Antimicrobial resistance in equine endometritis cases in New Zealand

Lee Morris
EquiBreed ART Ltd, Te Awamutu

Endometritis is an important cause of infertility in equine breeding programs. Since the late 1990s, intrauterine treatments of antibiotics have been used routinely after breeding or inseminating mares to prevent or treat persistent breeding induced endometritis. Consequently, commonly isolated bacteria are displaying resistance to different antibiotics. In line with the position of the NZVA to support the concept of One Health and to reduce the incidence of antimicrobial resistance, this paper aims to document the prevalence of endometritis and the status of antimicrobial resistance in the New Zealand broodmare population. At the same time an opportunity will be created to engage with practitioners and stud master's to improve the use of diagnostics methods and implement treatment strategies in line with the NZVA and One Health goals to reduce antibiotic use in animals.

In two surveys of reproductive performance in New Zealand the first cycle conception rates were reported as 54% in thoroughbreds (Hanlon *et al*. 2012) and 61% in standardbreds (Tanner and Barrell 2023). This translates to a failure of pregnancy in 39–46% of breeding cycles. There are many factors potentially involved in the failure to establish pregnancy which can be attributed to either a failure to conceive or a failure to establish pregnancy. A major impediment to the establishment of pregnancy is "endometritis" if the uterus is inflamed or infected, it provides a hostile environment that both kills spermatozoa and prevents the establishment of pregnancy.

Equine endometritis is cited as a major cause of subfertility and infertility in mares and contributes to significant wastage in the breeding industry worldwide (Heil *et al*. 2023; Rasmussen *et al.* 2015). There is, however, a lack of any published data on the prevalence of endometritis in the broodmare herd of New Zealand. A review of global literature cites authors that report endometritis is observed in 15–30% of mares, however these studies were not based on epidemiological methods and therefore may misrepresent the prevalence of the condition (Morris *et al*. 2020). Studies in Kentucky, USA, reported that when diagnostic cytology, culture and sensitivity on endometrial samples was performed, the isolation of microorganisms was associated with reduced pregnancy rates, even in mares with no apparent presence of inflammation (Riddle *et al*. 2007). In a Scandinavian study, of 76 clinically healthy standardbred mares, subclinical endometritis was diagnosed in 28.6% of mares despite having no overt signs of inflammation or infection (Rasmussen *et al.* 2015). More recently in a European study (Díaz-Bertrana *et al*. 2021), 89% of 363 problem mares were diagnosed with active endometritis, and of further concern was that these cases involved bacteria resistant to commonly used β-lactam antimicrobials.

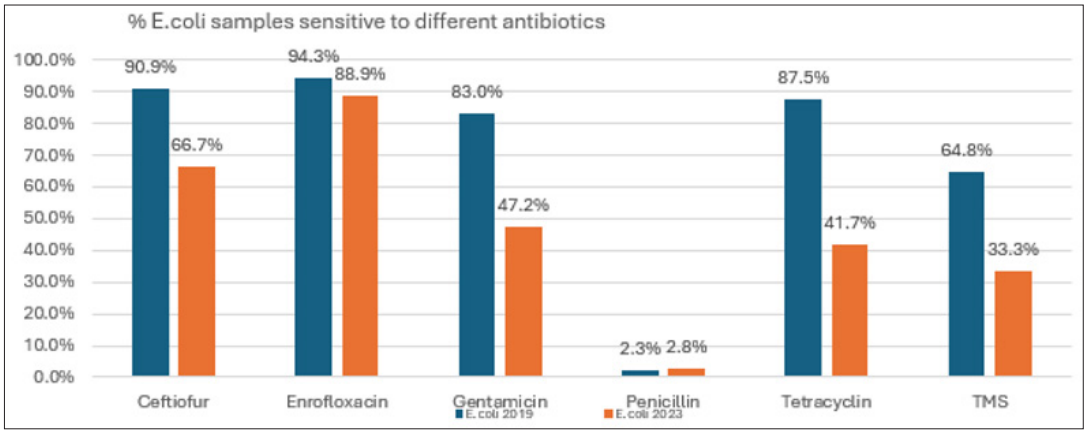
In the Northern Hemisphere, stud farms in the UK and Central Kentucky (USA) typically require mares to have a veterinary certificate stating that no uterine pathogens were isolated from the mare prior to mating. This is not a routine requirement in the thoroughbred or standardbred industry in New Zealand. One of the obstacles in New Zealand, is that uterine swabs are often couriered to the laboratory and bacterial culture takes 48–72h for the full identification and antimicrobial sensitivity results to be available. Even if uterine samples are cultured "in house" at the veterinary clinic, the antimicrobial sensitivity results take at least 48h.

