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Feasibility and Proof-of-Concept Evaluation on a Real-Time, Portable, Fluorescence-Based Device for Assessing Perineal Bacterial Contamination in Broodmares

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Abstract

Research on the reproductive tract microbiology of broodmares has primarily focused on the uterus, with a limited set of tools for the rapid detection of pathogenic bacteria in the perineal region. Accurate, real-time identification of bacterial contamination could improve the diagnosis and management of post-breeding infectious endometritis. In this proof-of-concept study, we evaluated the ability of a portable MolecuLight i:X fluorescence imaging device for the rapid, non-invasive detection of potentially pathogenic perineal bacteria in healthy broodmares, comparing results with microbiological culture as the gold standard. Using ultraviolet-induced fluorescence imaging guided for swabbing and microbiological culture, the device demonstrated 80% sensitivity, 96% specificity, and 91% accuracy in differentiating potential pathogenic from commensal bacteria in clinically healthy broodmares. These preliminary findings may represent the basis for further assessment of the real-time, fluorescence-based technology in diseased or symptomatic broodmares, potentially aiding timely clinical decision-making. Further multicentred studies with larger inclusion of mares with confirmed endometritis are needed to strengthen the relevance of this technology and to expand the device's application in equine reproductive health.

Keywords: fluorescence imaging; bacterial endometritis; MolecuLight i:X; broodmare reproductive health; antibiotic resistance

1. Introduction

In recent years, research on the reproductive tract of mares has primarily focused on uterine microbiology, largely due to the significant impact of endometritis on breeding performance [1]. More recently, studies have highlighted that the entire reproductive tract, not just the uterus but the vulva and clitoris, plays a crucial role in achieving successful breeding outcomes [2–6].

Gil-Miranda et al. suggested that some bacteria typically associated with disease may, in fact, be normal inhabitants of the mare's reproductive tract. However, under certain conditions—such as after breeding—these bacteria can colonize the uterus and contribute to the development of bacterial endometritis [7].

In cyclic mares, the uterine environment can be easily disturbed by the invasion of opportunistic and commensal microorganisms, which may enter the uterus during natural mating, artificial insemination, reproductive examination, or parturition. Additionally,

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poor anatomical perineal conformation and a relaxed cervix during the postpartum period may favour air intake (pneumovagina) and urine pooling, further increasing the risk of infection [8].

Under field conditions, the diagnosis of post-breeding infectious endometritis rarely includes detailed cytological, microbial, or histological examinations. Moreover, because the optimal window for uterine treatment is within 48 h post-breeding—before the cervix closes after ovulation—culture and antibiotic susceptibility testing results from uterine samples are often unavailable in time to guide clinical decisions [6].

In field conditions, the first-cycle pregnancy rate in Thoroughbreds is reported to be 53–68%, implying conception failure in 32–47% of cycles [9,10]. Mares that fail to conceive, particularly older mares, are more likely to receive post-breeding uterine treatment within 48 h [11,12]. This practice has become increasingly common, with studies reporting intrauterine treatment rates of up to 26.3% in young mares and 51.3% in mares over 18 years of age [11,12]. Furthermore, Allen et al. [12] reported that antibiotics comprised 24.6% of post-service treatments. To address the growing issue of antibiotic resistance resulting from overuse, the 'One Health' approach has been adopted in veterinary medicine, recommending that antibiotics be used only in cases of bacterial endometritis confirmed by positive bacterial culture [6]. Therefore, accurate diagnosis and effective treatment of post-breeding infectious endometritis (PBIE) are now more important than ever [13,14].

In a clinical setting, the ability to rapidly and accurately assess the presence of pathogenic bacteria that may impair reproductive performance could help identify cases requiring further diagnostic or therapeutic intervention [15–17].

