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VOLUME 2

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CHAPTER 136

BACTERIAL GENITAL SYSTEM INFECTIONS OF HORSES

Elcin GUNAYDIN, Gulsen GONCAGUL, Kemal YILMAZ

In this chapter, you can find helpful information based on etiology, transmission ways, clinical symptoms, recommendations of prevention and control strategies about venereal transmitted bacterial diseases caused by *Tylorella equigenitalis*, agent of Contagious Equine Metritis (CEM), *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. To help the breeders and owners of the horses in conjunction with their veterinarians is aimed in this chapter.

Contagious Equine Metritis (CEM)

CEM is an acute, highly contagious, venereal disease of horses. It is characterized by profuse, mucopurulent vaginal discharge. In most affected mares early return to oestrus and temporarily infertility are observed. Infected stallions and chronically infected mares show no clinical signs. The causative agent of CEM, *Tylorella equigenitalis* (*T. equigenitalis*) is gram negative, non-capsulated, non-spore microorganism. There are 2 biotypes of the agent: streptomycin resistant and streptomycin sensitive (OIE, 2012). It was first reported in the UK in 1977 (Crowhurst, 1977). The spread of the infection between the countries occurs by imported horses and sperm. Outbreaks of CEM lead to loosing export status of the country. In order to hamper the epidemic, breeders should pay attention to take preventive precautions and control its spread. If any suspected case occurs, breeders should inform the owners of the horses which are at probable risk of infection and the veterinary surgeons. Contact with the affected horses/premises causes spread of the infection. Early warning enables to take precautions in order to prevent further spread of the infection among the horses, implement control measures and treat the affected horses (OIE, 2012).

If a case is finalized as CEM, the mandatory requirements are emphasized below:

1. Samples are collected from affected premises, infected places; information is obtained from the breeders, owners and veterinary surgeons to find the source and the extent of the disease.
2. Movement of horse (mares and stallions of any breed of horse or pony), carcass or other related item is prohibited or controlled.
3. Breeding activities including natural mating, teasing, collection and insemination of the semen of the suspected horses in the same premises are banned.
4. Infected materials, equipment and articles are disinfected and destructed.
5. Vehicles and premises are cleaned and disinfected.

Klebsiella pneumonia (*K. pneumonia*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) Infections

The clinical signs and transmission of the infection caused by *Klebsiella pneumonia* and *Pseudomonas aeruginosa* are similar to CEM. In addition to reproductive system, the infections caused by *K. pneumonia* and *P. aeruginosa* are seen in bladder and urinary system as well. Isolation of *K. pneumonia* and/or *P. aeruginosa* from reproductive system is not notifiable by law. However, if any clinical symptoms are seen in stallions, it is proposed to inform the veterinarians.

K. pneumonia is an encapsulated, gram negative bacterium. Simply, *K. pneumonia* gains the ability of causing infection by the capsule. Capsule type of *K. pneumonia* determines whether the isolated *K. pneumonia* is a venereal pathogen or not. Since, most of *K. pneumonia* is not considered to be a venereal pathogen. Capsule type 1, 2, 5 are the venereal pathogens, sexually transmitted. Therefore, following *Klebsiella* isolation, it is necessary to define the capsule type.

All *Pseudomonas aeruginosa* isolates are considered to be potential venereal pathogens due to difficulty of differentiating venereal and other *P. aeruginosa* isolates. Breeders should not ignore *P. aeruginosa* infections because it is difficult to eradicate if it spreads to stallion's penis. If the stallion is infected by two agents, informing the national breeder association is proposed.

ETIOLOGY

Identification of the Agent

Harmless commensals exist on the urogenital membranes of horses may interfere with the culture of *T. equiganitalis*. Overgrowth of the commensals both on swabs taken from horses which transferred to the laboratory in inappropriate conditions inhibits the growth of *T. equiganitalis* on culture plates. In this context, transport medium with activated charcoal such as Amies Transport Medium to absorb inhibitory by-products to bacterial metabolism is preffered (Swerczek, 1978). Swabs must be transfferred to laboratory in cold chain and plated out at the laboratory no later than 48 hours they were taken (Sahu *et al*., 1979, Swerczek, 1978). In addition to this, systemic antibiotic treatment and local antibiotic cure must be ceased at least 7 days and 21 days, respectively before swabbing.

