Section 3.6

Equidae

CHAPTER 3.6.2

CONTAGIOUS EQUINE METRITIS

SUMMARY

Description and importance of disease: *Contagious equine metritis is an inflammatory disease of the proximal and distal reproductive tract of the mare caused by* Taylorella equigenitalis*, which usually results in temporary infertility. It is a non-systemic infection, the effects of which are restricted to the reproductive tract of the mare.*

When present, clinical signs include endometritis, cervicitis and vaginitis of variable severity and a slight to copious mucopurulent vaginal discharge. Recovery is uneventful, but prolonged asymptomatic or symptomatic carriage is established in a proportion of infected mares. Direct venereal contact during natural mating presents the highest risk for the transmission of T. equigenitalis *from a contaminated stallion or an infected mare. Direct venereal transmission can also take place by artificial insemination using infective raw, chilled and possibly frozen semen. Indirectly, infection may be acquired through fomite transmission, manual contamination, inadequate observance of appropriate biosecurity measures at the time of breeding and at semen-collection centres. Stallions can become asymptomatic carriers of* T. equigenitalis*. The principal sites of colonisation by the bacterium are the urogenital membranes* (*urethral fossa, urethral sinus, terminal urethra and penile sheath*)*. The sites of persistence of* T. equigenitalis *in the majority of carrier mares are the clitoral sinuses and fossa and infrequently the uterus. Foals born of carrier mares may also become carriers. The organism can infect equid species other than horses, e.g. donkeys.*

Detection and Identification of the agent: *Swabs should be taken from designated genital sites. Culture or real-time polymerase chain reaction* (*PCR*) *should be used for agent identification. To avoid loss of viability for culture, individual swabs should be fully submerged in Amies charcoal medium and transported to the testing laboratory under temperature-controlled conditions for plating out within 48 hours of collection. Growth of* T. equigenitalis *is likely to take 3–6 days at 37°C on specialised media in an atmosphere of 5–10% CO_2. An incubation time of at least 7 days is advisable before certifying cultures negative for* T. equigenitalis*. Identification should include biochemical characterisation, antigenic testing using specific antibodies and molecular genotyping. The fastidious nature of* T. equigenitalis *makes it difficult to isolate. Two real-time PCR tests are also available for agent identity testing and have some advantages over culture. Swabs for real-time PCR do not need to be inserted into bacterial transport medium. Other PCR assays are available and additional detection assays such as the immunofluorescent antibody test have been developed and, ultimately, test-breeding of stallions for detection of the carrier state has been used as an adjunct to agent identity testing.*

Serological tests: *Serology has been used for detecting recent, but not chronic, infection in the mare. Serum antibody to* T. equigenitalis *can be detected in mares for 3–7 weeks after infection. It may also be demonstrated in the occasional carrier mare, but never in the stallion. No individual serological test described to date has been shown reliably to detect infection. Serological tests can*

be used as an adjunct to culture for T. equigenitalis *in screening mares recently bred to a carrier stallion, but must not be used as a substitute for culture.*

Requirements for vaccines: *Effective vaccines are not yet available.*

A. INTRODUCTION

1. Description and impact of the disease

Contagious equine metritis was first described in the United Kingdom (UK) in 1977, after which it was diagnosed in a number of countries world-wide. It first presented as disease outbreaks characterised by a mucopurulent vaginal discharge originating from inflammation of the endometrium and cervix, resulting in temporary infertility. Mares may experience more than one episode of the disease in a short period. Most mares recover uneventfully, but some may become carriers of the causal organism, *Taylorella equigenitalis*, for many months. infection does not always adversely affect conception and abortion due to *T. equigenitalis* is a very rare occurrence. Many primary cases are subclinical, and a frequent indicator of infection is the mare returning in oestrus prematurely after being bred to a putative carrier stallion. infection in a stallion is subclinical.

The carrier state plays an important role in the dissemination of the bacterium. The urogenital membranes of the stallion become contaminated at coitus or by contact with fomites typically employed in semen collection. The carrier state may persist for many months or years. Most carrier mares are clitoral carriers and poor hygienic measures when breeding may also spread the organism. Prior infection is not fully protective as the serum antibody only persists for a few weeks after infection so control of infection has relied solely on prevention of transmission. The organism can be eliminated by treatment with antibiotics combined with antiseptic washing and cleaning of the affected sites. *Taylorella equigenitalis* is not known to infect humans and it should be handled in the laboratory with appropriate biosafety and containment procedures as determined by biorisk analysis (see Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*).

