BACTERIOLOGICAL STUDIES OF ENDOMETRITIS AS A MAIN CAUSE FOR REPRODUCTIVE AND FERTILITY PROBLEMS IN SHE-CAMEL

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ABSTRACT

A total of 54 female camels with a history of conception failure were examined through transrectal palpation, ultrasonography and vaginal explorations. Animals were categorized according to type of uterine infection (endometritis \( n=26 \) animals) and (metritis \( n=28 \) animals). Several types of both Gram negative and Gram positive bacteria were isolated from diseased animals. Presence of bacteria were detected in samples (87.5 %) in cases of endometritis in contrast to (92.5%) from metritis cases. Several microorganisms were isolated from infected camels. The microorganisms associated with endometritis were identified as Staphylococcus aureus, 16 isolates (40 %), Corynebacterium Spp., 8 isolates (20 %), E.coli, 6 isolates (15 %) and Salmonella spp. 5 isolates (12.5 %). In metritis cases the isolated bacteria were identified as Corynebacterium Sp,12 isolates (30 %), Proteus Sp. 10 isolates (25 %), Klebsiella Sp. 8 isolates (20 %) and Salmonella Sp. 7 isolates (17.5 %). According to the line of treatment, female camels were divided into four groups for endometritis and metritis cases. In endometritis cases the groups treated as follows: G.1: \( n = 7 \) she-camel were treated with gentamycine sulphate I/M injections combined with intrauterine infusions of acriflavine solution. G.2: \( n = 6 \) she-camels were treated with Cefquinome intramuscularly and acriflavine solution. G.3: \( n = 7 \) received lotagen1 solution with gentamicin sulphate I/M. G.4: \( n = 6 \) received lotagen solution intrauterine wash with Cefquinome I/M. Meanwhile animals suffering from metritis treated as follows: group 1 \( n = 8 \) were washed with acriflavin solution intrauterine with Enrofloxacine I/M injections, group 2 \( n = 6 \) received acriflavin solution as intrauterine wash with Synulox, group 3 \( n = 7 \) received lotagen solution intrauterine with Enrofloxacine I/M and group 4 \( n = 7 \) received lotagen solution intrauterine with Synulox I/M. Our study revealed that the best conception rates was done in camels treated by gentamycine with acriflavine in endometritis cases, while in metritis the highest conception rates were cleared in she camel treated by synulox in combination with lotagen intra uterine wash.

Key words: Endometritis, She-Camel, Bacterial isolates.

INTRODUCTION

Uterine infections were considered to be the most common cause of reproductive failure in female camels (Werny and Kumar, 1994). The major contributing factors were overbreeding, postpartum complications, and unsanitary gynecological manipulation (Tibary, 2004). Arcanobacterium pyogenes, Streptococcus pyogenes, and Staphylococcus aureus, Corynebacterium, E.Coli as well as Proteus were frequently isolated from female camels with uterine infections (Ali et al., 2010). The subfertility associated with uterine infections involved the effects of uterine damage as well as disruption of ovarian function (Sheldon and Dobsonb 2006). There is a lot of confusion about the definitions of uterine infections, because the same conditions may receive different names, the examinations and criteria for the diagnosis of uterine infections may differ or are often not well specified.

The depth of inflammation of the uterine wall distinguishes uterine infection into metritis and endometritis (Sheldon et al., 2006). Diagnosis of uterine infections by rectal palpation was probably the basis for treatment of most cows in the field (Le Blank, 2008). Vaginal speculum examination enhances sensitivity for detecting endometritis (Dohmen et al., 1995; Le Blank et al., 2002 and Barlund et al., 2008). Manual vaginal examination was probably more practical and used simple techniques (Sheldon et al., 2002). For the treatment of
uterine infection in female camels, the use of PGF2a has been recognized in veterinary routine therapy (Miller et al., 1980). Also, an intrauterine treatment with antiseptics or antibiotics has been found effective (Fredrikson et al., 1988).

To date, there is no clinical trial evaluating the effectiveness of different treatments of uterine infections in female camels. Most veterinarians in practice use treatments proposed for the bovine or equine species.

This work was conducted to investigate the effect of antibiotic and antiseptic treatment on conception rates in she-camels based upon available famous antiseptics and antibiotics in the veterinary market used in combination together.

MATERIALS and METHODS

Animals and classification
A total of 54 female camels (Camelus dromedarius) aged 5–12 years were included in this study. The animals were presented in special farms in Egypt during the breeding season with a history of conception failure in spite of breeding more than 3 times with fertile males. All examined females were generally healthy with no systemic signs of diseases.

