

Strangles: Identification of carriers of *Streptococcus equi*

Identification of Animal Carriers

Streptococcus equi (*S. equi*) is the bacterium that causes Strangles in horses. Some horses are carriers of *S. equi*, meaning that they are infected with *S. equi* but do not show any signs of the disease, and these animals can be a source of 'Strangles' in susceptible individuals. Young horses (weanlings and yearlings) are particularly susceptible, but 'Strangles' may occur in horses of any age. Severe infections may result in death.

Outbreaks of 'Strangles' typically occur with movement of horses and mixing of groups that contain one or more carriers. Therefore, identification and treatment of carriers eliminates potentially infectious horses and can significantly reduce the probability of Strangles outbreaks.

The Animal Health Trust has investigated convalescent cases and their contacts in several outbreaks of Strangles. In most outbreaks we have identified a healthy long-term carrier of *S. equi*. Carriage is most commonly found in the guttural pouch, which is usually inflamed. However, in some cases inflammation may not be visible and *S. equi* can be in the shape of a thin, unobvious layer of bacteria (*biofilm*). Carriage did not resolve spontaneously in any of the cases we studied. In fact one untreated animal remained infected for 5 years.

When to Test for Carriers

Testing of individuals prior to introduction to a yard can significantly reduce the probability of Strangles outbreaks. New arrivals should be kept in quarantine until testing is complete and *S. equi* infection has been ruled out.

Following an outbreak of Strangles the best time to detect carriers is a minimum of 30 days after the last clinical signs are seen, as most horses cease to be infective within this time. Prior to and during the testing period all affected horses and their in-contacts should be kept in strict isolation with the highest possible standards of hygiene.

Diagnosis of *S. equi* carriage requires that horse owners are committed to follow-up with appropriate veterinary care and management to either isolate the carrier(s) permanently or, preferably, isolate the carrier(s) and treat to eliminate the infection.

Failure to test for carriers after an outbreak, or failure to treat or isolate known carriers, implies acceptance of the possibility that some of the convalescent cases and their in-contacts continue to present a danger of transmitting *S. equi* to other horses.

Detection of carrier animals is not useful unless horse owners are able to commit to proceed with either permanent isolation of the carrier or, preferably, through treatment and follow-up testing to demonstrate elimination of infection.

Laboratory Diagnosis of *Streptococcus equi*

- In animals showing acute, typical clinical signs of Strangles, diagnosis may be confirmed by bacterial culture/quantitative polymerase chain reaction (qPCR) of *S. equi* from nasal pus or abscessed lymph nodes or from nasopharyngeal swabs.
- At the end of a Strangles outbreak (at least 30 days after), horses that showed clinical signs should be tested in order to establish their infectious status. The gold standard method for investigating possible persistent **carriers** is culture AND qPCR of both guttural pouch (GP) lavage AND nasopharyngeal swab taken at the same time - this has the advantage of also

allowing visualisation of the GPs and targeted sampling of chondroids. After this, 3 nasopharyngeal swabs collected one week apart and tested by culture AND qPCR is the next best alternative. Culture only of 3 nasopharyngeal swabs collected one week apart is useful and better than not sampling or taking of single swabs. Culture will confirm presence of viable *S. equi*, which are required for onward transmission; however there is a small but unquantifiable risk of missing animals carrying lower numbers of *S. equi* in their GPs and which can act as the source of new outbreaks.

- In animals that have no clinical signs and need to be screened (i.e. prior to the introduction into a new herd, prior to movement, etc.), and also at the end of a Strangles outbreak (at least 30 days after) and only in the animals that showed no clinical signs, these animals can be tested using the ***S. equi* ELISA** to determine if they have evidence of an antibody response to *S. equi*, which could mean that these horses are sub-clinical *S. equi* carriers despite showing no signs of disease. If any of these animals are positive via ELISA, they should go through the same culture/qPCR testing process that is outlined above to determine if they are truly **carriers**.

Sample collection and submission

Nasopharyngeal swabs

Nasopharyngeal swabs are the specimen of choice for the initial confirmation of Strangles, and also in the investigation and identification of *S. equi* carriers when there are a large number of cases and potential in-contacts.

It is important that the swabs sample the back of the pharynx adequately. Therefore we recommend the use of our Nasopharyngeal Swabs, which have extra long shafts and an absorbent head (horse or foal/pony size). Swabs and bacterial transport medium (green top) are available by request from the Diagnostic Laboratory Services Customer Service Department (01638 552 993).

