CHAPTER 2.5.2.

CONTAGIOUS EQUINE METRITIS

SUMMARY

Definition of the 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: *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 and may take up to 14 days, but usually does not take longer than 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 and test-breeding of stallions for detection of the carrier state has been used as a valuable adjunct to cultural examination.*

Another species of Taylorella, T. asinigenitalis, *has been isolated from male donkeys and horse mares and stallions in the United States of America and a number of European countries. This bacterium has not been associated with naturally occurring disease; it has been detected in the genital tract of male donkeys and would appear to be passed to other donkeys and horses during mating.*

Serological tests: *Serology is of value in 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

Contagious equine metritis was first described in the United Kingdom (UK) in 1977 (Crowhurst, 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. The fastidious growth characteristics and slow growth of the causative bacterium, *Taylorella equigenitalis*, caused difficulties in initial attempts at culture (Platt *et al*., 1977), but the disease was reproduced by experimental challenge with isolated laboratory-grown bacteria (Platt *et al.,* 1978; Swaney & Sahu 1978). Using appropriate culture conditions, *T. equigenitalis* can be isolated from infective vaginal discharge. Mares may experience more than one episode of the disease in a short period of time (Timoney *et al.,* 1977; 1979). Serum antibody persists for 3–7 weeks after infection, but often it is not detectable for up to 15–21 days after recovery from acute infection in the mare (Dawson *et al.,* 1978). Most mares recover uneventfully, but some may become carriers of *T. equigenitalis* for many months (Platt *et al.,* 1978). Colonisation by *T. equigenitalis* in the mare is most consistently demonstrated by swabbing the clitoral fossa and recesses of the clitoral sinuses; it may be recovered from the cervix and endometrium in pure culture (Platt *et al.,* 1978). Sampling of the clitoral sinuses should be done with mini-tipped swabs, not the standard type of bacteriological swab. Carriage does not always adversely affect conception (Timoney *et al.,* 1978), and in such cases pregnancy may proceed to term. The offspring of such pregnancies may become contaminated during passage through the birth canal, and such foals may become long-term subclinical carriers (Timoney & Powell, 1982). Many primary cases of infection with *T. equigenitalis* in the mare are subclinical, and a frequent indicator of infection is a mare returning in oestrus prematurely after being bred to a putative carrier stallion.

Carrier mares and stallions act as reservoirs of *T. equigenitalis*, but stallions, because they mate with numerous mares, play a much more important role in dissemination of the bacterium. The urogenital membranes of stallions become contaminated at coitus, leading to a carrier state that may persist for many months or years (Schluter *et al.,* 1991). Failure to observe appropriate hygienic measures when breeding mares and stallions may also spread the organism. Other sites of the horse's body are not known to harbour *T. equigenitalis*. Most carrier mares are clitoral carriers of *T. equigenitalis*. Long-term persistence of the organism in the uterus, though uncommon, can occur. To detect such carriers of *T. equigenitalis,* an endometrial or deep cervical swab sample should be taken routinely in addition to sampling the clitoral area of all mares. Abortion in the mare due to *T. equigenitalis* is a very rare occurrence.

Another species of *Taylorella*, *T. asinigenitalis* (Jang *et al.,* 2001) has been isolated from male donkeys and horse mares and stallions in the United States of America (Katz *et al.,* 2000; Meade *et al.,* 2010)*,* and a number of European countries (Baverud *et al.,* 2006; Breuil *et al.,* 2011; Franco *et al.,* 2009). This bacterium, which has not been associated with naturally occurring disease, resides in the genital tract of male donkeys and can be passed to donkeys and horses during mating.

Taylorella equigenitalis is not known to infect humans and it should be handled in the laboratory at biosafety level 2.

- **Disease control**

Prior infection or vaccination are not fully protective (Fernie *et al.,* 1980; Timoney *et al*., 1979), and failure of antibody to persist has meant that control of infection has relied entirely on prevention of transmission through the detection of *T. equigenitalis* on swabs of the reproductive tract of stallions and mares. In spite of difficulties in culturing *T. equigenitalis*, screening mares and stallions prior to and while on the stud farm has successfully eliminated the disease from thoroughbred horses in countries using a voluntary code of practice. These have been based on the widely adopted UK's Horserace Betting Levy Board's Code of Practice (www.hblb.org.uk/codes.htm), which is reviewed and updated annually and will reflect current information. The key recommendations of that Code of Practice are summarised below.