The reproductive tract of each animal was examined through transrectal palpation, ultrasonography (Dynamic imaging LTD, Scotland-UK attached to 7.5 MHz transducer), and manual vaginal exploration. Size of the uterine horns, accumulation of uterine fluid, and vaginal contents were recorded. Catarhal (turbid mucus), mucopurulent (turbid mucous with flakes of pus), or purulent (profuse pus) vaginal discharges were regarded as clinical sings of uterine infections. The females were categorized according to type of uterine infection (endometritis n = 26 vs. metritis n = 28).

Where the criteria used to differentiate endometritis from metritis are shown in (Table 1) according to (Sheldon et al., 2006 and Kahn, 2004). Conception rate (CR): the number of pregnant females /total number of females conceived x 100.

Table 1: Diagnosis and distinguishing of endometritis and metritis of she camels.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Endometritis</th>
<th>Metritis</th>
</tr>
</thead>
<tbody>
<tr>
<td>History</td>
<td>Repeat breeder</td>
<td>Repeat breeder</td>
</tr>
<tr>
<td>Vaginal discharge</td>
<td>Catarrhal</td>
<td>Mucopulent/purulent</td>
</tr>
<tr>
<td>Transrectal palpation of uterus</td>
<td>No palpable uterine changes</td>
<td>The uterus enlarged and thickened with or without intrauterine fluids.</td>
</tr>
</tbody>
</table>

Samples:--

Two uterine swabs from each case were collected under complete aseptic conditions, one inoculated into nutrient broth tube and other swab into selenite F-broth tube, labeled and transported to the laboratory of mastitis and neonatal diseases department (ARRI) for examinations. The nutrient broth swabs were streaking directly but the selenite F-broth swabs were incubated for 18-24 hrs at 37°C before streaking on general media of bacteriology (blood agar (Oxoid), nutrient agar (Oxoid), MacConkey agar (Oxoid), S.S agar, Mannitol salt agar, Edward agar, Phenylalanin agar and Dorset egg medium) and all were incubated aerobically at 37°C for 24 and 48 hrs.

All isolates were identified further according to Cowan's tests (Cowan and Steel, 1985). Sensitivity antibiotic test, Different pure colonies at primary isolation were picked up aseptically, sensitivity tests was performed for each isolate esparately and suitable antibiotics for treatment were detected according to (Fredriksson et al., 1988).

Treatment regimes
Treatment was carried out during the breeding season. The animals were randomly assigned to one of four intrauterine treatments according to sensitivity results of bacterial infections.

In case of endometritis the animals were classified to four groups, group 1 (n = 7) received 100 mL 1.0 % acriflavin solution as intrauterine wash (C14N14CIN3, Fluka chemie EG, Buchs, Switzerland) with gentamycine sulphate I/M injections 2.5ml/100kg.B.W.for 5dayes (Egyptian European Company), group 2 (n = 6) received 100 mL 1.0 % acriflavin solution as intrauterine wash with Cefquinome 2ml/50kg B.W. I/M for 3 consecutive days (MSD Co.,U.S.A.), group 3 (n = 7) received 100 mL 0.5% lotagen solution (metacresolsulphonic acid and formaldehyde, Schering-Plough Animal Health, Segre-France) with 2.5ml/kg B.w gentamicin sulphate I/M for 5days and group 4 (n= 6) received100 mL 0.5% lotagen solution intrauterine wash with Cefquinome 2ml/50kg B.W. I/M for 3 consecutive days (MSD Co.,U.S.A.).

Meanwhile animal suffering from metritis treated as follows: group 1 (n = 8) were washed with 100 mL 1.0 % acriflavin solution intrauterine with Enrofloxacin 0.5 ml/20 kg B.W. I/M injections (ADWIA, Egypt), group 2 (n = 6) received 100 mL 1.0 % acriflavin solution as intrauterine wash with
Synulox 1ml/20kg B.W. I/M for 3 consecutive days (Pfizer, Haupt pharma, Italy), group 3 (n = 7) received 100 mL 2% lotagen1 solution intrauterine with Enrofloxacin 0.5 ml/20 kg B.W. I/M injections (ADWIA, Egypt). and group 4 (n = 7) received 100 mL 2% lotagen1 solution intrauterine with Synulox 1ml/20 kg B.W. I/M for 3 days.

All treated animals were exposed for natural mating with fertile males. All mated females received an intramuscular injection of 5000 IU hCG (Pregnyl1, N.V. Organon, Oss, Holand) at mating time. The conception rates (C.R.) were recorded for all treated endometritis and metritis groups.

