Received: 13 December 2023 | Accepted: 21 June 2024

DOI: 10.1111/evj.14201

Equine Veterinary Journal WILEY

NARRATIVE REVIEW

Review of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* as venereal pathogens in horses

Melanie Scholtz[1] | Alan John Guthrie[1] | Richard Newton[2] | Martin Lance Schulman[3]

[1]Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

[2]Department of Veterinary Medicine, University of Cambridge, Cambridge, UK

[3]Section of Reproduction, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

Correspondence
Melanie Scholtz, Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa.
Email: mel.scholtz@gmail.com

Funding information
University of Pretoria's Equine Research Centre

[Correction added on 13 August 2024, after first online publication: Section headings '4.1 to 7' have been renumbered to '5 to 9' in this version.]

Abstract

Three bacteria extensively acknowledged as venereal pathogens with the potential to induce endometritis include *Taylorella equigenitalis*, the causative agent of contagious equine metritis (CEM), specific strains of *Pseudomonas aeruginosa*, and certain capsule types of *Klebsiella pneumoniae*. The United Kingdom's Horserace Betting Levy Board recommends pre-breeding screening for these bacteria in their International Codes of Practice and >20 000 samples are tested per annum in the United Kingdom alone. While the pathogenesis and regulatory importance of CEM are well established, an evaluation of the literature pertaining to venereal transmission of *P. aeruginosa* and *K. pneumoniae* was lacking. The aim of this review was to evaluate published literature and determine the significance of *P. aeruginosa* and *K. pneumoniae* as venereal pathogens in horses. Literature definitively demonstrating venereal transmission was not available. Instead, application of molecular typing methods suggested that common environmental sources of contamination, such as water, or fomites be considered as modes of transmission. The presence of organisms with pathogenic potential on a horse's external genitalia did not predict venereal transmission with resultant endometritis and reduced fertility. These findings may prompt further investigation using molecular technologies to confirm or exclude venereal spread and investigation of alternative mechanisms of transmission are indicated.

KEYWORDS
horse, *Klebsiella pneumoniae*, pre-breeding screening, *Pseudomonas aeruginosa*, venereal

1 | INTRODUCTION

Venereally transmissible bacterial pathogens, while not the most common cause of endometritis,[1–4] contribute to the estimated prevalence of 10%–15% of persistent breeding-induced endometritis (PBIE) reported in intensively managed Thoroughbred mares.[5] Endometritis results in lowered reproductive performance,[6–8] with consequent significant economic and genetic losses.[9,10] Further economic losses may result due to restrictions on international trade and movement of horses and semen and embryo shipments, as in the case of contagious equine metritis (CEM).[11]

The three bacteria most widely considered as transmissible venereal pathogens capable of causing endometritis are *Taylorella equigenitalis*, the causative organism of CEM, certain strains of *Pseudomonas*

aeruginosa and *Klebsiella pneumoniae* with certain capsule types. Outbreaks of endometritis associated with these three bacterial species have been reported.[12–19] These bacteria are all similarly harboured as colonists of the epithelia in smegma associated with predilection sites within the external genitalia, producing a subclinical carrier state which is challenging to detect.[13,17,20–25] These sites are the clitoral fossa and sinuses of mares and the urethra, urethral fossa and preputial *lamina interna* of stallions.[13,20–24,26]

This insidious pathogenesis and the undoubted dramatic impacts on reproductive performance of the initially-reported CEM outbreaks in the late 1970's[19,27,28] swiftly prompted several national Thoroughbred authorities to develop various 'stud health' programmes and 'codes of practice'. These measures aimed to detect affected horses, limit their impact at the population level, and improve reproductive performance. Contagious equine metritis is a World Organisation for Animal Health (WOAH) listed disease[29] and consequently a controlled and, or notifiable disease in several countries, including but not limited to Australia, Japan, South Africa, the United Kingdom and the United States.[30–34] Although neither *P. aeruginosa* nor *K. pneumoniae* are notifiable by law in these countries, they are regarded by certain regulatory authorities as important venereal pathogens that warrant pre-breeding screening of stallions and mares to control venereal disease.[35–37] For example, pre-breeding testing for *T. equigenitalis*, *P. aeruginosa* and *K. pneumoniae* is advocated by the voluntary Codes of Practice of the UK's Horserace Betting Levy Board (HBLB)[35] and >20 000 samples were tested per annum in the United Kingdom from 2016 to 2023.[38] These samples comprise reproductive tract samples, including those submitted for pre-breeding screening, pre-export testing, and those obtained from horses with clinical disease. Multiple samples may originate from a single horse. These Codes have more recently been branded as 'international' in light of them being adopted by multiple countries, including France, Germany, Ireland and Italy. Pre-breeding screening for these above-mentioned bacteria is conducted by sampling genital predilection sites in mares and stallions and subjecting samples to either bacterial culture or PCR assays to confirm or rule out the subclinical carrier state.[21,35,39] Surprisingly, however, there is a paucity of reports linking both *P. aeruginosa* and *K. pneumoniae*, when obtained from samples derived from genital swabs, with either venereal transfer or disease occurrence. Managemental inputs and costs associated with testing are significant when viewed collectively and it is therefore imperative that such costs are substantiated by appropriate evidence. Recently, a focused review examined the evidence concerning venereal transmission of *P. aeruginosa* and emphasised the necessity for comprehensive assessments of both *P. aeruginosa* and *K. pneumoniae* as potential venereal pathogens.[40] Contagious equine metritis is extensively reported, with a well-established pathogenesis, clinical and regulatory importance[11,16,17,23,24,27,41–48] and as such it will not be reviewed here. This review will interrogate the literature reporting *P. aeruginosa* and *K. pneumoniae* as venereal pathogens.

2 | PERSISTENT BREEDING-INDUCED ENDOMETRITIS

The mare's cervix relaxes during oestrus, allowing for direct intrauterine deposition of semen by the stallion at ejaculation. This results in the mare's endometrium being challenged by the intra-uterine transfer of semen as well as bacteria found on the external genitalia of both the mare and stallion and endometritis may result.[49,50] All mares may be said to develop breeding-induced endometritis. This is typically short-lived and transient with most mares resolving the post-breeding uterine contamination and associated inflammation without intervention within 24–36 h.[51,52] If the inflammation is not resolved, persistent breeding-induced endometritis (PBIE) occurs.[5] Importantly, up to 15% of Thoroughbred mares may develop PBIE and require treatment.[5,6]

Bacterial endometritis is often not apparent and only evidenced by a failure to conceive, early embryonic death (EED) or a shortened luteal phase in affected mares. The consequence is subfertility or infertility arising from either conception or early gestational failure.[6,8,52–54] Persistence of infection in mares is affected by age, parity, barren years and uterine biopsy grade, with maiden mares being the most resistant to infection.[7,55–58]

3 | KEY CONCEPTS: MARE REPRODUCTIVE CATEGORISATION AND VENEREAL PATHOGENS

Typically, maiden and multiparous mares without evidence of previous foaling injuries or other abnormalities associated with disrupted normal genital anatomy, function or conformation are able to resolve the ensuing acute post-breeding endometritis caused by opportunistic bacteria and are termed 'resistant mares'. Mares with genital anatomical, functional or conformational abnormalities, myometrial abnormalities or injuries of the reproductive tract are unable to effectively resolve the ensuing post-breeding acute endometritis and are termed 'susceptible mares'.[49,53,54,56,58–67] Opportunistic bacteria are able to cause clinical endometritis in mares with compromised uterine defences[53,55,61,68–70] but do not produce epidemic disease and are therefore not considered venereal pathogens.[69] Venereal bacterial pathogens differ from opportunistic bacteria in that they are considered capable of causing acute endometritis, not only in susceptible mares, but also in resistant mares and may result in outbreaks of post-mating endometritis due to being transmitted between stallions and mares in either direction.[13,15,18,69,71]

4 | SEARCH METHODOLOGY

Systematic searches of electronic databases were performed for *P. aeruginosa* and for *K. pneumoniae* on 7 May 2023 and updated on 14 June 2023. Our search strategy is provided in Table S1.

