Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(4): 1422-1426

Received: 25-05-2019
Accepted: 27-06-2019

CT Nikhade
M.V. Sc. Scholar, Department of Animal Reproduction, Gynaecology and Obstetrics. Post Graduate Institute of Veterinary and Animal Sciences, Akola, Maharashtra, India

SG Deshmukh
Assistant Professor Department of Animal Reproduction, Gynaecology and Obstetrics. Post Graduate Institute of Veterinary and Animal Sciences, Akola, Maharashtra, India

SV Kuralkar
Professor and Head, Department of Animal Genetics and Breeding Post Graduate Institute of Veterinary and Animal Sciences, Akola, Maharashtra, India

AR Ratnaparkhi
Ph. D. Scholar, Department of Animal Reproduction, Gynaecology and Obstetrics. Post Graduate Institute of Veterinary and Animal Sciences, Akola, Maharashtra, India

SD Chepte
Assistant Professor, Department of Veterinary Surgery and Radiology Post Graduate Institute of Veterinary and Animal Sciences, Akola, Maharashtra, India

PS Bankar
Assistant Professor, Department of Animal Genetics and Breeding Post Graduate Institute of Veterinary and Animal Sciences, Akola, Maharashtra, India

Correspondence
SG Deshmukh
Assistant Professor Department of Animal Reproduction, Gynaecology and Obstetrics. Post Graduate Institute of Veterinary and Animal Sciences, Akola, Maharashtra, India

Comparative efficacy of different herbal extract on subclinical endometritis in postpartum cows

CT Nikhade, SG Deshmukh, SV Kuralkar, AR Ratnaparkhi, SD Chepte, PS Bankar

Abstract
The objective of the present research work was to study the prevalence of subclinical endometritis in an around Akola city and to study the therapeutic efficacy *Achyranthes aspera* and *Azadirachta indica* on subclinical endometritis in postpartum cows. Total 87 cows 30-60 days in milk were examined from surrounding farms of Akola city with cytobrush technique. Cows with ≥5% PMN cells in endometrial cytology were regarded as affected by subclinical endometritis and divided into three groups comprising ten cows in each group. In T_1 group cows treated with a 25 ml sterile hydromethanolic extract of *Azadirachta indica* intra uterine for three consecutive days. In T_2 group cows treated with a 20 ml (10mg/ml) sterile hydromethanolic leaf extract of *Achyranthes aspera* (200 mg) intrauterine for three consecutive days and T_3 group **c**ows showing PMN ≥5% in postpartum cows in uterine cytology were taken as a positive control group and given no treatment. In the present study the overall prevalence of subclinical endometritis at 30-60 days postpartum was 35.63%. The curative efficacy was higher in *Achyranthes aspera* treated cows followed by *Azadirachta indica* treated cows as compared to untreated control cow. The first service conception rate was higher in *Azadirachta indica* treated cows followed by *Achyranthes aspera* as compared to untreated cows. It was concluded that there was higher prevalence of subclinical endometritis in postpartum cows in surrounding farms of Akola city. The therapeutic efficacy was higher in *Achyranthes aspera* treated cows whereas first service conception rate was higher in *Azadirachta indica* treated cows.

Keywords: Subclinical, endometritis, cattle, *Achyranthes aspera*, *Azadirachta indica*

