



Testing for Strangles explained.

“Strangles” is an infection caused by the organism *Streptococcus equi subspecies equi* (*S. equi*), and shares almost 97% DNA identity with *Streptococcus equi subspecies zooepidemicus* (*S. zoo*), an organism that is often considered commensal in the upper respiratory tract of equines.

The similarity of *S. zoo* and *S. equi* makes it difficult to identify horses that have been exposed to *S. equi* in a background of almost ubiquitous exposure to *S. zoo*. Using the commercially available ELISA assay kits can lead to false positive results in as many as 23% of genuinely negative horses. The laboratory-based ELISA developed at the Animal Health Trust has been designed specifically with this differentiation in mind, and looks at two targets (which we call Antigens A and C). Our test incorrectly identified only 0.7% of genuinely negative horses, whilst detecting 93.3% of true positive horses allowing improved clinical interpretation.

The *S. equi* organism has evolved to exploit the anatomy of the horse. The bacterium enters via the respiratory tract and invades the mucosal surface, setting up an infection in the lymph nodes, which can be undetectable 24-48 hours post infection by microbiological and even PCR screening of a nasopharyngeal swab. Over time, infected horses develop clinical signs of strangles, shedding infectious material into their local environment, which enables the transmission of *S. equi* to in contact susceptible animals.

Following the resolution of clinical signs, up to 10% of recovered cases may remain persistently infected, harbouring *S. equi* mainly in their guttural pouches with the potential to intermittently shed the bacteria, enabling onward transmission to other animals and leading to future outbreaks of the disease.

The early identification and treatment of these persistently infected carriers is critical in breaking the *S. equi* “cycle of infection”. Testing of horses pre-movement or in quarantine prior to their introduction to other horses can identify apparently healthy carriers, enabling their treatment before they can trigger a new outbreak.

Approx. 98% of horses recover from strangles, and an adaptive immune response can be detected approximately 2 weeks post infection, with 75% of recovered horses developing a protective immunity.

So...what samples should I take and when???

Query: **As part of a pre-entry screen, a new yard requires animals to be tested for *S. equi* antibodies.**

Action: - If no clinical signs or suspicion of ongoing Strangles exists on the premises where the animal presently resides, or the animal has been isolated for 2 weeks prior to sampling.

A blood sample will show whether there are any antibodies to *S. equi* present.

A Negative result means the animal is considered safe to move. (Refer to the "interpreting your results" section).

- If there is suspicion of infection on the premises, but not in the animal concerned then it should be isolated for 2 weeks before a blood sample is taken, (as above).
- If there is a suspicion of recent Strangles infection in the animal concerned then *S. equi* serology will not be of any help as antibodies will not rise until 2 weeks post infection. The animal should be isolated and guttural pouch washes taken for qPCR and culture 4 weeks after the last clinical signs or after 4 weeks isolation, whichever is the longest.

Query: **You have been called to an animal showing clinical signs of strangles**

Action: If the animal is showing respiratory signs then a Nasopharyngeal swab should be taken (or swab of a ruptured abscess), and a blood sample. These should be tested by qPCR test and culture and ELISA.

If an animal is in the "acute phase" of a *S. equi* infection, serological changes will not be apparent for 2 weeks after infection. Should qPCR prove negative the blood sample taken in the acute phase can be paired with a second sample taken 2 weeks later to see if there is a rising antibody response, confirming the infection.

Query: **You have confirmed an outbreak of Strangles on a yard**

Action: Isolate the clinically affected animals.

- When clear of clinical signs for 4 weeks take guttural pouch washes for qPCR to prove the infection has been cleared.
 - o Blood samples for Strangles Serology should be taken from all animals that did not show any clinical signs 2-4 weeks after they have been separated from the affected animals. Animals that give a positive Serology result should have guttural pouch washes taken for qPCR.
 - o Animals showing a "grey area" Serology result should have repeat blood tests taken approx. 2 weeks later. (Refer to the "interpreting your results" section below).

Interpreting your Serology Results:

Single value:

- A **Negative** result (≤ 0.2) to both antigen A and C can give you confidence that the animal is not a carrier of *S. equi* and had not been exposed to *S. equi* 10 days prior to the sample being taken. It should be safe to move the animal to other premises if no in-contact horses are showing clinical signs.
- A **Positive** result (≥ 0.5) to antigen A and/or C indicates further testing is recommended and either guttural pouch washes, or 3 nasopharyngeal swabs (NPS) taken 1 week apart, should be submitted for qPCR. (Please note: should any of the NPS samples prove positive for *S. equi*, guttural pouch washes will still need to be taken).
- A result of **0.3 / 0.4** to either antigen A and/or C lies in the “**Grey area**” of the test. This may indicate the animal has recently been exposed to *S. equi* and may be incubating the disease, so we would recommend a repeat blood sample is taken approximately 2 weeks (10 – 21 days) later, looking for a positive or rising antibody level.

Paired Serology:

- If the first sample gives a Positive result, there is generally no point in taking a second sample. The animal should be screened for the presence or persistence of *S. equi*, to see if the animal is an infectious risk.
- Taking a second/paired sample following a negative or “grey area” result along with enhanced clinical monitoring can be useful for e.g. clearance of potentially exposed but asymptomatic animal(s), as part of outbreak control measures.
- Paired serology can prove helpful to identify carriers, and can often be most useful in group situations to highlight the presence of a carrier in the population that is intermittently shedding. Following identification of the presence of a potential carrier, all members of the group should be investigated using GP endoscopy and qPCR.

Note on Guttural Pouch washes:

It is important to sample both left and right guttural pouches, as potentially only one may be infected. (If necessary to reduce costs, these can be pooled for analysis, however this dilutes the overall sample, so may miss low positives).

When multiple animals are being examined and samples taken for testing, it is prudent to record the order in which the samples are being taken and ceasing collection if an overtly affected carrier is identified. Adequate disinfection of the equipment, environment and personnel should then be carried out to avoiding any risk of onward transmission.

Further reading:-

- Getting a grip on Strangles: Recent progress towards improved diagnostic and vaccines. *Waller et al.* The Veterinary Journal: 173 (2007) 492-501
- Combining two serological assays optimises sensitivity and specificity for the identification of *Streptococcus equi* subsp. *equi* exposure. *Robinson et al.* The Veterinary Journal: 197 (2013) 188-191.
- New Perspectives for the Diagnosis, Control, Treatment and prevention of Strangles in Horses. *A Waller.* Vet Clin Equine: Vol 30 Issue 3 (2014) 591-607.