CODE OF PRACTICE FOR CONTAGIOUS EQUINE METRITIS (CEM), *KLEBSIELLA PNEUMONIAE* AND *PSEUDOMONAS AERUGINOSA*

CODE OF PRACTICE FOR CEM, *KLEBSIELLA PNEUMONIAE* AND *PSEUDOMONAS AERUGINOSA*

This Code of Practice covers diseases caused by three species of bacteria:

- **Taylorella equigenitalis** (*T. equigenitalis*, the contagious equine metritis organism - CEMO)

  Contagious equine metritis (CEM), caused by this organism, was first diagnosed in 1977 in Newmarket. The cause, *T. equigenitalis*, was until then unknown. Following the development and application of this Code of Practice, CEM was eradicated in UK and has occurred only sporadically since. It occurs widely in the non-Thoroughbred population, and to a limited extent in Thoroughbreds, in mainland Europe.

- **Klebsiella pneumoniae** (*K. pneumoniae*)

  This organism is found in the environment and can cause infections in a variety of animal species, and in humans. There are many capsule types of *K. pneumoniae*, most of which do not cause equine venereal disease. However, types 1, 2 and 5 may be sexually transmitted in horses. Therefore, when *K. pneumoniae* is identified from breeding stock, tests to determine the capsule type(s) present must be undertaken.

- **Pseudomonas aeruginosa** (*P. aeruginosa*)

  This organism is also found in the environment and can cause infections in a variety of animal species, and in humans. Not all strains of *P. aeruginosa* cause equine venereal disease but there is no reliable method to differentiate between the strains. Therefore, all isolates should be considered as potential venereal pathogens.

Both *K. pneumoniae* and *P. aeruginosa* occur sporadically within Europe.

Notification Procedures

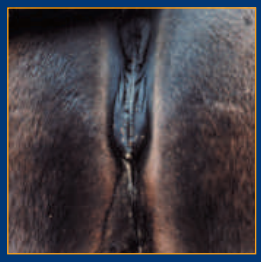
Contagious Equine Metritis (CEM)

In Great Britain (England, Scotland and Wales), isolation of an organism known or suspected to be *T. equigenitalis* (CEMO) must be notified under the Infectious Diseases of Horses Order 1987 to the Animal & Plant Health Agency (APHA). Please see Appendix 1 for APHA contact details.

Laboratories that have notified a suspect isolation are required to send the swab sample and/or the swab extract for PCR testing to the APHA Veterinary Investigation Centre Penrith (Merrythought, Calthwaite, Penrith, Cumbria CA11 9RR; tel: 01768 885314; email: penrith@apha.gsi.gov.uk) for official confirmation or negation of a suspected positive diagnosis of CEM.

Following an extended consultation between Government and the equine industry a new industry-led Contagious Equine Metritis (CEM) control protocol came into effect on 1 February 2018 as an initial pilot scheme in England, Scotland and Wales; there was no change in the arrangements for control of CEM in Northern Ireland (for more details please see Appendix 11). CEM remains notifiable throughout the UK and as such the disease will continue to be confirmed by the APHA Veterinary Exotic and Notifiable Diseases Unit (VENDU)

on the instructions of the Chief Veterinary Officer (CVO) in the country in GB where the disease is suspected. In 2021 the status of the industry-led CEM control protocol was changed from a pilot to an established protocol.



The basis of this industry-led scheme in GB is that when APHA is notified by a laboratory of a suspect *T. equigenitalis* isolation, APHA will inform the owner/agent of the horse of the suspicious result and ask the owner/agent whether, if CEM is confirmed and they are compliant with the Code of Practice, they wish to take part in the protocol.

If they elect to take part in the protocol further investigation will be carried out by an approved veterinary surgeon (AVS) appointed by a specialist advisor to the equine industry. A list is held by the British Equine Veterinary Association (BEVA). The owner or agent has 24 hours to inform that advisor of their agreement and to provide them with the required information about the infected horse(s), the premises and the attending veterinary surgeon involved. The specialist advisor will appoint an appropriate specialist in agreement with the owner/keeper, who, if CEM is confirmed, will visit the positive horse and premises and will advise the advisor whether the situation is compliant with the Code of Practice or not. The specialist may be the attending veterinary surgeon if he/she is on the BEVA Approved Veterinary Surgeon (AVS) list and if he/she consents to this responsibility.