Publications addressing venereal transmission and or genetic relatedness of *P. aeruginosa* or *K. pneumoniae* in genital swab samples or in semen were included, as were reports of outbreaks of endometritis caused by either *P. aeruginosa* or *K. pneumoniae*. Studies reporting only the prevalence of these bacteria in genital swabs, endometrial, or semen samples but not addressing venereal transmission or genetic relatedness have not been discussed below (Figure S1 and Text S1).[72] The lack of uniformity in study design, study populations, sampling sites and diagnostic methods employed precluded the possibility of conducting a meta-analysis. A summary of the studies included in this review are provided in Tables S2 and S3.

5 | *PSEUDOMONAS AERUGINOSA*

5.1 | Transmission by carrier stallions

Isolation of *P. aeruginosa* from the genital tract of mares was first described by Hughes et al. in 1966[63] and the bacterium was subsequently described as an equine reproductive tract pathogen in 1967[73] and in 1975.[74] Furthermore, it has been listed as a venereal bacterial pathogen in various texts.[69,75–77]

To document effects of *P. aeruginosa* on fertility, Hughes et al.[63] bred nearly 800 maiden, barren and foaling mares to *P. aeruginosa* carrier-stallions. Mares were tested by pre-breeding cervical and uterine cultures and those with negative results were bred without treatment. Those with clinical signs of infection and positive cultures were treated with intrauterine antibiotics prior to breeding. Subsequent to covering, *P. aeruginosa* was recovered from the cervices or uteri in approximately 10% of mares. In the view of Hughes et al.,[63] this established the occurrence of venereal transmission. It was however, also suggested that obtaining a positive *P. aeruginosa* culture from the mare's reproductive tract in the absence of clinical signs of inflammation, infection or pneumovagina, should be considered incidental as *P. aeruginosa* was recovered from the anterior genital tract in healthy mares that had not been bred.[63] Further observations were limited to 70 infected mares for which breeding and clinical records were available. Post-breeding, maiden mares readily eliminated *P. aeruginosa*, whereas foaling and barren mares appeared more susceptible to establishment of *P. aeruginosa* in their reproductive tracts, with resultant endometritis and subfertility. It was further noted that breeding a susceptible mare to a *P. aeruginosa* carrier-stallion with establishment of a *P. aeruginosa* infection might prevent conception. Interestingly, nearly 50% of mares treated with antibiotics prior to breeding were positive on post-breeding bacterial culture, which, arguably, may indicate that antimicrobial treatment reduced numbers of commensal bacteria, allowing the more resistant *P. aeruginosa* to proliferate. Notably, mares were sampled from the cervix and uterus and not the clitoral predilection sites. Thus, it is uncertain whether mares were inapparent carriers of *P. aeruginosa* prior to breeding. Furthermore, common environmental sources of *P. aeruginosa*, such as contaminated water, were not excluded from consideration.[78] Testing for *P. aeruginosa* was solely by bacterial culture without application of typing methods and prevented confirmation that the organism isolated from both stallion and mare were of the same type. While venereal transmission was reported, it was also emphasised that mares are exposed to many potential pathogens at the time of breeding and that resistant mares were less likely to be affected by this exposure than susceptible mares.[63]

In 1967, Hughes et al.[73] reported that conception rates of 25 stallions harbouring *P. aeruginosa* (isolated from urethral swabs or semen samples) ranged from poor to good, with approximately 25% having conception rates of 71% or higher. Venereal transmission of *P. aeruginosa* with resultant endometritis thus cannot be assumed to occur in all instances. Furthermore, consideration of the role of mare status (susceptible or resistant) was lacking in determining whether exposure to potential pathogens during coitus resulted in infection and the extent to which this status may affect mare fertility, irrespective of *P. aeruginosa* exposure. It was suggested that stallions from which *P. aeruginosa* was recovered should not be considered unfit for breeding but that these stallions should be viewed as a potential source of contamination to susceptible mares. This emphasised the benefit of having resistant mares in a breeding programme. This is frequently not the case in Thoroughbred breeding systems, however, with many of the high value mares retained in breeding programmes being multiparous and older and thus more likely to be classified as susceptible.[79]

Contradictory observations were reported in 1975, in two stallions with consistent isolation of *P. aeruginosa* from their semen.[56] Most multiparous mares bred to the first stallion developed clinical infections from which *P. aeruginosa* was isolated, whereas all maiden mares conceived. These observations suggested that stallions which harbour *P. aeruginosa* have poor conceptions rates when bred to multiparous mares and supported previous works demonstrating maiden mares were at lower risk of developing mating induced infections.[63,73] The second stallion bred a larger number of mares, with over 78% confirmed as pregnant and 67% produced live foals. This suggested that stallions harbouring *P. aeruginosa* may have acceptable pregnancy rates, comparable to the 53%–100% cumulative pregnancy rates recorded for Thoroughbred stallions in the United Kingdom.[80,81] However, the mares were of unknown reproductive status and age and these factors may individually and cumulatively have profoundly affected both conception and foaling rates.[7,80,82–89] Furthermore, the first stallion covered fewer mares, which limited valid comparison of the conception rates of these stallions.[56]

A stallion, diagnosed with seminal vesiculitis due to *P. aeruginosa*, was bred to mares by artificial insemination (AI) with raw or polymyxin-B sulfate-extended semen.[90] First cycle pregnancy rates were 10% for mares bred with raw semen, compared with 78% for those bred with extended semen.[90] Interestingly, none of the mares that returned to oestrus post-AI showed isolates of *P. aeruginosa* recovered from culture of uterine swabs or biopsies. Unfortunately, mare age and reproductive status were unavailable and their effects on differences reported between the two groups of mares is unknown. This stallion had an internal infection with leukospermia

that consistently yielded a heavy growth of *P. aeruginosa*. Consequently, these findings should be extrapolated with caution to the more commonly reported scenario of transient colonisation of the external genitalia.[91]

A more recent case study attempted to determine whether *P. aeruginosa* harboured on a stallion's external genitalia would be transmitted during natural service or AI.[92] Mares were confirmed negative for *P. aeruginosa* prior to breeding by bacterial culture of samples collected from the clitoral fossa, vaginal vestibule and endometrium. While the report lacked clarity, the authors seemingly stated that approximately 50% of mares bred by natural service were positive for *P. aeruginosa* post-breeding and thus concluded that venereal transmission had occurred.[92] Owing to the confusing nature of the report, the results were challenging to interpret. Furthermore, definitive demonstration of venereal transmission using molecular methods was lacking.

5.2 | Typing of genital isolates

Typing of *P. aeruginosa* strains plays an important role in elucidating routes of transmission of these bacteria amongst horses. Prior to 2009, the only reported typing of genital *P. aeruginosa* strains in horses was in 1982 by Atherton and Pitt[13] with serological typing, phage typing and haemagglutination techniques used to type bacterial isolates and in 1993 by Kenney et al.[93] who used serotyping. An overriding conclusion of the 1982 report was that correlation between strain type and pathogenicity could not be established, and consequently all strains of *P. aeruginosa* should be considered potentially pathogenic.[13] An additional description of three outbreaks seemingly supported the assumption of venereal transmission.[13] One stallion and six mares with clinical signs of endometritis were involved in the first outbreak. *Pseudomonas aeruginosa* type 03, H3 phage type A was isolated from cervical swabs from all mares and the same strain was recovered from the penis of the stallion that bred them. One stallion and four mares without clinical endometritis signs were involved in the second outbreak. *Pseudomonas aeruginosa* type 03, H3 phage type A was isolated from clitoral swab samples and the penis of the stallion. The *P. aeruginosa* types isolated from the first and second outbreaks were indistinguishable, with no obvious links between them. The third outbreak involved one stallion and nine mares without clinical endometritis. *Pseudomonas aeruginosa* was recovered from the stallion, the cervix of five mares, and clitoris of four mares. Isolates were type 09, H5 with identical phage patterns.[13] The belief in the significance of any genital isolate of *P. aeruginosa* for venereal transmission has endured over time, as evidenced by the HBLB International Codes of Practice.[35] This persistence remains despite the report being limited by the diagnostic and strain typing methods available at the time,[78] which consequently could not definitively demonstrate venereal transmission based on authoritative genotyped transmission data. Furthermore, the report did not specify epidemiological links between horses, farms or outbreaks, and a common environmental source of contamination or fomite transmission in the first and second outbreak cannot be ruled out.[12,78] A later observation from non peer-reviewed conference proceedings that serotypes of *P. aeruginosa* isolated from mare uteri, in most instances, differed to those isolated from stallions, was inconsistent with venereal transmission.[93] These two opposing findings, using different methodologies, highlight the need for studies applying currently available molecular technology, preferably to the level of discrimination of the whole genome sequence.