**Statistical analysis**

Data were analyzed for effects of treatment (acriflavin and lotagen with different regimes), type of uterine infection (endometritis and metritis), and C.R.. The level of significance was tested at P < 0.05. A statistical program (SPSS.Copyright 2007) was used to perform the statistical analysis.

**RESULTS**

Our obtained results revealed that there were bacterial isolates and sensitivity differences between endometritis and metritis as shown in Table 2, 3 and 4.

The effect of each type of treatment and severity of uterine infection and CR was shown in Table 5 and 6.

**Isolation and identification of bacteria:**

The types and frequency of bacteria isolated from the uterine swabs are listed in Table (2). Four specific and non-specific different genus of bacteria were identified in each type of infection (Endometritis and Metritis). It was observed that Staphylococcus aureus isolates were recorded the highest % of total isolates (40 %) followed by Corynebacterium sp (20 %), E.coli (15 %) and ended by salmonella sp. (12.5 %) in cases of Endometritis. Meanwhile Corynebacterium and Proteus sp. Were recorded the highest % of isolates (30 % and 25 % for each respectively) followed by Klebsella sp. (20 %), while Salmonella sp. Was isolated in lowest % (17.5 %) in cases of Metritis. Staph. aureus was not isolated from all metritis cases.

In table 3&4 our obtained results revealed that from 10 antibiotics used in sensitivity test, there were Gentamycine and Cefquinome getting a highly sensitive results in case of endometritis bacterial isolates, while Ciproflxacine and Amoxycilline+Claveolininic acid were getting the highest sensitivity results in metritis bacterial isolates.

Table 5: The results obtained after treatment of she-camel endometritis cleared that using of acriflavine intrauterine doingh accompanied with gentamycine sulphate intramuscular injection (group 1) gives the highest percentage of conception rates (85.7%) followed by (group 3) which getting 71.4% conception rates, while group 2 and groupe 4 gave the lowest conception rates (66.7%, 62.5%, and 57.1% respectively).

On the other hand the conception rates in metritis cases of she-camel after treatment (Table 6) showed that lotagen intrauterine douching with synulox intramuscular injection (group 4) revealed the highest conception rate followed by group 2, but group 1 and group 3 denoted the lowest conception rates (71.4, 66.7, 62.5, and 57.1% respectively).

**Table 2:** Types and percentages of bacteria isolates from affected she-camels.

<table>
<thead>
<tr>
<th>Type of disease</th>
<th>Isolates</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometritis</td>
<td>-Staphylococcus aureus</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>-E.coli</td>
<td>6</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>-Corynebacterium sp.</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>-Salmonella</td>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td>Metritis</td>
<td>-Corynebacterium sp.</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>-Klebsella sp.</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>-Proteus sp.</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>-Salmonella sp.</td>
<td>7</td>
<td>17.5</td>
</tr>
</tbody>
</table>
Table 3: Antibiotic sensitivity guide for isolated bacteria

<table>
<thead>
<tr>
<th>Name of the antibiotic discs</th>
<th>P</th>
<th>AML</th>
<th>GN</th>
<th>OT</th>
<th>S</th>
<th>Enr</th>
<th>Cef</th>
<th>E</th>
<th>AMC</th>
<th>AM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Types of diseases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometritis isolates</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Metritis isolates</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>

P (Penicillin G.), AML (Amoxicillin), GN (Gentamycin), OT (Oxytetracyclin), S (Streptomycin), Enr (Enrofloxacin), Cef (Cefquinome), E (Erythromycin), AMC (Amoxicillin + Clavulanic acid) and AM (Amoxicillin)

Table 4: Drug of choice according to sensitivity test.

<table>
<thead>
<tr>
<th>Types of Diseases</th>
<th>Drug of choice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometritis isolates</td>
<td>1- Gentamycin 2- Cefquinome</td>
</tr>
<tr>
<td>Metritis isolates</td>
<td>1- Enrofloxacin 2- Amoxicillin + clavulanic acid</td>
</tr>
</tbody>
</table>

Table 5: Conception rates after treatment of endometritis with different regimes in she-camels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Type of treatment</th>
<th>C.R.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>Acriflavine + Gentamycine</td>
<td>6/7</td>
<td>85.7 a</td>
</tr>
<tr>
<td>Group 2</td>
<td>Acriflavine + Cefquinome</td>
<td>4/6</td>
<td>66.7 c</td>
</tr>
<tr>
<td>Group 3</td>
<td>Lotagen + Gentamycine</td>
<td>5/7</td>
<td>71.4 b</td>
</tr>
<tr>
<td>Group 4</td>
<td>Lotagen + Cefquinome</td>
<td>4/6</td>
<td>57.1 d</td>
</tr>
</tbody>
</table>

*Means with different subscripts (a,b,c,d) in the same column were significantly different at P<0.05.