If the owner does not wish to take part in the protocol or the premises is not compliant with the Code of Practice, the advisor will advise Defra of this and APHA will take over the investigation and may make charges. Defra may serve Statutory Notices on the affected premises, declaring them an infected place and impose mandatory requirements, including:

- taking samples or obtaining information to establish the source and extent of disease;
- prohibiting or controlling movement of any horse, carcase or other item;
- prohibiting the breeding activities of any implicated horses;
- disinfection or destruction of infected articles or materials;
- cleansing and disinfection of premises and vehicles.

In the event of statutory powers being invoked, Defra would nominate the laboratories to undertake the testing of all samples required by the subsequent investigation.

Failure to comply with Statutory Notices is an offence under the Animal Health Act 1981 and may lead to prosecution.

If *T. equigenitalis* is isolated, it is advisable for owners, or a person authorised to act on their behalf, to inform the appropriate national breeders' association.

Thereafter, the outbreak and any contacts will be investigated, infected horses treated and re-tested as recommended by this Code of Practice until all infected horses are shown to have been repeatedly tested negative. If the specialist advisor is involved, they will then notify Defra that this is the case and VENDU will, if the appropriate person is satisfied, declare the outbreak over.

Klebsiella pneumoniae* and *Pseudomonas aeruginosa

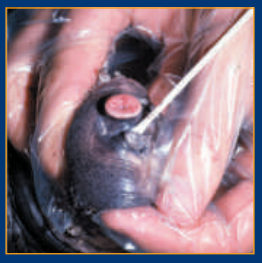
In the UK, isolation of *K. pneumoniae* or *P. aeruginosa* is not notifiable by law. However, if infection occurs in stallions, it is advisable for the owner, or a person authorised to act on their behalf, to inform the national breeders' association.

Clinical Signs

Mares

For all three bacteria, the severity of disease in mares varies. There are two states of infection:

- the active state in which the main outward sign is a vulval discharge which may range from very mild to extremely profuse;
- the carrier state in which there are no outward signs of infection. However, the mare remains capable of transmitting infection because the bacteria are established on the surface of the clitoris, in the clitoral fossa and sinuses and, in the case of *K. pneumoniae* and *P. aeruginosa,* sometimes in the urethra and bladder.



Stallions

Remember: 'stallion' means mating stallions, teasers and stallions used for AI:

- Infected stallions do not usually show clinical signs of infection but the bacteria are present on their penis, sheath and, in the case of *K. pneumoniae* and *P. aeruginosa*, sometimes in the urethra and bladder. These stallions can infect mares during mating, teasing or AI.
- Occasionally, in the case of *K. pneumoniae* and *P. aeruginosa* infections, the bacteria may invade the stallion's sex glands, causing pus and bacteria to contaminate the semen.

Note
Also see the
AI Guidelines
on page 87

Transmission of Disease

For all three bacteria, infection can be transmitted between horses in any of the following ways:

- direct transmission during natural mating;
- direct transmission during teasing. An infected teaser can transmit disease to mares through contact with his genitalia;
- indirect transmission during teasing. A teaser can transmit infected vulval discharge between mares through genital or naso-genital contact;
- transmission to mares if semen used for AI comes from infected stallions or has been contaminated with the bacteria during semen collection or processing;
- indirect transmission via the hands and equipment of staff or veterinary surgeons who have handled the tail or genitalia of an infected horse.

Prevention of Disease

Prevention is by far the most important means of control of venereal disease for the horse population. The most important means of preventing infection are:

- establishing freedom from infection before commencing breeding activities;
- checking that horses remain free from infection during breeding activities;
- exercising strict hygienic measures during breeding activities.

No vaccines against these bacterial diseases are available.

Freedom from infection

Establishing freedom from infection before, and checking that horses remain free from infection during, breeding activities involves a veterinary surgeon taking samples ('swabs') from the genitalia of mares and stallions for testing ('culturing' and/or PCR testing) in a laboratory. The laboratory will test for the presence of *T. equigenitalis*, *K. pneumoniae* and *P. aeruginosa*. If the results are negative, the horse is free from infection and breeding activities may take place. If the results are positive, the horse is infected and must be treated, re-tested and cleared. The horse must not be used for breeding activities at this time. If a swab is positive for the CEMO, the Notification Procedures on page 6 also apply, and an investigation of the source and extent of the disease will be undertaken.