Method of Collection for Nasopharyngeal Swabs

1. Prior to the introduction of the swab it can be moistened within thawed bacterial transport medium, provided that both the swab and the transport medium remain free of contamination and that there's enough transport medium left.
2. The swab is introduced via the ventral nasal meatus to the back of the throat. The distance is approximately equal to that from the nostril to the eye.
3. The swab is gently moved to sample the mucosa. Swallowing indicates the correct depth of swab penetration.
4. Following movement and induction of swallowing, the swab is removed.
5. The swab is immediately placed into thawed, cool bacterial transport medium (green top).
6. The swab is detached from the wire either using wire cutters or by repeated bending. Do not dissect the swab.
7. Be sure to cap each container securely.

The swab in bacterial transport medium (AHT green top) should be delivered as soon as possible to the Diagnostic Laboratory Services. Samples should be sent guaranteed next day delivery although specimens 48 hours old on receipt are acceptable. A completed submission form with details of the submitting veterinary surgeon, animal owner and clinical history should accompany all specimens.

When diagnosing carriers, a series of 3 nasopharyngeal swabs, collected 1 week apart, will result in detection by a positive culture on at least one of the swabs in approximately 60% of carrier animals. Concurrent testing of these swabs by qPCR increases the likelihood of detection to over 90% of carrier animals.

If the number of animals to be tested is small, it would be worth considering submitting a single wash sample from each guttural pouch.

Guttural Pouch Washes

For those animals in which a positive result is obtained by culture or qPCR on a nasopharyngeal swab, continued strict isolation and additional work-up is required. Endoscopic examination of the guttural pouches is essential to gauge the extent of inflammation present, which is a preliminary step in treatment. Bilateral guttural pouch washes should be collected and submitted for *S. equi* culture and qPCR.

Method of Collection for Guttural Pouch Washes

1. Pass the endoscope through the nares of the same side of the head as the guttural pouch to be sampled. Extend the endoscope up the ventral nasal meatus to the level of the common pharynx. The pharyngeal openings of the guttural pouches are visible as mucosal flaps on either side of the pharynx.
2. Pass the biopsy forceps or a similar 'guide' instrument through the biopsy channel and under the flap of the guttural pouch and extend the endoscope into the pouches. Slightly twisting the endoscope helps entry to the pouch.
3. Once inside the pouch, make a thorough assessment of all parts of the pouch.
4. Replace the 'guide' instrument in the biopsy channel with a sterile polythene catheter.
5. Ensure that the horse's head is elevated before instilling and aspirating approximately 50 ml of sterile phosphate-buffered saline into the pouch from a syringe attached to the catheter.
6. The entire wash should be placed in a leak-proof universal container and labelled with animal's and owner's name and location of aspirate (right or left guttural pouch). Do not just submit a swab of the wash.

The wash in phosphate buffered saline should be delivered as soon as possible to the Diagnostic Laboratory Services. Samples should be sent guaranteed next day delivery although specimens 48 hours old on receipt are acceptable. A completed submission form with details of the submitting veterinary surgeon, animal, owner and clinical history should accompany all samples.

S. equi ELISA test

This test identifies antibodies to two antigens that are unique to *S. equi*, and which are targeted by the equine immune system following exposure to *S. equi*. Horses that have been exposed to *S. equi* in the previous six months (including long-term carriers) will develop antibody titres to one or both antigens and therefore will have a positive result on the ELISA test.

There are two situations in which we envisage the *S. equi* ELISA test being used:

1. Testing horses prior to their introduction into a new herd:
 - If results are positive then perform a guttural pouch endoscopy or three nasopharyngeal swabs (at weekly intervals) and test samples via culture and qPCR.
 - If results are negative, a second sample would still be recommended in order to rule out exposure to *S. equi* around the time of the first sample.
 - If results are inconclusive (grey area**), take a second sample in 14 days to rule out exposure to *S. equi* around the time of the first sample.
2. At the end of a Strangles outbreak (at least 30 days after the last clinical signs were seen) and only in the animals that showed no clinical signs (even the animals without a direct/indirect contact with clinically affected cases), and NOT to be used in the clinically affected cases[#]:
 - If results are negative, the horse can be considered negative.
 - If results are positive, then perform a guttural pouch endoscopy or three nasopharyngeal swabs (at weekly intervals) and test samples via culture and qPCR.
 - If results are inconclusive (grey area**), take a second sample in 14 days to rule out a late response to *S. equi*.