1. Introduction
Subclinical endometritis is the common cause of infertility and subfertility in high producing dairy cattle delaying the onset of ovarian cyclic activity after parturition, extending luteal phase and reducing conception rate is (Sheldon *et al*., 2009) [1]. Uterine cytology or ultrasonography can be used for the diagnosis of subclinical endometritis. Cytobrush method (Barlund *et al*., 2008; Kasimanickam *et al*., 2004 and Kasimanickam *et al*., 2005) [2-4] can be used for the collection of samples for uterine cytology. An increased proportion of PMN is prognostic for impaired subsequent reproductive performance (Barlund *et al*., 2008; Gilbert *et al*., 2005 and Kasimanickam *et al*., 2004) [2, 5, 3]. Four to eighteen percent of PMN cells (Barlund *et al*., 2008; Galvao *et al*., 2009; Gilbert *et al*., 2005; Kasimanickam *et al*., 2004) [2, 6, 5, 3] is the threshold value that defines subclinical endometritis.
(Plontzke *et al*., 2010) [7] reported the overall prevalence of subclinical endometritis with endometrial cytology was 38% with more than 5% neutrophils. (Nibret and Jebar, 2012) [8] observed that the prevalence of subclinical endometritis in, cows with lack of hygienic condition, conceived heifers, cows those were inseminated, cows having good body condition score and cows in small scale farms were 71.4%, 100%, 69.8%, 75% and 86.7%, respectively with statistically non significant difference (p>0.05) among parity, hygienic condition, body condition score, method of service and farm scales, respectively.
In India as traditional ayurvedic and folklore medicine, Neem (*Azadirachta indica*) has been widely used for the treatment of various diseases (Bandyopadhyay *et al*., 2002) [9]. It has been proved to have immunomodulatory, anti-inflammatory, antifungal, antibacterial, antiviral, and antioxidant properties (Subapriya and Nagini, 2005) [10]. Due to its better antibiotic and immunomodulatory property of hydro alcoholic extract of neem oil, it can be used as a therapy for endometritis in repeat breeding cows and also observed as a better clinical recovery and conception rate with hydro alcoholic extract of neem (Harendra *et al*., 2013) [11].

Achyranthes aspera (Aghada) is distributed as a weed throughout India, tropical Asia and other parts of the world. The plant is reported to be used as oestrogenic, antimicrobial, immunostimulant, anti-inflammatory, antioxidant, diuretic, cardiac stimulant, antispasmodic and hepatoprotective (Dey 2011and Sharma *et al.,* 2013) [12, 13]. Immunomodulatory activity of the plant on elicitation of antigen-specific murine antibody response has been reported (Vasudeva *et al*. 2002) [14]. The estrogenic and uterotrophic activity of the extracts has successfully detected in mice (Vasudeva and Sharma, 2007) [15] & Shibeshi *et al*., 2006) [16]. Achyranthes aspera a potent immunostimulating plant for traditional medicine (Srivastava, 2014) [17].

Keeping in view the economic losses due to subclinical endometritis faced by the farming community & lack of cost-effective treatment for subclinical endometritis the present study was designed with the following objective.

2. Materials and Methods

The study was conducted on post partum cows between 30 to 60 days in milk were selected from ILFC, PDKV, Akola and surrounding Gorakshans of Akola city and were screened for subclinical endometritis. A total 87 cows 30-60 days in milk were examined from surrounding farms of Akola city with cytobrush technique. Cows with ≥5% PMN cells in endometrial cytology were regarded as affected by subclinical endometritis.

Out of 87 cows, total of 30 cows showing PMN cells ≥ 5% threshold level in postpartum cows were selected and divided into three groups comprising ten cows in each group. In T_1 group cows treated with a 25 ml sterile hydromethanolic extract of *Azadirachta indica* intra uterine for three consecutive days. In T_2 group cows treated with a 20 ml (10mg/ml) sterile hydromethanolic leaf extract of *Achyranthes aspera* (200 mg) intrauterine for three consecutive days and T_3 group **c**ows showing PMN ≥5% in postpartum cows in uterine cytology were taken as positive control group and given no treatment. For curative efficacy endometrial cytology was performed in all 30 cows from all the groups on consecutive oestrus by using cytobrush technique. Cows exhibited spontaneous estrus from all groups were artificially inseminated as per AM and PM rule. The conception rate in all the groups was determined by per-rectal (P/R) examination on 60 day post A.I.

3. Results and Discussion

3.1 Prevalence of Subclinical Endometritis in Postpartum Cows

A total 87 cows from 30-60 days in milk (DIM) without any vaginal discharge were enrolled from different farms and Gorakshan sanstha around Akola city.

The prevalence of subclinical endometritis in different herds is given in Table 1. The overall prevalence of subclinical endometritis with 5% threshold level from 30-60 days in milk was 35.63% (31/87). Herd level prevalence was 30.00% (6/20), 44% (11/25), 27.27% (3/11), 33.34% (4/12), 30% (3/10) and 44.45% (4/9) on farms ILFC, PDKV, Gorakshan akola, Rashtria shala, Nimbi shetki shala, Guldhi and Gow Sanstha Dabki road, respectively.