2. Nature and classification of the pathogen

Taylorella equigenitalis is a Gram-negative, non-motile, bacillus or cocco-bacillus that is often pleomorphic (up to 6 µm long) and may exhibit bipolar staining. It is catalase positive, phosphatase positive, and strongly oxidase positive. It is otherwise inert in tests for biochemical activity.

3. Differential diagnosis

The fastidious slow growing organism can be isolated in the laboratory from swabs of colonisation sites in the reproductive tract of stallions and mares (urethral fossa, urethral sinus, terminal urethra and penile sheath; clitoral fossa, clitoral sinuses and endometrium) using the correct atmospheric conditions and is currently the preferred procedure for international trade or movement. Designated swabbing sites are usually specified for international movement, by the competent authorities.

Molecular testing methods such as polymerase chain reaction (PCR) and particularly real-time PCR are now commonly used to detect *Taylorella* both from swabs and culture plates. They have the advantage of speed of result and can usually differentiate between *T. equigenitalis* and *T. asinigenitalis*.

B. DIAGNOSTIC TECHNIQUES

Table 1. Test methods available for the diagnosis of contagious equine metritis and their purpose

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection and identification of the agent						
Bacterial isolation and identification	+++	+++	+++	+++	+++	–
IFAT	+	+	+	+	+	–
Real-time PCR	+++	+++	+++	+++	+++	–

Key: +++ = recommended for this purpose; ++ recommended but has limitations;

+ = suitable in very limited circumstances; – = not appropriate for this purpose.

IFAT = indirect fluorescent antibody test; PCR = polymerase chain reaction.

1. Detection and identification of the agent

1.1. Culture techniques

Sampling and transport prior to *Taylorella* isolation and identification needs special attention. Swabs must be placed in a transport medium with activated charcoal, such as Amies medium, to absorb inhibitory by-products of bacterial metabolism (Swerczek, 1978). The numbers of viable *T. equigenitalis* decline on swabs over time, and this effect is more pronounced at higher temperatures (Sahu *et al.,* 1979). Swabs must be kept cool during transportation and should arrive and be plated out at the laboratory no later than 48 hours after they were taken.

Various bacteria exist on the urogenital membranes of horses as harmless commensals that may interfere with the culture of *T. equigenitalis* by obscuring its presence. Washing and antibiotic treatment may control this problem but may sublethally damage *T. equigenitalis,* allowing it to persist on the urogenital membranes but rendering it impossible to grow on laboratory media. Swabbing for *T. equigenitalis*, therefore, should not recommence until at least 7 days (systemic treatment) or 21 days (local treatment) following treatment.

The fastidious nature of T. *equigenitalis* makes it difficult to isolate. Test breeding of stallions has been used to increase the sensitivity of detection of the carrier state and it has been a valuable adjunct to cultural examination. The numbers of *Taylorella* present on the external genitalia of stallions can be very low and may be missed by culturing alone, but can be detected after multiplication in the mare that has been test bred. The use of test breeding as an additional diagnostic tool can be especially important in countries that are considered free from contagious equine metritis.

Culture media is produced by heating reconstituted agar base containing 5% (v/v) lysed horse blood to 70–80°C for 12 minutes ('chocolate' blood agar), which is cooled to 45–50°C and trimethoprim (1 µg/ml), clindamycin (5 µg/ml), and amphotericin B (5–15 µg/ml) is added (Timoney & Powell, 1982). Lysed horse blood contains thymidine phosphorylase, which will inactivate thymidine, thus allowing the trimethoprim to exert its selective effect. This is the preferred medium for isolating *T. equigenitalis* therefore each swab must be inoculated onto this medium. It will successfully isolate both streptomycin