Table 6: Conception rates after treatment of metritis with different regimes in she-camels.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Type of treatment</th>
<th>C.R.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td></td>
</tr>
<tr>
<td>Group 1</td>
<td>Acriflavine + Enrofloxacin</td>
<td>5/8</td>
<td>62.5 c</td>
</tr>
<tr>
<td>Group 2</td>
<td>Acriflavine + Synulox</td>
<td>4/6</td>
<td>66.7 b</td>
</tr>
<tr>
<td>Group 3</td>
<td>Lotagen + Enrofloxacin</td>
<td>4/7</td>
<td>57.1 d</td>
</tr>
<tr>
<td>Group 4</td>
<td>Lotagen + synulox</td>
<td>5/7</td>
<td>71.4 a</td>
</tr>
</tbody>
</table>

*Means with different subscripts (a,b,c,d) in the same column were significantly different at P<0.05.

DISCUSSION

Detection of uterine infection is very important in the prevention of venereal transmission of infection to other animals. In addition, identification of the causative agents and determination of its sensitivity to different drugs allows the practitioner to choose the most efficient treatment.

Various uterine disorders have been described in camelids and may play an important role in reduced fertility in these species (Tibary and Anouassi, 1997). Like so many domestic animal species, uterine infections are the most common of these disorders Fowler (1998); Johnson (1989); Nur (1984); Tibary and Anouassi (1997); Tibary and Anouassi (2000);
The bacteria responsible for endometritis in our results were Staphylococcus aureus, E.coli, Corynebacterium sp.and Salmonella sp, this agreement with the result revealed by Nawito (1973); Wernery (1991); Wernery and Wernery (1992); Wernery and Kumar (1994) where they found these bacteria in the equine and bovine species; the most common one to be isolated from camels with endometritis is Escherichia coli (E. coli). Other bacteria that have been isolated are Streptococcus zooepidemicus, -haemolytic Streptococci, Enterococcus, coagulase negative Staphylococcus, Proteus spp., Enterobacter aerogenes, Klebsiella pneumoniae and Arcanobacter pyogenes (Chauhan et al., 1987; Enany et al., 1990). Nawito (1973); Powers et al. (1990); Wernery, J. (1991); Wernery and Kumar (1994). Pseudomonas aeruginosa, Campylobacter fetus, and Trichomonas fetus have been isolated from infertile camels and may be associated in infertility or abortion outbreaks (Wernery and Wernery, 1992).

Left untreated uterine infections can lead to irreversible changes and complications such as salpingitis, resulting in a total loss of fertility (Tibary and Anouassi 2000). There is no clinical trial comparing the efficacy of different treatments of endometritis in camelds. Most practitioners use treatments proposed for the bovine and equine species, which include uterine lavage or flushing, intrauterine antibiotic infusion, systemic antibiotic treatments or a combination of these. In the present study we found that treatment of endometritis using of acriflavine intrauterine douching accompanied with gentamycin sulphate intramuscular injection gives the highest percentage of conception rates (85.7%). On other hand Intrauterine infusion of homologous blood plasma (twice at 24 hour intervals) has also been used in llamas and alpacas (Johnson, 1989).

Meanwhile, our results revealed that in metritis cases the isolated organisms in she camel were Corynebacterium and Proteus sp. recorded the highest percentages of isolates followed by Klebsella sp., while Salmonella sp. was the lowest isolates. On the other hand, the treatment regime in this study recorded that the highest results were detected after using lotagen intrauterine douching with synulox intramuscular injection which revealed the highest conception rate (71.4%).

Accordingly, the action of lotagen is not pathogen specific, but can attack a broad spectrum of pathogens Snyder et al. (1990). Gentamycin seems to be less efficient in treating female camels with metritis. With regard to the use of antibiotics to eliminate bacterial infections, one should remember that bacterial cultures and antibiotic susceptibilities are the best way to approach the problem of an efficacious antibacterial selection. Furthermore, the basic question to be addressed is what tissues are involved in the uterine infection being treated? If the infection involves deeper layers of the uterus and other genital tissues, systemic therapy would be necessary. If, however, the infection is limited to the endometrium, then local therapy is probably warranted due to very high-sustained levels of antibiotic in the lumen and endometrium (Youngquist and Shore, 1997).