At the start of the breeding season, swabs are taken from all stallions, including those in their first breeding season, from the urethra, urethral fossa and sinus, penile sheath and pre-ejaculatory fluid, on two occasions no fewer than 7 days apart. Mares are classified according to the degree of risk that they represent, and the frequency of sampling is adjusted accordingly. High-risk mares are defined in the Code of Practice as: (a) those from which *T. equigenitalis* has been isolated (the high risk status will remain until three sets of negative swabs have been taken at three different oestrous periods in each of 2 years); (b) mares that have visited any premises on which *T. equigenitalis* has been isolated within the previous 12 months; (c) mares arriving from countries currently listed in the Code of Practice as higher risk; d) all mares that have been in countries that are currently listed in the Code of Practice as higher risk within the last 12 months.

High risk stallions are defined as: (a) stallions that have not previously been used for breeding purposes; (b) stallions from which *T equigenitalis* has been isolated (the 'high risk' status will remain until treatment has been undertaken and required swab results are negative); (c) stallions that have been , on any premises on which *T equigenitalis* has been isolated within the last 12 months; (d) stallions that have mated a mare which has not been swabbed negative in accordance with the Code of Practice. 'Low risk' stallions are any stallions not defined as 'high risk'. There is a potentially serious problem with contagious equine metritis in this group of horses, especially those belonging to non-thoroughbred breeds.

Results of laboratory tests for *T. equigenitalis* should be entered on an officially approved certificate, which is sent to the veterinarians and stallion stud farm managers who supervise the breeding. The certificate should record the animal's name, the sites and date of swabbing, the name of the veterinarian taking the swabs, identity of the testing laboratory, the date the swabs were received and cultured by the laboratory, and whether the swabs were negative or positive, or whether the culture was overgrown by other bacteria to an extent that the laboratory could not be confident that small numbers of *T. equigenitalis* would be detected and, therefore, requiring another set of swabs to be collected.

Difficulties with the culture of *T. equigenitalis* due to its fastidious nature necessitate the use of a quality control system that should be approved before a laboratory is permitted to undertake official testing for contagious equine metritis and to issue certificates of the test results. The task of quality control should be undertaken by an experienced, reliable, and impartial microbiology laboratory authorised for the purpose.

Any mares with an abnormal vaginal exudate, or returning to oestrus prematurely, should be investigated and managed as though infected with *T. equigenitalis* until results of laboratory testing prove otherwise. Other causes of endometritis include *Pseudomonas aeruginosa*, *Streptococcus zooepidemicus* and certain capsule types of *Klebsiella pneumoniae*. Swabs should be examined for these bacteria, and an attempt made to culture and identify *K. pneumoniae* and *P. aeruginosa* so as to establish a differential diagnosis.

If carriers of *T. equigenitalis* are detected, the organism can be eliminated by treatment with systemic and/or local antibiotics combined with antiseptic washing of the sites of persistence in the mare and the stallion. Particular attention should be paid to the clitoral fossa and cleansing the recesses of the clitoral sinuses of mares, where colonisation by *T. equigenitalis* is frequently found in carrier animals. A course of treatment may take several weeks and may need to be repeated before swabbing consistently fails to recover *T. equigenitalis* from the treated stallion or mares (Crowhurst *et al.,* 1979). A variable number of carrier mares can be refractive to several courses of treatment. These may require surgery and ablation of the clitoral sinuses for permanent elimination of the carrier state in such animals.

Control measures for countries regarded as free from *T. equigenitalis* infection should be based on the screening of animals prior to importation and/or during a post-importation quarantine period using swabbing and testing regimes broadly based on those described above for breeding populations.

B. DIAGNOSTIC TECHNIQUES

1.   Identification of the agent (the prescribed test for international trade)

Various bacteria may be present on the urogenital membranes of horses as harmless commensals and may interfere with the culture of *T. equigenitalis*. Some may initially be present in small numbers, but multiply on the swab before it is cultured depending on the ambient temperature the swab is exposed to in transit to the testing laboratory. Overgrowth of these organisms on the culture plates may obscure the presence of *T. equigenitalis*. 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. Negative culture results from swabs plated out more than 48 hours after they were taken are unreliable. Antibiotic treatment for whatever cause should cease at least 7 days (systemic treatment) or 21 days (local treatment) before swabbing. The presence of antibiotics may sublethally damage *T. equigenitalis,* which nonetheless persists on the urogenital membranes but cannot be grown on laboratory media.

