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Serum Haptoglobin Concentration to Monitor Recovery from Postpartum Sub-Clinical Endometritis in Murrah Buffaloes

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ABSTRACT

A total of 150 postpartum apparently healthy buffaloes were screened. Out of these 30 buffaloes were found to be positive for sub-clinical endometritis were selected and divided into five groups. The group wise treatment allotted were: Group I (cloprostenol,), Group II (cloprostenol + benzathine cephapirin, single I/U infusion), Group III (100 ìg *E. coli* LPS, single I/U infusion), Group IV (500 mg Oyster glycogen, single I/U infusion) and Group V (0.25% Lugol's iodine 20 ml, single I/U infusion). All the animals were subjected to trans-rectal ultrasonography, endometrial cytology, microbial assay and blood sampling for serum haptoglobin concentration before and after treatment. Total viable bacteria count revealed non-significant ($p > 0.05$) difference within pre- and post-treatment samples between different treatment groups. Post-treatment total viable bacteria count significantly ($p < 0.05$) reduced to zero in treatment groups III and IV. Out of 30 pre-treatment uterine lavage samples obtained from all the treatment groups, 25 (83.33%) samples were found positive. *E. coli* 08 (30.76%) was highly prevalent followed by 07 (26.92%) *Staphylococcus* spp., 03 (11.53%) *Streptococcus* spp., 03 (11.53%) *Proteus* spp., 03 (11.53%) *Acinetobacter* spp. and 01 (07.69%) *Bacillus* spp. Serum haptoglobin concentration in different treatment groups ranged from 76.62 ± 1.58 to 85.83 ± 2.12 ìg/ml prior to treatment and 26.37 ± 0.86 to 42.57 ± 9.08 ìg/ml post-treatment. Significant reduction ($p < 0.05$) was observed in haptoglobin concentration between pre- and post-treatment values in all the treatment groups. It was concluded that assessment of haptoglobin concentration in sub-clinical endometritic postpartum buffaloes can be used to monitor course of treatment at different points of time.

Keywords: Sub-clinical endometritis, serum haptoglobin, acute phase protein

Acute phase proteins (APP) are group of hepatic glycoproteins which are stimulated by inflammatory mediators and respond to initial reaction to infection, inflammation or trauma in animals (Marinkovic *et al.* 1989). The function of APP is to promote immunoglobulin production and tissue repair, preventing further injury and recycling useful molecules and debris (Kent, 1992).

Ruminants differ significantly with other species in their acute phase response as haptoglobin (Hp) is the major acute phase protein. In healthy cattle the serum haptoglobin concentration is < 20 mg/L, but can increase upto > 2 g/L in 2 days of infection. Many studies have indicated that haptoglobin is a clinically useful parameter for measuring the occurrence and severity of inflammatory responses particularly in cattle with

postpartum uterine disorders (Hirvonen *et al.* 1999; Wen-Chan *et al.* 2004). Elevated serum haptoglobin concentrations in the first week postpartum are found to be associated with metritis, cytological endometritis and purulent vaginal discharge (Dubuc *et al.* 2010). In bovines, inflammatory processes are not always followed by an increase in the leucocyte population (Taylor, 2006). Therefore, haptoglobin assay can also be considered as better assay than hematological tests to differentiate chronic and acute inflammation in cattle. Serum haptoglobin concentrations at different points of time could also serve as reliable biomarker for the diagnosis and monitoring of clinical endometritis (Biswal *et al.* 2014).

In buffaloes, postpartum sub-clinical endometritis (SE) is defined as an endometrial inflammation occurring 21 days or more after parturition without any clinical signs whereas clinical endometritis (CE) is indicated by the presence of purulent/mucopurulent discharge (Sheldon *et al.* 2006). Postpartum endometritis has a negative effect on reproductive performance, causing an increase in the number of services per pregnancy and in the length of calving-conception interval (Bell and Roberts, 2007). There are limited reports on measuring the serum haptoglobin and other APPs concentrations in cattle while no such reports were observed in buffaloes suffering from sub-clinical endometritis.

Keeping this in view, the present study was undertaken to determine whether evaluation of serum haptoglobin concentration at different point of time following different treatment of sub-clinical endometritis can be used to monitor course of treatment.

