Case study on Klebsiella pneumoniae (kindly contributed by Kate Colles, Avonvale Veterinary Practice, Ratley Lodge, Banbury, Oxfordshire)

A mare originating from France and which had had a difficult foaling at a local stud was subsequently shown to be carrying Klebsiella pneumoniae on a routine clitoral swab. The isolate was typed as a venereally non-pathogenic capsule type 7 and as such was not subject to restrictions under the HBLB Codes of Practice for venereally transmitted bacterial diseases (capsule types 1, 2 and 5 are considered pathogenic). An endometrial swab taken at the foaling heat showed the presence of inflammatory cells and Klebsiella pneumoniae capsule type 7 again. Klebsiella pneumoniae was also recovered from an endometrial swab taken at the next oestrus cycle. A third endometrial swab was clear. On all three occasions, the isolate showed resistance to Ampicillin, Gentamicin, Penicillin G and Sulphamethoxazole/Trimethoprim. This multiple antibiotic resistance is not commonly encountered with Klebsiella pneumoniae isolates in the experience of this laboratory.

Focus Article - Endometrial cytological and bacteriological examinations in equine stud farm practice (Professor Sidney Ricketts, Rossdale and Partners, Newmarket)

The equine species is unusual in that the mare’s cervix relaxes during oestrus allowing the stallion to ejaculate semen into her uterus. In most other species, the female’s cervix remains closed and male ejaculation occurs into the vagina. Therefore, the mare’s endometrium is normally challenged at natural mating by seminal plasma and the contaminating microflora of the stallion’s penile skin and the mare’s perineal skin and vaginal mucosa. The endometrium responds to this challenge by mounting a normal protective/repairing inflammatory response (endometritis). In mares with otherwise healthy genitalia and associated natural defence mechanisms, the endometritis successfully resolves within 2-3 days so that when the fertilised ovum enters the uterus from the fallopian tube at approximately 5 days post-ovulation, the endometrium is healthy in preparation for pregnancy. In some mares, resolution does not occur and pregnancy does not ensue, because:

The mare’s natural defence mechanisms are defective and the endometritis persists to frank infection. This may occur when:

a) The mare has been mated at first post-partum oestrus before her uterus has fully involuted and the competence of its natural defence mechanisms has not been restored.

b) The mare’s perineal conformation deteriorates with advancing age and pneumovagina leads to cervicitis and endometritis.

c) The mare’s uterine and abdominal musculature fail in their competence to eject uterine lumenal fluid accumulations, through the mare’s cervix.

These problems, singly or in combination, perhaps with other as yet inadequately defined genital inadequacies, predispose to persistent post-mating or post-parturient endometritis associated with the common opportunist equine genital pathogens, most commonly Streptococcus zooepidemicus, Escherichia coli and Staphylococcus aureus.

The microorganism is one that is able to overcome the mare’s natural defence mechanisms and frank infection develops. Such infections may be sexually transmitted from carrier mare to stallion and onto other mares in a true venereal manner. Bacteria recognised as potential equine venereal disease producers are Taylorella equigenitalis.
(the cause of Contagious Equine Metritis), *Klebsiella pneumoniae* (capsule types 1, 2 and 5) and *Pseudomonas aeruginosa*.

Veterinary surgeons in equine stud farm practice therefore need to be able to assess the genital health of mares routinely at the first post-partum oestrous period, at other oestrous periods pre-mating and when mares have failed to conceive or pregnancy fails. They do this by assessing the external and internal genitalia for signs of discharge, inflammation, injury and conformational competence, by visual inspection externally, by vaginascopic examination, by rectal palpation, ultrasound imaging and videohysteroscopic examination, by the cytological and bacteriological examination of endometrial smear and swab samples and by the histological examination of endometrial biopsy samples, as appropriate to the individual mare.

Endometrial smear and swab samples are collected during oestrus, via a sterile vaginascope, by passing suitably extended swabs through the mare’s relaxed cervix into her uterine lumen.

(1) Collecting an endometrial swab or smear via a sterile vaginascope through the open cervix of an oestrous mare.

