



## Isolation and Identification of Pathogenic *Streptococcus pyogenes* from Vaginal and Cervical Cavity of Arabian Mares in Al-Zawraa Animals Park

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### Abstract

THE herein study was designed in order to determine the cause of abortion that happened in Al-Zawraa Animal Park and evaluate the healthy and capability of using all mares present in Arabian equine breeding and care center breeding inside Al-Zawraa Zoo Park before starting of breeding season with studying the presence of *Streptococcus pyogenes* as suspected cause. Vaginal and cervical swabs were taken from twenty-five mares during February 2023 the ages of mares distributed into three pubertal mares were under three years and sixteen mature mares were between four to twenty years as breeding mares and the rest three above twenty-one years as aged female. The routine bacterial isolation and identification of different bacterial type were done and then using PCR technique to detect *Streptococcus pyogenes* species. Eighteen positive isolations of five different bacteria from vagina, and twelve positive isolations from cervix of four different bacterial types. *Streptococcus pyogenes* was isolated from mature and aged mares and the aborted mares give positive result of this bacterium in addition to *Klebsilla* spp. that isolated from mature female only. Those two bacterial species were isolated from both (vaginal and cervical) swabs. We concluded that the cause of abortion in mares was due to infection with *Streptococcus pyogenes*, meanwhile others two aged female suffer from contamination with *Streptococcus pyogenes* and *Klebsilla* spp. which must be treated before using it for breeding. The second conclusion is the use of bacterial evaluation as point of choice before breeding season and subjected of only healthy mares for breeding and treats the infected one.

**Keywords:** Arabian mares, *Streptococcus pyogenes*, abortion, breeding.

### Introduction

The horse was been since many decades age one of most important domestic species in various ways [1]. The fertility of mare considered as a major factor in equine breeding industry by reducing early foal production [2]. The horses considered as one of the lowest fertility rates among animals. The evaluation of mares fertility was important procedures in equine breeding practice, bacterial and cytological exam will improve diagnostic performance [3]. Iraqi horses suffer from about 37.5% of infertility [4]. Pathogenic bacteria presence and accumulation of inflammatory uterine fluid considered as one of affecting factors [5]. The uterine infections of mares caused by opportunistic microbes [6], this type of infection

mostly occur during luteal phase and the uterus more resist to infectious bacteria during follicular phase [7]. Endometritis was the changes in endometrium associated with decrease fertility and originated about 25-60% of economic losses [2]. The maintenance of uterine environment was essential following aerobic and anaerobic invasion during natural breeding or other causes [8]. The positive result of  $\beta$ -hemolytic Steptococci species was highly correlated with neutrophil presence inside uterus, this help in diagnosis of endometritis [9]. Most isolated types from mare endometritis were  $\beta$ -hemolytic Steptococci [6,10], with percent reach about 50% of endometritis cases [11,12]. The bacterial

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pathogenicity of Streptococci depend upon their ability to adhere to endometrium and then stopping the clearance mechanism of uterus this happened through fibronectin-binding protein and hyaluronic acid capsule, also the Streptococci was resist to phagocytosis [13]. Sexual transmitted disease result from bacterial contamination of uterus mares with fecal flora accompanied with decrease of uterine defense mechanism, as soon as these bacteria colonized the mucus membrane show endometritis [14]. Several studies were done to detect the infection with *Streptococcus pyogenes* bacterium without dealing with equine species like Al-Athary [15], meanwhile some of studies deals with equine infection due to different causes like Mahmood *et al.* [16]; Al-Ajeeli [17] and Eabasha and Al-Awadi [18], others deals with evaluation of healthy of mares without using bacteriological detection techniques like Omran and Rasheed [19], the author had an investigation of bacterial contamination Mohammed [20]. The aim of this herein study was plain as a result of abortion of two mares in order to investigate the presence of *Streptococcus pyogenes* bacteria and others bacterial species inside vagina and cervix of Arabian mares in Al-Zawra Zoo Park and to evaluate the healthy and its portability to breeding during breeding season.