The MolecuLight i:X (MolecuLight Inc., Toronto, ON M5G 1T6, Canada) is a portable, advanced diagnostic device that enables clinicians to perform real-time wound imaging and measurement at the point of care, instantly detecting and revealing the location of commensal saprophytic and potentially pathogenic levels of selected bacteria, depending on their known capability to produce fluorescence [15,18–20]. To pick-up red/cyan (from bacteria defined as potentially pathogenic) or green (commensal) fluorescence, the device emits a low-intensity, safe violet light at a wavelength of 405 nm, which induces fluorescence in tissues and bacteria when used in a dark environment. Darkness is necessary to clearly visualize the fluorescence signals emitted by bacteria and other biological components on the device's video screen. The colour of fluorescence depends on the substrate; skin components (such as collagen, fibrin, and intact skin) and commensal saprophytic bacteria produce green fluorescence, while opportunistic pathogens with a load greater than 10^4 CFU/mL are associated with red or cyan fluorescence [21–23]. Bacteria known to produce fluorescence under violet light, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Clostridium difficile*, are listed on the manufacturer's website (https://us.moleculight.com/faq/ accessed on 14 November 2025). MolecuLight i:X has been shown to accurately detect clinically significant numbers of common and potentially harmful bacterial species in acute and chronic wounds in both human and veterinary patients and, at the best of the authors' knowledge, no attempt to apply such technology for detecting bacteria on intact skin has been performed yet [18,24].

Based on the current literature, the aims of this study were to estimate the prevalence of bacterial fluorescence in the healthy perineal region and to evaluate the diagnostic accuracy of bacterial fluorescence imaging. Specifically, we assessed the sensitivity, specificity, positive predictive value, and negative predictive value of the MolecuLight i:X device in detecting potentially pathogenic bacteria on the perineal region of broodmares.

2. Materials and Methods

2.1. Animal Selection and Procedures

All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Camerino (Body Responsible for Animal Welfare (OPBA) of the University of Camerino, approval date: 10 October 2023; Approval code: 10/2023) and were in accordance with the standards recommended by EU Directive 2010/63/EU for experiments on animals.

A total of 6 clinically healthy and regularly bred Quarter Horse broodmares (Table 1) conducted to the Veterinary Teaching Hospital of the University of Camerino for reproductive purposes were included in this study with the informed owners' consent.

Table 1. Signalment data for the enrolled patients. * BCS: Body condition score, is a tool used to assess body fat and potential nutritional imbalances; it ranges from 1 (emaciated) to 9 (too fat), with an ideal scoring of 5.

Patient #	Age (Years)	Weight (kg)	BCS *
1	10	420	7
2	14	400	7
3	12	440	8
4	11	410	7
5	15	450	9
6	10	418	8

A perineal area examination was carried out with the mares standing in examination stocks, with the tail wrapped and secured laterally. To be eligible, the mares had to have a normal perineal conformation and no recent history of diarrhea. The perineal assessment and sampling were performed prior to any cleaning of the perineal area and vulva with soap and tepid water for reproductive interventions.

The mares had received no antibiotic therapy during the six months preceding this study. Fluorescence imaging was performed during early estrus (days 1–3), prior to any reproductive procedure.

The study mares had been maintained on farms in the Marche region (central Italy) under similar management conditions.

2.2. Sampling Procedure

Images of the perineal area were captured using a MolecuLight i:X device before cleansing of the perineal area for the reproductive procedure. First, a picture of the perineal area was taken in normal lighting conditions (Figure 1). Immediately after this, the ultraviolet light of the lamp was turned on and the room was darkened until the lamp on the device indicated that the dark was enough to enable ultraviolet light to highlight any fluorescent material, then another picture of the perineal area was taken (Figure 2).