No horse should be used for breeding activities until or unless all swab results are available and negative.

Different types of swab and culture are recommended for different circumstances in this Code of Practice. For further information on the types of swab, taking and submission of swabs, culture and return of results, see 'Diagnosis' on page 13.

More detailed recommendations for establishing freedom from infection in mares and stallions before breeding activities commence, and for checking that horses remain free from infection during breeding activities, follow.

Hygiene measures

Staff should be made aware of the risk of direct and indirect transmission of infection. They should always wear disposable gloves when handling the tail or genitalia and change gloves between each horse. Separate sterile and, where appropriate, disposable equipment and clean water should always be used for each horse.

Biosecurity protocols specific to AI are described in further detail in the Guidelines on AI.

Prevention recommendations

These are minimum recommendations. Mare owners should check whether the stallion stud, boarding stud or local breeders' association (e.g. NSFA) has any additional requirements. If so, these should be respected.

References to swab testing means appropriate testing by culture and/or PCR, where a laboratory is registered by BEVA for this method

Mares

After 1st January in any year and before a mare is mated, teased or inseminated, the following should be undertaken:

- Ascertain whether the mare is 'high risk' or 'low risk' (see Appendix 2);
- Complete a Mare Certificate (see Appendix 3) and send it to the stallion owner/manager;
- Arrange for a veterinary surgeon to take the appropriate swabs (see protocol on page 10) and send them to an appropriate laboratory for testing;
- Distribute the resulting Laboratory Certificates (see Appendix 4) in accordance with the protocol on page 10.

References to swab testing means appropriate testing by culture and/or PCR testing where a laboratory is registered by BEVA for this method following satisfactory Quality Assurance testing.

If the results are negative, the mare is free from infection and breeding activities may commence. If they are positive, she is infected and must not be mated, teased or inseminated until she has been treated and cleared from infection under the direction of the attending veterinary surgeon and, in the case of the CEMO, with Defra/APHA or the BEVA Approved Veterinary Surgeon (AVS) (see Appendix 11).

References to swab testing means appropriate testing by culture and/or PCR, where a laboratory is registered by BEVA for this method

Swabbing protocol for mares temporarily or permanently resident at stallion stud (pre-breeding)

Mare status	Type of swab	When/where taken	Culture/PCR
Low risk	Clitoral	Home premises or stallion stud	Aerobic and microaerophilic
	Endometrial	During oestrus at stallion stud	Aerobic
High risk (not known to have been infected)	Clitoral	Before arrival at stallion stud	Aerobic and microaerophilic
	Clitoral	On arrival at stallion stud (at least 7 days after the previous swab)	Aerobic and microaerophilic
	Endometrial	During oestrus at stallion stud	Aerobic and microaerophilic

High risk (not known to have been infected) mares usually come from countries other than UK, Ireland, France Germany and Italy, i.e. the signatories for these Codes. For high risk (after treatment for infection) mares, please see Confirmation of Freedom from Disease, later.

Swabbing protocol for walking-in mares (pre-breeding) or for mares being presented for AI (considered as 'low risk')

The following applies to mares which will not be resident on the same premises as the stallion, but will be 'walked in', either from their home premises or from a boarding stud. If 'high risk' walking-in mares are going to a boarding stud, that stud should either be under the control of, or meet full approval of, the stallion owner/manager. The veterinary surgeons involved should liaise closely to ensure adherence to the Code of Practice and to arrange any additional precautions that may be required.

Mare status	Type of swab	When/where taken	Culture/PCR
Low risk	Clitoral	Home premises or boarding stud	Aerobic and microaerophilic
	Endometrial	During oestrus at home premises or boarding stud	Aerobic
High risk (not known to have been infected)	2 x clitoral	At least seven days apart at home premises or boarding stud	Aerobic and microaerophilic
	Endometrial	During oestrus at home premises or boarding stud	Aerobic and microaerophilic

Protocol for distribution of Laboratory Certificates

The timely provision of acceptable Laboratory Certificates is essential to satisfy the needs of stallion stud managers. Laboratory Certificates relating to pre-breeding swabs taken from mares at the home premises should be sent in advance to the stallion owner/manager and, where appropriate, to the boarding stud. **For 'walking in' mares, acceptable Laboratory Certificates**

must at least accompany the mare at the time of presentation for mating. Certificates relating to pre-breeding swabs taken from mares at boarding studs should be sent in advance to the stallion owner/manager.