resistant and sensitive biotypes of the pathogen; suppress the growth of many commensal bacteria and inhibit fungal growth. As inhibiters may prevent the isolation of some strains of *T. equigenitalis*, swabs should also be inoculated on to 5% 'chocolate' blood agar with a rich peptone agar base containing additional cysteine (0.83 mM), sodium sulphite (1.59 mM) and a fungicide (5–15 µg/ml amphotericin B). *Taylorella equigenitalis* can be cultivated on blood agar, but will grow better on 'chocolate' blood agar as described above. Some manufacturers produce a peptone agar base that supports the growth of *T. equigenitalis*. An important feature of all good *T. equigenitalis* media is the absence of fermentable carbohydrates. The fermentation of carbohydrates by other bacteria inhibits *T. equigenitalis* growth (Atherton, 1983; Fernie *et al.,* 1980). A third medium containing streptomycin sulphate (200 µg/ml) can be used to inhibit the growth of other bacteria that might obscure *T. equigenitalis* (Swerczek, 1978); however, the streptomycin-sensitive biotype will not be detected on this medium; and it should only be used in conjunction with medium without streptomycin. All culture media should be subjected to quality control and must support growth of a small inoculum of the suspect organism before their use on suspect samples. The reference strain of *T. equigenitalis* must also be cultured in parallel with the test samples to ensure that the culture conditions are optimal for isolation of this organism.

Plates must be incubated at 35–37°C in 5–10% (v/v) CO_2 in air or by use of a candle jar. At least 72 hours is normally required before colonies of *T. equigenitalis* become visible, after which time daily inspection is needed. Rarely, visual detection of colonies may take up to 14 days (Ward *et al.,* 1984). A standard incubation time of at least 7 days is advisable before certifying cultures negative for *T. equigenitalis*. Plates should be examined for contaminants after the first 24 hours' incubation. Colonies of *T. equigenitalis* may be up to 2–3 mm in diameter, smooth with an entire edge, glossy and yellowish grey. Laboratories should be aware that certain countries may require the prolonged incubation period as a standard procedure and should therefore ascertain the particular import requirements of those countries and/or indicate the incubation period on which their cultural findings are based. Growth of other bacteria, for example *Proteus mirabilis*, may be so extensive that the laboratory cannot issue a negative result. In this event, further swabs should be requested.

If a slow-growing organism is isolated that fits the description for cellular morphology and that is strongly oxidase and catalase positive, it can be tested for reactivity with *T.-equigenitalis*-specific antiserum.

1.2. Serotyping methods

A variety of serotyping tests has been developed to confirm that a culture is *T. equigenitalis,* ranging in complexity from slide agglutination to direct or indirect immunofluorescence. Each method has its advantages and disadvantages. The disadvantage of the slide agglutination test is that occasionally autoagglutination of isolates occurs. Culturing in bottled CO_2 in air, as opposed to in a candle jar, may reduce autoagglutination (Ter Laak & Wagenaars, 1990). Immunofluorescence may be of value in the identification of auto-agglutinating isolates; a validated indirect immunofluorescence test for the detection of *T. equigenitalis* in swabs from the reproductive tract of stallions and mares is commercially available.

Antiserum is produced by vaccinating rabbits with killed *T. equigenitalis*. A standard strain, such as NCTC 11184[1], should be used for immunisation. However, the most important consideration is the specificity of the antiserum produced. It should agglutinate *T. equigenitalis*, but fail to agglutinate other bacteria that might be cultured from horse urogenital membranes. In particular, it should not agglutinate any oxidase-positive and Gram-negative rods, such as *Mannheimia haemolytica*, *Actinobacillus equuli, Bordetella bronchiseptica* (to which *T. equigenitalis* is closely related, see Bleumink-Pluym *et al.* (1993), *Oligella urethralis* and *Pseudomonas aeruginosa*. *Taylorella asinigenitalis* has similar, though not identical, colonial appearance and cultural characteristics and gives identical biochemical test results to those used to confirm the identity of *T. equigenitalis*. There is even serological cross-reactivity between the two organisms. Differentiation of *T. asinigenitalis* from *T. equigenitalis* is possible using the PCR or 16S rDNA sequencing and biochemical reactivity (Baverud *et al.,* 2006; Breuil *et al.,* 2011; Duquesne *et al.,* 2007; Wakeley *et al.,* 2006). Monoclonal antibodies are available commercially that provide a highly specific means of identifying *T. equigenitalis*.