CONCLUSION

We can conclude that the relatively high incidence of recovery of endometritis and metritis cases in our study may reflect the importance of isolated micro-organisms in inducing genital tract infections in female camels. Gentamycin I/M injection seems to be more efficient in treating female camels with endometritis in combination with acriflavine intra uterine wash. While Synulox I/M has the best choice of treatment of metritis cases in combination with lotagen intra uterine douches. With regard to the use of antibiotics to eliminate bacterial infections, one should remember that bacterial cultures and antibiotic susceptibilities are the best way to approach the problem of an efficacious antibacterial selection.

REFERENCES


دراسات بكتيرولوجية على التهابات بطاقة الرحم كأسباب المشاكل التناسلية وقلة الخصوبة في إناث الجمال

أشرف تيماء محمد، رشاد حمد عمان

أجريت الدراسة على عدد 45 من إناث الجمال التي تعاني من التهابات بطاقة الرحم والتهابات الرحم. تم تقسيم إناث الجمال بعد فحص الرحم عن طريق المستقيم، للمواجات فوق الصوتية والاستكشاف المهني إلى قسمين: القسم الأول يشمل على عدد 26 إناث جمل تعاني من التهابات بطاقة الرحم، أما القسم الثاني فكان يعاني من التهابات رحمية ويشمل 19 إناث جمال. تم عزل اندواع مختلفة من البكتيريا سلالة الجرام و أخرى موجبة.

الجرام من الحيوانات المصابة، بنسبة 42.5 % في حالات التهابات بطاقة الرحم. كانت الميكروبات المعزولة من الحيوانات المصابة بالتهاب الرحم عبارة عن الميكروبات المعزولة ذهبي بعدد 16 ممزولة ونسبة 40 % من إناث المعزول. بينما كانت البكتيريا الكورني، الميكروبات الميكرولولو، وميكروبا السالمونيلا باعداد 8 و 6 و 5 ممزولة ونسب عزل و 100 و 15 و 12.5 % بالتباط. بينما كانت الميكرولولو وبيكروب السالمونيلا (20%) و كي و 10% والثاني. تبع لروتوكول العلاج الذي تم تطبيقه أثناء الدراسة، مما يقسم الحيوانات المصابة إلى أربع مجموعات: بالنسبة للحيوانات التي تعاني من التهابات بطاقة الرحم كما في: المجموعة الأولى: تشتمل 7 حيوانات، من إناث الجمال تم علاجها بإعطاء مضادات حيوية سلالة الجرامينين عن طريق الحق العضلى مع عامل كيرفلاتين. المجموعة الثانية: تحتوي على عدد 6 حيوانات، تم علاجها بإعطاء مضادات حيوية سلالة السلاحين مع عامل رومي ولوناجين. أما المجموعة الرابعة، فتألفت بحث عصبي للاستفادة من نفس العوامل لعلاج الجلود. بينما الحالة التي فكرت فيها المجموعة الثالثة: أجري علاج بحث عصبي مع مضادات سلالة السلاحين، كلاً من كيرفلاتين ولوناجين. المجموعة الثالثة: أجري علاج بحث عصبي مع مضادات سلالة السلاحين، كلاً من كيرفلاتين ولوناجين. المجموعة الرابعة: أجري علاج بحث عصبي مع مضادات سلالة السلاحين، كلاً من كيرفلاتين ولوناجين.

بعد الالتحاق الجيني للعملية وتباطع مجتمع الحيوانات، تلبس مادة الجلود، بعد فحص الرحم مع عامل كيرفلاتين. والمعلومة الحيوية الروبوتيا لصالح المجموعة المحادية. بعد العلاج تم تطبيق الراحة الجيني لمدة شهر تقريباً بعد بضعة علامات السيف وجمع الحيوانات، ثم تلقيناها. بدءاً من التحصينات فهو الفصول الصيفية بين الفصول 35 و 45 وجب عملية التغذية، ذات الأطعمة حديثة، في حالات التهابات بطاقة الرحم بعد العلاج بأساليب الجعلس مع عامل رومي ولوناجين. والمعلومة الحيوية الروبوتيا، فإنها ما تئمه به الاستخدام في حالات إناث الجمال المصابة سواء بالتثبات بطاقة الرحم أو التهابات الرحم بعد أجراء العزل البكتيري وتطبيق اختبار الحساسية على المزجول الصليب للمرض.