

## CONTAGIOUS EQUINE METRITIS

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### SUMMARY

*Contagious equine metritis is an inflammation of the endometrium of mares 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.*

*If present, the chief clinical signs are a slight to copious mucopurulent vaginal discharge and a variable cervicitis and vaginitis. Recovery is uneventful, but prolonged asymptomatic carriage is established in a proportion of infected mares. Taylorella equigenitalis is most frequently transmitted by sexual contact with carrier stallions, which are always asymptomatic and in which the principal sites of T. equigenitalis colonisation are the urogenital membranes (urethral fossa, urethral sinus, urethra and penile sheath). Inadequate hygiene during the cleansing or examination of the genitalia of horses can also be responsible for the transmission of infection. The sites of persistence of T. equigenitalis in the mare are urogenital membranes, principally in the clitoral sinuses and fossa and very infrequently in the uterus. Foals born of carrier mares may also become carriers. The organism can infect equid species other than horses, e.g. donkeys.*

*Washing with disinfectants combined with local and systemic antibiotic treatment can eliminate T. equigenitalis. Vaccination has been found to be ineffective. The principal means of control is through preventing transmission by establishing that stallions and mares are free from T. equigenitalis before breeding commences. Determination of the carrier state depends on the detection of T. equigenitalis on urogenital swabs of mares and stallions and its accurate identification. Serum antibody to T. equigenitalis can be detected in mares for 3–7 weeks after infection and can also be demonstrated in the occasional carrier mare, but never in the stallion. Serology is of value in detecting recent, but not chronic, infection in the mare, but the emphasis for control of the disease should be on the detection of carriers by culture.*

**Identification of the agent:** *Swabs must be transported to the laboratory with precautions to avoid loss of viability. The swab should be fully submerged in Amies charcoal medium and transported to the testing laboratory, preferably in temperature-controlled conditions, for plating out within 48 hours of collection. Growth of T. equigenitalis is likely to take at least 72 hours and may take up to 14 days, but usually does not take longer than 6 days at 37°C on medium enriched with heated blood and an atmosphere of 5–10% CO<sub>2</sub>. An incubation time of at least 7 days is advisable before certifying cultures negative for T. equigenitalis. After 72 hours under appropriate culture conditions colonies may be small – up to 2–3 mm in diameter – watery to opaque and yellowish grey, and are smooth with an entire edge. Taylorella equigenitalis is a Gram-negative, small, coccoid rod that is sometimes pleomorphic and exhibits bipolar staining. It produces catalase and phosphatase, and is strongly oxidase positive. It is otherwise unreactive biochemically, and identification is finally dependent on antigenic characterisation of an isolate using specific antibodies. 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.*

*Recently another species of Taylorella, T. asinigenitalis, has been isolated from male donkeys and horse stallions in the United States of America and from stallions in Europe. This bacterium has not been associated with naturally occurring disease; it resides in the genital tract of male donkeys and can be passed to other donkeys and horses during mating.*

*Antibody to whole killed T. equigenitalis and a latex agglutination kit employing these antibodies may be used. Specificity for the organism and evidence of its failure to react with other oxidase*

positive/catalase positive, Gram-negative bacteria that might be cultured from the urogenital tract of horses is essential. Monoclonal antibodies have been developed that can be used successfully to identify *T. equigenitalis* and distinguish it from strains of *T. asinigenitalis*.

**Serological tests:** No serological test described to date will, by itself, reliably detect infection for diagnosis and control purposes. However, 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 and diagnostic biologicals:** Effective vaccines that protect against contagious equine metritis or prevent colonisation by *T. equigenitalis* are not yet available.

## A. INTRODUCTION

Contagious equine metritis was first described in the United Kingdom (UK) in 1977 (12), 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 nature and slow growth of the causative bacterium, *Taylorella equigenitalis*, caused difficulties in initial attempts at culture (22), but the disease was reproduced by experimental clitoral challenge with isolated laboratory-grown bacteria (21, 23, 27). Using appropriate culture conditions, *T. equigenitalis* can be isolated from infective vaginal discharge. Mares may encounter more than one episode of the disease in a short period of time (32). 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 mares (14). Most mares recover uneventfully, but some may become carriers of *T. equigenitalis* for many months (21). Colonisation by *T. equigenitalis* is most consistently demonstrated by culture of swabs taken from the recesses of the clitoral fossa and sinuses, but it may be recovered from the cervix and endometrium in pure culture (21). Carriage does not always affect conception (33), and in such cases pregnancy may proceed so that foals are born, become infected by passage down the birth canal, and thereby also become long-term and subclinical carriers (30). 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 transmission 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 (25). Unhygienic examination of mares and unsanitary washing of the stallion's penis 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*, cervical or endometrial swab samples should be taken routinely in addition to sampling the clitoral area of all mares. *Taylorella equigenitalis* can cause abortion in the mare but this is a rare occurrence.

## B. DIAGNOSTIC TECHNIQUES

Prior infection and vaccination are not fully protective (15), 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 urogenital membranes. 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 (16), which is reviewed annually and updated as necessary; the key recommendations of the Code 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 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 Canada, France, Germany, Ireland, Italy, the UK and the USA that have been mated during the last breeding season with stallions resident outside these countries; (d) all mares that have been in countries other than Canada, France, Germany, Ireland, Italy, the UK and the USA within the last 12 months. 'Low risk' mares are any mares not defined as 'high risk'.