Before a mare is mated, the mare owner/manager is advised to request a Laboratory Certificate confirming the stallion's disease-free status in the current breeding season.

Mare owners/managers should not accept semen for AI without obtaining evidence that the donor stallion was free from infection when the semen was collected. In the UK, this evidence would be provided by a Laboratory Certificate confirming the stallion's disease-free status in the current breeding season. When importing semen, it should be accompanied by documentary evidence of freedom from infection with all three bacteria and the original import certificate.

If the mare does not conceive on first (or subsequent) matings, and her return to oestrus is normal, she should be swabbed again before being re-mated to check that she is not infected as a result of the previous mating or indirect transmission, according to the protocol below.

The mare may be re-mated on the basis of negative swab results. If the results are positive, she is infected and must not be mated, teased or inseminated until this has been investigated, she has been treated and cleared under the direction of the attending veterinary surgeon, and, in the case of *T. equigenitalis,* with Defra/APHA or the BEVA Approved Veterinary Surgeon (AVS) (see Appendix 11).

References to swab testing means appropriate testing by culture and/or PCR, where a laboratory is registered by BEVA for this method

Swabbing protocol for mares temporarily or permanently resident at stallion stud (repeat matings)

Mare status	Type of swab	When/where taken	Culture/PCR
Low risk	Endometrial	During oestrus at stallion stud	Aerobic
High risk	Endometrial	During oestrus at stallion stud	Aerobic and microaerophilic

Swabbing protocol for walking-in mares (repeat matings)

The following swab recommendations apply to mares which will not be resident on the same premises as the stallion, but will be 'walked in', either from their home premises or from a boarding stud. If 'high risk' walking-in mares are going to a boarding stud, it should either be under the control of, or meet full approval of, the stallion owner/manager. The veterinary surgeons involved should liaise closely to ensure adherence to the Code of Practice and to arrange any additional precautions that may be required

Mare status	Type of swab	When/where taken	Culture/PCR
Low risk	Endometrial	During oestrus at home premises or boarding stud	Aerobic
High risk	Endometrial	During oestrus at home premises or boarding stud	Aerobic and microaerophilic

Protocol for distribution of Laboratory Certificates

Laboratory Certificates relating to repeat swabs taken from mares at the home premises should be sent in advance to the stallion owner/manager

and, where appropriate, to the boarding stud. **For 'walking in' mares, acceptable Laboratory Certificates must at least accompany the mare at the time of presentation for mating.** Certificates relating to repeat swabs taken from mares at boarding studs should be sent in advance to the stallion owner/manager.

If any mare returns to oestrus at an unusual (especially shorter than normal) time (in the opinion of the attending veterinary surgeon), this may be because she is infected. Repeat clitoral and endometrial swabs should be taken and cultured under aerobic and microaerophilic conditions or by PCR test.

If any mare changes stallions between matings, repeat clitoral and endometrial swabs should be taken at least seven days after mating by the original stallion and cultured under aerobic and microaerophilic conditions or by PCR test.

These are minimum recommendations. Mare owners should check whether the stallion stud, boarding stud or local breeders' association (e.g. NSFA) has any additional requirements, e.g. requirements for microaerophilic culture or PCR testing for repeat matings.

References to swab testing means appropriate testing by culture and/or PCR, where a laboratory is registered by BEVA for this method.

Stallions

After 1st January in any year and before a stallion is used for mating/teasing or semen collection, the owner/manager should:

- ascertain whether the stallion is 'high risk' or 'low risk' (see Appendix 2);
- arrange for swabs to be taken by a veterinary surgeon in accordance with the protocol below;
- ensure that a Laboratory Certificate (see Appendix 4) confirming the mare's disease free status in the current breeding season, and a current Mare Certificate (see Appendix 3) are received for each mare to be mated, teased or inseminated at the stallion's premises;
- ensure that a Laboratory Certificate confirming the stallion's disease-free status in the current breeding season is made available to mare owners/managers.

References to swab testing means appropriate testing by culture and/or PCR testing where a laboratory is registered by BEVA for this method following satisfactory Quality Assurance testing.