However, some molecular typing methods have been key to confirming routes of transmission, occurrence of venereal spread and possibly, variations in strain pathogenicity.[12,78,94] In human medicine, various molecular typing techniques have revealed the epidemiology of *P. aeruginosa* populations.[95] These techniques include multilocus sequence typing (MLST), pulsed field gel electrophoresis (PFGE), random amplified polymorphic DNA analysis, repetitive extrapalindromic PCR analysis and restriction fragment length polymorphic DNA analysis.[95] Additionally, the use of genome sequencing has elucidated characteristics of *P. aeruginosa* associated with pathogenicity and identified strains considered non-pathogenic to humans.[94] There are limited reports of the application of such molecular techniques to populations of *P. aeruginosa* in horses, with Tazumi et al.,[96] Kidd et al.[78] and Allen et al.[12] reporting on the genetic relatedness of equine *P. aeruginosa* isolates.

In 2009, Tazumi et al.[96] examined the genetic relatedness of 63 *P. aeruginosa* isolates recovered from 63 horses in Ireland over a 5-year period using an enterobacterial repetitive intergenic consensus (ERIC) random amplification of polymorphic DNA (RAPD) PCR. Swabs were originally collected from various sites, including the urogenital tract and from semen. Twenty-four distinct genotypes were obtained, thus revealing substantial clonal heterogeneity, although certain genotypes appeared to persist and recur within this population. The authors reported good repeatability of results when examining isolates with ERIC2.[96] Unfortunately, it was not clear how many isolates originated from the urogenital tract or from semen, nor was it stated whether an association between genotypic clustering and sampling site existed. Furthermore, information regarding whether horses were epidemiologically linked was not provided,[78] thus no inference could be made regarding the genetic relatedness of *P. aeruginosa* isolates that originated from the urogenital tract and semen samples based on the report of Tazumi et al.[96] Similarly, in a more recent analysis, no inference could be drawn regarding the genetic relatedness of equine genital isolates. Amongst the 41 isolates from various species subjected to whole-genome sequencing (WGS), 15 originated from equine genital sources. Unfortunately, information regarding the genetic relatedness or epidemiological links of the sampled horses was not available. Overall, the strains exhibited considerable diversity.[97]

Kidd et al.[78] examined samples collected from 2040 mares and 18 stallions from 66 stud farms in south-east Queensland, from 2005 to 2009, to determine the relatedness of genital *P. aeruginosa* isolates. Mare samples were either clitoral or uterine swabs obtained from those with suspected infection or for routine screening and stallion samples were collected from the urethral fossa. Isolates of *P. aeruginosa* obtained on bacterial culture of samples further underwent qPCR, ERIC-PCR strain typing and antimicrobial susceptibility testing. Of the 2040 mares tested, 93 (4.6%) were positive for

P. aeruginosa while none of the 18 stallions were positive.[78] The observation that no stallions tested positive may suggest that transmission by coitus did not play a major role, that sample technique was inadequate or that stallion management practices prevented colonisation of the external genitalia.[78] The genital tracts of Thoroughbred mares from that region were found to harbour a diverse range of unrelated *P. aeruginosa* organisms, but 53/93 (57%) mares did share clonal complexes of *P. aeruginosa*. It was suggested that transmission of the organism via fomites or a common environmental source should be considered or alternatively, that some *P. aeruginosa* strains may have a tropism for the equine reproductive tract.[78] These findings were similar to those of Tazumi et al.[96] in that both found a high degree of clonal heterogeneity with some small clusters of shared clonal complexes, but dissimilar in that Kidd et al.[78] reported a single dominant genotype[78] This may be because Tazumi et al.[96] examined samples collected from various sites, whereas Kidd et al.[78] limited sample collection to the reproductive tract.

In 2011, Allen et al.[12] assessed the usefulness of PFGE in determining venereal transmission of *P. aeruginosa*. Investigation of an outbreak of endometritis revealed that the same PFGE strain of *P. aeruginosa* was isolated from the external genitalia of three stallions and the uteri of four mares. However, as these three stallions had bred different mares, venereal transmission could not account for all instances of spread of the organism, and thus a common environmental source of contamination was suspected. These findings contradicted assertions by Atherton and Pitt,[13] that multiple outbreaks of *P. aeruginosa* endometritis occurred due to venereal transmission. Interestingly, transmission of *P. aeruginosa* to all mares served by affected stallions did not occur and there were no subsequent infections in mares following the implementation of a new water source for cleaning.[12]

In alignment with the proposals by Kidd et al.[78] and Allen et al.,[12] to consider a shared environmental source of *P. aeruginosa* contamination, a recent investigation of *P. aeruginosa* sequence types (STs) from mares with endometritis and their corresponding drinking water reported instances of identical STs in both sources. This implied the potential for bidirectional contamination between mares and water supplies, underscoring the need to consider water's role in mare hygiene, particularly before breeding procedures.[98]

In summary, contradicting the arguably circumstantial evidence in earlier reports,[56,63,73,92] more recent findings based on typing isolates of *P. aeruginosa*[12,78,93,98] suggested that venereal transmission may not be the main mechanism of transmission of related *P. aeruginosa* genital isolates amongst horses. Rather, fomites or common environmental sources should be considered. While Atherton and Pitt[13] described outbreaks of similar strain types in mares and stallions, they were limited by the lack of molecular technology, as employed in later studies.[12,78] Thus, although *P. aeruginosa* is often referred to as a venereal pathogen, substantive evidence to this effect appears to be limited.[12,78,93] The lack of molecular methodologies in earlier years, limitations associated with conventional bacterial isolation and typing, and limited characterisation of strains involved in endometritis, might have resulted in the assumption that venereal transmission had occurred for a solitary strain of *P. aeruginosa*.

