Haemophilus equigenitalis: The Agent of CEM

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The Thoroughbred industry became threatened by a new genital infection in 1977. This new disease, contagious equine metritis (CEM), was first reported during the 1977 breeding season in England.[2] Shortly thereafter, the disease was reported in Ireland,[9] Australia, and France as well.[6] Cases of the disease had been seen in Ireland in 1976 but apparently had not been reported.[8]

The United States was added to the list of countries reporting CEM in February of 1978.[18] The disease was apparently brought into the United States by two stallions which had been imported from France late in 1977.[18] The outbreak resulted in a two-week moratorium on breeding in Kentucky.[18] At least 45 Thoroughbred mares and five stallions were involved in the outbreak.[18]

The infection is characterized by an endometritis associated with an inflammation of the cervix and vagina. A copious, mucopurulent discharge normally occurs three to five days after the mare is covered.[2,15] The secretions may be seen as a vulval discharge or may pool on the floor of the vagina.[2] The exudate, which appears to originate in the uterus, is opaque, nonodorous, and usually tenacious.[10] It may be seen as early as 24 hours and may last as long as 18 days, with cervicitis persisting for longer periods.[8,10]

Postmortem findings of several pony mares with artifically induced CEM indicated enlarged uteri with greyish mucopurulent fluid[15] and widespread endometritis with focal destruction of the endometrial epithelium.[12] No evidence of an inflammatory reaction was seen in the ovaries, uterine tube, perimetrium, or bladder.[12] No other signs of systemic involvement were observed.

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The histopathological changes associated with CEM include proliferation of the luminal epithelial cells as early as two days after infection.[14,15] Intercellular vacuolation of the basal region of the luminal epithelium also occurs at this time and may persist for up to 37 days.[14,15] Stromal neutrophilic leukocyte infiltrations are very marked at 24 hours, but by 48 hours the predominant cellular infiltration of the stroma is mononuclear cells, including many plasma cells.[14]

Characteristically, mares affected with CEM return to estrus (approximately 3-12 days) after a shortened diestrous period.[2,18,21]

The degree to which stallions are affected by the CEM organism is unknown, but they do not have clinical signs of disease.[4,18] Stallions function as carriers of the disease.[18]

Spread of the disease is primarily by venereal transmission but the disease is extremely contagious and can be spread by other means as well.[4,2] Transmission is also possible by people who wash the genitalia of infected mares and by contaminated specula and instruments used to examine mares.[10]

It appears that CEM may also be spread in the breeding shed from stallion to stallion by handlers as well as from contaminated floors.[18] The teaser stallion's muzzle may also be a route of transmission.[5]

Contagious equine metritis has been experimentally induced by both vaginal exudate and cultures of the organism.[15,12] Pony stallions have been infected by introduction of pure cultures of the organism into the urethral fossa on swabs. The organism survives for varying periods of time without giving any signs of the infection.[11] An experimentally infected stallion has transmitted the infection to a healthy mare by coitus.[11]

Smears made of the vaginal and cervical discharge from mares with CEM reveal a Gram-negative coccobacillus bacterium.[12]

Conventional aerobic culture of these cervical swabs do not yield a recognizable pathogen,[20] and additional cultural methods need to be employed.

The organism is fastidious, growing satisfactorily in microaerophilic conditions of 90% H_2 and 10% V/V CO_2 or 5-10 percent carbon dioxide in air. A high degree of humidity does not appear to improve growth. Growth is observed over the temperature range of 30°C to 41°C; with an optimum at 37°C.[20]

Optimum growth of the CEM organism is seen on chocolate agar plates consisting of Eugonagar base with 10% horse blood,[18] and chocolate columbia blood agar.[20] Other media which support varying degrees of growth include unheated blood agar, serum agar, starch agar, casein agar, egg yolk (LV) agar, and Casman's blood agar.[20]

The CEM colonies from field cases grown in Eugonagar and 10% horse blood, are not observed until 48 hours and at that time as tiny pinpoint colonies (1 mm in diameter), that are shiny, smooth, and slightly gray.[18] At 72 hours and beyond, the colonies appear white to gray, raised, shiny with elevated, slightly opaque centers. The colonies have a wax-like appearance and can be moved freely with a loop over the agar surface.[18]