Protocol for swabbing (pre-breeding)

After 1st January and before any breeding activity is commenced, two sets of swabs (see definition on page 13) should be taken from all stallions at an interval of no less than seven days and cultured aerobically and microaerophilically or tested by PCR.

If the results of swab testing are negative, the stallion is free from infection and breeding activities may commence. If they are positive, he is infected and must not be used for mating, teasing or semen collection until he has been treated and cleared under the direction of the attending veterinary surgeon and, in the case of the CEMO, with Defra/APHA or the BEVA Approved Veterinary Surgeon (AVS) (see Appendix 11).

The following should be carried out during the breeding season to check that the stallion has not become infected:

'High risk' stallions and any other stallion(s) standing at a stud for the first time warrant additional precautions. The first four mares mated with them

should be screened for *T. equigenitalis, K. pneumoniae* (capsule types 1, 2 and 5) and *P. aeruginosa* by taking a clitoral swab two days after mating. If the mare subsequently returns to oestrus, an endometrial swab should be taken at that time. These swabs should always be tested aerobically and microaerophilically or by PCR test.

In stallions, bacterial growth or PCR detection of *T. equigenitalis* is generally more easily achieved after mating. Swabbing of all stallions after their first few matings in any season should therefore be considered in conjunction with the attending veterinary surgeon. In addition, mid-season swabbing should be considered for all stallions and teasers. These swabs should always be tested by aerobic and microaerophilic culture and/or by PCR testing.

Remember: 'stallion' means mating stallions, teasers and stallions used for AI.

Diagnosis

Laboratory diagnosis is essential to confirm the presence or absence of *T. equigenitalis, K. pneumoniae* and *P. aeruginosa* in swabs taken from mares and stallions.

References to swab testing means appropriate testing by culture and/or PCR, where a laboratory is registered by BEVA for this method

Types of swab

Mares

There are two types of swab:

Clitoral swab: taken from two sites; the clitoral fossa and the clitoral sinuses using mini tip swabs to ensure appropriate sampling of sinus contents, at any point during the reproduction cycle to demonstrate whether these sites are free from infection.

In the case of **pregnant mares who have had difficult foalings** requiring veterinary attention and antibiotic treatments, additional clitoral swabs should be taken after foaling and more than 7 days after antibiotic treatment has finished, in addition to routine endometrial swabs, in order to rule out acquired *K. pneumoniae* and *P. aeruginosa* infections. Providing the pre-foaling clitoral swab was certified negative for *T. equigenitalis*, the additional post-foaling clitoral swab may be tested by aerobic culture only, or by PCR.

Endometrial swab: taken during oestrus from the lining of the uterus via the open cervix to demonstrate whether the uterus is free from infection.

Mare swabs taken for disease prevention purposes should be tested according to the recommendations on pages 9–12.

Note: These are minimum recommendations. Mare owners should check whether the stallion stud, boarding stud or local breeders' association (e.g. NSFA) has any additional requirements.

Stallions

Swabs should be taken from three sites; the urethra, urethral fossa and penile sheath, plus pre-ejaculatory fluid when possible. Separate swabs should be used for each site and tested by aerobic and microaerophilic culture and/or by PCR test, in all circumstances.

Further information on how to collect equine genital swabs in stud practice for the prevention of venereal diseases, as recommended by the Codes of Practice, is available online at: http://codes.hblb.org.uk/index.php/page/40

Taking swabs

All swabs should be taken by a veterinary surgeon, who should:

- Pay attention to the PowerPoint presentation 'How to Collect Equine Genital Swabs in Stud Practice' by Professor Sidney Ricketts FRCVS https://www/rossdales.com/wp-content/uploads/2018/01/Collecting-Swabs-2017.pdf
- Clitoral swabs must be taken using a micro tip (mini tip) swab to get deep into the clitoral sinuses.
- Submerge the swabs in Amies Charcoal Transport Medium (which must be within the expiry date) to protect them from the damaging effects of light, which will readily kill any *T. equigenitalis, K. pneumoniae* or *P. aeruginosa* present.
- label them clearly to show the date and time they were taken, the horse's name and the site of swabbing;
- indicate clearly whether aerobic, microaerophilic or both cultures, and/or PCR test are required;
- submit them to a BEVA registered Laboratory for testing. Culture must commence within 48 hours of the swab being taken.