6 | *KLEBSIELLA PNEUMONIAE*

6.1 | Transmission by carrier stallions

Venereal transmission of *K. pneumoniae* was first reported in 1972 when 10 of 32 mares became infected after being bred to a persistently infected stallion.[14] Those infected consisted of foaling ($n = 4$), maiden ($n = 1$) and barren ($n = 5$) mares. This result suggested that both potentially resistant and susceptible mares were affected. The *K. pneumoniae* isolated from the stallion and all infected mares had identical colony morphology, capsule type and antibiotic sensitivity and resistance. Consequently, it was concluded that venereal transmission had taken place. This report was widely cited to support the supposition that *K. pneumoniae* is a venereal pathogen.[18,56,69,99–101] Of the 10 infected mares, only the five barren mares were examined bacteriologically prior to breeding and found to be negative. While not explicit, it appeared that only cervical swabs were obtained, thus not excluding an inapparent carrier status in the mares. This report differed from others in that the stallion was shown to be persistently infected. Both topical and parenteral antimicrobial treatment were ineffective in eliminating the organism that colonised the stallion's external genitalia.[14] In this case, *K. pneumoniae* was isolated as far proximal as the renal pelvis and bladder on post-mortem examination. This implied establishment of a virulent strain in the genitourinary tract of the stallion and not transient contamination or colonisation of the external genitalia which is suggested as the more common scenario.[18,55,91]

6.2 | *Klebsiella pneumoniae* capsule types implicated in outbreaks of endometritis

It has frequently been stated that *K. pneumoniae* capsule types 1, 2 and 5 are capable of venereal transmission, whereas other capsule types may be part of the normal flora of equine external genitalia.[14,18,35,69,102] Capsule types 1 and 5 were those most commonly associated with outbreaks of *K. pneumoniae* endometritis in Newmarket between 1967 and 1975.[18] In 1972, however, capsule types 2 and 5 were reportedly isolated from all samples obtained from outbreaks of endometritis and submitted to the Equine Research Station, Newmarket, UK.[14] Outbreaks associated with capsule type 2 were previously reported by the Equine Research Station in 1963 and 1964.[18] During the period 1967 to 1975, stallions that served mares with confirmed *K. pneumoniae* infection were sampled via preputial swabs and capsule type 7 was most frequently isolated. Capsule type 7 was considered as a possible component of the normal genital flora as it did not appear to be associated with uterine infections in mares.[18] In one instance, a stallion was apparently temporarily infected with *K. pneumoniae* capsule type 5 after serving a subclinically infected mare and it was suggested that the stallion transmitted this infection to another mare via the venereal route.[18] It was concluded that venereal transmission had occurred based solely on serotyping to determine capsule type. Considering the above, it would seem reasonable to conclude that capsule types 1, 2 and 5 appeared to be pathogenic in terms of their ability to cause uterine infections. It is similarly

reasonable, however, to conclude that venereal transmission was not proven, as serotyping was used in all instances to determine capsule type and this method is associated with several limitations, including extensive cross reactivity amongst certain serotypes and subjective interpretation of results.[101,103–105] Furthermore, fomites or a common environmental source of contamination were not considered.

In a Japanese survey of cervical, faecal and nasal swabs obtained from mares and genital swabs from stallions, it was found that *K. pneumoniae* capsule type 1 predominated in cases of endometritis.[99] Capsule types 1 and 7 and those that were un-typable were isolated from stallions. As only capsule type 1 was implicated in endometritis and capsule type 7 was not, these findings supported those of Platt et al.[18] that postulated that capsule type 7 was part of the normal genital flora and not typically associated with bacterial endometritis. The Japanese survey[99] presumed that venereal transmission had occurred, as only mares bred to stallions carrying heavily encapsulated capsule type 1 developed endometritis, whereas those bred to stallions carrying less heavily encapsulated capsule type 1 did not. It was further presumed that the increase in *K. pneumoniae* isolation from stallions for 2 years after the increase in *K. pneumoniae* endometritis cases was due to venereal spread of the pathogen. Venereal transmission, however, was not definitively demonstrated, as genotyping was unavailable, and common environmental sources or fomites were not investigated. Furthermore, clitoral swabs were not obtained prior to mating to determine whether mares were carriers of heavily encapsulated capsule type 1. Interestingly, the number of reported *K. pneumoniae* endometritis cases had increased concurrently and in the same area as an outbreak of CEM.[99] Speculatively, the use of antibiotics in the treatment of CEM may have altered the genital flora, favouring the growth of more resistant organisms such as *K. pneumoniae*, resulting in the observed increase in its isolation from mares and stallions. Similar observations of such disruption were made during the more recent South African CEM outbreak.[106] Later, the presence of similar plasmid profiles in *K. pneumoniae* capsule type 1 isolated from infected mares and from stallions seemingly supported the concept of venereal transmission.[107] In contrast, plasmid profiles of capsule type 7 isolated from mares and stallions were varied.[107] It has, however, been demonstrated that identical plasmids can be disseminated amongst diverse bacterial strains of *K. pneumoniae* via horizontal gene transfer.[108] Thus, distantly related bacteria may share certain plasmids. A greater degree of certainty regarding genetic relatedness should be attained through MLST or other more discriminatory molecular techniques such as WGS.

In summary, although various sources referenced this bacterium as a venereal pathogen,[35] literature definitively demonstrating venereal transmission of *K. pneumoniae* is not yet available, and information regarding phylogenetic proximity of strains isolated from equine genital tracts is limited.

7 | IMPLICATIONS FOR EVIDENCED-BASED BREEDING MANAGEMENT

In summary, mares and stallions may harbour potentially pathogenic bacteria in the clitoral fossa or sinuses of mares, and on the penis or in the urethral fossa and urethra of stallions, and these may be introduced into the mare's uterus during breeding, whether by natural or artificial means.[13,35,55,56,91,109–112] Although not definitively demonstrated, several studies have suggested that despite the presence of potential pathogens on the external genitalia of stallions and the associated potential for their coital transmission, the majority of mares did not become infected and, or, pregnancy rates were unaffected.[55,56,99,111] Notably, mares bred to stallions positive for potential pathogens on the external genitalia did not exhibit an elevated frequency of positive post-breeding uterine cultures, and bacteria isolated from these uteri often differed from those isolated from stallions.[55] Whether or not this demonstrated a lack of venereal transmission or more simply the mares' ability to clear the organisms from their reproductive tracts post-breeding cannot be inferred, especially as information on mare parity and classification as resistant or susceptible was typically not provided. It may therefore be concluded that during natural mating the presence of organisms with pathogenic potential on stallions' external genitalia does not predict venereal transmission with resultant endometritis and reduced fertility. Furthermore, some stallions carrying *P. aeruginosa* or *K. pneumoniae* on their genitalia achieved acceptable pregnancy rates, and not all mares exposed to these pathogens during the course of being bred subsequently became infected.[12,14,55,63,73,99,111] Resistant mares have demonstrated the ability to conceive even when challenged with these pathogens on a stallion's genitalia.[56,63] The detection of either of these bacteria from genital swabs thus provides insufficient evidence of the presence of venereally transmissible pathogenic bacteria capable of causing disease. Interestingly, a recent brief review of venereal transmission of *P. aeruginosa*, suggested this organism should be categorised as an opportunistic, not venereal, pathogen and managed similarly to β-haemolytic *Streptococci* and *E. coli*, which are considered part of the normal microflora of a horse's genitalia and may be mechanically transmitted during coitus.[40]

8 | FUTURE DIRECTIONS

The limited evidence supporting venereal transmission of *P. aeruginosa* or *K. pneumoniae*, coupled with the observational nature of studies, highlight the need for contemporary investigations utilising advanced molecular technologies to definitively confirm or dismiss venereal transmission. In this regard, experimental studies employing molecular techniques such as WGS are imperative to elucidate the transmission dynamics, encompassing both venereal and fomite-mediated routes, as well as potential environmental sources of contamination. Recent findings from France, employing a genomic approach, revealed that hypervirulent and, or multidrug-resistant (MDR) strains of *K. pneumoniae* were undetected by a PCR assay targeting capsule types 1, 2, and 5 in equine genital swabs collected for pre-breeding screening,[113] suggesting the assay may not be comprehensive enough to detect all potentially pathogenic types of *K. pneumoniae*. These findings underscore the necessity of employing

modern techniques. Future research and surveillance may wish to consider the detection and transmission of MDR strains of *P. aeruginosa* and *K. pneumoniae*, along with hypervirulent variants of *K. pneumoniae*, given the challenges they may pose in treatment or eradication efforts.[14,97,113–117]

9 | CONCLUSIONS

This is the first extensive review of literature concerning venereal transmission for both *P. aeruginosa* and *K. pneumoniae*. Although both *P. aeruginosa* and *K. pneumoniae* are frequently referred to as venereal pathogens, data supporting this assertion are limited and often predated the advent of current methodologies including molecular typing technologies.[13,14,18,56,63,73,93,107] The inconclusive evidence for *P. aeruginosa* and *K. pneumoniae* as important venereal pathogens strongly supports further investigation using molecular typing technologies to either confirm or exclude venereal spread of specific bacterial strains and genotypes amongst horses and facilities. The possibility of transmission of these bacteria by fomites or a common environmental source warrants additional investigation.[12,78,98]

FUNDING INFORMATION

This study was supported by the University of Pretoria's Equine Research Centre.

