIS

- **Serology** (virus neutralization (VN), ELISA – red top tube)
  - A four-fold increase in antibody titres in serum samples collected two to four weeks apart offers evidence of active infection. The virus neutralisation (VN) test may be problematic in horses recently vaccinated against EHV1 and so the ELISA test may be preferable as a screening test.
  - Stable or declining seropositive titres in samples collected 2-3 weeks apart in geldings and mares is innocuous and simply reflects recovered infection in the past. However, seropositive unvaccinated stallions present further concerns as a proportion of these might be persistently infected carriers.

- **Virus identification** (Nasopharyngeal and conjunctival swabs (in viral transport medium), EDTA-blood samples, semen or placentae)
  This should be performed on any horses showing respiratory signs following breeding, and also in stallions found to be seropositive in the absence of a solid vaccination record:
  - Virus isolation (VI) test can be used to test stallion’s semen for international trade purposes.
  - PCR can be used on respiratory swabs, blood, semen and placentae. Semen testing must be carried out in a Defra laboratory. Two ejaculates are examined 7 days apart; if either is positive then the stallion is a shedder; if both are negative then test mating is recommended to ensure the stallion is clear.
  - Test mating is used for final confirmation for shedding status in a stallion (if negative by PCR). This must be done in strict isolation and under veterinary supervision. The following procedure should be followed:
    - Identify 2 seronegative mares;
    - Take and store blood samples from each and then isolate the mares;
    - Consult the testing laboratory about storage conditions;
    - Mate each mare twice a day with the stallion on 2 consecutive days;
    - Keep the mares in isolation;
    - After 28 days, take blood samples and send them, with the pre-isolation samples, to the laboratory.
  - If the mares remain seronegative, the stallion is unlikely to be a shedder and can be released after a clinical examination. If one or more mares become seropositive, the stallion is a shedder. He must be kept in isolation and not be used for breeding activities while he is shedding, unless permitted under an official licence issued by Defra.
  - Seropositive mares must remain in isolation until they have a stable or declining antibody level in two sequential blood tests taken at an interval of at least 14 days.

VACCINATION

Vaccination (Equip Artervac, Zoetis) is available for protection against EVA infection following documentation of seronegativity in blood samples. Following the initial primary course (2 doses 3-6 weeks apart) it is imperative that regular **6 monthly boosters** must be maintained or subsequently documented seropositivity may require expensive PCR and test mating to confirm that it is indeed vaccine-related.
CONTAGIOUS EQUINE METRITIS (CEM)

CEM may be caused by 3 particular bacteria:

- *Taylorella equigenitalis* (contagious equine metritis organism (CEMO))
- *Klebsiella pneumoniae* (capsule types 1, 2 and 5 only)
- *Pseudomonas aeruginosa* - although not all *P. aeruginosa* strains are venereal pathogens, it must be assumed that they are in the absence of any current laboratory methods to differentiate them.

In the UK, isolation of *T. equigenitalis* is notifiable under the Infectious Diseases of Horses Order 1987. *K. pneumoniae* and *P. aeruginosa* are not notifiable although it is advised that infection in stallions is reported to the national breeders' association.

**CLINICAL SIGNS**

Although infection in mares may cause a vulval discharge and early return to oestrus post-breeding, mares can also carry the infection (primarily clitoris, clitoral fossa and sinuses and sometimes urethra/bladder) without detectable clinical signs. Stallions generally carry infection silently on their penis, sheath and sometimes in the urethra and bladder although may rarely show clinical signs of purulent semen.