Endometrial cytology can be performed as part of the diagnostic process, but not all cases are associated with positive cytology if the bacteria inhibit inflammatory cells or hide in the endometrial glands. Therefore, once we have determined the prevalence and bacterial populations involved in endometritis then we will be able to evaluate rapid diagnostic methods for New Zealand conditions.

Many studies have shown that the bacteria most commonly isolated from the uteri of mares after mating are *Streptococcus spp.* and *E. coli* (LeBlanc 2010). Despite that in the healthy mares the presence of these bacteria may be transient and an infection is not established, routine use of antibiotics to prevent post breeding endometritis has been implemented on stud farms since the studies by Pycock and Newcombe in 1996 (Pycock and Newcombe 1996) which illustrated the benefits of combining oxytocin with antibiotics post breeding. Therefore, typically, post breeding endometritis is treated with intrauterine infusions of antibiotics accompanied by multiple treatments of oxytocin to contract the uterus and aid evacuation of inflammatory products from the uterus (LeBlanc 2010). Likely because of this practice, the most commonly isolated bacteria after breeding in the mare, *Streptococcus spp.* and *E. coli*, have developed resistance to routinely used broad spectrum antibiotics such as penicillin, tetracyclines and sulphonamides. These bacteria are now often only sensitive to orange and red light antimicrobials[1], such as cephalosporins, aminoglycosides and fluoroquinolones (Benko *et al.* 2015) their antibiotic resistance patterns, and selected reproductive indices in English thoroughbred mares during the foal heat cycle with the latter not available for clinical veterinary use in New Zealand. Further, it remains unknown if different factors influence the bacterial populations involved in endometritis, e.g. geographical region, age of mare, lactation status or the type of semen sued (natural service vs artificial insemination with fresh or frozen semen).

Recently EquiBreed ART and Matamata Veterinary Services analysed their data from endometrial swabs taken in 2019 (n = 287) and 2023 (N = 153) in the Waikato and found disturbing patterns of increased antimicrobial resistance in Gram negative bacteria over the four-year period.

Figure 1. Changing patterns of AMR to E.coli isolated from equine endometritis cases between 2019 and 2023.



Non-antibiotic alternatives have been evaluated for intrauterine treatment and include the use of anti-inflammatory and antibacterial treatments such as Manuka Honey (Morris *et al.* NZERF project), mannose infusions (King *et al*. 2000) platelet enriched plasma (Ghallab *et al*. 2023, Segabinazzi *et al*. 2021, Mazzuchini *et al*. 2023), Botukiller™, DMSO (Mazzuchini *et al*. 2023), hydrogen peroxide (Mazzuchini *et al*. 2023), Coke® (Mazzuchini *et al*. 2023) Ozon, and stem cell infusions (Navarrete *et al.* 2020). Mazzuchini *et al.* reported that Botukiller™ and hydrogen peroxide showed the greatest *in vitro* antimicrobial activity against Staphylococcus spp., *E.coli, Pseudomonas* spp., *Klebsiella* spp. and *Candida* spp. while PRP showed selective efficacy against *Staphylococcus* spp., *E.coli*, *Klebsiella* spp (Mazzuchini *et al*. 2023). In a recent study funded by the NZERF, the efficacy of Manuka Honey was investigated as a treatment for equine endometritis, and it was found that 100% concentration of MGO 500+ honey produced a significantly large zone of inhibition during culture on agar with bacteria isolated from cases of endometritis. The infusion of Manuka Honey into the uterus was not detrimental to the uterine health, but it was spermicidal.

Further work is required to determine the MIC of Manuka Honey and other non-antibiotic alternatives to verify their efficacy against the different causative agents of equine endometritis in New Zealand.

[1] https://nzva.org.nz/assets/Policies-Guidelines-Resources/AMR

We propose that further work is required in New Zealand to determine:

1. The prevalence of endometritis, the efficacy of diagnostic methods and treatment practices in New Zealand
2. How effective are our antibiotic treatment options in New Zealand? We need to describe the antimicrobial resistance patterns of the bacterial populations involved in endometritis in New Zealand
   a. define the bacterial genera most prevalent in equine endometritis
   b. the status of antimicrobial resistance (AMR)
   c. investigate non-antibiotic alternatives to treatment
3. To involve the veterinarians in the data collection, analysis and education to enhance their understanding and contribution to the development of plans to improve diagnostic methods and targeted treatment options.