ACKNOWLEDGEMENTS

We thank Myleen Oosthuizen from the University of Pretoria, Faculty of Veterinary Science library, for her assistance with database searches and sourcing full text records.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Melanie Scholtz: Conceptualization; methodology; investigation; data curation; writing – original draft; writing – review and editing; visualization; project administration. **Alan John Guthrie:** Conceptualization; methodology; resources; writing – review and editing; supervision; funding acquisition. **Richard Newton:** Methodology; writing – review and editing; validation; supervision. **Martin Lance Schulman:** Conceptualization; methodology; writing – review and editing; visualization; supervision; project administration; funding acquisition.

DATA INTEGRITY STATEMENT

No new data were created or analysed in this study.

ETHICAL ANIMAL RESEARCH

Not applicable.

INFORMED CONSENT

Not applicable.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/evj.14201.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

ORCID

Melanie Scholtz https://orcid.org/0000-0002-3198-2022
Alan John Guthrie https://orcid.org/0000-0001-7729-9918
Martin Lance Schulman https://orcid.org/0000-0003-0285-8679

REFERENCES

1. 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. 2021;11(5):1416. https://doi.org/10.3390/ani11051476
2. LeBlanc MM, Magsig J, Stromberg AJ. Use of a low-volume uterine flush for diagnosing endometritis in chronically infertile mares. Theriogenology. 2007;68(3):403–12. https://doi.org/10.1016/j.theriogenology.2007.04.038
3. Rasmussen CD, Petersen MR, Bojesen AM, Pedersen HG, Lehn-Jensen H, Christoffersen M. Equine infectious endometritis—clinical and subclinical cases. J Equine Vet. 2015;35(2):95–104. https://doi.org/10.1016/j.jevs.2014.12.002
4. Walter J, Neuberg KP, Failing K, Wehrend A. Cytological diagnosis of endometritis in the mare: investigations of sampling techniques and relation to bacteriological results. Anim Reprod Sci. 2012;132(3):178–86. https://doi.org/10.1016/j.anireprosci.2012.05.012
5. Zent WW, Troedsson MH, Xue JL. Postbreeding uterine fluid accumulation in a normal population of thoroughbred mares: a field study. Proceedings of the annual convention of the American Association of Equine Practitioners. Lexington, KY: American Association of Equine Practitioners; 1998. p. 64–5.
6. Pycock JF, Newcombe JR. Assessment of the effect of three treatments to remove intrauterine fluid on pregnancy rate in the mare. Vet Rec. 1996;138(14):320–3. https://doi.org/10.1136/vr.138.14.320
7. Riddle WT, LeBlanc MM, Stromberg AJ. Relationships between uterine culture, cytology and pregnancy rates in a thoroughbred practice. Theriogenology. 2007;68(3):395–402. https://doi.org/10.1016/j.theriogenology.2007.05.050
8. Alghamdi A, Troedsson M, Laschkewitsch T, Xue J. Uterine secretion from mares with post-breeding endometritis alters sperm motion characteristics in vitro. Theriogenology. 2001;55(4):1019–28. https://doi.org/10.1016/s0093-691x(01)00462-9
9. Amat JP, Hendrikx P, Tapprest J, Leblond A, Dufour B. Comparative evaluation of three surveillance systems for infectious equine diseases in France and implications for future synergies. Epidemiol Infect. 2015;143(14):3122–33. https://doi.org/10.1017/S0950268815000217
10. Bosh KA, Powell D, Neibergs JS, Shelton B, Zent W. Impact of reproductive efficiency over time and mare financial value on economic returns among thoroughbred mares in central Kentucky. Equine Vet J. 2009;41(9):889–94. https://doi.org/10.2746/042516409X456059
11. Luddy S, Kutzler MA. Contagious equine metritis within the United States: a review of the 2008 outbreak. J Equine Vet. 2010;30(8):393–400. https://doi.org/10.1016/j.jevs.2010.07.006