Table 1: Prevalence of subclinical endometritis in different farms in and around Akola city

Name of Farm	No of animals screened	Positive for SCE	Herd level prevalence (%)
ILFC, PDKV	20	6	30.00%
Gorakshan, Akola	25	11	44.00%
Rashtria shala,	11	3	27.27%
Nimbi shetki shala	12	4	33.34%
Guldhi	10	3	30.00%
Gow sanstha Dabki road	09	4	44.45%
Total	87	31	35.63%

The overall prevalence of subclinical endometritis in the present study is in accordance with previously published data from confinement housing systems. Kasimanickam *et al.* (2004) [3] diagnosed subclinical endometritis in clinically healthy dairy cows and described a prevalence of 35% and 34% for 20 to 33 days postpartum and 34 to 47 days postpartum, respectively. Green *et al.* (2009) [18] reported a prevalence of 37% subclinical endometritic cows. Cheong *et al.* (2011) [19] reported a herd-level prevalence of SCE of 36.1%. Senosy and Osawa (2013) [20] revealed the prevalence of SCE to be 33.3% and 30.4% in cold and temperate season, respectively at ≥5% threshold level. Lima *et al.* (2013) [21] observed prevalence of SCE of 29.5% with 5% threshold level. Melcher *et al*. (2014) [22] revealed the prevalence of SCE of 33% by counting 100 cells. Carneiro *et al.* (2014) [23] reported a prevalence of SCE of 26%. Singh *et al.* (2016) [24] observed the prevalence of SCE was 29.4% at ≥4% threshold level. Dini *et al*. (2015) [25] reported the prevalence of SCE 30 days postpartum as 38.5% using the threshold of 18%. Syed (2016) [26] reported prevalence of 36.14% in post partum cows in and around Akola city showing herd-level prevalence of 30% (6/20), 46.66% (7/15), 40% (8/20), 30% (3/10), 33.34% (4/12) and 33.34% (2/6) in (ILFP), Dr Punjabrao Deshmukh Krishi Vidyapeeth, Akola; Shri Gorakshan Sanstha Akola, Shri Gorakshan Sanstha Mhaispur, Hanuman Goseva Gorakshan Sanstha, Kumbhari and Shri Gorakshan Sanstha (Umri).

In contrast to present findings, a lower prevalence of SCE obtained by Dubuc *et al.* (2010) [27] who reported 13.5% prevalence for cytological endometritis at ≥6% threshold level of PMN cells. Kaufmann *et al.* (2010) [28] who observed the overall prevalence of SCE was 12.4% at 18% threshold level of PMN cells. Ribeiro *et al.* (2013) [29] reported 13.4% prevalence in subclinical endometritic cows at ≥5% threshold level and Barrio *et al.* (2015) [30] who observed 14.9% prevalence of SCE in 35-45 days in milk.

The difference in percentage of prevalence with earlier findings may be due to different threshold level of PMN cells, difference in postpartum day of diagnosis, variation in season, variation in Body Condition Score and different counting methods for PMN cells (Senosy and Osawa 2013, Carneiro *et al.*, 2014 and Melcher *et al.*, 2014) [20, 23, 22].

3.2 Therapeutic Efficacy of *Azadirachta indica* and *Achyranthes aspera* in Subclinical Endometritic Cows

The therapeutic efficacy of *Azadirachta indica* and

Achyranthes aspera in subclinical endometritic cows were shown in Table 2. The therapeutic efficacy was measured on the basis of curative percentage by endometrial cytology on the subsequent heat after the treatment.