### **Experimental**

**Location:** The resent study was done at Al-Zawraa Animal Park inside equine care center. Twenty-five mares were used in this study, in different physiological status. The study was performed during the spring season (February 2023). Two of mares were aborted due to unknown causes.

**Bacteriological isolation and identification:** isolation of *Streptococcus pyogenes* bacteria and others species as a primary step was done using blood agar. Gram stain and biochemical tests will identify the bacteria. This was done according to Quinn *et al.* [21].

**Identification by PCR:** PCR technique was used to identify the presence of *Streptococcus pyogenes* performed by DNA isolates for the specific gene (Spy 1258) using pair of primers (F 5'-AAAGACCGCCTTAACACCT-3' and R 5'-TGGCAAGGTAACTTCTAAAGCA-3'). The protocol was done according to Liu *et al.* [22]. The analysis was done using gel electrophoresis under UV trans-illuminator.

**Samples collection:** Samples of swabs were collected from vagina cavity and cervix cavity according to the methods described by Rasheed [1]. All samples were taken in the same day.

**Experiment designed:** Twenty-five pure Arabian mares were used in this study, during February 2023; mares were in different physiological status and ages. Three mares were pregnant between seventh to ninth month aged were (9, 12 and 19) years respectively,

three mares in pubertal age (under three years of age), and sixteen mature mares in transition period aged between four to twenty years and the last group was three mature mares above twenty-one years.

**Statistical analysis:** Statistical test was done by using Q square test in order to detect the variation between percentage of groups at ( $P < 0.01$  and  $P < 0.05$ ). This was done using system of SAS [23].

### **Results and Discussion**

The recent study indicated that the swabs from Arabian mares show significant increases ( $P < 0.05$ ) in vaginal samples of positive bacterial isolation 18 (72%) than negative isolation 7 (28%) (Table 1). On the contrary the cervical samples show significant decrease ( $P < 0.05$ ) in percentage of positive bacterial isolation than negative samples (Table 1).

This study listed that bacterial isolation results presence of several types of bacteria from different tracts of genital system of Arabian horses (Table 2). In this regard five types of bacteria included *Streptococcus pyogenes* species in addition to *Klebsilla* spp., *E. cloi*, *Staphylococcus* spp. and *Staphylococcus aureus* were isolated from vaginal samples (Table 2). While the same bacterial types were isolated except *Staphylococcus* spp. was not isolated from cervical samples only (Table 2). *Streptococcus pyogenes* bacteria appear with 16.7% in vaginal swabs in about 3 of 18 positive isolation and its increase in vaginal positive samples to reach 25% in 3 of 12 swabs (Table 2). The *E. cloi* bacteria appear in both positive samples in about 33.3% from 6 of 18 positive samples in vaginal samples, while in increases in cervical samples to reach 50% from 6 of 12 positive samples (Table 2). *Klebsilla* spp. and *Staphylococcus aureus* recorded in both positive isolation from vagina and cervix samples to reach 5.6% (1 of 18) and 8.3% (1 of 12) respectively for *Klebsilla* spp. positive samples and 11.1% (2 of 18) and 16.7 % (2 of 12) respectively for *Staphylococcus aureus* bacteria (Table 2). The *Staphylococcus* spp. appear only in vaginal positive swabs sample to record 33.3% (6 of 18) (Table 2).

The results which obtained from vaginal samples isolation of *Streptococcus pyogenes* and *E. cloi* bacteria from mature and ages mares (Table 3). The *Klebsilla* spp., *Staphylococcus* spp. and *Staphylococcus aureus* isolated from vagina samples in mature age only (Table 3).

The results which obtained from cervical swabs isolation of *Streptococcus pyogenes* and *E. cloi* bacteria from mature and ages mares (Table 4). The *Klebsilla* spp. and *Staphylococcus aureus* isolated from cervix swabs in mature age only (Table 4).