MATERIALS AND METHODS

A total of 150 postpartum (28 to 45 days) apparently healthy buffalo cows with normal calving history and free from peripartum disorders from college livestock farm and organized dairy farms in and around Jabalpur were screened. After recording history all the animals were subjected to gynaeco-clinical examination, White side test, transrectal ultrasonography and endometrial cytology by cytobrush technique. Out of these 30 postpartum buffaloes found to be positive for sub-clinical endometritis were selected and were randomly divided into five groups and were subjected to different treatment regimens as follows:

Sample collection

All the animals were subjected to aseptic collection of uterine fluid by low volume lavage technique for microbial assay, total viable bacteria count before treatment and post-treatment successive estrus. These samples were collected aseptically in autoclaved Brain Heart infusion (BHI) broth tubes and brought to laboratory in ice boxes. These tubes were incubated for

Table 1. Distribution of groups in the study

Treatment Groups	No. of animals	Treatment given
Group I	06	Single intramuscular injection of 500 mcg cloprostenol after CL palpation
Group II	06	Combination therapy of single intramuscular injection of 500 mcg Cloprostenol after CL palpation and 72 hours later followed by 500 mg single intrauterine infusion of cephapirin benzathin
Group III	06	*E. coli* lipopolysaccharide (M/s Sigma-Aldrich Inc., USA) at the dose rate of 100 mcg in 30 ml sterile phosphate buffer saline solution (PBS, pH=7.0, M/s HI-MEDIA laboratories Pvt. Ltd., Nasik, India), single intrauterine infusion
Group IV	06	Oyster glycogen (M/s Sigma-Aldrich Inc., USA) at the dose rate of 500 mg in 30 ml sterile phosphate buffer saline solution (PBS, pH=7.0, M/s HI-MEDIA laboratories Pvt. Ltd., Nasik, India), single intrauterine infusion
Group V	06	0.25 per cent, 20 ml Lugol's Iodine solution, single intrauterine infusion

Table 2: Total viable bacteria count in different treatment groups of sub-clinical endometritic postpartum buffaloes

Sub-clinical endometritis treatment groups (n=06 per group)		Total viable bacteria count (10^6 CFU/ml)
Group I	Pre-treatment	0.33 ± 0.08
	Post- treatment	0.05 ± 0.05
Group II	Pre-treatment	0.10 ± 0.03
	Post- treatment	0.08 ± 0.08
Group III	Pre-treatment	0.21 ± 0.16
	Post- treatment	0.00 ± 0.00
Group IV	Pre-treatment	0.22 ± 0.14
	Post- treatment	0.00 ± 0.00
Group V	Pre-treatment	0.06 ± 0.03
	Post- treatment	0.01 ± 0.01

The means with the same superscript did not differ significantly (p>0.05)

6-8 h using nichrome loops. It was then gently steaked on BHI, MLA and EMB agar medium in petri dishes and incubated for 48 h at 37°C (Quinn *et al*. 1999a). Isolation and identification of bacteria were based on the morphology, cultural characters and biochemical tests as described by Quinn *et al*. (1999b). All the isolates were characterized morphologically using Gram staining (Quinn *et al*. 1999b). Total viable bacterial count was calculated using spread plate technique as per the methods described by Koshy and Padmanaban (1989) and Sarkar *et al*. (1996) with minor modifications. The pin head shaped colonies were counted with the help of digital colony counter (M/s Environmental and Scientific Instrument Co. Pvt. Ltd., Model No. 361) and the results were interpreted as standard plate count/ ml. The total number of colonies was determined as follows:

Total number of colonies = No. of colonies/ plate × Ratio of dilution

Blood samples (05 ml from jugular vein) were collected aseptically on the day of examination and post treatment on first successive estrus/day 21 post treatment. Evacuated tube (without anticoagulant) (VAKU-8®, Clot accelerator, Hindustan syringes and medical devices Ltd., Faridabad, India) were used for serum. All the samples were kept in the ice box and were brought to the laboratory. Serum was separated from sample by centrifuging at 1500 rpm for 10 minutes and stored in labeled vials in deep freezer at -20 C until assayed. Haptoglobin was estimated in serum samples collected from the animals using Standard commercial ELISA kit for bovine haptoglobin assays (Bio-X Diagnostics, Belgium). The standard procedure as recommended by suppliers for quantitative direct sandwich test for blood serum was followed to analyze the samples. Absorbance and concentration was measured at 450 nm using ELISA reader (ATOM Maroche Pvt Ltd., Italy). The optical density readings for the unknown serum samples were plotted on the calibration curve to determine the samples concentrations.