References to swab testing means appropriate testing by culture and/or PCR, where a laboratory is registered by BEVA for this method

A list of laboratories in UK, Ireland, Germany and France registered by BEVA for the purposes of testing for the CEMO, *K. pneumoniae* and *P. aeruginosa* is available from:

https://www.beva.org.uk/Portals/0/Documents/ResourcesForVets/Lab%20Scheme/BEVA%20Laboratory%20Reg%20Scheme%20June%202022.pdf?ver=2022-07-27-160433-357

Submitting swabs to BEVA registered laboratories

The BEVA registered laboratories must set up swabs for conventional microaerophilic culture for *T. equigenitalis* within 48 hours of them being taken from the horse as this organism is short lived, even in bacteriological transport medium. Veterinary surgeons submitting swabs by routine postal services are, therefore, advised not to take swabs on Fridays, Saturdays or Sundays as they may not arrive in time. If weekend or bank holiday swabbing is unavoidable, the veterinary surgeon should ensure that the laboratory is open and able to commence cultures within the 48 hours. In this event, a suitable courier service should be used to deliver the swabs. If a swab does not arrive in time, the laboratory should reject it and advise the veterinary surgeon to repeat the swabbing.

Note
The term 'at risk' relates to any horse which may have become infected as a result of direct or indirect transmission of the disease.

However, time constraints do not apply to swabs submitted to laboratories that are registered to run PCR tests for *T. equigenitalis* as specific DNA from non-viable organisms can be detected for long periods.

Experience suggests that swabs cultured aerobically for *K. pneumoniae* and *P. aeruginosa* are not so time sensitive and these organisms have a long life in bacteriological transport medium, as they do in the environment.

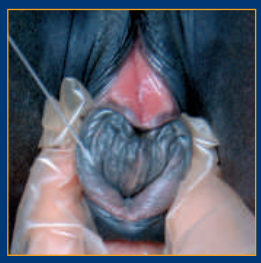
Laboratory culture of swabs

Laboratories can culture swabs in two ways: aerobically and microaerophilically (see Glossary, Appendix 10). The results of culture will be returned by the

laboratory on an official Laboratory Certificate. When planning the timing of breeding activities, breeders and veterinary surgeons should be aware that the results of microaerophilic culture will not be available for at least seven days. Aerobic swabs require 24 hour (overnight) culture before the initial result can be reported. If these results are satisfactory, 'low risk' mares may then be mated. However, final aerobic culture results will not be available for 48 hours (to exclude the possibility of slow-growing *P. aeruginosa* organisms), so mating before these results are available is at the stallion stud's own risk. PCR test results do not have these time delays.

Other laboratory tests

Polymerase chain reaction (PCR) testing of swabs for T. equigenitalis, *K. pneumoniae* and *P. aeruginosa* is validated for industry screening purposes. PCR testing is not recognised for import/export testing in the UK. **Breeders and veterinary surgeons may find PCR test results helpful, as they may be available on the same day that a sample is received at a laboratory that is able to undertake PCR testing**. Positive PCR test results will need to be further investigated by conventional culture to help determine their significance and, in the case of *K. pneumoniae*, for capsule typing, unless being tested, in the same laboratory, for capsule typing by PCR test. Positive PCR test results for *T. equigenitalis* must be reported to Defra/APHA (see Appendix 11).



The immunofluorescence test (IFT) for *T. equigenitalis,* which is available only in France, is not acceptable on its own, although it may be used in addition to culture.

Preparing mares for covering

It has been brought to the Codes of Practice Committee's attention that some mares are now being presented for walk-in coverings with only PCR test certification and sometimes incomplete gynaecological preparation.

The Code of Practice recommends PCR testing of uterine swabs from mares because this is currently the best method to rule out the contagious endometritis infections caused specifically by T. equigenitalis, K. pneumoniae and P. aeruginosa infection, which is the principal objective of this Code. However, this test is not a substitute for traditional aerobic cultures and a proper full genital examination of all mares, for signs of other uterine endometritis infections or non-infectious endometritis, or other significant genital abnormality. This examination must be performed when the mare is in early oestrus, when she will have a sufficiently relaxed cervix to allow proper swabbing, and should include:

1. Visual inspection of the vulva, vestibule and vagina to confirm adequate vulval closure, correct post-partum healing of vulval tears or Caslick repairs and the absence of signs of pneumovagina, which will need correction, prior to covering.