Chocolate agar plate containing 5% heated blood is preffered media for the isolation. Addition of trimethoprim (1 μg/ml), clindamycin (5 μg/ml), and amphotericin B (5-15 μg/ml) are proposed by Timoney *et al*. (Timoney *et al*., 1982). In bacteriological media containing peptone, thymidine which will inactivate trimethoprim is present. Lysed horse blood contains thymidine phosphorylase which will inactivate thymidine, thus allowing the trimethoprim to exert its selectivity. This medium is successfully used for the isolation of both streptomycin resistant and sensitive biotypes of the pathogen and to suppress the growth of many commensal bacteria and inhibit fungal growth (Timoney *et al*., 1982).

After swabs streaked onto agar plates, plates must be incubated at 35-37 °C in 5-10% CO_2 in air or by use of candle jar at 72 hours normally required before *T. equigenitalis* become visible. After 72 hours, if the colonies are not seen, daily inspection is needed till 14 days (Ward et al., 1984). Colonies of *T. equigenitalis*

are 2-3 mm in diameter, smooth with an entire edge, yellowish grey and glossy. *T. equigenitalis* is a Gram-negative, non-motile, bacillus or coco-bacillus and may exhibit bipolar staining. The biochemical characteristics of the agent is catalase positive, phosphatase positive and strongly oxidase positive, it should be tested for reactivity with *T. equigenitalis*-specific antiserum (OIE, 2012).

A variety of serotyping tests have been developed such as slide agglutination, indirect immunofluorescence. Slide agglutination test results with auto-agglutination (Ter Laak & Wagenaars, 1990). In order to overcome auto-agglutination, immunofluorescence can be used to solve the auto-agglutination problem. However, some workers reported cross-reaction with *Mannheimia haemolytica.* If any cross-reaction is suspected, it is recommended to retest with adsorbed antisera (Ter Laak & Wagenaars, 1990). Yet monoclonal antibodies are not available. Nevertheless polyclonal and monoclonal antibodies can be used in discrimination of *T. assinigenitalis* and *T. equigenitalis* (Breuil *et al.*, 2012).

The colonial appearance, cultural properties and biochemical test results of *T. assinigenitali*s and *T. equigenitalis* are very similar. There is even serological cross-reactivity between two organisms. For discrimination of two, Polymerase Chain Reaction (PCR) or 16S rDNA sequencing is a useful tool (Bavured *et al*., 2006, Breuil *et al*., 2011, Duquesne *et al*., 2007, Wakeley *et al*., 2006).

For the isolation of *K. pneumonia* and *P. aeruginosa, MacConkey agar and Pseudomonas agar are* proposed, respectively. After isolation procedure, pure cultures should be prepared from the suspected colonies and biochemical tests must be done. In order to discriminate whether the isolated *K. pneumonia* is venereal or not should be proven by capsule typing (OIE, 2012). Also, multiplex-PCR is defined to differantiate capsule 1, 2, 5 by Turton *et al.* (Turton et al., 2008).

CLINICAL SIGNS

a) Mares: '*Symptoms of disease are solely seen in mares*'. Two states of infection are observed. (OIE, 2012).