Table 2: Therapeutic efficacy of *Azadirachta indica* (T_1) and *Achyranthes aspera (*T_2*)* in subclinical endometritic cows

Treatments Groups (n=6)	No of Animals Treated	No of Animals Negative in endometrial cytology	Percentage (%)
T_1	10	7	70.00%
T_2	10	8	80.00%
T_3	10	2	20.00%

The therapeutic efficacy on the basis of curative percentage in groups *Azadirachta indica* and *Achyranthes aspera* were 70.00% (7/10) and 80.00% (8/10) after the treatment and in untreated group it was 20.00% (2/10), respectively. From the present study it was revealed that the curative efficacy was higher in *Achyranthes aspera* treated cows followed by *Azadirachta indica* treated cows as compared to untreated control cows. The higher curative percentage of *Achyranthes aspera* and *Azadirachta indica* might be due to immunomodulatory, anti inflammatory and antibacterial as well as estrogenic properties of *Achyranthes aspera.*

The therapeutic efficacy of *Azadirachta indica* observed in the present study is in accordance with Kumar *et al.* (2013) [31] who reported 75% therapeutic efficacy with hydro acetoin neem bark extract, whereas higher therapeutic efficacy was observed by Kumar *et al.* (2013) [31] and Harendra *et al.* (2013) [11] 96.02% with hydroalcholic neem bark and methanolic extract of neem oil, respectively which are in not accordance with present study.

The difference in therapeutic efficacy of *Azadirachta indica* with earlier findings might be due to difference in breed, postpartum time period of treatment, season, body condition score and variation in PMN cells counting. (Senosy and Osawa 2013, Carneiro *et al*., 2014 and Melcher *et al*., 2014) [20, 23, 22].

Therapeutic efficacy of *Achyranthes aspera* observed in present study is in close accordance with Syed (2016) [26] who reported 66.66%, therapeutic efficacy of hydromethanolic extract of *Achyranthes aspera* in postpartum cows, whereas 20% cows observed negative for subclinical endometritis in untreated cows which are in aggregment with Kumar *et al.* (2013) [31] and Harendra *et al.* (2013) [11] both of whom reported 25% cows to be negative for subclinical endometritis cows.

3.3 First Service Conception Rate in Experimental cows

All the cows exhibited spontaneous estrus in groups treated with *Azadirachta indica*, *Achyranthes aspera* including untreated groups they were artificially inseminated as per Am and Pm rule. In the present study, 5 out of ten cows were found to be pregnant with a 50 percent conception rate in *Azadirachta indica* group, 4 out of ten cows were found to be pregnant with 40.00 percent conception rate in the *Achyranthes aspera* group. Whereas, out of ten cows only two cows were found to be pregnant with 20.00 percent in untreated groups, respectively. (Table 3)

Table 3: First service conception rate in subclinical endometritic cows with different treatments

Treatments Groups (n=10)	Number of Animals Treated	Number of Animals Inseminated	Number of Animals Pregnant	First Service Conception Rate (%)
T_1	10	10	5	50.00
T_2	10	10	4	40.00
T_3	10	10	2	20.00

From the present study, it was observed that cows treated with *Azadirachta indica* showed higher first service conception rate followed by *Achyranthes aspera* treated cows as compared to untreated cows.

The higher first service conception rate observed in cows from treatment groups as compared to untreated group might be due to better therapeutic efficacy of *Azadirachta indica* or *Achyranthes aspera* in subclinical endometritis which had a detrimental effect on fertility causing an increase in calving to conception and a decrease in the rate of cows who become pregnant (Ricci *et al.* 2016) [32].

The first service conception rate in *Achyranthes aspera* group observed in present study is in close agreement with Syed (2016) [26] who recorded 50% first service conception rate in subclinical endometritis cows treated with methanolic extract of *Achyranthes aspera.*

The first service conception rate in *Azadirachta indica* observed in present study is in accordance with Kumar *et al.* (2013) [31] who reported 50% conception rate in endometritic cows treated with 30 ml hydroacetonic neem bark, whereas Kumar *et al.* (2013) [31] and Harendra *et al.* (2013) [11] reported higher conception rate 62.50% and 71.42% in endometric cows treated with hydroalcholic and methanolic fraction of neem oil, respectively which is not accordance with present study.

A lower first service conception rate than the present study for *Azadirachta indica* observed by Harendra *et.al* (2013) [11] 25% in endometritic cows treated with ethanolic fraction of neem seed powder.