A latex agglutination kit is available commercially for the antigenic identification of *T. equigenitalis*. It is based on polyclonal antibodies produced using methods similar to those described above. This is widely used by routine testing laboratories for the confirmation of the identity of colonies growing on selective medium that give a biochemical reaction consistent with *T. equigenitalis*. As *T. equigenitalis* is antigenically relatively distinct, and small amounts of cross-reactive antibody are easily absorbed during production of the reagent, the test has proved

to be highly specific and sensitive. It should be emphasised that it will not necessarily distinguish strains of *T. equigenitalis* from *T. asinigenitalis*.

1.3. Immunofluoresence methods (IFAT)

Antibody-based methods can also be used for the direct detection of *T. equigenitalis* in swabs taken from sampling sites. Both in-house and commercially available indirect immunofluorescence antibody tests (IFAT) have been described (Breuil *et al.,* 2010). Reported sensitivity and specificity are 93% and 100%, respectively (Breuil *et al.,* 2010). It is important that kits used have been fully validated in accordance with Chapter 1.1.6. *Validation of diagnostic assays for infectious diseases of terrestrial animals*. Kits should preferably be selected from those listed on the WOAH Register[2].

1.4. Matrix-assisted laser desorption ionisation-time-of-flight mass spectrometry (MALDI-TOF MS)

Matrix-assisted laser desorption ionisation-time-of-flight mass spectrometry (MALDI-TOF MS) has also been described as a reliable method for identification of *Taylorella* culture colonies and for the differentiation of *T equigenitalis* and *T. asinigenitalis* with appropriately expanded reference spectra, using direct spotting of 48-hour suspect colonies (Petry *et al.,* 2019).

1.5. Molecular methods

Molecular testing methods such as PCR and real-time PCR have been applied to the detection of *T. equigenitalis* both directly (using swabs taken from sampling sites) and indirectly (on cultures grown from swabs). To mitigate the possibility of false negative results, it is recommended that, whenever possible, several colonies suspected of being of the *Taylorella* genus are selected for confirmation by PCR. In Japan the field application of the PCR was evaluated for the eradication of contagious equine metritis. It was demonstrated that the PCR was more sensitive than culture for the detection of *T. equigenitalis* from genital swabs of horses (Anzai *et al.,* 1999; 2002). Laboratory ring trial data also indicate improved detection of *T. equigenitalis* by PCR compared with culture (Mawhinney, 2020; Petry *et al*., 2018).

1.5.1. Real-time PCRs

A real-time PCR was developed for use directly on genital swabs and compared with culture (Wakeley *et al.,* 2006, Mawhinney *et al*., 2019); it has subsequently been used for pre-breeding screening studies (Ousey *et al.,* 2009) and surveillance and eradication schemes in various countries (Belloy *et al.,* 2012; Jeoung *et al.,* 2018; May *et al.,* 2016). A second real-time PCR was developed and used successfully for the diagnosis of CEM (Nadin-Davies *et al.,* 2015). These two real-time PCRs have been shown to be highly specific and reproducible in different laboratories with slightly higher sensitivity than culture; they are the recommended PCR assays.

i. Real-time PCR 1 (Wakeley et al., 2006)
This PCR has been used widely for detection of CEM. It has been shown to be robust to minor modifications of methodology and different DNA extraction techniques when validated in-house (May *et al.,* 2016; Petry *et al.,* 2018). This real-time PCR uses two separate PCRs, one that specifically amplifies a portion of the 16S rDNA of *T. equigenitalis* and *T. asinigenitalis,* and a second control PCR that amplifies a region of the 16S rDNA of many commensal bacteria found in the genital tract of horses and that acts to confirm inoculation of the swab and DNA extraction. Two probes, TEquiFAM and TAsiniHEX, corresponding to the two species of *Taylorella*, are labelled with different fluorophores to discriminate the amplicons and used in the *Taylorella*-specific PCR (Table 2a). In the control PCR, another probe labelled with a different fluorophore is used for each sample.