Swabs were obtained under the ultraviolet guide (swabbing in the zone of maximum luminosity, two swabs for any fluorescent area) using the Levine method [25]. In detail, using one charcoal (Cliniswab® TS swab Amies Charcoal with wood shaft/cotton swab, APTACA Spa, Canelli, Italy) and one plain (Cliniswab® DS swab Amies Charcoal with wood shaft/cotton swab, APTACA Spa, Canelli, Italy), swabs were taken from regions of fluorescence red and green coloured on the perineal surface with MolecuLight i:X device guide. All the swabs were submitted for microbiological analysis, and samples were labelled with consecutive numbers, so a blinded microbiological analysis was performed to avoid any possible detection and selection bias.

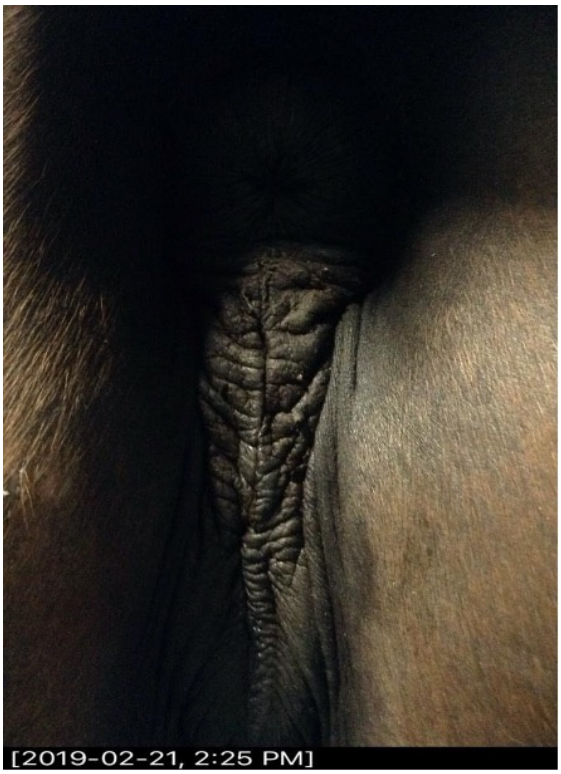

Figure 1. Perineal area captured using MolecuLight i:X device under normal lighting condition.

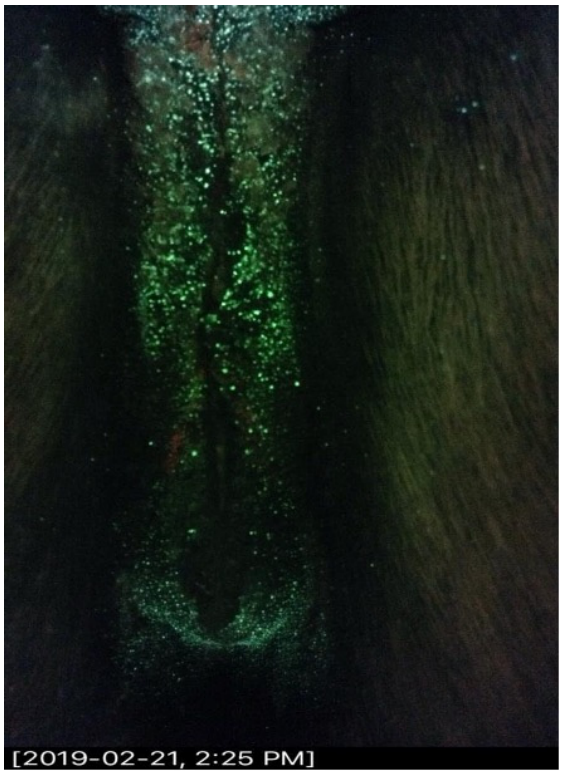

Figure 2. Same perineal area of Figure 1; image captured in a darkened room using the MolecuLight i:X device in ultraviolet mode. It is possible to note the green and red areas of fluorescence.