**HIGH RISK OR LOW RISK?**

Low risk mares and stallions are any not defined as high risk as below:

**High risk mares:**

- *T. equigenitalis, K. pneumoniae* capsule types 1, 2 or 5 or *P. aeruginosa* has been isolated.
- visited any premises on which *T. equigenitalis* has been isolated within the previous year;
- mated during the last breeding season with stallions resident outside France, Germany, Ireland, Italy and the UK
- been in countries other than France, Germany, Ireland, Italy and the UK within the last 12 months

**High risk stallions:**

- Stallions which have not previously been used for breeding
- *T. equigenitalis, K. pneumoniae* capsule types 1, 2 or 5 or *P. aeruginosa* has been isolated
- been at any premises on which *T. equigenitalis, K. pneumoniae* capsule types 1, 2 or 5 or *P. aeruginosa* has been isolated in the last year
- mated a mare which has not been swabbed negative in accordance with the Code of Practice.
SWABBING MARES

After 1st January, and before a mare is mated/teased/inseminated, negative swabs must be obtained as follows:

Mares at stud (pre-breeding)

Low Risk
- clitoral swab (aerobic and microaerophilic or PCR) collected at home or at the stud
- endometrial swab (aerobic) during oestrus while at the stud

High Risk
- clitoral swab (aerobic and microaerophilic or PCR) collected at home
- clitoral swab (aerobic and microaerophilic or PCR) collected at the stud
- endometrial swab (aerobic and microaerophilic or PCR) during oestrus while at the stud

Walking-in mares (pre-breeding) or mares for AI

Low Risk
- clitoral swab (aerobic and microaerophilic or PCR) collected at home or at the stud
- endometrial swab (aerobic) during oestrus at home or at the stud

High Risk
- 2 x clitoral swabs (aerobic and microaerophilic or PCR) ≥7 days apart collected at home
- endometrial swab (aerobic and microaerophilic or PCR) during oestrus while at home

If the mare does not conceive and returns to oestrus, she should be swabbed again before being re-mated according to the protocol below.

Mares at stud (repeat matings)

Low risk
- endometrial swab (aerobic) during oestrus while at the stud

High Risk
- endometrial swab (aerobic and microaerophilic or PCR) during oestrus while at the stud
CONTAGIOUS EQUINE METRITIS (CEM)

Walking-in mares (pre-breeding) or mares for AI

Low Risk
- endometrial swab (aerobic) during oestrus at home

High Risk
- endometrial swab (aerobic and microaerophilic or PCR) during oestrus while at home

If any mare returns to oestrus at an unusual (especially shorter than normal) time, repeat clitoral and endometrial swabs should be taken (aerobic and microaerophilic or PCR).

If any mare changes premises, or stallions, repeat clitoral and endometrial swabs should be taken at least seven days after mating by the original stallion (aerobic and microaerophilic or PCR).

SWABBING STALLIONS

After 1st January and before a stallion is used for mating/teasing/semen collection, 2 sets of negative swabs taken ≥7 days apart must be obtained from the urethra, urethral fossa and penile sheath, plus pre-ejaculatory fluid when possible (cultured aerobically and microaerophilically or PCR).

'High risk' stallions and stallions standing on a stud for the first time warrant additional precautions during the breeding season. The first four mares mated with them should be screened for *T. equigenitalis*, *K. pneumoniae* capsule types 1, 2 and 5 and *P. aeruginosa* by taking a clitoral swab two days after mating. If the mare subsequently returns to oestrus, an endometrial swab should be taken at that time. These swabs should always be tested aerobically and microaerophilically or PCR. In addition, mid-season swabbing should be considered for all stallions and teasers.

Submitting swabs

All swabs should be taken by a veterinary surgeon, who should:

- place swabs in Amies Charcoal Transport Medium (must be within the expiry date)
- label with
  - date and time collected
  - horse’s name
  - site of swabbing;
- submit them to a HBLB Registered Laboratory for testing (Liphook Equine Hospital is HBLB registered laboratory for both culture and PCR)
CONTAGIOUS EQUINE METRITIS (CEM)

- Swabs for microaerophilic culture for *T. equigenitalis* must arrive in the laboratory within 48 hours of them being taken (time constraints do not apply for PCR).
- Microaerophilic culture results (*T. equigenitalis*) will not be available for at least seven days and aerobic culture results (*K. pneumoniae, P. aeruginosa*) will not be available for 48 hours.
- PCR test results should be available within 24 hours of arrival at a laboratory (Monday-Friday).