References

Benko T, Boldizar M, Novotny F, Hura V, Valocky I, Dudrikova K, Karamanova M, Petrovic V. Incidence of bacterial pathogens in equine uterine swabs, their antibiotic resistance patterns, and selected reproductive indices in English thoroughbred mares during the foal heat cycle. *Veterinarni Medicina* 60**:** 613–620, 2015

Díaz-Bertrana ML, Deleuze S, Pitti Rios L, Yeste M, Morales Fariña I, Rivera del Alamo MM. Microbial prevalence and antimicrobial sensitivity in equine endometritis in field conditions. *Animals (Basel),* 11, 2021

Ghallab RS, El-Beskawy M, El-Shereif AA, Rashad AMA, Elbehiry MA. Impact of intrauterine infusion of platelets-rich plasma on endometritis and reproductive performance of Arabian mare. *Reprod Domest Anim* 58: 622–629, 2023

Gores-Lindholm AR, LeBlanc MM, Causey R, Hitchborn A, Fayrer-Hosken RA, Kruger M, Vandenplas ML, Flores P, Ahlschwede S. Relationships between intrauterine infusion of N-acetylcysteine, equine endometrial pathology, neutrophil function, post-breeding therapy, and reproductive performance. *Theriogenology* 80: 218–227, 2013

Hanlon DW, Stevenson M, Evans MJ, Firth EC. Reproductive performance of Thoroughbred mares in the Waikato region of New Zealand: 1. Descriptive analyses. *NZ Vet J* 60: 329–334, 2012

Heil BA, Van Heule M, Thompson SK, Kearns TA, Oberhaus EL, King G, Daels P, Dini P, Sones JL. Effect of sampling method on detection of the equine uterine microbiome during estrus. *Vet Sci* 10, 2023

King SS, Young DA, Nequin LG, Carnevale EM. Use of specific sugars to inhibit bacterial adherence to equine endometrium in vitro. *Am J Vet Res* 61: 446–449, 2000

LeBlanc MM. Advances in the diagnosis and treatment of chronic infectious and post mating induced endometritis in the mare. *Reprod Domest Anim* 45: 21–27, 2010

Mazzuchini MP, Segabinazzi LG, Canisso IF. Antimicrobial activity of alternative therapies for microorganisms causing chronic endometritis in mares. *Journal of Equine Veterinary Science* 125: 104730, 2003

Morris LH, McCue P, Aurich C. Equine endometritis: a review of challenges and new approaches. *Reproduction* 160: R95–R110, 2020

Navarrete F, Saravia F, Cisterna G, Rojas F, Silva PP, Rodríguez-Alvarez L, Rojas D, Cabezas J, Mançanares ACF, Castro FO. Assessment of the anti-inflammatory and engraftment potential of horse endometrial and adipose mesenchymal stem cells in an in vivo model of post breeding induced endometritis. *Theriogenology* 155: 33–42, 2020

Pycock JF, Newcombe JR. Assessment of the effect of three treatments to remove intrauterine fluid on pregnancy rate in the mare. *Vet Rec* 138: 320–323, 1996

Rasmussen CD, Petersen MR, Bojesen AM, Pedersen HG, Lehn-Jensen H, Christoffersen M. Equine Infectious Endometritis—Clinical and Subclinical Cases. *Journal of Equine Veterinary Science* 35: 95–104, 2015

Riddle WT, LeBlanc MM, Stromberg AJ. Relationships between uterine culture, cytology and pregnancy rates in a Thoroughbred practice. *Theriogenology* 68: 395–402, 2007

Segabinazzi L, Canisso IF, Podico G, Cunha LL, Novello G, Rosser MF, Loux SC, Lima FS, Alvarenga MA. Intrauterine blood plasma platelet-therapy mitigates persistent breeding-induced endometritis, reduces uterine infections, and improves embryo recovery in mares. *Antibiotics (Basel),* 10, 2021

Tanner JC, Barrell GK. Reproductive performance of a cohort of Standardbred mares under a commercial breeding system. *Equine Veterinary Journal,* 1–10, 2023