2.3. Microbiological Analysis

Swabs were submitted to the Medical Microbiology and Infectious Disease Laboratory at the School of Biosciences and Veterinary Medicine, University of Camerino, for bacteriological culture and total bacterial count evaluation. Direct culture onto nutritive and

selective media was performed. Specifically, Columbia blood agar with 5% sheep blood (Liofilchem®, Roseto degli Abruzzi, Italy), Columbia CNA modified agar with sheep blood 5% (Liofilchem®, Roseto degli Abruzzi, Italy), Chromatic Staph aureus (Liofilchem®, Roseto degli Abruzzi, Italy), MacConkey agar (Liofilchem®, Roseto degli Abruzzi, Italy), Hektoen enteric agar (Liofilchem®, Roseto degli Abruzzi, Italy), and Cetrimide agar (Liofilchem®, Roseto degli Abruzzi, Italy were used following the standard dilution plating technique by sterile loop and incubated at a controlled temperature of 36 °C ± 1 °C for 24 to 48 h under aerobic and anaerobic atmosphere. Bacterial strains were identified at the species-level using Matrix-Assisted Laser Desorption/Ionization—Time of Flight Mass Spectrometry (MALDI-TOF MS, Bruker Daltonics, Fahrenheitstr, Bremen, Germany), following the Standard Operating Procedure (SOP) Direct Transfer (DT) method. Briefly, each bacterial pure colony was first inoculated in a MALDI-TOF MS target plate, and subsequently 1 µL of α-Cyano-4-hydroxycinnamic acid matrix solution (Bruker Matrix HCCA, Billerica, MA 01821, USA) was added to the sample. For the MALDI-TOF MS analysis, mass spectra were processed using flexAnalysis (version 3.4; Bruker Daltonics, Fahrenheitstr., Bremen, Germany) and Biotyper® software (version 3.1; Bruker Daltonics, Fahrenheitstr, Bremen, Germany). The identification was based on the score values released by the equipment's instructions. Specifically, score values below 1.7 indicated a non-reliable identification, between 1.70 and 1.99 a probable genus identification, and a score of ≥2.0 indicated a secure genus identification and a highly probable species-level identification. The row spectra obtained were compared with those present in the Biotyper database and log (score) ≥ 2.0 was considered to be a match.

The total bacterial count per swab was calculated following the standard method [26]. Samples were serial-diluted and plated in triplicate onto Columbia blood agar (sheep blood 5%) (Liofilchem®, Roseto degli Abruzzi, Italy) and incubated at 36 °C ± 1 °C for 24 to 48 h under aerobic and anaerobic conditions. The target count for enumeration tests was in the range of 0 to 300 colony-forming units (CFU) per plate. Antibiogram testing was not performed since this was out of the scope of the present study and potential clinical application of the tested device.

The results were returned listing the isolated bacterial organism(s) and their burden in colony-forming units per swab (CFU/swab). Subsequently, the principal investigators coupled the results of the microbiological investigation with the type of fluorescence generated (if red or green).

2.4. Statistical Analysis

All equine perineal regions were divided in areas in relation to the fluorescence (green or red area). Categorical variables were expressed as percentages of commensal (saprophytic) or opportunistic pathogen bacteria for each different fluorescent area, while cardinal variables are presented as colony-forming unit/swab (mean ± standard deviation). The statistical differences were compared using the Yates's corrected chi-squared test and Student's *t* test, for nominal and continuous variables, respectively. To assess the performance of the MolecuLight i:X device, sensitivity, specificity, and positive and negative predictive values were calculated in comparison to the microbiological analysis as the gold standard test. When at least one opportunistic pathogen bacterium was isolated, the area was considered positive microbiologically. GraphPad Prism 10.0.0 for Apple version 10.1.1-270 (GraphPad Software Inc., San Diego, CA, USA), and Stata software, version 13.0 (© StataCorp LLC, College Station, TX, USA) were used. The significance level threshold was set at a p-value < 0.05.

3. Results

A total of six broodmares were included in this study and underwent MolecuLight i:X device examination plus microbiological swabbing. All mares exhibited a total microbial count in the perineal area that was above the threshold limit of detection for the MolecuLight i:X device, which is $>10^4$ CFU/mL.