Each swab must be inoculated on to 5% (v/v) heated blood, ('chocolate'), agar plates, produced by heating the liquid medium, containing blood, at 70–80°C for 12 minutes. When cooled to 45–50°C, trimethoprim (1 μg/ml), clindamycin (5 μg/ml), and amphotericin B (5–15 μg/ml), is added to the medium (Timoney *et al.,* 1982). Thymidine, which will inactivate trimethoprim, is present in bacteriological media containing peptone, so it is important to add 5% lysed horse blood at this stage. 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;* this medium has been used successfully to isolate equally well both streptomycin resistant and sensitive biotypes of the pathogen and to 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 plates of 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). While *T. equigenitalis* can be cultivated on blood agar, it will grow better on 'chocolate' blood agar as described above.

Some manufacturers[1] produce a peptone agar base that is quality controlled for its ability to support the growth of *T. equigenitalis*. The quality of the commercial agar should be confirmed by the testing laboratory. An important feature of all good *T. equigenitalis* media is the absence of fermentable carbohydrates. These do not enhance the growth of *T. equigenitalis*, but their fermentation by other bacteria inhibits *T. equigenitalis* growth (Atherton, 1983; Fernie *et al.,* 1980). A third medium containing streptomycin sulphate (200 µg/ml) is sometimes used as some isolates of *T. equigenitalis* are resistant to this concentration of antibiotic, which serves to reduce the extent of growth of other bacteria that might otherwise obscure the presence of small numbers of *T. equigenitalis* (Swerczek, 1978). However, a streptomycin-sensitive biotype is now the most common strain isolated and will not be detected on this medium; consequently, it should only be used in conjunction with medium without streptomycin. Growth by other bacteria, for example *Proteus mirabilis*, however, may be so extensive that the laboratory should report that they cannot issue a negative result. In this event, further swabs should be requested in the hope that the problem will not recur.

Occasionally, the urogenital membranes of stallions or mares will be persistently colonised by another bacterium that interferes with detection of *T. equigenitalis.* It may be necessary to attempt to eliminate this by washing and antibiotic treatment. Swabbing for *T. equigenitalis* shall not recommence until at least 7 days (systemic treatment) or 21 days (local treatment) after treatment has stopped. The use of Timoney's medium (Timoney *et al.,* 1982), described above, should overcome this difficulty in most cases

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.

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.

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 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.

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. 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.

A variety of serotyping tests have been developed 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). It has been suggested that immunofluorescence can be used to identify autoagglutinating isolates, some workers have reported cross-reaction with *Mannheimia haemolytica* but this is very rare. If a cross-reaction is suspected, it may be necessary to repeat the test using adsorbed antisera (Ter Laak & Wagenaars, 1990). The specificity of the immunofluorescence test can be improved by the use of monoclonal antibodies that are now available. Nevertheless polyclonal and monoclonal antibodies can be used in the differentiation of *T. equigenitalis* and *T. asinigenitalis* (Breuil *et al.,* 2010) [2].

Antiserum is produced by vaccinating rabbits with killed *T. equigenitalis*. A number of different immunisation regimes can be employed, ranging from those used for producing *Escherichia-coli*-typing antisera to immunisation together with an adjuvant, such as Freund's incomplete. Monoclonal antibodies are available commercially that

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1   For example, Mast Diagnostics, Mast House, Derby Road, Bootle, Merseyside L20 1EA, United Kingdom (UK), and Lab M, Tomley House, Wash Lane, Bury BL9 6AU, UK.
2   Institut Pourquier, 326 rue de la Galera, Parc Euromedicine, 34090 Montpelier, France. E-mail: info@institut-pourquier.fr

provide a highly specific means of identifying *T. equigenitalis*. A standard strain, such as NCTC 11184[3], 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, even if rarely. 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. Differentation of *T. asinigenitalis* from *T. equigenitalis* is possible using the polymerase chain reaction (PCR) or 16S rDNA sequencing and biochemical reactivity (Baverud *et al.,* 2006; Breuil *et al.,* 2011; Duquesne *et al.,* 2007; Wakeley *et al.,* 2006).

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*.

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) (Bleumink-Pluym *et al.,* 1994). 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.

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 not yet available.

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

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NB: There are OIE Reference Laboratories for Contagious equine metritis
(see Table in Part 3 of this *Terrestrial Manual* or consult the OIE Web site for the most up-to-date list:
http://www.oie.int/en/our-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