**EXPORT**

Swabs taken for export must be sent to the APHA laboratory, Bury St Edmunds.
LYME DISEASE AND ANAPLASMOSIS

Lyme disease (or Borreliosis) and Anaplasmosis are recognised increasingly as causes of clinical disease in horses. Both organisms are transmitted by *Ixodes* ticks (that are common in many parts of the UK) and are obligate intracellular organisms that may infect multiple species. Some of the clinical signs are summarised below.

**LYME DISEASE**

Lyme disease is caused by the spirochete *Borrelia burgdorferi*. *B. burgdorferi* infects leucocytes and synovial lining cells and triggers an inflammatory (and potentially autoimmune) response. The persistence of the bacteria within both synovial structures and tendons in humans leads to the prolonged treatment required.

*B. burgdorferi* has been reported to cause neuroborreliosis leading to the clinical signs of ataxia, hyperaesthesia and mentation changes and this can be diagnosed based on CSF samples.

High rates of *Borrelia* seropositivity have been recorded in horses from many regions of the UK and it is likely that seropositivity for Anaplasma is similar. A study performed in 1994 indicated low levels (<7%) of seropositivity in Newmarket, Ireland, Yorkshire and Scotland but quite high levels (30-35%) in South Coast areas and East Anglia.

**ANAPLASMOSIS**

Anaplasmosis is caused by the Rickettsial organism *Anaplasma phagocytophilum* (formerly *Ehrlichia equi*). *Anaplasma phagocytophilum* infects neutrophils and eosinophils resulting in neutropaenia and anaemia. Clusters of intracellular organisms may be visible as blue-grey spoke-wheel inclusions during the initial phase of infection. The presence of visible intracellular organisms tends to correlate with the presence of pyrexia, which usually lasts for around 10 days after infection. The majority of acute infections will lead to a marked pyrexia.

<table>
<thead>
<tr>
<th>Lyme disease</th>
<th>Anaplasmosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild pyrexia</td>
<td>Pyrexia</td>
</tr>
<tr>
<td>Lethargy</td>
<td></td>
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<tr>
<td>Anorexia/Weight Loss</td>
<td>Anorexia/Weight loss</td>
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<tr>
<td>Stiffness/Lameness</td>
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<tr>
<td>Muscle Soreness</td>
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<tr>
<td>Synovial Effusions</td>
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<td>Laminitis</td>
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<td>Uveitis</td>
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<tr>
<td>Somnolence/Altered mentation</td>
<td>Somnolence/Altered mentation</td>
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<tr>
<td>Hyperaesthesia</td>
<td></td>
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<tr>
<td>Ataxia</td>
<td>Ataxia (collapse/recumbency)</td>
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<tr>
<td>Ventral oedema</td>
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</tr>
<tr>
<td>Anaemia</td>
<td>Petechiae/Ecchymotic haemorrhages</td>
</tr>
</tbody>
</table>

**DIAGNOSIS**

Definitive confirmation of Lyme disease or Anaplasmosis is problematic and currently diagnosis is based upon finding a positive antibody titre for the suspected organism in a horse with suspicious clinical signs in an area where the disease, or at least *Ixodes* ticks, are known to be endemic. This has several limitations however. Firstly, with standard test methods it may take up to 3 months following infection for horses to seroconvert – meaning that many early cases will be ‘negative’ on serology. Secondly, horses may become infected and seroconvert without showing any clinical signs – hence many healthy horses or horses with other conditions could be misdiagnosed with Lyme disease on the basis of serology. Thirdly, horses that are successfully treated may still remain seropositive for a very long time thereafter – complicating interpretation of successful resolution.
LYME DISEASE AND ANAPLASMOSIS

The ELISA method used at the LEH for detection of *Borrelia* targets antibodies against the *Borrelia* surface protein V1sE. In experimental infections, animals became seropositive to V1sE within 3-5 weeks of infection, well before clinical signs arose. Additionally, infected horses that were successfully treated showed waning antibody titres more rapidly than with other test methods (although this may still be a matter of months). Furthermore, the method was able to detect some seropositive cases that had been missed using standard Western Blot techniques. The diagnostic accuracy of the ELISA method used at the LEH for the identification of seroconversion to *Borrelia* has been evaluated in 3 studies with a sensitivity of 65% - 100% and a specificity of 95% - 100%.