According to the fluorescence detected by the MolecuLight i:X device, all mares presented areas of both green and red fluorescence, indicating the simultaneous presence of opportunistic pathogens and resident saprophytic bacterial populations. In total, 12 areas were identified and included in the statistical analysis.

A microbiological investigation detected a total of 34 bacterial species in all samples, and all mares presented colonization with more than one microorganism.

Supplementary Table S1 reports for each mare the bacteria isolated from perineal swabs, their total microbial count, and their fluorescence.

A microbiological examination detected microorganisms in all samples, including both opportunistic pathogen bacteria and microorganisms of the perineal microbiota of the mares. The pathogenic bacteria isolated included *Streptococcus equi* ssp. *zooepidemicus* in three samples (one of which as a false negative), *Streptococcus dysgalactiae* in three samples (one of which as a false negative), *Streptococcus equinus* in two samples, and *Streptococcus haemolyticus* and *Escherichia coli* in one sample each. Microorganisms of the microbiota were *Lactobacillus ruminis* in four samples, *Lactobacillus plantarum* in three samples, *Bacillus clausii* in three samples, *Streptococcus thoraltensis* in three samples, *Lactobacillus paracasei* in two samples (one of which as a false positive), *Aerococcus viridans* in two samples, while *Lactobacillus salivarius*, *Bacillus pumilus*, *Micrococcus flavus*, *Lactobacillus nageii*, *Lactobacillus coryniformis*, *Solibacillus silvestris* and *Arcanobacterium haemolyticum* were isolated in one sample each. Despite a observed 100-fold higher mean bacterial load in red areas, the difference was not statistically significant ($p = 0.8244$) due to high variability and limited sample size.

Coupling the results of fluorescence patterns by the MolecuLight i:X with those obtained by the microbiological investigations, there were one false positive and two false negative results. This resulted in a positive predicting value (PPV) and negative predicting value (NPV) of 83% and 67%, respectively (Table 2).

Table 2. Diagnostic performance of the MolecuLight i:X device considering the perineal area's fluorescence in comparison to saprophytic or opportunistic pathogenic bacteria (microbiological analysis).

MolecuLight i:X	**Microbiological Culture**		
	Positive Area—Pathogenic Bacteria	**Negative Area—Normal Resident Flora**	
Positive area (red fluorescence)	5 (true positive)	1 (false positive)	PPV 83.33% ($CI_{95\%}$ 43.65–96.99%)
Negative area (green fluorescence)	2 (false negative)	4 (true negative)	NPV 66.67% ($CI_{95\%}$ 30–90.32%)
	Sensitivity 71.43% ($CI_{95\%}$ 35.89–91.78%)	Specificity 80% ($CI_{95\%}$ 37.55–96.38%)	Accuracy 75% ($CI_{95\%}$ 46.77–91.11%)

PPV = Positive predictive value; NPV = Negative predictive value. Yates's corrected chi-squared test $X^2 = 1.371$, $p = 1.371$.

Using the MolecuLight i:X device, it was possible to differentiate between colonization and infection in four mares, as confirmed by the microbiological results. The probability of isolating at least one opportunistic pathogen in the fluorescent green areas was low (20% to 25%); whereas, in the fluorescent red areas, it was always 100% (Supplementary Table S1).

Considering the total number of bacterial strains cultured and evaluating their presence in relation to their pathogenicity and the fluorescent colour of the area, the MolecuLight i:X device's accuracy increased to 91%, with sensitivity of 80%, specificity of 96%, and positive predicting value (PPV) and negative predicting value (NPV) of 89% and 92%, respectively (Table 3).

Table 3. Diagnostic performance of the MolecuLight i:X device considering the number of different opportunistic pathogenic and saprophytic bacterial species (microbiological analysis) in each fluorescent area (green, red).