The herein study indicated that the positive bacterial isolation samples were higher in vaginal samples than in cervical one. This fact was become in agree partially with the study of Rasheed [1] who stated that there was high significant differences

between positive and negative swabs isolated from same sites. The several bacterial types which were isolated during this study were typical with study of [1] whom recorded several bacterial types from genital samples that taken from mares' genital system. *Streptococcus pyogenes* a bacterium was recorded in this study from aborted mares indicated that this type was highly pathogenic to pregnant mares. This fact fit with the study of Gilday *et al.* [24] and da Silva *et al.* [25]. This explain that the ability of this organism to be localized in different parts of reproductive tracts. This was in agreement with Rasheed [1]. While the contamination with these bacterial types during coitus give good chance to these organisms to spread through the reproductive tracts. This become agrees with Rasheed [1] and Noakes *et al.* [7]. Some of important pathogenic bacteria such as *Streptococcus pyogenes* and *Klebsilla* spp. were isolated from different swabs samples from different genital sites. This was fit with the statement of Rasheed [1] and Noakes *et al.* [7]. While the reset of isolated bacteria was lining the reproductive tracts such as *E. cloii*, *Staphylococcus aurous* and *Staphylococcus* spp., this finding was similar to Rasheed [1]. The isolation of different bacteria from mature and aged females due to distribution of bacteria inside reproductive tracts during breeding season with ability to transport through genital tracts as ascending infection. This result agree with Rasheed [1] and Zaid [26]. The presence of *E. cloi*, *Staphylococcus aurous* and *Staphylococcus* spp. may explain as bacterial ability to transport and localized as a normal microflora. This becomes fit with Rasheed [1] and Noakes *et al.* [7]. While the as *Streptococcus pyogenes* and *Klebsilla* spp. localization may explained according

to the type of endometrial infection. Uterine defense mechanism plays an important role in the control the development of endometritis and many factors like coitus and pneumovagina cause recurrent infection and irritation to endometrium and other reproductive tracts [1]. The isolation of *Staphylococcus* spp. in one site may explain as the intact uterus must be clean from normal microflora. This is similar to the finding of Rasheed [1].

### Conclusions

We could be concluded that the aborted mares suffer from endometritis caused specifically from *Streptococcus pyogenes* infection, and the other mares that contaminated with *Streptococcus pyogenes* and *Klebsilla* spp. may suffer from low fertility rate. The second point of is the use of bacteriological evaluation of all mares before breeding is a profound technique to detect and scoring the intrauterine contamination and only the eligible mare should be subjected and use to breed with application of treatment choice of infected mares.

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### Conflicts of interest

There are no conflicts to declare.

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**TABLE 1. Percentage of bacterial swabs isolation from different sites of reproductive tracts of Arabian mares.**

Swabbing sites	Positive	Negative	Total	Significant
Vaginal swabs	18 (72%)	7 (28%)	25	*
Cervical swabs	12 (48%)	13 (52%)	25	Nil
Significant	*	*		

\*Significant differences at P<0.05.

**TABLE 2. Isolated bacteria from different sites of reproductive tracts of Arabian mares.**

Isolated bacteria	Vaginal swabs	Cervical swabs	Significant
<i>Streptococcus pyogenes</i>	3 (16.7%)	3 (25%)	Nil
<i>Klebsilla</i> spp.	1 (5.6%)	1 (8.3%)	Nil
<i>E. coli</i>	6 (33.3%)	6 (50%)	Nil
<i>Staphylococcus</i> spp.	6 (33.3%)	0 (0%)	*
<i>Staphylococcus aurous</i>	2 (11.1%)	2 (16.7%)	Nil
Total	18 (100%)	12 (100%)	*
Significant	*	*	

\*Significant differences at P<0.05.

**TABLE 3. Isolated bacteria from different ages of Arabian mares from vaginal swabs.**

Isolated bacteria	Pubertal age	Pregnant	Mature age	Aged mare	Total	Significant
<i>Streptococcus pyogenes</i>	0 0%	0 0%	2 66.7%	1 33.3%	3 100%	*
<i>Klebsilla spp.</i>	0 0%	0 0%	1 100%	0 0%	1 100%	*
<i>E. coli</i>	0 0%	0 0%	4 66.7%	2 33.3%	6 100%	*
<i>Staphylococcus spp.</i>	0 0%	0 0%	6 100%	0 0%	6 100%	*
<i>Staphylococcus aureus</i>	0 0%	0 0%	2 100%	0 0%	2 100%	*
<b>Total</b>	0 0%	0 0%	15 83.3%	3 16.7%	18 100%	*
<b>Significant</b>	Nil	Nil	*	*	*	

\*Significant differences at P<0.05.