We also now have a PCR assay targeting the OspA antigen of *Borrelia* confirming current infection.

The ELISA test used at the LEH to diagnose *Anaplasma* targets antibodies against peptides derived from the immunodominant p44 protein of *Anaplasma phagocytophilum*.

**TREATMENT**

Intensive treatment can include 7-10 days of oxytetracycline (5 mg/kg IV SID/BID) followed by either oral doxycycline (10 mg/kg PO BID) or oral minocycline (4mg/kg PO BID) for 1-2 months for Lyme disease. Anaplasmosis can be treated with a shorter course of either oxytetracycline, doxycycline or minocycline. While treating with these antibiotics renal values should be closely monitored. Ceftiofur (2-4 mg/kg IM BID) has also been recommended.

Total eradication of organisms from clinical cases can be problematic and clinical signs can recur following apparently successful treatment. Although vaccines are available for Borrelia in other countries, there are no licensed products available in the UK. Tick control is also an important component of management in endemic areas.

**FURTHER READING:**


PIROPLASMOSIS

Infection with *Theileria equi* and/or *Babesia caballi* is referred to as piroplasmosis. These are protozoan parasites that reside within erythrocytes. Both parasites can coexist in the same horse. Infection is present in France, Sweden, Spain, central and south America, the middle east, Asia and Africa. Following infection horses tend to carry the intra-erythrocytic parasites for years and perhaps for life. Cases are sometimes encountered in horses in UK following importation of previously infected horses but transmission within the UK is thus far unrecognised.

CLINICAL SIGNS

*T. equi* infection is generally more severe than *B caballi*. Piroplasmosis is a cause of a haemolytic anaemia in any age of foal or adult horses, mainly in the summer/autumn associated with tick activity.

- Pyrexia
- Lethargy, anorexia, dullness, weakness
- Tachycardia, tachypnoea
- Pale/jaundiced/petechiated membranes
- Systolic heart murmur associated with anaemia
- (haemoglobinuria/bilirubinuria)
- (Mild colic/diarrhoea)
- (Oedema)

DIAGNOSIS

Haematology

- Decreased PCV, RBC and Hb concentrations is typical.
- Haemolysis may lead to increased MCH (*Hb/RBC*) and MCHC (*Hb/PCV*) due to anaemia with free haemoglobin
- Increased MCV is often seen as part of a regenerative response.
- Thrombocytopenia is also often seen
- White cell numbers are variable (high, low or normal)

Microscopy

- Blood smears from acute clinical cases should be stained with Romanowsy stains such as Diff-Quick, Giemsa or Wrights. This may demonstrate intra-erythrocytic parasites although failure to identify infection is not uncommon unless great care and time is taken. Identification of parasites in non-clinical carrier animals is unlikely.
- With *T equi*, typically only 1-5% of red cells will be affected. Oval trophozoites or 4 x pyriform merozoites ("maltese cross") may be seen. Massive parasitaemias may be seen in neonatal cases (eg 50% of red cells).
- With *B caballi* oval trophozoites or paired pear-shaped merozoites may be seen although often <0.1% of red cells are parasitized.
PIROPLASMOSIS

Biochemistry
- High serum fibrinogen and SAA reflect the infection and inflammatory response.
- High serum bilirubin (>100 umol/L) is common.
- Mild increases in liver and muscle enzymes and urea and creatinine may reflect hypoxaemia/dehydration

Serology
- The most reliable serologic tests are the Indirect Fluorescent Antibody Test (IFAT) assay and ELISA
- The Complement Fixation test (CFT) is not very sensitive and should probably not be used

PCR
- May be used in both clinical cases and carriers to identify the presence of parasites.
A notifiable disease is a disease named in section 88 of the Animal Health Act 1981 or an Order made under that Act. Section 15(1) of the Act says that:

“any person having in their possession or under their charge an animal affected or suspected of having one of these diseases must, with all practicable speed, notify that fact to a police constable.”