1. Vulval discharge ranges from mild to mucopurulent can be seen in **active state** of the infection, approximately 10-14 days. Edema, hyperemia of endometrium, endocervix, and vaginal mucosa are the lesions observed in the course of infection (Timoney et al., 1977; 1979).
2. There is no sign on the **carrier state** of the infection. Discharge subsides after a few days, finally mare exhibit no clinical signs in the carrier state. Once infected, mare remains capable of transmitting infection for several months since *T. equgenitalis* which is a causative agent of CEM, *K. pneumonia*, *P. aeruginosa* are determined on the surface of clitoris, the clitoral fossa and sinuses. In the carrier state, most of the mares do not conceived. CEM results in temporary infertility (Plat *et al*., 1978). If they conceive, their foals may be infected at the birth or shortly after the birth, when they reach the sexual maturity, foals become carriers of CEM (Timoney & Powell, 1982, Timoney *et al*., 1978).

b) Stallions: Stallions used for mating, teasing, or semen collection for artificial inseminationare described here. Although no clinical signs are observed in stallion, 3 agents are established on their penis and sheath. Since the stallion is carrier,

mares are infected during mating, teasing and artificial insemination (Schluter *et al*., 1991). After sex glands are invaded by bacteria, subsequently bacteri causes pus and contaminate semen (OIE, 2012).

TRANSMISSION of THE DISEASE

Infection is transmitted primarily at mating, but infected instruments and fomites play a role during transmission as well. Overlooked infected mares and stallions are the major source of outbreaks. Due to harboring microorganisms in the smegma of the prepuce, surface of the penis, especially urethral fossa, transmission rate to mares by the infected stallions is very high (Schluter *et al*., 1991, Plat *et al*., 1977)

Those agents are transmitted directly and indirectly (Schluter *et al*., 1991)

-The direct transmission ways are as follows:

1) During mating or teasing, infection can spread through direct transmission from mares to stallions and teasers or from stallions and teasers to mares.

2) The semen from infected stallions is the possible transmission and spread factor at the time of artificial insemination.

-Breeders should pay attention to the indirect transmission of the infection, also. If an example is given;

1) Discharges from the genital area of mares, and from penis of stallions can contaminate the water, instruments, utensils, easily.

2) Carelessness of the staff and veterinary surgeons can cause transmission of the bacteria. If the staff and veterinary surgeon do not pay necessary attention to clean the hands or change the gloves after handling tail and genital area of mares or the penis of stallions and teaser, infection transmit from horse to horse via the hands.

3) Between the horses, genital to genital or nose to genital contact is inevitable. If the breeders see any suspicious discharge from the genital area or penis of the horses, keeping the horse in a separate place is advisable.

If one of three is isolated from genital swabs of mares, the breeders should treat the mares according to veterinary advice.

Isolation of *K. pneumonia* and/or *P. aeruginosa* from the swabs of stallions or teasers, the breeders should stop mating/ teasing and collecting and inseminating semen. Treatment of the stallions and teasers should be done under veterinary direction (www.hblb.org.uk/codes.htm)

Definition of High Risk and Low Risk Mares and Stallions

Screening mares and stallions prior and while on the stud farm has successfully eliminated the disease from thoroughbred horses in countries using a voluntary code of practice. UK's Horserace Betting Levy Board's Code of Practice (www.hblb.org.uk/codes.htm) is reviewed and annually updated. In this section, you can find the summarized information about *T. equigenitalis, K. pneumonia, P. aeruginosa* according to UK's Horserace Betting Levy Board's Code of Practice.

It is thought that knowing the high risk and low risk mares/stallions will help the breeders to take effective and efficient preventive-control precautions,

diagnose, treat and hamper the outbreaks. In this concept, differentiating which mare/stallion termed as high risk or low risk is the main item to combat against *T. equigenitalis, K. pneumonia, P. aeruginosa.*

1. High Risk Mares:

-Mares from which *T. equigenitalis*, *K. pneumonia* (capsule type 1, 2, 5), *P. aeruginosa* have been isolated are termed as high risk. Only way to use high risk mares in future breeding activities is taking three sets of swabs at 3 different oestrus periods in each of two years (type of swabs, where to take are in the diagnosis section in detail). As predicted, all the laboratory results must be negative.

- Mares which have visited the premises that the *T. equigenitalis* has been isolated within the previous 12 months are defined as high risk.

- If the mares are mated with stallions during the last breeding season with the stallion resident outside France, Germany, Ireland, Italy, and UK, those are high risk until proven to be free from infection.