12. Allen JL, Begg AP, Browning GF. Outbreak of equine endometritis caused by a genotypically identical strain of *Pseudomonas aeruginosa*. J Vet Diagn Invest. 2011;23(6):1236–9. https://doi.org/10.1177/1040638711425589
13. Atherton JG, Pitt TL. Types of *Pseudomonas aeruginosa* isolated from horses. Equine Vet J. 1982;14(4):329–32. https://doi.org/10.1111/j.2042-3306.1982.tb02446.x
14. Crouch JR, Atherton JG, Platt H. Venereal transmission of *Klebsiella aerogenes* in a thoroughbred stud from a persistently infected stallion. Vet Rec. 1972;90(2):21–4. https://doi.org/10.1136/vr.90.2.21
15. Dimock WW, Edwards PR. Pathology and bacteriology of the reproductive organs of mares in relation to sterility. Research bulletin. Volume 286. Lexington, KY: Kentucky Agricultural Experiment Station; 1928. p. 157–237.
16. Erdman MM, Creekmore LH, Fox PE, Pelzel AM, Porter-Spalding BA, Aalsburg AM, et al. Diagnostic and epidemiologic analysis of the 2008–2010 investigation of a multi-year outbreak of contagious equine metritis in the United States. Prev Vet Med. 2011;101(3–4):219–28. https://doi.org/10.1016/j.prevetmed.2011.05.015
17. May CE, Guthrie AJ, Keys B, Joone C, Monyai M, Schulman ML. Polymerase chain reaction-based national surveillance programme to determine the distribution and prevalence of *Taylorella equigenitalis* in South African horses. Equine Vet J. 2016;48(3):307–11. https://doi.org/10.1111/evj.12439
18. Platt H, Atherton JG, Ørskov I. *Klebsiella* and *Enterobacter* organisms isolated from horses. J Hyg. 1976;77(3):401–8. https://doi.org/10.1017/S0022172400055789
19. Timoney PJ, Ward J, Kelly P. A contagious genital infection of mares. Vet Rec. 1977;101(5):103. https://doi.org/10.1136/vr.101.5.103-a
20. Acland HM, Allen PZ, Kenney RM. Contagious equine metritis: distribution of organisms in experimental infection of mares. Am J Vet res. 1983;44(7):1197–202.
21. Anonymous. Chapter 3.6.2. Contagious equine metritis. Manual of diagnostic tests and vaccines for terrestrial animals 2022. Paris, France: World Organisation for Animal Health (WOAH); 2022 [cited 2023 May 31]. Available from: https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.06.02_CEM.pdf
22. Anzai T, Eguchi M, Sekizaki T, Kamada M, Yamamoto K, Okuda T. Development of a PCR test for rapid diagnosis of contagious equine metritis. J Vet Med Sci. 1999;61(12):1287–92. https://doi.org/10.1292/jvms.61.1287
23. May CE, Schulman ML, Keys B, Guthrie AJ. The distribution of *Taylorella equigenitalis* on the external genitalia of carrier stallions in South Africa. Clin Theriogenol. 2013;5:399.
24. Parlevliet JM, Bleumink-Pluym NMC, Houwers DJ, Remmen JLAM, Sluijter FJH, Colenbrander B. Epidemiologic aspects of *Taylorella equigenitalis*. Theriogenology. 1997;47(6):1169–77. https://doi.org/10.1016/S0093-691X(97)00097-6
25. Timoney P. The asymptomatic carrier stallion: critical role in venereal disease transmission. Equine Dis Q. 2016;25(4):4.
26. Stratton LG, Corstvet R, Brown J, Corley L. Isolation of *Klebsiella pneumoniae* from the urogenital tract of experimentally infected mares. J Reprod Fertil. 1979;(27):317–20.
27. Eaglesome MD, Garcia MM. Contagious equine metritis: a review. Can Vet J. 1979;20(8):201–6.
28. Platt H, Atherton JG, Simpson DJ, Taylor CE, Rosenthal RO, Brown DF, et al. Genital infection in mares. Vet Rec. 1977;101(1):20. https://doi.org/10.1136/vr.101.1.20
29. Anonymous. Chapter 1.3. Diseases, infections and infestations listed by WOAH. Terrestrial code. Paris, France: World Organisation for Animal Health; 2023 [cited 2023 Oct 9]. Available from: https://www.woah.org/en/what-we-do/standards/codes-and-manuals/terrestrial-code-online-access/?id=169&L=1&htmfile=chapitre_oie_listed_disease.htm#chapitre_oie_listed_diease
30. Anonymous. Contagious equine metritis. Australia: Department of Primary Industries, New South Wales Government; [cited 2024 Feb 8]. Available from: https://www.dpi.nsw.gov.au/about-us/services/laboratory-services/veterinary/contagious-equine-metritis
31. Anonymous. Ministerial Ordinance for Enforcement of the Act on Domestic Animal Infectious Diseases Control, Japan; [cited 2024 Feb 8]. Available from: https://www.maff.go.jp/e/policies/ap_health/animal/attach/pdf/index-6.pdf
32. Anonymous. Contagious equine metritis. United States of America Department of Agriculture; 2023. [cited 2024 Feb 8]. Available from: https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/equine/cem
33. Anonymous. List of controlled and notifiable animal diseases in terms of the Animal Diseases Act, 1984 (Act no. 35 of 1984), South Africa; 2023. [cited 2024 Feb 8]. Available from: https://old.dalrrd.gov.za/vetweb/Disease%20Control/List%20of%20Controlled%20and%20Notifiable%20Animal%20Diseases%202023.pdf
34. Anonymous. The infectious diseases of horses order 1987, United Kingdom; 1987. [cited 2024 Feb 8]. Available from: https://www.legislation.gov.uk/uksi/1987/790/article/2/made
35. Anonymous. International codes of practice. The Horserace Betting Levy Board; 2023. [cited 2023 Jun 1]. Available from: https://codes.hblb.org.uk/
36. Anonymous. Reglement du stud book français du cheval de pursang. L'Institut français du cheval et de l'équitation (IFCE); 2023. [cited 2023 May 31]. Available from: https://www.ifce.fr/wp-content/uploads/2021/12/SIRE-RSB-Pur-Sang-2023.pdf
37. Anonymous. Stud health scheme. National Horseracing Authority; 2023. [cited 2023 May 31]. Available from: https://www.nhra.co.za/index.php/studbook/stud-health/stud-health-scheme
38. Anonymous. Surveillance reports. Equine Infectious Disease Surveillance; 2023. [cited 2023 Feb 9]. Available from: https://equinesurveillance.org/landing/
39. Crabtree J. Prebreeding examination of the stallion 1. Phys Exam Pract. 2010;32(1):22–8. https://doi.org/10.1136/inp.b5503
40. Storme J, Brookes VJ, Gunn A. Is there evidence demonstrating venereal transmission of clitoral *Pseudomonas aeruginosa* in horses? Vet Rec. 2023;192(11):444–6. https://doi.org/10.1002/vetr.3140
41. May CE, Guthrie AJ, Schulman ML. Direct culture-independent sequence typing of *Taylorella equigenitalis* obtained from genital swabs and frozen semen samples from South African horses. J Vet Diagn Invest. 2019;31(5):792–4. https://doi.org/10.1177/1040638719871089
42. May CE, Guthrie AJ, Schulman ML. Addition of an intramammary antimicrobial formulation markedly decreased the intervals to elimination of *Taylorella equigenitalis* in a carrier mare and gelding. J Equine Vet. 2016;39:S62. https://doi.org/10.1016/j.jevs.2016.02.134
43. May CE, Schulman ML, Keys B, Guthrie AJ. The distribution of carrier status of *Taylorella equigenitalis* in stallions and exposed mares in South Africa. Clin Theriogenol. 2013;5:398.
44. Meldrum KC, Newton JR. Contagious equine metritis: a 2020 vision on control of a notifiable equine disease in the United Kingdom. Equine Vet J. 2020;52(3):347–8. https://doi.org/10.1111/evj.13247
45. Schulman ML, May CE, Keys B, Guthrie AJ. Contagious equine metritis: artificial reproduction changes the epidemiologic paradigm. Vet Microbiol. 2013;167(1):2–8. https://doi.org/10.1016/j.vetmic.2012.12.021
46. Timoney PJ. Contagious equine metritis. Comp Immunol Microbiol Infect Dis. 1996;19(3):199–204. https://doi.org/10.1016/0147-9571(96)00005-7
47. Timoney PJ, Powell DG. Contagious equine metritis—epidemiology and control. J Equine Vet. 1988;8(1):42–6. https://doi.org/10.1016/S0737-0806(88)80109-6