Colonies from horses with active infections of CEM will usually be visible within 48 hours, whereas those from carrier horses, when the organism has become dormant, may take several days to grow.[18] In one report, growth was not seen until day 15.[18] Differences in morphology may be observed in isolates of organisms made from mares when discharging and when clinically normal. Positive cultures from the latter category tend to show a greater degree of pleomorphism with bacillary as well as coccobacillary forms present.[23]

It is now known that the two stallions imported into the United States from France were carrying different strains of the organism.[18] One strain is streptomycin sensitive and the other is streptomycin resistant.[18] Isolates from Ireland and England show only the streptomycin resistant strain. This fact suggests that it is essential to use two different culture media, one with antibiotics and one without.[18]

The bacteria, isolated from pure cultures, are Gram-negative, non-acid fast, non-motile, short rods with occasional filaments 5-6 μm in length,[20] and no flagella.[12] It is suggested that the organism is not dependent upon X, V, or XV factors,[20] but on nutrient agar plates a distinct halo of growth is seen around X and XV discs.[17] Some stimulation by X factor is observed but the D-amino-laevulinic is positive which indicates lack of X dependency.[20]

In biochemical tests, the organism is rather unreactive and is positive only in catalase, cytochrome oxidase and phosphatase tests. Also, it appears that the bacterium is asaccharolytic in all media tested.[20] Tests for cellular enzymes by means of an APIZYM strip reveals the presence of acid and alkaline phosphatases, leucine aminopeptidase, phosphoamidase and esterase.[20] The DNA base composition estimated from the melting temperature is 36.1 percent GC.[20]

A compliment fixation test for antibody to the contagious equine metritis organism has been investigated.[3] Results show that non-specific titers up to 4/2 occur in a high proportion of horses not exposed to the CEM organism. Titers of 1/4 to 3/4 may be considered as inconclusive and titers of 4/4 or higher as specific and positive.[3]

Slide agglutination tests have been developed and appear to be very sensitive, accurate, and rapid.[18] In experimental mares positive reactions were first observed seven days post-infection. Titers reached a peak at 20 days post-infection and slowly declined thereafter.[3] A titer of 1:16 and higher appears to be positive evidence of CEM exposure.[3]

Table 1 shows the antimicrobial susceptibility of the streptomycin resistant strain of the CEM organism using the diffusion test and minimum inhibitory concentrations.[20] Chlorhexidine was effective in dilutions up to 1 in 125,000 W/V.[20]

Naming of the organism which causes contagious equine metritis caused difficulty because it is so unreactive in conventional biochemical tests. It was suggested that rather than create a new genus, containing only one species and defined on essentially negative test results, that it would be preferable to regard the causative organism of CEM as a new species in the genus Haemophilus.[20] The organism has been officially classified as *Haemophilius equigenitalis,* with two strains,

Table 1—Antimicrobial Susceptibility of the CEM Organism

Antimicrobial agent	Susceptibility by diffusion test	Minimum inhibitory concentration (mg/l)
Penicillin	S	<0.25
Ampicillin	S	0.5
Cephaloridine	S	NT
Carbenicillin	S	NT
Tetracycline	S	1
Erythromycin	S	<0.06
Clindamycin	R	16
Lincomycin	R	32
Gentamicin	S	0.25
Kanamycin	S	1
Neomycin	S	1
Streptomycin	R	>512
Amikacin	S	NT
Tobramycin	S	NT
Chloramphenicol	S	NT
Nalidixic acid	S	4
Nitrofurantoin	S	1
Polymixin B	S	0.25
Fusidic acid	S	NT
Sulphamethoxazole	R	32
Trimethoprim	R	4
Cotrimoxazole	I	NT
Metronidazole	R	NT

NT – not tested
R – resistant
S – susceptible
I – intermediate

one resistant and one sensitive to streptomycin.[1]