The preparation of DNA samples from isolated bacteria as well as genital swabs has been described by Wakeley *et al.* (2006). Isolated bacteria are suspended in 1.5 ml PBS (pH 7.4), transferred to Eppendorf tubes and heated at 95–100°C for 20 minutes in a heating block, after which the tubes are centrifuged at 18,000 ***g*** for 1 minute in a microcentrifuge. Each swab is immersed and manually agitated in 0.2 ml of 0.1 M PBS (pH 7.4) in a 1.5 ml Eppendorf tube for 5 seconds, a negative extraction control is also prepared using 0.2 ml of nuclease free water. The Eppendorf tube is then centrifuged at 18,000 ***g*** in a microcentrifuge for 30 seconds to pellet the bacteria. The supernatant is aspirated and disposed of and the pellet is re-suspended in 100 µl nuclease free water. The re-suspended pellet is heated at 95–100°C for 15 minutes in a heat block prior to centrifugation at 18,000 ***g*** for 1 minute in a microcentrifuge. This

supernatant is the DNA extract or lysate. The lysate can be used immediately or stored at −20°C. Each reaction uses 2–5 µl of the lysate.

Alongside the samples, a *T. equigenitalis*, *T. asinigenitalis* and a no template control are tested on each plate. Cycling involves initial denaturation for 2 minutes at 94°C followed by 40 amplification cycles of denaturation for 5 seconds at 94°C, primer annealing for 10 seconds at 60°C and extension and data collection for 15 seconds at 72°C. A control real-time PCR for bacterial 16S rDNA (BactUniF, ACTA CGT-GCC-AGC-AGC-C; BactUniR, GGA-CTA-CCA-GGG-TAT-CTA-ATC-C) using the 16SrDNA ROX, TGT-TTG-CTC-CCC-ACG-CTT-TCG-CAC-BHQ2 probe, is run in parallel in a separate well to ensure the extraction and test process has worked. Minor variations from the published method, such as DNA extraction methods using proprietary kits, master mix compositions or internal controls, should be laboratory validated (in-house). The PCR is performed on a suitable real-time PCR machine. A positive test has a CT value ≤ 40 for the specific probe. A valid *Taylorella* PCR test requires the control 16S rDNA PCR to be positive for the sample in question.

ii. Real-time PCR 2 (Nadin-Davies et al., 2015)

This real-time PCR has not been as widely used, but is also highly reproducible when used as described. The assay is composed of a single primer set with two probes specific for *T. equigenitalis* (EQUI-VIC; if available HEX can also be used) and *T. asinigenitalis* (ASINI-FAM) (Table 2a). A third probe, HA5-CY5, TCT-ACG-AGA-GAA-CCT-CTC-CGA-GCT-CAG-CT-BHQ2, is specific to a cloned sequence in a spiked-in plasmid and is used as an internal positive control (IPC). The spiked-in plasmid contains the TAYQ primer sequences flanking a 148 bp sequence from human adenovirus 5 corresponding to bases 28297 through 28407 of GenBank Accession number M73260.

To prepare the test sample, each swab is extracted and placed into 1.0 ml sterile nuclease free water in a microcentrifuge tube, vortexed for 5 seconds, and allowed to stand for 5 minutes. At the same time, a negative extraction control is also prepared using 1.0 ml of nuclease free water. After standing in the microcentrifuge tube, the swab is returned to its original vial. The sample is then centrifuged for 5 minutes at 17,949 ***g***, the supernatant aseptically removed and discarded, and the pellet re-suspended in 20 µl of 10 mM TE buffer pH 8.0. Suspensions are heated for 15 minutes at 100°C then cooled. Prior to testing, samples are centrifuged for 5–10 seconds to remove any liquid from the lid and tested immediately or stored at −20°C until tested. Stored samples are centrifuged for 30 seconds prior to testing to pellet debris. The supernatant is the DNA extract or lysate.

Each reaction is composed of 2 µl of supernatant added to 18 µl of mastermix containing 500 nM each of TayQF and TayQR, 250 nM of EQUI-VIC, and 125 nM of ASINI-FAM. Minor variations in the mastermix or concentrations must be laboratory validated (in-house). The described assay includes an IPC consisting of a manufactured sequence that is added to each well together with the sample DNA and negative extraction control and specific probe. The IPC is detected by a corresponding probe included in the master mix. Failure to amplify this control indicates inhibition of the PCR. Each run includes a standard curve (five dilution series) of DNA template controls for *T. equigenitalis* together with IPC to demonstrate PCR efficiency. Individual wells each containing *T. asinigenitalis* PCR control and IPC, *Oligella urethralis* and IPC, IPC alone, and a no template control are also included on every plate, to demonstrate PCR specificity and fitness for purpose. Samples are tested in duplicate. PCR cycle conditions are as follows: 95°C for 10 minutes followed by 45 cycles of 95°C for 15 seconds and 55°C for 1 minute. A CT value ≤ 40 for the specific probe is considered positive.