48. Wakeley PR, Errington J, Hannon S, Roest HIJ, Carson T, Hunt B, et al. Development of a real time PCR for the detection of *Taylorella equigenitalis* directly from genital swabs and discrimination from *Taylorella asinigenitalis*. Vet Microbiol. 2006;118(3):247–54. https://doi.org/10.1016/j.vetmic.2006.08.007
49. Christoffersen M, Woodward E, Bojesen AM, Jacobsen S, Petersen MR, Troedsson MHT, et al. Inflammatory responses to induced infectious endometritis in mares resistant or susceptible to persistent endometritis. BMC Vet Res. 2012;8(1):41. https://doi.org/10.1186/1746-6148-8-41
50. Kotilainen T, Huhtinen M, Katila T. Sperm-induced leukocytosis in the equine uterus. Theriogenology. 1994;41(3):629–36. https://doi.org/10.1016/0093-691X(94)90173-G
51. Katila T. Onset and duration of uterine inflammatory response of mares after insemination with fresh semen. Biol Reprod. 1995;52(1):515–7.
52. Troedsson MHT, Desvousges A, Macpherson ML, Pozor MP. Persistent breeding-induced endometritis. Pferdeheilkunde. 2008;24(1):56–60. https://doi.org/10.21836/PEM20080112
53. Leblanc M, Causey R. Clinical and subclinical endometritis in the mare: both threats to fertility. Reprod Domest Anim. 2009;44(Suppl 3):10–22. https://doi.org/10.1111/j.1439-0531.2009.01485.x
54. Woodward E, Christoffersen M, Campos J, Betancourt A, Horohov D, Scoggin K, et al. Endometrial inflammatory markers of the early immune response in mares susceptible or resistant to persistent breeding-induced endometritis. Reproduction. 2013;145(3):289–96. https://doi.org/10.1530/REP-12-0452
55. Cerny KL, Little TV, Scoggin CF, Coleman RJ, Troedsson MHT, Squires EL. Presence of bacteria on the external genitalia of healthy stallions and its transmission to the mare at the time of breeding by live cover. J Equine Vet. 2014;34(3):369–74. https://doi.org/10.1016/j.jevs.2013.07.008
56. Hughes JP, Loy RG. The relation of infection to infertility in the mare and stallion. Equine Vet J. 1975;7(3):155–9. https://doi.org/10.1111/j.2042-3306.1975.tb03256.x
57. Waelchli RO. Endometrial biopsy in mares under nonuniform breeding management conditions: prognostic value and relationship with age. Can Vet J. 1990;31(5):379–84.
58. Woodward E, Christoffersen M, Campos J, Squires E, Troedsson MH. Susceptibility to persistent breeding-induced endometritis in the mare: relationship to endometrial biopsy score and age, and variations between seasons. Theriogenology. 2012;78(3):495–501. https://doi.org/10.1016/j.theriogenology.2012.02.028
59. Canisso IF, Stewart J, Coutinho da Silva MA. Endometritis: managing persistent post-breeding endometritis. Vet Clin North Am Equine Pract. 2016;32(3):465–80. https://doi.org/10.1016/j.cveq.2016.08.004
60. Fumuso EA, Aguilar J, Giguère S, Rivulgo M, Wade J, Rogan D. Immune parameters in mares resistant and susceptible to persistent post-breeding endometritis: effects of immunomodulation. Vet Immunol Immunopathol. 2007;118(1):30–9. https://doi.org/10.1016/j.vetimm.2007.04.009
61. Heilkenbrinker T, Schubert TS, Oetjen J, Pózvári M, Frerking H. Consequences of a surgical correction of an insufficient closure of the vulva on genital flora and conception rate in mares. Dtsch Tierarztliche Wochenschr. 1995;102(5):183–7.
62. Hughes JP, Loy RG. Investigations on the effect of intrauterine inoculations of *Streptococcus zooepidemicus* in the mare. Proceedings of the annual convention of the American Association of Equine Practitioners. Lexington, KY: American Association of Equine Practitioners; 1969. p. 289–92.
63. Hughes JP, Loy RG, Asbury AC, Burd HE. The occurrence of pseudomonas in the reproductive tract of mares and its effect on fertility. Cornell Vet. 1966;56(4):595–610.
64. LeBlanc MM, Neuwirth L, Asbury AC, Tran T, Mauragis D, Klapstein E. Scintigraphic measurement of uterine clearance in normal mares and mares with recurrent endometritis. Equine Vet J. 1994;26(2):109–13. https://doi.org/10.1111/j.2042-3306.1994.tb04346.x
65. Nikolakopoulos E, Watson ED. Uterine contractility is necessary for the clearance of intrauterine fluid but not bacteria after bacterial infusion in the mare. Theriogenology. 1999;52(3):413–23. https://doi.org/10.1016/S0093-691X(99)00139-9
66. Troedsson MHT, Liu IK, Ing M, Pascoe J, Thurmond M. Multiple site electromyography recordings of uterine activity following an intrauterine bacterial challenge in mares susceptible and resistant to chronic uterine infection. Reproduction. 1993;99(2):307–13. https://doi.org/10.1530/jrf.0.0990307
67. Troedsson MH, Liu IK. Uterine clearance of non-antigenic markers (51Cr) in response to a bacterial challenge in mares potentially susceptible and resistant to chronic uterine infections. J Reprod Fertil Suppl. 1991;44:283–8.
68. Lu KG, Morresey PR. Reproductive tract infections in horses. Vet Clin N Am Equine Pract. 2006;22(2):519–52. https://doi.org/10.1016/j.cveq.2006.03.010
69. Ricketts SW. Contagious equine metritis and other equine venereal infections. In: Mair TS, Hutchinson RE, editors. Infectious diseases of the horse. Fordham, Cambridgeshire, UK: Equine Veterinary Journal; 2009. p. 217–25.
70. Troedsson MHT. Uterine clearance and resistance to persistent endometritis in the mare. Theriogenology. 1999;52(3):461–71. https://doi.org/10.1016/S0093-691X(99)00143-0
71. Platt H, Atherton JG, Simpson DJ. The experimental infection of ponies with contagious equine metritis. Equine Vet J. 1978;10(3):153–9. https://doi.org/10.1111/j.2042-3306.1978.tb02245.x
72. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann Intern Med. 2009;151(4):264–9. https://doi.org/10.7326/0003-4819-151-4-200908180-00135
73. Hughes JP, Asbury AC, Loy RG, Burd HE. The occurrence of *Pseudomonas* in the genital tract of stallions and its effect on fertility. Cornell Vet. 1967;57(1):53–69.
74. Youngquist RS. *Pseudomonas* metritis in a mare. Vet Med Small Anim Clin. 1975;70(3):340–2.
75. Markey BK, Leonard F, Archambault M, Cullinane A, Maguire D. Enterobacteriaceae. Clinical veterinary microbiology. 2nd ed. Edinburgh: Elsevier; 2013. p. 269–74.
76. Tibary A, Pearson LK, Fite CL. Reproductive tract infections. In: Sellon DC, Long MT, editors. Equine infectious diseases. 2nd ed. St. Louis: W.B. Saunders; 2014. p. 84–8.
77. Timoney PJ. Infectious diseases and international movement of horses. In: Sellon DC, Long MT, editors. Equine infectious diseases. 2nd ed. St. Louis: W.B. Saunders; 2014. p. 544–51.
78. Kidd TJ, Gibson JS, Moss S, Greer RM, Cobbold RN, Wright JD, et al. Clonal complex *Pseudomonas aeruginosa* in horses. Vet Microbiol. 2011;149(3):508–12. https://doi.org/10.1016/j.vetmic.2010.11.030
79. Scoggin CF. Not just a number: effect of age on fertility, pregnancy and offspring vigour in thoroughbred brood-mares. Reprod Fertil Dev. 2015;27(6):872–9. https://doi.org/10.1071/RD14390
80. Morris LHA, Allen WR. Reproductive efficiency of intensively managed thoroughbred mares in Newmarket. Equine Vet J. 2002;34(1):51–60. https://doi.org/10.2746/042516402776181222
81. Allen WR, Wilsher S. The influence of mare numbers, ejaculation frequency and month on the fertility of thoroughbred stallions. Equine Vet J. 2012;44(5):535–41. https://doi.org/10.1111/j.2042-3306.2011.00525.x
82. Allen WR, Brown L, Wright M, Wilsher S. Reproductive efficiency of Flatrace and National Hunt Thoroughbred mares and stallions in