In practice, if you suspect signs of any of the notifiable diseases in the table below, you must immediately notify your local Animal and Plant Health Agency (APHA) office (03000 200301).

**AFRICAN HORSE SICKNESS (AHS)** - UK threat: MODERATE Last seen in UK: Never
AHS is caused by an Orbivirus spread via blood either by Culicoides midges or via contaminated equipment or blood products. If it entered the UK we do have vectors that are capable of spreading the disease. African Horse Sickness could potentially enter the UK through importation of infected equines, importation of infected blood products or entry of infected midges into the UK. Midges could enter the UK along with imported plants, flowers or other products. The disease is endemic in sub-Saharan Africa and has spread to Morocco and The Middle East. Currently, infected midges are not considered close enough to the UK to be carried on the wind.

**Clinical Signs:**
- Marked pyrexia
- Respiratory distress
- Coughing
- Nasal discharge
- Head swelling (especially periorbital region)
- Colic
- Sudden death (ultimately fatal in 50-95% of cases)
- Donkeys show similar, but less severe signs

**ANTHRAX** - UK threat: LOW Last seen in UK: 2006 (in cattle)
Occurs sporadically following soil disturbance and infection with the bacterium Bacillus anthracis. Horses are less susceptible than farm animals.

**Clinical Signs:**
- Often sudden death but horses can die more slowly than farm animals
- Swelling around the larynx
- Colic

**AUJESKY’S DISEASE** - UK threat: LOW Last seen in UK: 2009 (in pigs)
Also known as Pseudorabies, the disease was identified in the UK in 1979 and eliminated by 1991. Pigs are the only natural host but other species may become infected.

**Clinical Signs:**
- CNS disease
NOTIFIABLE DISEASES

**CONTAGIOUS EQUINE METRITIS (CEM)** - UK Threat: HIGH Last seen in UK: 2012
A constant threat caused by bacterial infection with *Taylorella equigenitalis* and spread by sexual or fomite contact. See the HBLB codes of practice for more information.

Clinical signs:
- Stallions tend to be silent carriers
- Mares may exhibit uterine inflammation and vulval discharge or be silent carriers

**DOURINE** - UK threat: MODERATE Last seen in UK: Never
Dourine is widespread worldwide and was present in Italy last year. Caused by *Trypanosoma equiperdum*, a protozoan. Could enter the UK via importation of infected horses or semen. Spread via mating or AI.

Clinical Signs:
- Localised inflammation of genitalia
- Cutaneous plaques
- Neurological signs

**EPIZOOTIC LYMPHANGITIS** - UK Threat: LOW Last seen in UK: 1906
Fungal skin disease caused by *Histoplasma farciminosum* formerly called *Cryptococcus farciminosum*. Endemic in Africa and the Middle East.

Clinical Signs:
- Suppurating skin eruptions that are often associated with a wound and follow lymphatics

**EQUINE INFECTIOUS ANAEMIA** - UK Threat: HIGH Last seen in UK: 2012
Caused by EIA virus and also known as “swamp fever” the disease is present worldwide including parts of mainland Europe. A major outbreak occurred in Ireland in 2006 and was last identified (and contained) in the UK in 2012. EIA could enter the UK in imported animals, blood products or contaminated equipment.

- Pyrexia
- Tachycardia / tachypnea
- Ataxia
- Jaundice
- Anaemia / thrombocytopenia
- Depression
- Haemorrhage
- Oedema
- Weight loss
- Infected horses may become infective carriers without exhibiting any clinical signs of disease.
NOTIFIABLE DISEASES

**EQUINE VIRAL ARTERITIS** - UK Threat: HIGH Last seen in UK: 2012
Caused by equine arteritis virus which is present worldwide including parts of mainland Europe. Spread during mating or AI, via contact with material from abortion/parturition and via respiratory droplets. Stallions may shed the virus without exhibiting clinical signs. A constant threat to the UK via importation of infected animals or semen.