-In the last 12 months, mares which have been resident outside France, Germany, Ireland, Italy, and UK, are high risk.

2. Low Risk Mares: are any mares not defined as high risk

3. High Risk Stallions:

-Stallions which have not previously been used for breeding activities.

- Stallions from which *T. equigenitalis, K. pneumonia* (capsule type 1, 2, 5), *P. aeruginosa* have been isolated are termed as high risk.

-Until the treatment has been finished and required swab results are negative (type of swabs, where to take are in the diagnosis section in detail), stallions remains high risk status.

- Stallion which have been at the premises which the *T. equigenitalis, K. pneumonia* (capsule type 1, 2, 5), *P. aeruginosa* have been isolated within the previous 12 months are defined as high risk.

-If the stallion mated with a mare which has been not been controlled by swab culture or swab results not proven as negative is high risk.

4. *Low Risk Stallion*: Stallions are any stallions not defined as high risk.

DIAGNOSIS

Clinical signs are not enough to diagnose the diseases. In mares active stage takes short time and resembles any genital infections. As emphasized, stallions do not exhibit any clinical symptoms, they are carriers. Laboratory diagnosis is essential to confirm the presence or absence of *T. equigenitalis*, *K. pneumonia* (capsule type 1, 2, 5), *Pseudomonas aeruginosa* in swabs taken from mares and stallions. Types of swabs, site of swabbing, and at which intervals the swabs are collected, submitting conditions to the laboratory are summarized here (www.hblb.org.uk/codes.htm).

'*Briefly, stallions, teasers and mares are controlled twice a year before mating and after mating. Stallions are controlled after 2 years old because till 2 years old, stallions*

are foal. Mares are controlled after 2 years twice a year however against the possibility of maternal infection (mother-infected infection) foals are controlled at 6 months.'

Types of Swabs: All swabs should be taken by a veterinarian. Taken swabs submerge in Amies Charcoal Transport Medium (breeders pay attention to the expiry date of the swabs and keep enough the swabs according to the time of sampling for the control). Amies Charcoal Transport Medium protects the microorganisms from the damage of light and continuation of vitality of *T. equigenitalis, K. pneumonia, P. aeruginosa*. Routine bacteriological transport mediums also used for *K. pneumonia, P. aeruginosa* for submerging swabs, except *T. equigenitalis*. The swabs were labelled. Name of the horse, swabbing date and time, site of swabbing should be clearly written on the label (www.hblb.org.uk/codes.htm).

Site of Swabbing: Site of swabbing differs for mares and stallions due to gender.

In stallions, swabs should be taken from 3 sites: 1) Urethra 2) Urethral fossa 3) Penil sheath. In addition to 3 sites, pre-ejaculatory fluid if it is possible to take is helpful to examine. Separate should be used for each site and tested aerobic, microaerophilic culture and/or PCR (OIE, 2012).

In mares, clitoral (clitoral fossa, clitoral sinus) and endometrial swabs (vagina, uterus, cervix) are taken. 1) At any point during reproductive cycle, clitoral fossa and clitoral sinus must be free from infection. If the mare is pregnant and has a difficult foaling, veterinary attention and antibiotic treatment is required. In pregnant mares clitoral swabs should be taken after foaling and at least 7 days after antibiotic treatment. In this way, pro-foaling clitoral swabs are certified negative for CEM. Post foaling clitoral swabs may be taken for *K. pneumonia* and *P. aeruginosa*. 2) Endometrial swabs are taken during oestrus. The swabs are taken from the lining of uterus via the open cervix to demonstrate whether the uterus is free from infection (OIE, 2012).

After 1st January in any year, and before a mare is mated/teased/inseminated, the swabs must be examined for the presence of *T. equigenitalis*, *K. pneumonia*, *P. aeruginosa*. If the results are negative the mare is free from infection and the breeding activities commence. If the results are positive, you should stop breeding activities until treatment ended and the mare cleared up (www.hblb.org.uk/codes.htm).