Alternative laboratory validated variations of controls have been used, however every PCR plate should include a no template negative extraction control, *T. equigenitalis* and *T. asinigenitalis* positive controls and an IPC control, as a minimum. A control test to show the presence of bacteria or bacterial DNA to confirm that the swabs have been inoculated is also advisable.

Table 2. Primer sequences for use in recommended real-time PCR tests

Primer 1 (forward)		Primer 2 (reverse)		Probe (only for real-time PCRs)		Reference
Name	Sequence (5' → 3')	Name	Sequence (5' → 3')	Name	Sequence (5' → 3')	
Tay377 for	CCG-CGT-GTG-CGA-TTG-A	Tay488 rev	TTT-GCC-GGT-GCT-TAT-TCT-TCA	Tequi FAM-probe TasiniHEX-probe	6FAM-AAA-GGT-TTG-TGT-TAA-TAC-CAT-GGA-CTG-CTG-ACG-G-BHQ1 HEX-AAA-GTT-TTA-GGA-TAA-TAC-CCT-AGG-ATG-CTG-ACG-G-BHQ1	Wakeley *et al.*, 2006
TAYQF	CGC-GTG-TGC-GAT-TGA-A	TAYQR	GCC-GGT-GCT-TAT-TCT-TCA	EQUI-VIC (or HEX)	(HEX) VIC-AGG-TTT-GTG-TTA-ATA-CCA-TGG-ACT-GCT-GAC-QSY7 (BHQ1 if using HEX)	Nadin-Davies *et al*, 2015
				ASINI-FAM	FAM-AGT-TTT-AGG-ATA-ATA-CCC-TAG-GAT-GCT-GAC-GGT-BHQ1	

If using HEX in these two real-time PCRs, the *Taylorella* species that use the HEX and FAM labels are opposite (Table 2).

Maintaining the viability of the organism is not necessary for the recommended real-time PCRs, so swabs may be transported at ambient temperature. For PCR testing it is not necessary to use transport medium to convey swabs to the laboratory. Swabs in plain sleeves without transport medium can be used. It is recommended that swabs for PCR should be tested no more than 7 days after sampling.

The direct detection of *T. equigenitalis* by real-time PCR has several advantages over isolation of the bacteria by culture. First, PCR is less vulnerable to contaminating flora, which reduces the number of false-negative results. Secondly, the turnaround time of the PCR is much shorter than the minimum 7-day culture time with isolation. Thirdly, as only DNA is detected rather than viable organisms, the need for rapid transport of specimens to the laboratory is reduced. Fourthly these PCRs also differentiate *T. equigenitalis* from *T. asinigenitalis*. A strict PCR regime to avoid DNA cross contamination should be deployed in diagnostic laboratories.

1.5.2. Other PCRs

Other conventional and real-time PCRs have been developed and commercial PCR kits are available for the detection of *T. equigenitalis.* Before use in a diagnostic laboratory these must be fully validated to WOAH Standards (Chapter 1.1.6) as fit for use for defined purposes.

1.5.3. Sequencing-based methods

Sequencing-based methods, such as sequencing of the 16s rRNA gene, have been used to confirm the identification of *Taylorella* spp. (Erdman *et al*., 2011). Advances in whole genome sequencing have led to additional identification and typing tools as well as improved molecular characterisation of *Taylorella* spp. The multi-locus sequence typing (MLST) scheme has been used for typing of *Taylorella* spp. and defining clonal complexes that further describe genetic relationships between the sequence types (Duquesne *et al.,* 2013). Recent studies have also demonstrated the use of MLST, single nucleotide polymorphism analysis, and other genomic methods to characterise strains, evaluate detailed genetic relationships between isolates, and generate phylogenetic trees, which may prove useful in understanding transmission patterns and for epidemiological tracing (Duquesne *et al*., 2020; Hicks *et al.,* 2018,). New research has explored targeted amplification from semen and swabs for culture independent sequence typing using MLST (May *et al.,* 2019).