MolecuLight i:X	Microbiological Culture		
	No. of Pathogenic Bacterial Species	**No. of Saprophytic Bacterial Species**	
Positive areas (red fluorescence)	8 (true positive)	1 (false positive)	PPV 88.89% ($CI_{95\%}$ 56.5–98.017%)
Negative areas (green fluorescence)	2 (false negative)	23 (true negative)	NPV 92% ($CI_{95\%}$ 75.03–97.78%)
	Sensitivity 80% ($CI_{95\%}$ 49.02–94.33%)	Specificity 95.83% ($CI_{95\%}$ 79.76–99.26%)	Accuracy 91.18% ($CI_{95\%}$ 77.04–96.95%)

PPV = Positive predictive value; NPV = Negative predictive value. Yates's corrected chi-squared test $X^2 = 17.14$, $p = 0.00002$.

4. Discussion

This manuscript reports a preliminary, proof-of-concept investigation evaluating the feasibility of using portable fluorescence imaging technology to detect bacterial fluorescence on the intact perineal skin of broodmares. The primary aim of this exploratory study was not to establish clinical diagnostic performance, but rather to assess whether fluorescence imaging—originally developed and validated for human wound care—can detect bacterial colonization on equine intact skin under field conditions.

To the best of the authors' knowledge, this is the first proof-of-concept study to use fluorescence technology and the MolecuLight i:X device on intact skin to assess the presence of pathogenic bacteria in healthy mares.

The only study conducted in veterinary medicine highlighted that the MolecuLight i:X device accurately detected the presence of bacteria on the surface of postoperative and traumatic wounds in dogs and cats, confirming clinically relevant wound infections at the time of evaluation in all cases. However, the sensitivity and specificity of the device for bacterial detection were not assessed in that study [27].

The novelty of the present study is represented by the application of portable fluorescence imaging technology in equine patients and potential scenarios that the results may open. In the present study, the MolecuLight i:X device was able to detect fluorescence patterns associated with both commensal and opportunistic pathogens and bacteria colonizing the intact perineal area of selected broodmares.

In human medicine, the MolecuLight i:X device has demonstrated a positive predictive value (PPV) between 95% and 100% for detecting wound infections in vivo, particularly for burn wounds and military trauma-related wounds [28,29]. Additionally, several studies have found the device to have a negative predictive value (NPV) and sensitivity of 100% [30–33].

In the present exploratory study, the MolecuLight i:X device was able to detect the presence of bacteria in the perineal region of healthy broodmares, including potential pathogens such as Streptococci (*S. equi* ssp. *zooepidemicus*, *S. dysgalactiae*, *S. equinus*, *S. haemolyticus*) and *Escherichia coli*. These microorganisms are among the most frequently

associated with impaired uterine health and endometritis in broodmares [1,34–38]. A recent study by Carvalho et al. [39] found that *Escherichia coli* and beta haemolytic *Streptococcus* spp. accounted for positive bacterial cultures in 65.4% of uterine lavage, biopsy, or swab samples from broodmares; they also reported concerning antimicrobial resistance patterns, particularly among Streptococci.

In the present study, although there was presence of pathogenic bacteria, no treatment was deemed necessary due to the lack of clinical signs of uterine inflammation/infection (i.e., vulvar discharge and accumulation of uterine fluid). Key indicators for mandatory treatment are represented by a positive diagnosis of pathogenic bacteria alongside signs of uterine inflammation (detected via ultrasound, cytology, or biopsy), repeated failure to conceive, especially with fluid accumulation after breeding (persistent mating-induced endometritis), and the presence of specific pathogens such as *Taylorella equigenitalis* or certain *Klebsiella* and *Pseudomonas* types [4–6]. The same bacteria isolated from the mares in the present investigation may be responsible for puerperal diseases in breeding mares, and it would be interesting to expand the possible application of the MolecuLight i:X device to safely and effectively drive the immediate management and treatment of cases.