2. Vaginascopic examination of the cervix and vagina to confirm a relaxed oestrous cervix and no signs of parturient tears or inflammation to suggest pneumovagina, which will need repair/resolution.

3. Rectal palpation and ultrasound examination to monitor ovarian follicular development to allow the optimal time for covering to be predicted and signs of ovarian and/or uterine abnormality (e.g. delayed uterine involution and/or excessive and/or turbid uterine fluid) to be ruled out. If found, these abnormalities should be treated and time allowed for resolution before the mare is sent for covering.

4. When the cervix is adequately relaxed, swabs should be taken for PCR testing (see above) **and for aerobic bacterial culture** and, ideally, at the same time, for a smear cytology test. This will allow endometritis, caused by any infectious

organism (most commonly with equine skin or environmental contaminants) or non-infectious endometritis (indicating inflammation rather than active infection) to be ruled out. The smear test will provide a more precise interpretation of the significance of bacteria cultured from the swab and will allow the diagnosis of non-infectious inflammation.

If any of these problems are detected, covering at this stage is more likely to result in failure of conception or early pregnancy failure. The mare will then require further, perhaps more prolonged, treatment to return her into a state for covering again and valuable time will have been lost out of the breeding season, for the mare to achieve a pregnancy. This is why such careful preparation of mares for covering is helpful for the mare and all concerned and is therefore recommended.

Control of Infection

If infection with any of the three organisms is suspected in any mare, stallion or teaser on the basis of clinical signs, all breeding activities must cease immediately. The affected horse(s) should be isolated and swabbed by the attending veterinary surgeon.

If *T. equigenitalis, K. pneumoniae* (capsule types 1, 2 or 5) or *P. aeruginosa* is subsequently isolated or confirmed by PCR from any mare, stallion or teaser:

1. Stop mating, teasing and collection and insemination of semen immediately;
2. Seek veterinary advice immediately;
3. Isolate and treat the infected horse(s) as advised by the attending veterinary surgeon. In the case of *T. equigenitalis*, the laboratory will have notified Defra/APHA, who will give directions on the next steps which must be followed (see Appendix 11);
4. Arrange swabbing of any in-contact horses, as advised by the attending veterinary surgeon or, in the case of *T. equigenitalis*, with Defra/APHA or the BEVA Approved Veterinary Surgeon (AVS) (see Appendix 11);

   The term 'at risk' relates to any horse that may have become infected as a result of direct or indirect transmission of the disease.
5. Disinfect all equipment used for breeding procedures
6. Inform all owners of mares booked to the stallion, including any that have already left the premises;
7. Inform people to whom semen from the stallion has been sent;
8. Inform the appropriate national breeders' association;
9. Arrange for one straw from every ejaculate of stored semen from infected and "at risk" stallions to be tested by a laboratory. If a straw from any ejaculate is infected, all straws from that ejaculate should be destroyed;
10. Any "at risk" pregnant mare must be foaled in isolation. The placenta must be incinerated. Foals born to these mares should be swabbed three times, at intervals of not less than seven days, before three months of age. These swabs should all be tested by aerobic and microaerophilic culture or by PCR test;
    - Filly foals: swab the clitoral fossa.
    - Colt foals: swab inside the penile sheath and around the tip of the penis.
11. Do not resume any breeding activity until freedom from disease has been confirmed in all infected horses (see below). The approval of the attending veterinary surgeon or, in the case of *T. equigenitalis*, of Defra/APHA, should be obtained before resumption of breeding activity.

Remember: in any suspected or confirmed disease situation, the implementation of strict hygienic measures is essential.

In the case of *T. equigenitalis*, if Defra/APHA or the BEVA Approved Veterinary Surgeon (AVS) do not believe voluntary compliance is sufficient to control infection, Defra will impose statutory requirements.

Swabbing of at-risk/suspect etc horses

Sites to be swabbed (single swabs only required from each site):

- Mares – clitoral fossa, clitoral sinus & endometrium (oestrus)
- Stallions – urethra, urethral fossa & prepuce. A swab of the pre-ejaculatory seminal fluid should also be submitted if possible.

Mares covered by the infected stallion will require at least three sets of screening swabs with negative results to be considered infection free.