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, within the last 12 months, on any premises on which *T. equigenitalis* has been isolated; (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* caused by 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, which is not involved in routine testing of contagious equine metritis swabs. At 6-month intervals, swabs inoculated into mixed cultures that are designed to test a laboratory's ability to recover and identify *T. equigenitalis* in the face of contaminants, as well as procedures for reporting results, should be sent to laboratories wishing to be approved for testing for this bacterium. A list of those laboratories satisfactorily passing the quality control should be published in a veterinary journal that is widely read by the national veterinarians. The veterinarians and stallion stud farm managers who supervise the breeding mares and stallions should accept only certificates provided by laboratories currently approved for that purpose.

Any mares with 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 outbreaks of endometritis include *Pseudomonas aeruginosa*, *Streptococcus zooepidemicus* and certain capsule types of *Klebsiella pneumoniae*. Swabs should be examined for these bacteria, and an attempt should be 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 antibiotics combined with disinfectant washing of exposed genital membranes (1). Particular attention should be paid to cleansing of the recesses of the clitoral fossa and 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 intensive swabbing consistently fails to recover *T. equigenitalis* in stallions and mares (13). A significant 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.

## 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. 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 (28). Over time, numbers of *T. equigenitalis* decline on swabs with time, and this effect is more pronounced at higher temperatures (24). Swabs must be kept cool during transportation and should arrive at the laboratory no later than 24–48 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 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 µg/ml), is added to the medium, Timoney *et al.* (31). 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 biotypes of this pathogen and to eliminate the growth of many commensal bacteria. As inhibitors 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 µg/ml amphotericin B). *Taylorella equigenitalis* will grow on blood agar, but it can tolerate less than ideal conditions better when grown on 'chocolate' blood agar as described above. Some manufacturers<sup>1</sup> 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 (4, 15). 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* (28). 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 record that they cannot issue a negative result. In this event, further swabs should be requested in the hope that the problem will not recur.

All culture media prepared should be subjected to quality control and must support growth of the suspect organism from a small inoculum, 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 serves as a valuable adjunct to cultural examination. The numbers of *Taylorella* mechanically carried by stallions can be very low and may be missed by culturing swabs, but can be detected after multiplication in the mare that has been test bred. The use of test breeding as a diagnostic tool can be especially important in countries that are considered free from contagious equine metritis.

Occasionally, the urogenital membranes of stallions or mares will be persistently colonised by another bacterium that interferes with diagnosis, and it will prove necessary to attempt to eliminate this by washing and antibiotic treatment. Swabbing for *T. equigenitalis* shall not recommence until at least 7 days after treatment has stopped. The use of Timoney's medium (31), described above, should overcome this difficulty in most cases. It is important to emphasise that alongside each day's tests, additional plates should be inoculated with a culture of *T. equigenitalis* to check that each batch of medium will support growth.

Plates must be incubated at 35–37°C in 5–10% (v/v) CO<sub>2</sub> 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. Visual detection of colonies may take up to 14 days (35). 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 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 (see ref. 5 for methods for examining catalase, phosphatase and oxidase activities). 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<sub>2</sub> in air, as opposed to in a candle jar, may reduce autoagglutination (29). 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 (29). The immunofluorescence test can be improved by the use of monoclonal antibodies, which are now available<sup>2</sup>.

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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 Euromedecine, 34090 Montpellier, France. E-mail: info@institut-pourquier.fr

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 (26), to immunisation together with an adjuvant, such as Freund's incomplete. Monoclonal antibodies are available commercially that provide a highly specific means of identifying *T. equigenitalis*. A standard strain, such as NCTC 11184<sup>3</sup>, 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 ref. 9), and *Pseudomonas aeruginosa*. Recently another species of *Taylorella*, *T. asinigenitalis*, has been isolated from male donkeys in the United States of America (17) and from a horse stallion in Europe (6). This newly described 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. Moreover it 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. At the National Veterinary Services Laboratories (NVSL) in Ames, Iowa, USA<sup>4</sup>, and at the Veterinary Laboratories Agency (VLA), Bury St Edmunds, United Kingdom<sup>4</sup>, differentiation of *T. asinigenitalis* from *T. equigenitalis* is possible using the polymerase chain reaction (PCR).

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*.

A PCR method has been used for detecting *T. equigenitalis* and was compared with culture methods in the Netherlands and Japan (2, 7, 10). In these studies, a much higher rate of detection by PCR was found than by culture, even among horses imported from a source without previous evidence of *T. equigenitalis* infection or clinical disease. The authors proposed that carriage is more widespread than previously believed, and that recently discovered genetic variation among strains (8, 18) may relate to differences in pathogenicity. The PCR has also been used in the UK (11). It was 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 of the equine urogenital tract. Recently in Japan, the field application of the PCR in the eradication of contagious equine metritis was evaluated. It was demonstrated that the PCR was more sensitive than culture for the detection of *T. equigenitalis* from genital swabs of horses in the field (2, 3, 19, 20). In 2004–2005, a real-time PCR was developed in the UK for use directly on genital swabs and compared with culture (34). 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*. This promising technique needs to be more fully and widely evaluated, especially for detection of the carrier state in the stallion.

## 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 suspect a carrier stallion.

### C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS

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.

4 For further information contact the NVSL at NVSL, P.O. Box 844, Ames, Iowa 50010, USA. E-mail: NVSL\_Concerns@aphis.usda.gov or the VLA at VLA, Rougham Hill, Bury St Edmunds, IP33 2RX, UK. E-mail: bury-st-edmunds@vla.defra.gsi.gov.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: [www.oie.int](http://www.oie.int)).