(2) A selection of commercially available equipment for collecting equine endometrial samples for cytological and bacteriological examinations: swabs with and without transport medium, one connected to a disposable intrauterine catheter for necessary extension of length, a disposable vaginascope and ‘penlight’ torch.

(3) Endometrial smear from a normal oestrous mare, stained with Pollack’s rapid trichrome stain, showing normal endometrial epithelial cells and no polymorphonuclear leucocytes (PMNs).

(4) Endometrial smear from an oestrous mare with acute endometritis, stained with Pollack’s rapid trichrome stain, showing degenerate epithelial cells and many degenerate polymorphonuclear leucocytes (PMNs).

(5) Endometrial biopsy from an oestrous mare with Candida endometritis, stained with Periodic Acid Schiff (PAS) stain, showing many yeast spores in the uterine lumen.

(6) *Taylorella equigenitalis* growing on specialised CEMO haemolysed blood agar after 5 days microaerophilic culture, showing typical colony formation.

(7) *Pseudomonas aeruginosa* growing on blood agar after 24 hours aerobic culture, showing a typical greenish pigmentation.

(8) *Klebsiella pneumoniae* growing on McConkey’s agar after 24 hours aerobic culture, showing typical mucoid colony formation.
Cytological examinations

Smear samples are rolled onto gelatin-coated slides, fixed with a suitable cytological fixative (e.g. polyethylene glycol) and stained with a suitable cytological stain (e.g. Pollack’s trichrome). For urgent or on-the-studfarm results, smears may be rolled onto pre-stained (Romanowski) slides (Testsimplets, BCL), incubated at room temperature for 3 minutes, washed off and cover-slipped. Smears are examined for the presence of endometrial epithelial cells to assure that the smear was reliably endometrial and for the presence or absence of polymorphonuclear leucocytes (PMNs) as indicators of inflammation, i.e. endometritis. The numbers of PMNs seen on the smear may be graded as +/- (the occasional scattered cell only), 1+ (a small but consistent number), 2+ (a moderate number) and 3+ (a large number), with 1+ and more considered a sign of significant endometritis. Fungal stains (e.g. Periodic Acid Schiff, PAS) may help to identify cases of mycotic endometritis.

Bacteriological examinations

Swab samples are collected into Amies charcoal transport medium and need to reach a Horserace Betting Levy Board designated laboratory within 48 hours of collection for official Code of Practice certification. Swabs are plated onto specialised haemolysed CEMO blood agar for up to 7 days microaerophilic (10% CO₂) culture for *T. equigenitalis* and onto blood and McConkey’s agar for up to 48 hours aerobic culture for *K. pneumoniae, P. aeruginosa* or opportunist pathogens. The organisms are identified by their cultural characteristics, by biochemical reactions and by specific latex agglutination testing (*T. equigenitalis*). *K. pneumoniae* isolates may be capsule typed by specialist laboratories using the Quellung method or more sophisticated analyses. In some cases, anaerobic culture may be used for the identification of the most common equine uterine anaerobe *Bacteroides fragilis*, which may act synergistically with opportunist aerobes to potentiate pathogenicity. In other cases swabs may be plated onto Sabaraud’s agar for fungal cultures. The most common equine mycotic endometritis isolates are *Candida* spp. and *Aspergillus* spp.. The laboratory isolation of *T. equigenitalis* in UK must be reported to DEFRA under the Infectious Diseases of Horses Order, 1987.

Concurrent smear and swab examinations

No matter how careful the clinician is when collecting endometrial swab samples for bacteriological examination and irrespective of the equipment and techniques are used, insignificant contaminant or commensal microorganisms may be collected and cultured by the laboratory, particularly in samples delayed by transit through the postal services. When opportunistic pathogens are concerned, the only way to determine the significance of the isolate (in the absence of clinical signs of inflammation and/or discharge) is to look for the presence or absence of inflammation (PMNs) in a concurrently collected endometrial smear. Without concurrent smear and swab tests, clinicians may misinterpret bacterial and mycotic culture results leading to false positive diagnoses of endometritis leading to the inappropriate treatment and management of the mare. Without endometrial smear tests, clinicians may miss cases of sterile endometritis or cases from which a pathogen is present but cannot be isolated, leading to failure to treat and inappropriate management of the mare.