However, where the risk is believed to be low e.g. other stallions that have not had direct contact, have not shared artificial breeding equipment and mares covered by these stallions, but not the infected stallion etc, then a single clearance set of samples from these animals would probably be considered sufficient to give assurance that there had not been any unexpected transmission.

Treatment

Any necessary treatment will be determined by the attending veterinary surgeon. In the case of *T. equigenitalis*, Defra/APHA or the BEVA Approved Veterinary Surgeon (AVS) will determine treatment (see Appendix 11).

Confirmation of Freedom from Disease

Following infection with any of the three bacteria, breeding activities should only be resumed with approval from the attending veterinary surgeon, and in the case of *T. equigenitalis*, the Veterinary Exotic Notifiable Diseases Unit (VENDU), who must be satisfied that infected and in-contact horses have been investigated, treated as appropriate and subsequently cleared on the basis of negative swabs.

The first post treatment swabs should be taken seven or more days after treatment has ended. All post treatment swabs should be tested by aerobic and microaerophilic culture **and** by PCR. All positive isolates of *K. pneumoniae* should be capsule typed where they are identified on post-treatment samples, irrespective of whether pathogenic *K. pneumoniae* was isolated prior to treatment. All positive *K. pneumoniae* PCR results will need culture tests also performed to provide bacteria for capsule typing, unless being tested, in the same laboratory, for capsule typing by PCR test.

Experience in various parts of the world from treating CEMO in mares and stallions, highlights that treatments may predispose to subsequent colonisation by *K. pneumoniae* or *P. aeruginosa* and, therefore, it is very important that screening post-treatment of one type of infection continues to include swabbing and testing for all three pathogens. It is emphasised that endometrial swabs, which are required for confirmation of freedom of disease, can only be successfully collected when the mare is in oestrus.

Mares

Starting not earlier than seven days after cessation of treatment for infection has ended, three clitoral swabs should be taken at intervals of at least seven days. For *K. pneumoniae* (capsule types 1,2 and 5) or *P. aeruginosa* two endometrial swabs should be taken during two oestrous periods and tested by both aerobic and microaerophilic culture **and** by PCR tests, **but** for *T.equigenitalis* three endometrial swabs should be taken during three oestrous periods and tested by both aerobic and microaerophilic culture **and** by PCR tests.

In respect of maiden mares whose pre-screening clitoral swab **only** (i.e. endometrial swab negative) was positive for *P. aeruginosa*, pre-treatment, three clitoral swabs must be taken but only one endometrial swab (taken in oestrus) is required. All swabs should be tested by both aerobic and microaerophilic culture and by PCR tests.

All results must be negative before any breeding activities resume. If any result is positive, further investigations should be undertaken in conjunction with the attending veterinary surgeon and for *T. equigenitalis* with Defra/ APHA or the BEVA Approved Veterinary Surgeon (AVS) (see Appendix 11).

Stallions

Starting not earlier than seven days after cessation of treatment for infection, three sets of penile swabs (see Diagnosis – types of swab) should be taken at intervals of at least seven days and tested by aerobic and microaerophilic culture **and** by PCR tests and negative results confirmed. All results in treated stallions must be negative before any breeding activities resume.

Thereafter, the first three mares mated naturally by the stallion should have clitoral swabs taken three times at intervals of at least seven days, starting two days after mating and tested by both aerobic and microaerophilic culture **and** by PCR tests. If any result in the mated mares is positive, breeding activities should cease again immediately, and further investigations should be undertaken in conjunction with the attending veterinary surgeon and, for *T. equigenitalis*, with Defra/APHA or the BEVA Approved Veterinary Surgeon (AVS) (see Appendix 11). For stallions intended solely for semen collection for artificial insemination, in addition to the set of penile swabs confirming freedom from infection, raw semen (i.e. with no semen extender and/or antibiotic added) should also be collected and tested by both aerobic and microaerophilic culture **and** by PCR tests, with all negative results confirmed before any semen is used for artificial insemination in mares.

Export Certification

Swabs taken for examination for *T. equigenitalis* from horses in the United Kingdom for the purpose of official export health certification must be sent to the designated laboratory within the APHA. This is the APHA Regional Laboratory, Penrith. In the case of horses that are to be exported from Northern Ireland, swabs should be sent to the Veterinary Science Division Laboratory, Belfast.