Statistical analysis

The data was analysed statistically by analysis of variance (ANOVA). The means were compared using Duncan's multiple range test (DMRT). Pre-treatment and post-treatment means were compared using t-test as per the standard method described by Snedecor and Cocharn (1994).

RESULTS AND DISCUSSION

Total viable bacteria count in different treatment groups of sub-clinical endometritic postpartum buffaloes are presented in table 1 and figure 1.

No significant ($p > 0.05$) difference was observed within pre- and post-treatment total viable bacteria within different treatment groups. Pre- and post-treatment total viable bacteria count did not vary significantly ($p > 0.05$) in treatment groups I, II and V, while total viable bacteria

Table 3. Bacterial isolates obtained from uterine lavage of sub-clinical endometritic buffaloes

Sub-clinical endometritis treatment groups (n=06 per group)		Bacterial isolates		Positive bacterial isolates			Types of bacterial isolates					
		-ve sample	+ve sample	Single type	Mixed type	Total	*Streptococcus* spp.	*Bacillus* spp.	*Pseudomonas* spp.	*E. coli*	*Staphylococcus* spp.	*Acinetobacter* spp.
Group I	Pre-treatment	1 (16.67)	5 (83.33)	4 (80.00)	1 (20.00)	6	1 (16.67)	1 (16.67)	2 (33.33)	2 (33.33)	0 (0.00)	0 (0.00)
	Post-treatment	5 (83.33)	1 (16.67)	1 (100.00)	0 (0.00)	1	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.00)	0 (0.00)	0 (0.00)
Group II	Pre-treatment	1 (16.67)	5 (83.33)	5 (100.00)	0 (0.00)	5	0 (0.00)	0 (0.00)	1 (20.00)	2 (40.00)	2 (40.00)	0 (0.00)
	Post-treatment	5 (83.33)	1 (16.67)	1 (100.00)	0 (0.00)	1	0 (0.00)	0 (0.00)	0 (0.00)	1 (100.00)	0 (0.00)	0 (0.00)
Group III	Pre-treatment	1 (16.67)	5 (83.33)	5 (100.00)	0 (0.00)	5	1 (20.00)	1 (20.00)	0	1 (20.00)	2 (40.00)	0 (0.00)
	Post-treatment	6 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)	0	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Group IV	Pre-treatment	1 (16.67)	5 (83.33)	5 (100.00)	0 (0.00)	5	0 (0.00)	0 (0.00)	0 (0.00)	1 (20.00)	2 (40.00)	2 (40.00)
	Post-treatment	6 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)	0	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Group V	Pre-treatment	1 (16.67)	5 (83.33)	5 (100.00)	0 (0.00)	5	1 (20.00)	0 (0.00)	0 (0.00)	2 (40.00)	1 (20.00)	1 (20.00)
	Post-treatment	4 (66.66)	2 (33.33)	2 (100.00)	0 (0.00)	2	1 (50.00)	0 (0.00)	0 (0.00)	1 (50.00)	0 (0.00)	0 (0.00)
Overall (n=30)	Pre-treatment	05 (16.67)	25 (83.33)	24 (96.00)	1 (4.00)	26	3 (11.53)	2 (7.69)	3 (11.53)	8 (30.76)	7 (26.92)	3 (11.53)
	post-treatment	26 (86.67)	4 (13.33)	4 (100.00)	0 (0.00)	4	1 (25.00)	0 (0.00)	0 (0.00)	3 (75.00)	0 (0.00)	0 (0.00)

* Figures in paranthesis indicate percentage.

count significantly ($p < 0.05$) reduced to zero in treatment groups III and IV. This post-treatment reduction in bacteria count in group III and IV (*E. coli* LPS and oyster glycogen treatment groups) to zero may be due to immunomodulatory effect of the drugs to eliminate bacterial infection by the chemotactic action that increased the PMN cell influx to uterus (Palanisamy *et al*. 2014; Biswal *et al*. 2014).