1.6. Use of agent identity tests

1.6.1. Testing post-treatment

After a horse has been treated to eliminate *T. equigenitalis*, either culture or PCR may be used to determine the continued presence of infection or carrier state. Detection by culture can be inhibited by carry-over of the antimicrobial agent in the swab onto the culture plate, sub-lethal damage to the organism preventing its growth *in vitro* or due to overgrowth by competing organisms. PCR detects the DNA of both viable and non-viable organism. If treatment does not involve adequate washing of debris from the anatomical sites used for swabbing, then DNA from the dead organism may persist. For these reasons it is common to test by either method at least 21 days after treatment. In most cases adequate treatment will result in negative tests using both methods. However if discordant results between culture and PCR are found, it is advisable to assume that infection may still be present and consider re-treatment to eliminate the organism.

1.6.2. Semen testing

Table 1 refers to the tests validated for use with genital swabs rather than semen. There are little available data on the relative diagnostic sensitivity of testing semen compared with genital swabs from the stallion (Al-Kass *et al.,* 2019; Erdman *et al.,* 2011). Semen testing is therefore usually used as an adjunct to rather than a substitute for swabbing the horse. There are limited data on the use of either culture or PCR in semen or the viability of the organism in transit. Published data indicate the organism can remain viable after freezing semen, and it can be readily recovered from fresh semen by culture. Its growth in culture is often completely inhibited by antibiotics in semen extender (Klein *et al.,* 2012; Olivieri *et al.,* 2011), but this is not always the case, and semen may remain infective in the presence of extender (Delerue *et al.,* 2019). Further data from a semen spiking study indicated that culture can be very sensitive if the semen is pure and fresh, but extended semen containing antibiotics showed a rapidly reduced detection level, and refrigerated storage of semen samples over many days resulted in poor detection of *T. equigenitalis* by culture for both pure and extended semen due to overgrowth with commensal organisms. A real-time PCR (Wakeley *et al.,* 2006) with a DNA extraction step used on the same samples was reasonably sensitive and not diminished by extender nor by storage. There is an inherent limit on the analytical sensitivity of PCR due to the quantity of semen that is used in the DNA extraction and reaction steps, but it is not known if this limits the diagnostic sensitivity in carrier horses as there are little data on expected viable cell counts of *T. equigenitalis* in semen from infected horses. A study using real-time PCR on frozen extended semen samples from a naturally infected horse indicated that testing multiple aliquots from each ejaculate may enhance the sensitivity in detecting whether the donor horse was infected (Schulman *et al.,* 2016). The sensitivity of culture from frozen extended semen has not been widely reported but the limitations of culture for fresh extended semen at least apply to frozen samples. Aliquots of semen and swabs dipped in semen have both been used as samples for culture and PCR, and the same conditions for transit are applied as for horse swab samples.

2. Serological tests

No serological test described to date will, by itself, reliably detect infection for diagnosis and control. However, the complement fixation test has been used successfully as an adjunct to culture for *T. equigenitalis* in screening mares between 21 and 45 days after being bred to a suspect carrier stallion.

C. REQUIREMENTS FOR VACCINES

Effective vaccines that protect against contagious equine metritis or prevent colonisation by *T. equigenitalis* are currently unavailable.

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NB: THERE ARE WOAH REFERENCE LABORATORIES FOR CONTAGIOUS EQUINE METRITIS (PLEASE CONSULT THE WOAH WEB SITE FOR THE MOST UP-TO-DATE LIST: HTTPS://WWW.WOAH.ORG/EN/WHAT-WE-OFFER/EXPERTISE-NETWORK/REFERENCE-LABORATORIES/#UI-ID-3). PLEASE CONTACT WOAH REFERENCE LABORATORIES FOR ANY FURTHER INFORMATION ON DIAGNOSTIC TESTS, REAGENTS AND VACCINES FOR CONTAGIOUS EQUINE METRITIS

NB: FIRST ADOPTED IN 1990. MOST RECENT UPDATES ADOPTED IN 2022.