England. Equine Vet J. 2007;39(5):438–45. https://doi.org/10.2746/042516407X1737581
83. Blanchard TL, Thompson JA, Brinsko SP, Varner DD, Love CC, Ramsey J, et al. Some factors associated with fertility of thoroughbred stallions. J Equine Vet. 2010;30(8):407–18. https://doi.org/10.1016/j.jevs.2010.05.005
84. Bosh KA, Powell D, Shelton B, Zent W. Reproductive performance measures among thoroughbred mares in central Kentucky, during the 2004 mating season. Equine Vet J. 2009;41(9):883–8. https://doi.org/10.2746/042516409x456068
85. Hanlon DW, Stevenson M, Evans MJ, Firth EC. Reproductive performance of thoroughbred mares in the Waikato region of New Zealand: 1. Descriptive analyses. N Z Vet J. 2012;60(6):329–34. https://doi.org/10.1080/00480169.2012.693039
86. Nath LC, Anderson GA, McKinnon AO. Reproductive efficiency of thoroughbred and standardbred horses in north-east Victoria. Aust Vet J. 2010;88(5):169–75. https://doi.org/10.1111/j.1751-0813.2010.00565.x
87. Ricketts SW, Alonso S. The effect of age and parity on the development of equine chronic endometrial disease. Equine Vet J. 1991;23(3):189–92. https://doi.org/10.1111/j.2042-3306.1991.tb02752.x
88. Schulman ML, Kass PH, Becker A, van der Merwe B. A predictive model for reproductive performance following abortion in thoroughbred mares. Vet Rec. 2013;172(2):44. https://doi.org/10.1136/vr.100670
89. Woods GL, Baker CB, Baldwin JL, Ball BA, Bilinski J, Cooper WL, et al. Early pregnancy loss in brood mares. J Reprod Fertil Suppl. 1987;35:455–9.
90. Blanchard TL, Varner DD, Love CC, Hurtgen JP, Cummings MR, Kenney RM. Use of a semen extender containing antibiotic to improve the fertility of a stallion with seminal vesiculitis due to *Pseudomonas aeruginosa*. Theriogenology. 1987;28(4):541–6. https://doi.org/10.1016/0093-691X(87)90259-7
91. Cerny KL, Little TV, Coleman RJ, Ball BA, Troedsson MHT, Squires EL. Variations of potentially pathogenic bacteria found on the external genitalia of stallions during the breeding season. J Equine Vet. 2015;35(2):170–3. https://doi.org/10.1016/j.jevs.2014.12.007
92. Tiago G, Júlio C, António R. Conception rate, uterine infection and embryo quality after artificial insemination and natural breeding with a stallion carrier of *Pseudomonas aeruginosa*: a case report. Acta Vet Scand. 2012;54(1):20–5. https://doi.org/10.1186/1751-0147-54-20
93. Kenney RM, Cummings MR, Zierdt CH, Umphenour N. *Pseudomonas aeruginosa*—somatic typing of genital tract isolates and colonization of the stallion penis: significance, diagnosis and treatment. Proceedings of the annual convention of the American Association of Equine Practitioners. Lexington, KY: American Association of Equine Practitioners; 1993. p. 601–8.
94. Wang Y, Li C, Gao C, Ma C, Xu P. Genome sequence of the nonpathogenic *Pseudomonas aeruginosa* strain ATCC 15442. Genome Announc. 2014;2(2):e00421-14. https://doi.org/10.1128/genomeA.00421-14
95. Speert DP. Molecular epidemiology of *Pseudomonas aeruginosa*. Front Biosci J Virtual Librar. 2002;7:e354–61. https://doi.org/10.2741/A929
96. Tazumi A, Maeda Y, Buckley T, Millar BC, Goldsmith CE, Dooley JSG, et al. Molecular epidemiology of clinical isolates of *Pseudomonas aeruginosa* isolated from horses in Ireland. Irish Vet J. 2009;62(7):456–9. https://doi.org/10.1186/2046-0481-62-7-456
97. Pottier M, Castagnet S, Gravey F, Leduc G, Sévin C, Petry S, et al. Antimicrobial resistance and genetic diversity of *Pseudomonas aeruginosa* strains isolated from equine and other veterinary samples. Pathogens. 2022;12(1):64. https://doi.org/10.3390/pathogens12010064
98. Mahmoud SF, Fayez M, Swelum AA, Alswat AS, Alkafafy M, Alzahrani OM, et al. Genetic diversity, biofilm formation, and antibiotic resistance of *Pseudomonas aeruginosa* isolated from cow, camel, and mare with clinical endometritis. Vet Sci. 2022;9(5):239. https://doi.org/10.3390/vetsci9050239
99. Kikuchi N, Iguchi I, Hiramune T. Capsule types of *Klebsiella pneumoniae* isolated from the genital tract of mares with metritis, extra-genital sites of healthy mares and the genital tract of stallions. Vet Microbiol. 1987;15(3):219–28. https://doi.org/10.1016/0378-1135(87)90076-9
100. Madsen M, Christensen P. Bacterial flora of semen collected from Danish warmblood stallions by artificial vagina. Acta Vet Scand. 1995;36(1):1–7. https://doi.org/10.1186/BF03547698
101. Turton JF, Baklan H, Siu LK, Kaufmann ME, Pitt TL. Evaluation of a multiplex PCR for detection of serotypes K1, K2 and K5 in *Klebsiella* sp. and comparison of isolates within these serotypes. FEMS Microbiol Lett. 2008;284(2):247–52. https://doi.org/10.1111/j.1574-6968.2008.01208.x
102. Greenwood RES, Ellis DR. *Klebsiella aerogenes* in mares. Vet Rec. 1976;99(22):439. https://doi.org/10.1136/vr.99.22.439
103. Ayling-Smith B, Pitt TL. State of the art in typing: *Klebsiella* spp. J Hosp Infect. 1990;16(4):287–95. https://doi.org/10.1016/0195-6701(90)90001-5
104. Jenney AW, Clements A, Farn JL, Wijburg OL, McGlinchey A, Spelman DW, et al. Seroepidemiology of *Klebsiella pneumoniae* in an Australian tertiary hospital and its implications for vaccine development. J Clin Microbiol. 2006;44(1):102–7. https://doi.org/10.1128/JCM.44.1.102-107.2006
105. Pan YJ, Lin TL, Chen YH, Hsu CR, Hsieh PF, Wu MC, et al. Capsular types of *Klebsiella pneumoniae* revisited by wzc sequencing. PLoS One. 2013;8(12):e80670. https://doi.org/10.1371/journal.pone.0080670
106. May CE. Molecular epidemiological investigation of contagious equine metritis in South Africa [PhD]. Pretoria: University of Pretoria; 2021.
107. Kikuchi N, Hiramune T, Blakeslee JR. Plasmid profiles of *Klebsiella pneumoniae* isolated from horses. J Vet Med Sci. 1995;57(1):113–5. https://doi.org/10.1292/jvms.57.113
108. Pedersen T, Tellevik MG, Kommedal Ø, Lindemann PC, Moyo SJ, Janice J, et al. Horizontal plasmid transfer among *Klebsiella pneumoniae* isolates is the key factor for dissemination of extended-Spectrum β-lactamases among children in Tanzania. mSphere. 2020;5(4):e00428-20. https://doi.org/10.1128/mSphere.00428-20
109. Choi SK, Lee SG, Yang JH, Cho GJ. Distribution and antimicrobial susceptibility patterns of bacteria isolated from genital tract in thoroughbred mares. J Vet Clin. 2007;24(1):19–25.
110. Lee S, Lee I, Lee SK, Lee IH. PCR-based determination of the prevalence of common venereal bacterial pathogens in breeding thoroughbreds of South Korea. J Vet Clin. 2019;36(5):245–7. https://doi.org/10.17555/jvc.2019.10.36.5.245
111. Malmgren L, Olsson Engvall E, Engvall A, Albihn A. Aerobic bacterial flora of semen and stallion reproductive tract and its relation to fertility under field conditions. Acta Vet Scand. 1998;39(2):173–82. https://doi.org/10.1186/BF03547790
112. Moore JE, Buckley TC, Millar BC, Gibson P, Cannon G, Egan C, et al. Molecular surveillance of the incidence of *Taylorella equigenitalis* and *Pseudomonas aeruginosa* from horses in Ireland by sequence-specific PCR [SS-PCR]. Equine Vet J. 2001;33(3):319–22. https://doi.org/10.2746/042516401776249750

113. Gravey F, Sévin C, Castagnet S, Foucher N, Maillard K, Tapprest J, et al. Antimicrobial resistance and genetic diversity of *Klebsiella pneumoniae* strains from different clinical sources in horses. Front Microbiol. 2024;14:141334555. https://doi.org/10.3389/fmicb.2023.1334555
114. De Vos D, Vilela CL, Pitt T, Oliveira M, Griffiths L, Craven K, et al. Antibiotic resistance profiling of phenotypic and genomic characterized *Pseudomonas aeruginosa* isolates from animal and human origin. Int J Infect Dis. 2008;12:E121–2. https://doi.org/10.1016/j.ijid.2008.05.302
115. Duchesne R, Castagnet S, Maillard K, Petry S, Cattoir V, Giard JC, et al. In vitro antimicrobial susceptibility of equine clinical isolates from France, 2006–2016. J Glob Antimicrob Resist. 2019;19:19144–53. https://doi.org/10.1016/j.jgar.2019.03.006
116. Ferris RA, McCue PM, Borlee GI, Glapa KE, Martin KH, Mangalea MR, et al. Model of chronic equine endometritis involving a *Pseudomonas aeruginosa* biofilm. Infect Immun. 2017;85(12):e0033217. https://doi.org/10.1128/IAI.00332-17
117. Sukmawinata E, Uemura R, Sato W, Htun MT, Sueyoshi M. Multidrug-resistant esbl/ampc-producing *Klebsiella pneumoniae* isolated from healthy thoroughbred racehorses in Japan. Animals. 2020;10(3):369. https://doi.org/10.3390/ani10030369

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Scholtz M, Guthrie AJ, Newton R, Schulman ML. Review of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* as venereal pathogens in horses. Equine Vet J. 2024. https://doi.org/10.1111/evj.14201