

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

A.M. NABIH* and R.H. OSMAN**

* Mastitis and neonatal diseases department

** Artificial Insemination and Embryo Transfer Department

Animal Reproduction Research Institute (ARRI), Al-Haram, Giza, Egypt

ABSTRACT

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A total of 54 femal 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 acriflavin solution. G.2: (n = 6) she-camels were treated with Cefquinome intramuscularly and acriflavin 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 Enrofloxacin I/M injections, group 2 (n = 6) received acriflavin solution as intrauterine wash with Synulox, group 3 (n = 7) received lotagen solution intrauterine with Enrofloxacin 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 Dobson 2004). Uterine infections also suppressed hypothalamic GnRH and pituitary LH (Herth *et al.*,

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

under the same conditions 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. Catarrhal (turbid mucus), mucopurulent (turbid mucous with flacks of pus), or purulent (profuse pus) vaginal discharges were regarded as clinical signs 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.

Criteria	Endometritis	Metritis
History	Repeat breeder	Repeat breeder
Vaginal discharge	Catarrhal	Mucopurulent/purulent
Transrectal palpation of uterus	No palpable uterine changes	The uterus enlarged and thickened with or without intrauterine fluids.

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 separately 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 gentamycin sulphate I/M injections 2.5ml/100kg.B.W.for 5days (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) received 100 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 (Pregnyll, 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 *Klebseilla 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 Ciproflxacin and Amoxycilline+ Claveolinic 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 douching 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% 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.

Type of disease	Isolates	No.	%
Endometritis	-Staphylococcus aureus	16	40 %
	-E.coli	6	15 %
	-Corynebacterium sp.	8	20 %
	-Salmonella	5	12.5 %
Metritis	-Corynebacterium sp.	12	30 %
	-Klebseilla sp.	8	20 %
	-Proteus sp.	10	25 %
	-Salmoneila sp.	7	17.5 %

Table 3: Antibiotic sensitivity guid for isolated bacteria

Types of diseases	Name of the antibiotic discs									
	P	AML	GN	OT	S	Enr	Cef	E	AMC	AM
Endometritis isolates	++	+	++++	-	++	++	++++	-	+	-
Metritis isolates	++	-	++	+	++	++++	++	-	++++	-

P (Penicillin G.), AML (Amoxycillin), GN(Gentamycin), OT (Oxytetracyclin), S (Streptomycin), Enr (Enrofloxacin), Cef (Cefquinone), E(Erythromycin), AMC (Amoxycillin+ Claviolinic acid) and AM (Ampicillin)

Table 4: Drug of choice according to sensitivity test.

Endometritis isolates	1- Gentamycine	2- Cefquinome
Metritis isolates	1- Enrofloxacin	2- Amoxicillin + clavulinic acid

Table 5: Conception rates after treatment of endometritis with different regimes in she – Camels.

Groups	Type of treatment	C.R.	
		No.	%
Group 1	Acriflavine +Gentamycine	6/7	85.7 a
Group 2	Acriflavine +Cefquinome	4/6	66.7 c
Group 3	Lotagen +Gentamycine	5/7	71.4 b
Group 4	Lotagen +Cefquinome	4/6	57.1 d

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

Table 6: Conception rates after treatment of metritis with different regimes in she – Camels.

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

*Means with different subscripts (a,b,c,d) in the same column were significantly differ 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);

Wernery and Wernery (1992); Wernery and Kumar (1994), but unlike other species, little is known about their pathogenesis and evolution in camelids. Consequently, many practitioners approach the diagnosis and treatment of endometritis and metritis in female camelids in the manner described for cows and mares.

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 with venereal transmission and should be considered 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 camelids. 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 gentamycine 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).

The success of treatment of endometritis is variable and depends on its duration and on endometrial changes observed in the uterine biopsy. Pregnancy rates after treatment vary from 30 to 60% (Powers *et al.*, 1990; Wernery and Kumar 1994). Treated females should always be re-examined after a period of sexual rest. Acriflavin is a fluorescent dye used as a biological stain and as a local antiseptic agent for the skin and mucous membranes and it is known to inhibit mitochondriogenesis (Ungar, Robinson 1944), this illustrated in our results in which the evaluated regimes were efficient in treating female camels with edometritis. This suggested that the uterine infections were mild or superficial that all regimes investigated gave good different effects.

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 *Klebsciilla* 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 Schnyder *et al.* (1990). Gentamicin 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 aciflavine intra uterine wash. While synalox I/M has the best choice of treatment of metritis cases in combination with lotagen intra uterine doches. 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.

