CHAPTER 3.5.2.

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 nonsystemic 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.*

Identification of the agent: *Swabs should be taken from designated genital sites. To avoid loss of viability, 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. Therefore additional detection assays such as polymerase chain reaction and 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 cultural examination.*

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 asymptomatic.

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 or vaccination 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 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, nonmotile, 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) 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 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 easily 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
Agent identification						
Bacterial isolation and identification	+++	+++	+++	+++	+++	n/a
IFAT	+	+	+	+	+	n/a
Real-time PCR	++	++	++	++	++	n/a
Detection of immune response						
CFT	–	+	+	-	+	-

Key: +++ = recommended method, validated for the purpose shown; ++ = suitable method but may need further validation;
+ = may be used in some situations, but cost, reliability, or other factors severely limits its application;
– = not appropriate for this purpose; n/a = purpose not applicable.
IFAT = indirect fluorescent antibody test; PCR = polymerase chain reaction; CFT = complement fixation test.

1. 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 positive, it should be tested for reactivity with *T.-equigenitalis*-specific antiserum.

1.2. Serotyping methods

A variety of serotyping tests have 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. *Principles and methods of validation of diagnostic assays for infectious diseases*. Kits should preferably be selected from those listed on the OIE Register (http://www.oie.int/en/scientific-expertise/registration-of-diagnostic-kits/background-information/).

1.4. 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 (from cultures grown from swabs). In studies carried out in the UK the PCR was shown to be highly specific and was able to detect very small numbers of *T. equigenitalis* in the presence of very large numbers of bacteria comprising the background flora harvested from culture plates inoculated with samples from the equine urogenital tract. 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.,* 2002; Moore *et al.,* 2001). A real-time PCR was developed in the UK for use directly on genital swabs and compared with culture (Wakeley *et al.,* 2006) and this has been subsequently used for pre-breeding screening studies (Ousey *et al.,* 2009). There was no significant difference in the performance of the direct PCR and culture, but the PCR had the added advantage of speed of result and also differentiated *T.* equigenitalis from *T. asinigenitalis*. Commercial PCR kits are available for the detection of *T. equigenitalis* and these may be used to enhance the testing capabilities of authorised laboratories. An overview of the primers and probes mentioned in publications on PCR detection of *T. equigenitalis* is given in Table 2.

1 Obtainable from the National Collection of Type Cultures, Colindale, London, UK.

Table 2. *Primer sequences for use in PCR tests*

Primer 1 (forward)		Primer 2 (reverse, sequence from 5' to 3')		Probe (only for real-time PCRs)		Reference
Name	Sequence from 5' to 3'	Name	Sequence from 5' to 3'	Name	Sequence from 5' to 3'	
Tay377for	CCG-CGT-GTG-CGA-TTG-A	Tay488rev	TTT-GCC-GGT-GCT-TAT-TCT-TCA	Tequi FAM-probe	6FAM-AAA-GGT-TTG-TGT-TAA-TAC-CAT-GGA-CTG-CTG-ACG-G-BHQ1	Wakeley *et al.*, 2006
CEM1 mod	GCA-GCA-TAA-GGA-GAG-CTT-GCT	CEM2 mod	GCT-CGA-CAG-TTA-GAA-ATG	CEM–FLU	GTA-AAA-GGT-CAT-CTC-TGA-TCC	Premanandh *et al.,* 2003
				CEM-LCR 640	CCT-CAG-GGC-GTA-TGC-GGT-ATT-AGC	
Primer 1	CAG-CAT-AAG-GAG-AGC-TTG-CTT-TTC-T	Primer 2	CTC-GAC-AGT-TAG-AAA-TGC-AGT	Probe	TCA-GAG-ATG-ACC-TTT-TAC-TA	Bleumink-Pluym *et al.,* 1994
FORW	AGG-TTT-GTG-TTA-ATA-CCA-TGG-ACT-G	REV	CAG-TCT-CAT-TAG-AGT-GCC-CAT-CTT-ACT-TG			Buckley *et al.*, 2005
Te1	CAG-CAT-AAG-GAG-AGC-TTG-CTT-TTC-T	Te2	GTC-CAT-GGT-ATT-AAC-ACA-AAC			Duquesne *et al*., 2007
TEQF	GGT-TTG-TGT-TAA-TAC-CAT-GGA-C	TEQR	TCG-CTA-CCA-AGA-CCC-G			Arata *et al.,* 2001

The direct detection of *T. equigenitalis* by 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. And thirdly, as only DNA is detected rather than viable organisms, the need for rapid transport of specimens to the laboratory is reduced. A strict PCR regime to avoid DNA cross contamination should be deployed in diagnostic laboratories.

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), Current advances in whole genome sequencing of *Taylorella* spp. could lead to improved identification, characterisation, and genotyping methods.

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 OIE Reference Laboratories for Contagious equine metritis
(see Table in Part 4 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list:
http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE 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 2018.