Clinical Signs:
- Pyrexia
- Lethargy
- Depression
- Limb oedema
- Conjunctivitis (pink eye)
- Periorbital/scrotal/mammary gland swelling
- Nasal discharge

**EQUINE VIRAL ENCEPHALOMYELITIS** - UK Threat: LOW Last seen in UK: Never
A collection of viral diseases which are transmitted by mosquitoes.

Clinical signs:
- Pyrexia
- Inappetance
- Hypersensitivity
- Hyperexcitement
- Blindness
- Muscle fasciculations
- Somnolence

**GLANDERS AND FARCY** - UK threat: LOW Last seen in UK: 1928
Bacterial disease caused by *Burkholderia mallei* eradicated form the UK in 1928 and no longer present in Europe.

Clinical Signs:
- Pyrexia
- Depression
- Nasal discharge
- Coughing
- Ulceration of nasal mucosa
- Nodules beneath skin, nasal mucosa and within organs
- Septicaemia
- Death
For exclusion of West Nile, samples may be tested for the presence of antibodies by submission of serum to the Animal and Plant Health Agency (APHA) without the need to report suspected cases to DEFRA.
MAKING USE OF MINIMUM INHIBITORY CONCENTRATIONS (MICs)

Minimum inhibitory concentrations (MICs) are considered the ‘gold standard’ for determining the susceptibility of bacteria to antimicrobials and tells you what minimum antimicrobial concentration you need to achieve to inhibit the growth of a micro-organism. Concentration dependent antimicrobials indicate that therapeutic success will be achieved when peak concentration of 8-10 x MIC is attained. Time-dependent antimicrobials aim to exceed the MIC for as long as possible (at least 50% of the time).

**MIC calculation.** All vials are inoculated. Turbidity (bacterial growth) present at $0.25 - 2$ microg/mL, but not at $4-32$ microg/mL. MIC = $4$ microg/mL which is the minimum target concentration for time dependent antimicrobials (8-10 x $4 = 36-40$ microg/mL for concentration dependent antimicrobials)

<table>
<thead>
<tr>
<th>Time dependent antimicrobials</th>
<th>Concentration dependent antimicrobials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aim: exceed MIC for &gt;50% of dosing interval</td>
<td>Aim: achieve peak concentration of 8-10 x MIC</td>
</tr>
<tr>
<td>Beta lactams – penicillins and cephalosporins</td>
<td>Aminoglycosides (gentamicin, amikacin)</td>
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<tr>
<td>Tetracyclines</td>
<td>Fluoroquinolones (enrofloxacin)</td>
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<tr>
<td>Sulphonamides</td>
<td>Metronidazole</td>
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<tr>
<td>Macrolides (azithromycin, clarithromycin)</td>
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MAKING USE OF MINIMUM INHIBITORY CONCENTRATIONS (MICs)

From combining knowledge of achievable systemic drug concentrations at standard doses, MIC “breakpoint concentrations” are determined – above which resistance (R) is predicted (as this represents unrealistic concentrations) and below which sensitivity (S) is predicted (as such concentrations can be readily achieved). Depending on the drug and MIC, it is possible that modification of the dose might achieve therapeutic success when the MIC value is only just above the S/R breakpoint.

Compared with simple binary results stating resistant/sensitive, MIC values give a more quantitative impression of how sensitive or how resistant an organism is (lower MIC is better – i.e. more sensitive). From the example above, if we know we can achieve tissue concentrations of 4 microg/mL with a particular antibacterial then this will be reported as sensitive (S). In contrast, if we know we can only achieve 0.25 microg/mL with a particular drug then this will be reported as resistant (R). However, if we know we can usually achieve around 2-3 microg/mL (reported as R) then we could use a higher than normal dose and still possibly achieve success (as long as higher doses aren’t toxic).

Low MIC values indicate a greater likelihood of achieving the relevant target concentrations with systemic treatment whereas high values indicate that systemic therapy is likely to fail, although adequate concentrations might still be reached with local treatments (e.g. intraocular, intrauterine) or circumstances where especially high concentrations can be achieved (e.g. intravenous regional perfusion). To make best use of MIC values, an idea of readily attainable systemic drug concentrations is required.