Details of bacterial isolates obtained from uterine lavage of different treatment groups are tabulated in table 2 and figure 2. In treatment group I, out of 6 pre-treatment uterine lavage samples screened for bacterial isolates, 5 samples were found to be positive for bacterial isolates. Out of these 5 samples, 4 (80.00%) samples yielded single bacterial isolates while 1 (20.00%) isolate were of mixed type. Among the 6 isolates obtained, 2 (33.33%) were *E. coli* and 2 were (33.33%) *Pseudomonas* species followed by 1 (16.67%) of *Streprococcus* species and 1 (16.67%) was of *Bacillus* species. In treatment group II, out of 6 uterine lavage samples, 5 samples were found to be positive. All the 5 samples obtained were of single type. Among the 5 isolates obtained, 2 (40.00%) were of *E. coli* and 2 (40.00%) of *Staphylococcus* species followed by 1 (20.00%) of *Pseudomonas* species. In treatment group III also, out of 6 uterine lavage samples, 5 samples were found positive. All the 5 samples obtained were of single type bacterial isolate. Among the 5 isolates obtained, 2 (40.00%) were of *Staphylococcus* species 1(20.00%) of *E. coli,* 1 (20.00%) of *Streptococcus* species and 1 (20.00%) was of *Bacillus* species.

Similarly in treatment group IV, only 5 samples were found to be positive out of 6 samples screened for bacterial isolates. All the 5 samples were single type bacterial isolates. Out of these 5 samples screened, 2 (40.00%) were of *Acinetobacter* species, 2 (40.00%) of *Staphylococcus* species and 1(20.00%) was of *E. coli*. In treatment group V, 5 out of 6 samples were positive. All the 5 positive samples were of single type. Out of these 5 bacterial isolates, 2 (40.00%) were of *E. coli*, 1 (20.00%) of *Staphylococcus* species, 1 (20.00%) of *Acinetobacter* species and 1 (20.00%) was of *Streptococcus* species. Out of 30 pre-treatment uterine lavage samples obtained from all the treatment groups of sub-clinical endometritic buffaloes, 25 (83.33%) samples were found positive while only 05 (16.67%) samples were negative for bacterial isolates. Among these 25 positive samples, 24 (96.00%) samples yielded single isolates while in only 01 (04.00%) sample mixed isolates were obtained. Among the 26 bacterial isolates 08 (30.76%) *E. coli* was highly prevalent followed by 07 (26.92%) *Staphylococcus* spp., 03 (11.53%) *Streptococcus* spp., 03 (11.53%) *Proteus* species, 03 (11.53%) *Acinetobacter* species and 01 (07.69%) *Bacillus* species. The prevalence of *E. coli* as observed in the present study was in accordance with the findings of Kusum *et al*. (2003), Udhayavel *et al*. (2013) and Biswal *et al*. (2014). Bhat and Bhattacharya (2012) isolated *Staphylococcus* spp., *E. coli*, *Bacillus* spp., *Corynebacterium* spp., *Pseudomonas* spp., *Proteus* spp., *Klebsiella* spp. and *Streptococcus* spp. from crossbred cows affected with metritis. The pre-treatment samples negative for any bacterial isolates may be due to non-infectious sub-clinical endometritis.

Out of 12 post-treatment uterine samples obtained from treatment groups I and II, only 01 sample (16.67%) each from both the groups (single bacterial isolate) was found to be positive. The isolated bacteria from both the samples were *E. coli* (100.00%). All the post-treatment uterine samples of treatment groups III and IV were found to be sterile. While out of 06 post-treatment uterine samples of treatment group V, 2 (33.33%) samples were found to be positive. Both the positive samples yielded single isolate. The prevalent bacteria type was *E. coli* (01, 50.00%) and *Streptococcus* spp. (01, 50.00%). Overall irrespective of groups, out of 30 post-treatment samples analysed, only 04 (13.33%) samples were found to be positive. All the 04 (100.00%) positive samples yielded single type bacterial isolates. Among the overall total of 04 single type bacterial isolates obtained from uterine lavage samples, *E. coli* and *Streptococcus* spp. were 03 (5.00%) and 01 (25.00%), respectively. The difference in the Lugol's iodine group and other groups with respect to bacterial isolates may be attributed to higher efficacy of the therapeutic agents used in treatment other groups. Moreover, the anti-bacterial spectrum of Lugol's iodine is limited and its therapeutic action is attributed more to its irritant quality than to its anti-bacterial spectrum covered.