Cultural sites for the CEM organism may vary slightly depending upon whether or not the mare is showing clinical signs of the disease. In active cases of CEM the cervix or uterus is the ideal location to obtain specimens because the bacterium is present in large numbers and there are less contaminating bacteria.[18] In mares not showing clinical signs, specimens may be collected from the cervix or uterus and also the urethra, clitoral fossa and clitoral sinuses.[18] The CEM organism may be isolated from the clitoris up to 35 days longer than from the cervix and uterus in some cases.[13] In mares not showing clinical signs, specimens should be collected while the mares are in estrus, preferably during the first part of the heat period. The CEM organism appears to increase during this time while the contaminating bacteria seem to decrease.[18]

When stallions are being examined by bacteriologic culturing for the CEM organism, pre-ejaculatory fluid, urethra, urethral fossa, urethral sinuses, and sheath should be cultured.[18]

After swabs have been taken they should be placed in transport media as soon as possible. Stuart's[4] and Amies transport media have both been used successfully.[10] Amies transport medium appears to be superior in that the bacteria live longer. The transport media should be held at 4°C or kept on ice and transported as soon as possible.[18] The transport media may also be frozen and transported cold with dry ice. It is important not to add any inhibitors or antibiotic to the transport media.[18]

A variety of methods and techniques have been employed for the diagnosis of CEM. Bacteriological swabbing is currently the most effective means of diagnosis. Suspect colonies may be picked and the catalase test performed, and if positive, the oxidase test peformed. If both tests are positive a Gram stain is made and examined. If a small Gram-negative, coccobacillary organism with the morphological characteristics of the CEM organism is present, a positive laboratory diagnosis is made.[18] Compliment fixation and slide agglutination tests may also aid in the diagnosis of suspect organisms.

Biolumenescence may also be of value in the rapid diagnosis of CEM.[22] Approximately 80% of the discharges from bacteriologically confirmed cases of CEM emit an apple-green fluorescence at 254 nm and 356 nm. Specimens from mares with other infections i.e., streptococcal, klebsiella, coliform, etc., are not positive at either wavelength.[22]

Different approaches have been taken to the problem of treating contagious equine metritis and they have resulted in varying amounts of success. One treatment used for stallions which seems to be quite successful consists of once a day for five days washing the penis (while erected) and the sheath with chlorhexidine surgical scrub and then rinsing with water.[18] The penis and sheath are then coated with nitrofurazone ointment. The stallions are also treated parenterally with antibiotics.[18]

A suggested treatment for mares includes intrauterine irrigation with chlorhexidine, nitrofurazone, ampicillin or benzyl penicillin daily for three to five days, accompanied by parenteral treatment with ampicillin or penicillin.[4] Another treatment which has been used is twice daily treatment with ampicillin intramuscularly for 10 days, with no local treatment. Then, 10 days are allowed to elapse without treatment followed by 10 days with treatment.[9]

In some field cases of CEM infected mares, the disease appeared to be prolonged when

antibiotics were used, resulting in carrier mares, while other mares spontaneously recovered without antibiotic therapy.[19] Antibiotics destroy the normal flora of the reproductive tract of mares and stallions which are apparently helpful in discouraging the growth of pathogens.[19] Over 12 different bacteria from the reproductive tract of mares and stallions have been identified as causing various degrees of inhibition to the CEM organism.[19] Therefore, it has been suggested by some, that no antibiotics should be given until a suitable antimicrobial agent is found that will prevent the CEM organism without damaging the normal flora of the reproductive tract.

Since contagious equine metritis is a new and highly contagious disease with relatively little known about it, the key to its control is prevention. Prevention can best be brought about through management by compliance with an established set of guidelines. Such a guideline was set forth in England by a scientific committee of inquiry established by the Horserace Betting Levy Board.[4] The following are recommendations contained in the code of practice for the control of contagious equine metritis.

1) Examination of mares before the commencement of the breeding season
2) Examination of all mares arriving at studs for covering
3) Examination of stallions before the commencement of the breeding season
4) Sampling and Culturing Procedures
5) Gynecological Veterinary Examination of mare and stud hygiene
6) Treatment of infected mares
7) Treatment of infected stallions

The value of a guideline was voiced by the deputy chairman of the board, Mr. John Marriage when he said, "Strict compliance with the code is absolutely essential if the whole future of our breeding industry both domestically and internationally is not to be jeopardized."[7]

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