The device also correctly identified non-pathogenic, mainly probiotic species, such as *Lactobacillus ruminis*, *L. plantarum*, *L. paracasei*, *L. coryniformis*, *L. salivarius*, *L. nageii*, *Bacillus clausii*, *B. pumilus*, *Micrococcus flavus*, *Solibacillus silvestris* and *Aerococcus viridans*, which are commonly associated with the resident equine uterine and perineal microbiota, although some have occasionally been implicated in endometritis [7,40–43]. *Arcanobacterium haemolyticum* and *Streptococcus thoraltensis* are unusual bacteria that, if in high load, have also been identified as causes of endometritis in horses [44,45].

Traditional antimicrobial isolation testing, routinely used in both clinical and research laboratories, is considered the gold standard but typically requires 24–48 h to yield reliable results. In laboratory settings, more rapid methods—such as time-lapse imaging, sensor arrays, ATP-bioluminescence assays, and molecular techniques—can significantly reduce the time required compared to traditional culture and sensitivity testing [46].

In clinical practice, especially under field conditions, it would be ideal to rapidly and accurately identify the presence of potentially pathogenic bacteria to reduce turnaround time. In this context, portable fluorescence imaging technology may play a crucial role in advancing the use of medical screening devices in veterinary medicine, although further research is needed to clarify its applications.

However, in the present study, both false positive and false negative results were observed.

One false positive case involved *Lactobacillus paracasei*, which was detected as a 'false' signal; however, it is possible that the device correctly detected a fluorescing bacterium that was not classified as a potentially opportunistic pathogen. Some lactobacilli are known to produce porphyrins and red fluorescence, which may explain this result. The observation that fluorescence detection may not always correlate directly with bacterial pathogenicity, as in the case of *L. paracasei*, highlights the need to develop a dedicated list of commensals and pathobiont bacteria for veterinary applications [47].

The false negative results involving *S. zooepidemicus* and *S. dysgalactiae* may be due to a bacterial load in the swabbed area that was below the device's detection threshold, leading to misinterpretation. Additionally, these specific strains have not been reported to produce sufficient porphyrins for red fluorescence under the device's settings, an aspect that warrants further investigation.

It is important to note that the designation of opportunistic pathogen bacteria by the MolecuLight i:X device in this study was based on a combination of the manufacturer's specified fluorescence profiles for known pathogens and a literature-based consensus

on bacteria commonly associated with equine endometritis. This approach simplifies a complex microbiological reality that differs between human and veterinary medicine, and further research is needed to better correlate bacterial pathogenicity with fluorescence characteristics. The false positive and negative results observed in this study underscore the need to further investigate the fluorescent properties of common equine pathogens. Not all fluorescence-producing bacteria are included in the manufacturer's list, and it would be beneficial to expand this list to include a dedicated section for veterinary applications.

Although this study provides promising preliminary data on the use of portable fluorescence imaging technology for the detection of bacteria in broodmares, several limitations of the findings must be recognized to put them into context.

Lack of a diseased cohort: this study was conducted solely on healthy mares. As such, the diagnostic performance of the MolecuLight i:X device was evaluated for bacterial colonization on intact skin, not active infection or clinically relevant endometritis diagnosis. This proof-of-concept study aimed to verify whether portable fluorescence imaging technology might be capable of detecting bacterial fluorescence signals on the intact skin of broodmares, a condition that has so far been poorly explored in equine veterinary medicine. Further clinical validation of the device necessarily requires the inclusion of mares with a confirmed diagnosis of infectious endometritis, as well as a direct comparison with standard diagnostic methods (e.g., bacterial culture, uterine cytology) as a fundamental step for the subsequent development and clinical application of the technology.