-If any mare returns to oestrus shorter than normal time, she is probably infected. Repeat clitoral and endometrial swabs are taken and examined for *T. equigenitalis, K. pneumonia, P. aeruginosa*.

-If any mares changes premises or stallions during mating, at least seven days after mating repeat clitoral and endometrial swabs are taken and examined for *T. equigenitalis, K. pneumonia, P. aeruginosa*.

After 1st January in any year, before a stallion is used for mating/teasing/semen collection, two protocols must be followed.

Swabbing Protocol for Stallions (Pre-breeding):

-Two sets of swabs should be taken from three sites of stallions (urethra, urethral fossa, penile sheath, pre-ejaculatory fluid when possible). The swabs are

taken at an interval of no less than seven days. All the swabs are examined for *T. equigenitalis, K. pneumonia, P. aeruginosa*.

-If the results are negative, it means that stallion is free from infection and stallion can be used for breeding activities. If the results are positive, it means he is infected. You should stop breeding activities until treatment ended and the stallion cleared up. If CEM is proven, do not forget to inform the veterinarian

Swabbing Protocol for Stallions (During breeding):

-High risk stallions or any other stallion standing on a stud for the first time, the first four mares mated with stallions screened for *T. equigenitalis, K. pneumonia, P. aeruginosa* by taking clitoral swab two day after mating in any season. In mid-season swabbing should be done for all stallions and teasers and all the swabs should be screened for *T. equigenitalis, K. pneumonia, P. aeruginosa*.

Submitting Swabs to the Laboratory: Before submitting swabs to the laboratory, breeders, veterinarian and laboratories must be in contact. Laboratories have been informed about the transporting date due to the routine postal services. It is advised not to take swabs on Friday, Saturday and Sunday as they may not arrive in time. Veterinary surgeon should ensure about the laboratory is open and ready to commence culture within 48 hours. Otherwise the swabs are rejected, swabbing procedure is repeated again. *T. equigenitalis* is time sensitive micro-organism, it does not protect its viability for a long time, so time is important for the culture. However, if the laboratory applies PCR, specific *T. equigenitalis* DNA from even nonviable microorganism can be detected for long periods. *K. pneumonia* and *P. aeruginosa* is not time sensitive, in bacteriological transport medium, they protect their viability for along as they do in enviroment (www.hblb.org.uk/codes.htm).

4) Laboratory Tests: Mares and stallions are usually controlled for *T. equigenitalis, K. pneumonia, P. aeruginosa* by culture and PCR.

Culture: Proven of a swab that CEM negative takes 7-14 days at microaerophilic conditions. Fastidious nature of *T. equigenitalis* makes it difficult to isolate. Proven a swap that is *K. pneumonia* and *P. aeruginosa* negative takes 2 days at aerobic conditions. After isolation of *K. pneumonia*, additional tests must be applied to define the capsule type (capsule 1, 2, 5 are the causative agent of genital infections).

Polymerase Chain Reaction: This diagnostic technique is validated and routinely used in laboratories for *T. equigenitalis*, *K. pneumonia, P. aeruginosa* (Moore *et al*., 2001; Turton *et al*., 2008) Breeders and veterinarians find PCR useful because the results are available after 24 hours arrival at the laboratory. PCR and real-time PCR have been used for the detection of *T. equigenitalis* both directly from swabs and indirectly from cultures grown on agar plates (Bleumink & Pluym, 1994). Especially studies carried in UK, PCR is considered to be highly specific since the fastidious growth characteristics of the agent and back ground flora already exist on the samples. In Japan it was demonstrated that PCR was more sensitive than culture from genital swabs of horse (Anzai *et al*., 2002, Moore *et al*., 2001). For screening pre-breeding studies, real-time PCR applied by Wakeley *et al*. and Qusey *et al*. are widely used (Wakeley *et al*., 2006, Qusey *et al*., 2001). PCR is used for pre-diagnostic purposes, morever it shortens time of results and also enables the differentiation of *T. equigenitalis* and *T. asinigenitalis*.