Haptoglobin is a glycoprotein having two á and two â chains connected by disulphide bridges. Haptoglobin binds to free haemoglobin released from erythrocytes with high affinity and reduced the oxidative damage to itself and albumin (Yang *et al*., 2003). Haptoglobin-haemoglobin complex reduced the availability of the iron residue for bacterial growth and therefore it had an indirect antibacterial activity (Murata *et al*. 2004). Haptoglobin also acts as a pro-inflammatory mediator. Serum haptoglobin concentration in different treatment groups is presented in table 3 and figure 3. The serum haptoglobin concentration in different treatment groups ranged from 76.62 ± 1.58 to 85.83 ± 2.12 ìg/ml prior to treatment and 26.37 ± 0.86 to 42.57 ± 9.08 ìg/ml after the treatment at subsequent estrus. No significant difference

Table 4: Serum haptoglobin concentration in different treatment groups of sub-clincial endometritic postpartum buffaloes

Sub-clinical endometritis treatment groups (n=06 per group)		Serum haptoglobin (µg/ml)
Treatment group I	Pre-treatment	83.85 ± 1.93[a]
	Post-treatment	33.73 ± 7.01
Treatment group II	Pre-treatment	76.62 ± 1.58[b]
	Post-treatment	26.37 ± 0.86
Treatment group III	Pre-treatment	85.22 ± 2.33[a]
	Post-treatment	26.99 ± 0.82
Treatment group IV	Pre-treatment	85.83 ± 2.12[a]
	Post-treatment	26.85 ± 0.68
Treatment group V	Pre-treatment	83.22 ± 1.90[a]
	Post-treatment	42.57 ± 9.08

*The means with the same superscript did not differ significantly (p>0.05).

($p > 0.05$) was observed in pre-treatment serum haptoglobin in treatment groups I, III, IV and V while significant difference ($p < 0.05$) was observed between treatment group I and II, II and III, II and IV and II and V. The difference in post-treatment serum haptoglobin concentration was non-significant ($p > 0.05$) in various treatment groups.

No significant difference ($p > 0.05$) was observed in pre-treatment serum haptoglobin in treatment groups I, III, IV and V while significant difference ($p < 0.05$) was observed between treatment group I and II, II and III, II and IV and II and V. This significant variation in pre-treatment serum haptoglobin concentration may be attributed to difference in inflammatory response as well as stage of infection in between animals of groups. Serum haptoglobin is positive reactive acute phase protein for inflammatory response as well as pathological damage due to infection (Biswal *et al.* 2014).

The difference in post-treatment serum haptoglobin concentration was non-significant ($p > 0.05$) between various treatment groups. This shows that the therapeutic response of various treatments done in different groups and their therapeutic efficacy to treat sub-clinical endometritis and reduce the inflammatory response which is reflected by lower haptoglobin values in these animals. Significant reduction ($p < 0.05$) was observed in serum haptoglobin concentration between pre- and post-treatment values in all the treatment groups. This significant reduction in haptoglobin concentration may be attributed to curative effect of various treatments in different treatment groups (Heidarpour *et al.* 2012; Biswal *et al.* 2014). In the present study, we could not collect sequential blood samples for analysis of haptoglobin concentration following treatment (at 24-72 hours post-treatment) due to limitations of field trial. However, the studies related to serum haptoglobin concentration in post-treatment subsequent estrus are lacking.

CONCLUSION

It may be inferred from the study that evaluation of serum haptoglobin concentration in postpartum buffaloes at different points of time following different therapeutic regimens in sub-clinical endometritis can be used to monitor course of treatment.

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