High homogeneity and sample size (same geographical area, similar dietary conditions, small number of healthy mares): this reduces the possibility of directly extending the results to more heterogeneous equine populations. The primary objective of this work was to demonstrate the technical and biological feasibility of portable fluorescence imaging technology for the real-time identification of bacterial presence on intact perineal skin in mares. The choice was deliberate and motivated by the intent to reduce confounding variability in an initial validation phase of the technology, allowing for a more controlled assessment of the fluorescence signal in relation to skin bacterial colonization. Factors such as breed, age, reproductive status (e.g., postpartum period or history of endometritis), as well as different management and feeding systems can influence the perineal microbiota and the signal detected by the device. The present results may not be representative of other breeds or mares with poor perineal conformation, with active reproductive pathology, or under different management systems. These aspects should be considered when future multicentred and longitudinal studies are outlined to improve the clinical transferability of the results.

The sensitivity and specificity of the device for distinguishing pathological from physiological uterine invasion in a symptomatic cohort are still unknown and crucial for ultimate clinical utility. A further step toward the clinical use of the MolecuLight i:X device should include an assessment of the device's performance in a cohort of mares affected by endometritis, alongside a direct comparison with standard diagnostic methods (e.g., bacterial culture, uterine cytology) as a fundamental milestone for the subsequent development and application of the device.

Reference standard and "pathogen" definition: the designation of opportunistic pathogens versus commensal flora was based on a consensus derived from the literature. This simplification may not be appropriate when considering the equine reproductive microbiome, which is complex and often context-dependent, where the pathogenicity of an isolate may depend on bacterial load, host immune status, and microbial synergies. The *Lactobacillus paracasei* false positive result is illustrative of this challenge, whereby some species demonstrate commensal and pathogenic behaviours.

Technical and interpretative challenges: the study design required a subjective identification of "areas of maximum luminosity" for swabbing. An inherent problem with the non-availability of a uniform, objective method to demarcate the boundaries of fluorescence increases the potential for operator-dependent variation in the results. Furthermore, a priori, the device detection threshold (>10^4 CFU/g, according to the manufacturer) is derived and calibrated for human wound bacteria. The exact bacterial burden required to produce fluorescence in equine skin-specific bacteria remains unidentified and may be a reason for the false negatives observed.

The extent of fluorescent bacteria: the fluorescence profiles from the device depend on a manufacturer-defined list that is largely validated for common human pathogens. The fluorescent properties of many equine-specific pathogens and commensals are uncharacterized. The present study identifies a need for a veterinary-specific database since fluorescence detection is a function of bacterial biochemistry (i.e., porphyrin production) rather than direct pathogenicity. These limitations point to the exploratory nature of this research. They do not invalidate the promising initial results but clearly define the necessary scope and design for future, larger-scale validation studies in establishing the device's role in clinical equine practice.

As stated above, these preliminary results suggest new possibilities for using portable fluorescence imaging technology to detect clinically relevant bacterial contamination or infection in real time.

Future research should focus on larger, multicentred validation studies involving different breeds, management systems, and—critically—mares with confirmed infectious endometritis. Such studies should incorporate standardized fluorescence interpretation protocols, quantitative bacterial load assessment, uterine cytology, and culture-based diagnostics to establish clinically relevant thresholds and to determine true diagnostic utility. In addition, the inclusion of comparative performances of the technology regarding antibiotic susceptibility testing to possibly expand clinical interpretation and antimicrobial stewardship implications can be of interest. The development of a veterinary-specific fluorescence database for equine pathogens and commensals will also be essential. Only through this staged validation process, the clinical relevance of the MolecuLight i:X device in equine reproductive practice can be appropriately determined. Such technology represents a future direction that deserves to be better ascertained for bringing rapid, non-invasive diagnostics and monitoring out of the laboratory and into the clinical field, enabling point-of-care decision-making for faster and customized treatment of patients.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/applmicrobiol6020026/s1, Table S1: Comparison between the microbiological results and the MolecuLight i:X fluorescence observed and the probability of opportunistic pathogens in each fluorescent area.

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