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دراسات بكتريولوجية على التهابات بطانة الرحم كأحد أسباب المشاكل التناسلية وقلة الخصوبة في إناث الجمال

أشرف نبيه محمد ، رشاد حامد عثمان

أجريت الدراسة على عدد ٥٤ من إناث الجمال التي تعاني من التهابات بطانة الرحم والتهابات الرحم. تم تقسيم إناث الجمال بعد فحص الرحم عن طريق المستقيم، الموجات فوق الصوتية والاستكشاف المهبلي إلى قسمين : القسم الأول يشتمل على عدد ٢٦ أنثى جمال تعاني من التهابات بطانة الرحم ، أما القسم الثاني فكان يعاني من التهابات رحمية ويشمل ٢٨ أنثى جمال. تم عزل أنواع مختلفة من البكتيريا سالبة الجرام وأخرى موجبة الجرام من الحيوانات المصابة، بنسب عزل إجمالية ٨٧,٥% في حالات التهابات بطانة الرحم و ٩٢,٥% في حالات التهابات الرحم . كانت الميكروبات المعزولة من الحيوانات المصابة بالتهابات بطانة الرحم عبارة عن ،الميكروب العنقودي الذهبي بعدد ١٦ معزولة وبنسبة ٤٠% من إجمالي المعزولات ، بينما كانت بكتيريا الكوريني ، الميكروب القولوني وميكروب السالمونيلا بأعداد ٨ و ٦ و ٥ معزولة وبنسب عزل ٢٠ و ١٥ و ١٢,٥% بالنتائج. بينما كانت أعداد ونسب العزل في الحالات المصابة بالتهابات رحمية متمثلة في ميكروب الكوريني، ميكروب البروتيس ،ميكروب الكليسيلا وميكروب السالمونيلا ١٢ (٣٠%) ، ١٠ (٢٥%) ، ٨ (٢٠%) و ٧ معزولات بنسبة (١٧,٥%) بالنتائج. تبعا لبروتوكول العلاج الذي تم تطبيقه أثناء الدراسة ،قمنا بتقسيم الحيوانات المصابة إلى أربع مجموعات : بالنسبة للحيوانات التي تعاني من التهابات بطانة الرحم كما يلي : المجموعة الأولى : تشمل ٧ حيوانات من إناث الجمال تم علاجها بمضاد حيوي سلفات الجنتاميسين عن طريق الحقن العضلي مع غسول رحمي أكريفلافين. المجموعة الثانية : تم تطبيقها على عدد ٦ حيوانات تم علاجها بمضاد حيوي سيفكينوم حقن عضلي مع أكريفلافين كغسول رحمي. المجموعة الثالثة : على عدد ٧ إناث جمال، كان العلاج بمضاد حيوي سلفات الجنتاميسين مع غسول رحمي لوتاجين. أما المجموعة الرابعة فكانت بحقن عضلي لمضاد سيفكينوم مع نفس الغسول الرحمي لوتاجين. بينما الحالات التي كانت تعاني من التهابات رحمية فقد تم علاجها كالاتي: المجموعة الأولى : بعدد ٨ حالات تم غسل الرحم لها بمحلول الأكريفلافين مع مضاد حيوي انروفلوكساسين للحقن العضلي. المجموعة الثانية: كان عدد الحيوانات المصابة ٦ تم علاجهم بنفس الغسول الرحمي أكريفلافين مع مضاد حيوي سينالوكس (أموكسيسيلين + حامض الكلايفوليك). المجموعة الثالثة والرابعة : كان العدد ٧ لكلا منهما وتم علاجهم بنفس الغسول الرحمي لوتاجين وبالمضادات الحيوية انروفلوكساسين للمجموعة الثالثة وسينالوكس للمجموعة الرابعة. بعد العلاج تم تطبيق الراحة الجنسية لمدة شهر تقريبا وبعد ظهور علامات الشيق لجميع الحيوانات تم تلقيحها بنكور ذات خصوبة عالية. تم تشخيص حالات الحمل بالموجات فوق الصوتية بين اليوم ٣٥ و ٤٥ بعد عمليات التلقيح. كانت أعلى معدلات حدوث حمل في حالات التهابات بطانة الرحم بعد العلاج بسلفات الجنتاميسين حقن عضلي مع غسول رحمي بمحلول الأكريفلافين ، بينما كان أعلى معدلات الخصوبة في الحيوانات التي تعاني من التهابات الرحم بعد العلاج بالغسول الرحمي لوتاجين مع المضاد الحيوي سينالوكس حقن عضلي، وهو ما ننصح به للاستخدام في حالات إناث الجمال المصابة سواء بالتهابات بطانة الرحم أو التهابات الرحم بعد إجراء العزل البكتيري وتطبيق اختبار الحساسية على المعزولات المسببة للمرض.