** Grey area: 0.3 and 0.4 for both Antigen A and Antigen C.

These horses would be expected to remain seropositive by ELISA for up to approximately 6 months due to the time it takes for antibody levels to decrease naturally over time. The ELISA, therefore, may not discriminate carriers from non-carriers in the 6 months after clinical strangles.

It is important to underline that the ELISA test will not discriminate responses to natural infection from those following vaccination with live attenuated vaccines that have been licensed anywhere to date.

When testing paired serology, please note that the original sample has to be re-tested with the second sample to provide a valid paired test result. Therefore there could be an inter-assay variation between the original results and the re-test of the first sample, which shouldn't exceed +/- 0.3. Only samples tested as pairs on the same test can be validly compared.

For this test clotted blood/serum is needed, if the sample is in our lab by 10:00 a.m., results will be issued the same working day (Mon – Fri).

Treatment Recommendations

Guidelines in Strangles are included in the Horserace Betting Levy Board (HBLB) Codes of Practice and are available on the HBLB website: www.hblb.org.uk.

All horses in which a positive culture or qPCR is obtained should be isolated from other horses. Strict hygiene to eliminate spread of secretions by aerosol, grooming utensils and human beings (boots, clothing and hands) should be observed.

Treatment requires a minimum of a single endoscopic examination of the guttural pouches.

The most effective treatment comprises:

Removal of inflammatory material harbouring infection

Inflammatory exudate should be removed by flushing. Solid material may need to be removed using endoscopically guided forceps or retrieval baskets. In some cases, inflammation may not be visible and *S. equi* may be in the shape of a thin, unobvious layer of bacteria (*biofilm*).

Instillation of topical antibiotic

5 million units of Penicillin G in approximately 30-50ml of a 3% gelatin solution is recommended.

Course of systemic antibiotics

7 days of Penicillin or Ceftiofur by injection

Repeat testing to confirm absence of infection

Continued isolation of animals for 30 days following treatment is recommended. A nasopharyngeal swab and bilateral guttural pouch washes should then be collected and submitted for *S. equi* culture.

If the horse appears healthy and a negative culture is obtained from all 3 sites, the treatment is considered to be effective and continued isolation is not required. Should any of the 3 sites give positive results, repeat treatment and efficacy testing is recommended.

Tests offered at Animal Health Trust

For information regarding prices, please contact Diagnostic Laboratory Services on 01638 552993.

Strangles Culture (S. equi Culture)

Bacterial culture from a nasopharyngeal swab/bilateral guttural pouch washings. This test can take up to 4 working days.

Strangles quantitative/real-time PCR (qPCR)

qPCR from a nasopharyngeal swab/bilateral guttural pouch washings. Please note that charcoal swabs are not suitable for qPCR testing. This test can take up to 4 working days.

Strangles Culture and qPCR profile

Consisting of culture and qPCR from a nasopharyngeal swab/bilateral guttural pouch washings. The results for both tests will be issued in approximately 4 working days.

ELISA

Antibody ELISA test carried out in a clotted blood/serum sample. If the sample is in our lab by 10:00 a.m., results are reported the same working day.

Strangles Culture, qPCR and ELISA

Profile consisting of culture and qPCR from a nasopharyngeal swab/bilateral guttural pouch washings, and ELISA test in a clotted blood/serum sample. This takes approximately 1 working day for the ELISA, 3 working days for the culture and up to 4 working days for the qPCR results. The results for the ELISA will be issued same day and the results for the culture and qPCR tests will be issued in approximately 4 working days

For Additional Advice and Information

Additional consultation regarding *S. equi*, outbreak investigation or testing may be obtained by contacting our epidemiologists on 01638 552993.

References

1. Newton, J.R., Wood, J.L.N. and Chanter, N. Strangles: long term carriage of *Streptococcus equi* in horses. Equine Vet. Educa. 9(2): 98-102, 1997.
2. Newton, J.R., Wood, J.L.N., Dunn, K.A., DeBrauwere, M.N. and Chanter, N. Naturally occurring persistent and asymptomatic infection of the guttural pouches of horses with *Streptococcus equi*. Vet. Record 140: 84-90, 1997.
3. Newton, J.R., Wood, J.L.N., DeBrauwere, M.N., Chanter, N., Verheyen, K. and Mumford, J.A. Detection and treatment of asymptomatic carriers of *Streptococcus equi* following strangles outbreaks in the UK. Equine Infectious Diseases VIII: Proceedings of the 8th International Conference, 1998.

Flow Diagram for Investigation of *S. equi* Carriers