Results of untreated cow control group are in agreement with Nibret and Jebar (2012) [8] and Ricci *et al.* (2015) [32] who observed first service conception rate of 21.4% and 13% in Subclinical endometritic cows, respectively. Similarly Barlund *et al.* (2008) [02] reported 14.2% conception rate in endometritic positive cows.

In the present study the difference in conception rate in earlier findings may be due to the variation in curative efficacy of different drugs used. Lima *et al.* (2013) [21] stated that the cows with subclinical endometritis from the early postpartum period have greater depression in measures of fertility at first AI.

4. Conclusion

The therapeutic efficacy was higher in *Achyranthes aspera* treated cows followed by Azadirachta *indica* treated cows as compared to untreated control cows. The first service conception rate was higher in *Azadirachta indica* treated cows followed by *Achyranthes aspera* treated cows as compared to untreated control cows.

5. References

1. Sheldon I, Price S, Cronin J, Gilbert R, Gadsby J. Mechanisms of Infertility associated with clinical and subclinical endometritis in high producing dairy cattle. Reprod. Dom. Animal. 2009; 44(3):1-9.
2. Barlund C, Carruthers T, Waldner C, Palmer C. A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. Theriogenology. 2008; 69:714-723.
3. Kasimanickam R, Duffield T, Foster R, Gartley C, Leslie K, Walton J *et al*. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology. 2004; 62:9-23.
4. Kasimanickam R, Todd F, Duffield, Robert A, Foster, Gartley J *et al*. A comparison of the cytobrush and uterine lavage techniques to evaluate endometrial cytology in clinically normal postpartum dairy cows. Can. Vet. J. 2005; 46:255-259.
5. Gilbert R, Shin S, Guard C, Erb H, Frajblat M. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology. 2005; 64:1879-1888.
6. Galvao K, Greco L, Vilela J, Sa Filho M, Santos J. Effect of intrauterine infusion of ceftiofer on uterine health and fertility in dairy cows. J Dairy Sci. 2009; 92:1532-1542.
7. Plontzke J, Madoz L, De la Sota R, Drillich M, Heuwieser W. Subclinical endometritis and its impact on reproductive performance in grazing dairy cattle in argentina. Ani. Reprod. Sci. 2010; 122:52-57.
8. Nibret M, Jebar A. Prevalence of Subclinical Endometritis and its Effect on Pregnancy in Crossbred Dairy Cows in Gondar. J of Reproduction and Infertility. 2012; 3(2):26-31.
9. Bandyopadhyay U, Biswas K, Chatterjee R, Bandyopadhyay D, Chattopadhyay I, Ganguly C *et al*. Gastro protective effect of Neem (*Azadirachta indica*) bark extract: Possible involvement of H+-K+-ATPase inhibition and scavenging of hydroxyl radical. Life Sci. 2002; 71:2845-2865.
10. Subapriya R, Nagini S. Medicinal properties of neem leaves: A review. Curr. `Med Chem. Anticancer Agents. 2005; 5(2):149-6.
11. Harendra K, Goawami T, Ranwat M. Use of neem preparations for the treatment of endometritis in cows. Advances in Animal and Vet. Sci. 2013; 1(6):194-196.
12. Dey A. *Achyranthes aspera*: phytochemical and pharmacological aspects. Int. J. of Pharmaceutical Sci. Review and Research Article. 2011; 9(2):72-82.
13. Sharma V, Singh R, Paliwal R, Chaudhary U, Agarwal A. MIC values of inflorescence and leaves extracts of *Achyranthes aspera* against usual pathogenic bacterial strains. Asian J. Pharm. Clin. Res. 2013; 6(1):185-187.
14. Vasudeva R, Duddukuri G, Sunil B, Athota R. Immunomodulatory Activity of *Achyranthes aspera* on the Elicitation of Antigen-Specific Murine Antibody Response. Pharmaceutical Biology. 2002; 40(03):175-178.