**TABLE 4. Isolated bacteria from different ages of Arabian mares from cervical swabs.**

Isolated bacteria	Pubertal age	Pregnant	Mature age	Aged mare	Total	Significant
<i>Streptococcus pyogenes</i>	0 0%	0 0%	2 66.7%	1 33.3%	3 100%	*
<i>Klebsilla spp.</i>	0 0%	0 0%	1 100%	0 0%	1 100%	*
<i>E. coli</i>	0 0%	0 0%	4 66.7%	2 33.3%	6 100%	*
<i>Staphylococcus aureus</i>	0 0%	0 0%	2 100%	0 0%	2 100%	*
<b>Total</b>	0 0%	0 0%	9 75%	3 25%	12 100%	*
<b>Significant</b>	Nil	Nil	*	*	*	

\*Significant differences at P<0.05.

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## عزل وتشخيص البكتريا العقدية المقيحة الأمراض من تجويف المهبل وعنق الرحم للأفراس العربية في حديقة حيوانات الزوراء. أنسام خالد محمد<sup>1</sup> و نزيه ويس زيد<sup>2</sup>

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### الخلاصة

تم تصميم الدراسة الحالية لمعرفة سبب حدوث حالات الأجهاض في الأفراس العربية الموجودة في حديقة حيوانات الزوراء وأيضا تقييم الحالة الصحية والقبالية التكاثرية للأفراس الموجودة في مركز تكثير الخيول العربية الأصيلة داخل حديقة حيوانات الزوراء قبل بدء الموسم التكاثري وكذلك وجود البكتريا العقدية المقيحة كسبب مسبب للأجهاض. تم أخذ مسحات مهبلية ومسحات من عنق الرحم من خمسة وعشرين فرس خلال شهر شباط 2023 وقسمت أعمار الأفراس لثلاثة أقسام: ثلاثة أفراس قبل البلوغ وكانت تحت عمر ثلاث سنوات وستة عشر فرس بالغة بين عمر أربعة وعشرين عاماً كأفراس تكاثر والبقية هي الأفراس الهرمة التي في عمر فوق واحد وعشرين سنة. تم إجراء العزل الروتيني وتشخيص الجراثيم للأفراس المختلفة ومن ثم استخدمت تقنية تفاعل البوليمريز المتسلسل لتشخيص جرثومة العقد المقيحة. ثمانية عشر عزلة موجبة تم عزلها لخمسة أنواع مختلفة من الجراثيم من المهبل، وأثنى عشر عزلة جرثومية موجبة من عنق الرحم لأربعة أنواع جرثومية. الجرثومة العقدية المقيحة تم عزلها من الأفراس البالغة والهرمة والأفراس المهضمة أظهرت نتيجة موجبة لهذه الجرثومة إضافة إلى وجود الكليسيلا والتي عزلت فقط من الأفراس الهرمة. هذه الجرثومتين عزلتا من كلا مسحات تجويفي المهبل وعنق الرحم. نستنتج من ذلك أن سبب الأجهاض في الأفراس حدث نتيجة الإصابة بالجرثومة العقدية المقيحة وفي نفس الوقت الأفراس الهرمة عانت من الإصابة بنوعي الجراثيم العقدية المقيحة والكليسيلا ولهذا يجب علاجها قبل استخدامها في التكاثر. الأستنتاج الثاني هو استخدام تقييم الجراثيم كأختيار قبل البدء بالموسم التكاثري للأفراس المستخدمة للتكاثر وعلاج المصابة منها.

**الكلمات الدالة:** الأفراس العربية، الجرثومية العقدية المقيحة، أجهاض، تكاثر.