Example 1.

- 10 day old septicemic foal
- Blood culture isolates Actinobacillus equuli
- Gentamicin MIC calculated to be 2 microg/mL
- a “concentration dependent” antibiotic needs to reach 8-10 x MIC (16-20 mg/mL)
- Gentamicin 6.6 mg/kg iv (typically achieves plasma concentrations >20 microg/mL)
Example 2
- Adult horse with peritonitis
- Peritoneal fluid isolates *Streptococcus zooepidemicus*
- Penicillin MIC calculated to be <0.06 microg/mL
- A "time-dependent" antibiotic needs to exceed MIC (0.06 mg/mL) for as long as possible
- Give procaine penicillin 22,000 IU/kg im q 12 hrs (typically achieves peritoneal concentrations 0.5 microg/mL)

Example 3
- Corneal ulcer
- Swab isolates Moraxella sp.
- Gentamicin MIC = 16 microg/mL (resistant)
- You must achieve 8-10 x MIC (130-160 microg/mL)
- Impossible to reach with systemic treatment
- However, 3 drops 0.5% gentamicin drops → >350 microg/mL in tear film
- Topical gentamicin should be effective despite lab prediction of resistance

Example 4
- Mare with bacterial cystitis
- E.coli isolated
- Gentamicin MIC = 16 microg/mL (‘resistant’)
- Need 8-10 x MIC (130-160 microg/mL)
- 6.6 mg/kg gentamicin → 20-30 microg/mL in plasma
- 2 mg/kg gentamicin → 425 microg/mL in urine
- Low dose systemic gentamicin should be effective despite lab prediction of resistance
DETERMINING APPROPRIATE GENTAMICIN CONCENTRATIONS

Gentamicin is a popular aminoglycoside antimicrobial in equine practice with good activity against many gram negative aerobes and also many Staphylococci. The standard recommended dose is 6.6 mg/kg IV q 24hrs based upon pharmacokinetic studies in healthy horses. Foals usually require higher doses to reach therapeutic levels. Dosing above effective levels risks renal tubular damage and is uneconomical; subtherapeutic dosing risks a lack of efficacy and the potential for development of bacterial resistance.

Gentamicin is a concentration-dependent antimicrobial i.e. its effectiveness relates directly to the magnitude of its peak plasma concentration. The optimal peak plasma concentration is 8-10 times the minimum inhibitory concentration (MIC) of susceptible bacteria.

Recently, Liphook Equine Hospital evaluated the peak and trough gentamicin concentrations of a large population of hospitalised horses (n=348) that received 6.6mg/kg IV q24hrs and found a median peak plasma concentration of 21.4µg/mL. Over a similar time period, the MIC of gentamicin in 536 bacterial isolates from ambulatory and hospital practice were measured. The vast majority of bacterial isolates were either very sensitive (MICs ≤2 µg/ml) or very resistant (MICs ≥8 µg/mL) to gentamicin and so a standard 6.6mg/kg SID IV dose rate of gentamicin should be effective in the majority of gram-negative and Staphylococcal infections encountered in equine patients. The MICs of most resistant isolates were so high that increasing the gentamicin dose rate probably wouldn’t be of clinical benefit; in fact, choosing an alternative antimicrobial might be more appropriate when resistant isolates are identified.

Additionally, this study found that a reasonable number (40/348 - 11.5%) of patients had a trough concentration >1 µg/mL. This emphasises the value in measurement of trough gentamicin concentrations in clinical practice to spot horses where an increase in the dose interval is needed to minimise the possibility of renal tubular damage.

Interestingly, this study also identified that bacterial isolates from ambulatory patients were more likely to be sensitive to gentamicin than hospitalised patients (approximately 90% vs 40% respectively). This finding highlights the importance of using bacterial culture and sensitivity (with MIC measurement) as often as possible to select appropriate antimicrobial therapy, rather than relying on likely susceptibility patterns.