15. Vasudeva N, Sharma S. oestrogenic and pregnancy interceptor effects of *Achyranthes aspera* Linn. Root. Afr. J Trad. Cam. 2007; 4(1):7-11.
16. Shibeshi W, Makonnen E, Debella A, Zerihun L. phytochemical, contraceptive efficacy and safety evaluations of the methanolic leaves extract of *achyranthes aspera* L. in rats Pharmacologyonline. 2006; 3:217-224.
17. Srivastava P. *Achyranthes aspera*: A potent immunostimulating plant for traditional medicine, Praveen KS. 2014; 5(5):1601-1611.
18. Green M, Ledgard A, Berg M, Peterson A, Back P. Prevalence and identification of systemic markers of sub-clinical endometritis in postpartum dairy cows. Proceedings of the New Zealand Society of Ani. Prod. 2009; 69:37-42.
19. Cheong S, Nydam D, Galvao K, Crosier B, Gilbert R. Cow-level and herd-level risk factors for subclinical endometritis in lactating Holstein cows. J Dairy Sci. 2011; 94:762-770.
20. Senosy W, Osawa T. Association among calving season and measures of energy status, resumption of ovulation and subclinical endometritis in early lactating dairy cows. Anim. Reprod. 2013; 10(1):24-31.
21. Lima F, Bisinotto R, Ribeiro E, Greco L, Ayres H, Favoreto M *et al*. Effects of 1 or 2 treatments with prostaglandin F_2 on subclinical endometritis and fertility in lactating dairy cows inseminated by timed artificial insemination. J Dairy Sci. 2013; 96:6480-6488.
22. Melcher Y, Prunner I, Drillich M. Degree of variation and reproducibility of different methods for the diagnosis of subclinical endometritis. Theriogenology. 2014; 30:1-7.
23. Carneiro L, Ferreira A, Padua M, Saut J, Ferraudo A, Maria dos Santos R. Incidence of subclinical endometritis and its effects on reproductive performance of crossbred dairy cows. Trop. Anim. Health Prod. 2014; 46:1435-1439.
24. Singh J, Honparkhe M, Chandra A, Kumar, Ghuman S, Dhindsa S. Diagnostic efficacy of uterine cytobrush technique for subclinical endometritis in crossbred dairy cattle. Indian Vet. J. 2016; 93(02):11-13.
25. Dini P, Farhoodi M, Akbari G, Hostens M, Pascottini O, Ataei O *et al*. Prevalence of subclinical endometritis in two dairy farms in Iran and consequences for further fertility. Research Opinions in Ani and Vet. Sci. 2015; 5(3):148-153.
26. Syed A. Therapeutic efficacy of *Tinospora cordifolia* and *Achyranthes aspera* on subclinical endometritis in cows. Unpublished M.V.Sc. thesis submitted to Maharashtra Animal and Fishery Sciences University, Nagpur. 2016, 57-81.
27. Dubuc J, Duffield T, Leslie K, Walton J, LeBlanc S. Definitions and diagnosis of postpartum endometritis in dairy cows. J Dairy Sci. 2010; 93:5225-5233.
28. Kaufmann T, Westermann B, Drillich M, Plontzke M, Heuwieser W. Systemic antibiotic treatment of clinical endometritis in dairy cows with ceftiofur or two doses of cloprostenol in a 14 day interval. Ani. Reprod. Sci. 2010; 121:55-62.
29. Ribeiro E, Lima F, Greco L, Bisinotto R, Monteiro A, Favoreto M. Prevalence of periparturient diseases and effects on fertility of seasonally calving grazing dairy cows supplemented with concentrates. J Dairy Sci. 2013; 96:5682-5697.
30. Barrio M, Vigo M, Quintela L, Becerra J, Garcia-Herradon P, Bello D *et al*. Influence of subclinical endometritis on the reproductive performance of dairy cows. Spanish J of Agricultural Research. 2015; 13(4):1-6.

31. Kumar A, Gupta H, Prasad S. Studies on the immunomodulatory and therapeutic efficacy of Neem (*Azadirachta indica*) on endometritis in repeat breeding crossbred cows. Indian J of Ani. Reproduction. 2013; 34(2):1-5.
32. Ricci A, Gallo S, Molinaro F, Dondo A, Zoppi S, Vincent L. Evaluation of subclinical endometritis and consequences on fertility in piedmontese beef cows. Reprod. Dom. Anim. 2015; 50:142-148.