Serological Tests: Serological tests is meaningfull in acute stage, but not chronic. Serum antibody to *T. equigenitalis* can be detected in mares for 3-7 weeks after infection. Due to this reason, no serological test has a value to detect antibody against *T. equigenitalis* reliably detect infection for diagnosis and control. Yet, Complement Fixation Test (CFT) has a value for a short time, screening mares between 21 and 45 days after being bred to a suspect carrier stallion (OIE, 2012).

PREVENTION and CONTROL

1. Before starting breeding activities, establishing freedom from infection is the best way.

2. During breeding activities, for being sure that the horses remain free from the infection is achieved by control of the horses at certain intervals.

3. Applying strict hygienic measure reduces contamination risk.

There is no vaccine against these bacterial agents.

Freedom from infection is achieved by checking horses at certain intervals. Genital swabs from mares and stallions are examined by laboratories. Laboratories check swabs for the presence of *T. equigenitalis, K. pneumonia, P. aeruginosa*. Different types of swab and laboratory examination is recommended. These are described in the diagnosis section.

If the final result is negative, the horse is free from infection and breeding activities takes place.

If the result is positive, infected horse is treated, re-tested. Till the result is negative, the horse leaves out breeding activities.

If it is *T. equigenitalis*, informing veterinarian is obligatory and then investigation of source and extent of the infection is done. Pay attention not to use horses on the same premises for breeding activities until all the swab results negative.

If mare owners pay attention to use semen collected from infection free stallions at the time semen collection, artificial insemination is a useful control measure.

***Hygiene Measures*:**

1. The staff should be taught the risk of direct and indirect transmission of infection. They should be informed that the all three bacteria are highly contagious.
2. They should wear disposable gloves when handling the genital region of mares, stallions and teasers.
3. They should change gloves between each horse. For each stallion/teaser, they should use separate utensils.
4. If mares are infected during pregnancy or foaling, hygienic measures should be taken as soon as possible because the discharges of mares and contaminated utensils are main source of the infection transmission.

When the infection is suspected or confirmed, until treatment has taken place and subsequent swabbing has proved that the infection has cleared up, mating, teasing, collection and insemination of semen should be stop. After treatment for the control purposes,

- The first swab should be taken 7 or 14 days after treatment ended.

-Repeat clitoral and penis swabs should be taken at intervals of 7 or more days.

-During the next 3 oestrous periods, repeat endometrial swabs should be collected.

When the *T. equigenitalis* is suspected or confirmed, informing veterinarian is necessary. According to advice of veterinarian, mares, stallions and teasers and collected semen should be localized in an isolated place in order to prevent transmission to healthy horses. Control of CEM depends on identification of infected carrier animals and on their treatment or elimination from breeding programs. Strict import regulations exist in many countries to avoid the introduction of CEM. Step by step, things to do are summarized below:

-You should stop mating, teasing, collecting and inseminating semen from stallions/teasers.

-You should isolate and treat the stallion/teaser according to veterinarian's direction.

- You should isolate and treat the mare according to veterinarian's direction.

-You should collect blood samples from the other mares deal with the outbreak and send the blood samples to the laboratory to check them.

-All the owners are informed about the situation incase infected stallion is used as a stud.

-Informing the people to whom semen from the stallion has been sent is a preventive precaution.

-After treatment of stallion/teaser at least 3 full post treatment sets of swabs have been obtained negative, stop breeding activities.

- Three mares mated to treated stallion should be swabbed 3 times at intervals of at least seven days.

Stallions can be treated by cleaning the extended penis with chlorhexidine surgical scrub and then applying nitrofurazone ointment. This should be repeated daily. Stallions retested at least 10 days after treatment (*Schluter et al*., 1991).

Most mares rid themselves of uterine infection after a few weeks. Those that become chronically infected, harbor *T. equigenitalis* in the clitoral fossa and sinuses. Mares can be treated by cleaning the clitoral area with chlorhexidine surgical scrub and then applying nitrofurazone ointment. In some mares excision of clitoral sinuses may be required to rid them of infection.

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