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BACTERIA ISOLATED FROM THE UTERUS OF COWS WITH FOETAL MEMBRANE RETAINED

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Abstract

The aim of the study was to define the species of microorganisms isolated from the uterus of healthy cows (control group) and cows affected with foetal membrane retained (FMR) before and after an experimental therapy with the use of an immunomodulator and antibiotics versus commonly used methods (antibiotic + ß-blocker or antibiotic + PGF2α). The examinations were carried out on three farms with similar system of rearing and nutrition on 95 cows with FMR. The control group consisted of 21 cows without postpartum disturbances. Smears from the uterus were taken at 0 and the 21st d of observation. Most frequently *Escherichia coli* and other species of *Enterobacteriaceae* family were isolated. These species were stated in 67.4% of sick cows and in 47.6% of healthy cows. The degree of *Arcanobacterium pyogenes* infection was almost identical in healthy and sick cows (8.4 and 9.5% respectively). *Streptococcus* sp. was isolated from 15.8% of FMR cows and from 19% of control ones. Staphylococci were isolated from 12.6% of FMR and from 9.5% of control cows. Other bacteria species were isolated from less than 10% of the examined cows. The best elimination of infections (65%) was noted in cows treated with intrauterinary inlocations of antibiotics (neomycin and ampicillin) combined with the lysozyme dimer i.m. injection. The worst results were noted as an effect of inlocation of these antibiotics only and antibiotics combined with ß-blocker (40%). The species of bacteria in the uterus were similar in healthy and sick cows. However, on 21st day after treatment *A. pyogenes* was isolated only from the uterus of the sick animals. This pathogen was identified in 10 cows (83.3%) from 12 eliminated because of infertility.

Key words: cows, retained placenta, bacteria.

Foetal membrane retained (FMR) is often the consequence of abortions or premature parturitions, immunological and hormonal disturbances, atony of the uterus, and some septic diseases as brucellosis, infectious rhinotracheitis, campylobacteriosis, trichomonadosis (1, 10, 28), neosporosis (12), leptospirosis, mycoplasmosis (19, 24) and yeast infection (6). At present only neosporosis constitutes an important problem, especially related to abortion (8, 14).

The decrease in local immunity, open cervix of the uterus, presence of afterbirth in the uterus and manual treatment are the main cause of complications and growth of pathogenic and opportunistic microorganisms. Infections are caused mostly by *Arcanobacterium pyogenes* and anaerobes, like *Fusobacterium necrophorum* and *Bacterioides melaninogenicus* (5, 10, 20, 21). These pathogens show synergic activity. *Fus. necrophorum* produces a toxin which decreases phagocyte cell activity. *Bac. melaninogenicus* and *Bac. fragilis* produce a substance decreasing also phagocyte cell activity. *A. pyogenes* secretes a growth factor for *Fus. necrophorum* (4, 20). The toxins of certain strains of *E. coli* and *A. pyogenes* decrease phagocytic activity of leukocytes (29). Neutrophil function is a determining factor for the development of FMR in dairy cattle. Also, depressed production of Il-8 may be a factor affecting neutrophil function in cows developing FMR (13, 27).

Manual help during parturition and manual treatment of FMR are often the cause of damage in uterine mucous membrane. This damage can be also a result of intrauterine inlocation of irritating drugs and disinfectant fluids. These substances lead to necrotic changes of the epithelium of mucous membrane and decrease the phagocytic activity of neutrophils. The fluid which contains fragments of afterbirth and afterbirth fluid is a very good nutrient medium for bacteria (22, 25).

The development of *metritis ichorosa* with heavy, toxic systemic symptoms can be the effect of infection of lochial fluid and foetal membranes. Another consequence of FMR is purulent metritis and then chronic endometritis (2, 16, 23).

The aim of the study was to define the species of microorganisms present in the uterus of healthy and sick cows before and after therapy of FMR with different methods.

Material and Methods

Field trials were carried out over 5 years on 3 farms on 95 primiparous and multiparous cows 2 – 8 years old, HFxblack/white breed, with foetal membrane retained. The control group consisted of 21 cows without postparturient disturbances. The average milk yield was 5-6.5 kg for the last lactation.

The cows with FMR were randomly qualified to one of the 5 groups:

- in the first group (30 cows) antibiotic product (Metrisan AN) containing ampicillin (200 mg) and neomycin (300 000 i.u.) was introduced intrauterinally once on the second day after parturition,
- in the second group (20 cows) only lysozyme dimer (Lydium KLP) was applied intramuscularly (*i.m.*), in a dose of 0.02 mg/kg,
- in the third group (20 cows) Metrisan AN was introduced intrauterinally together with Lydium KLP *i.m.* in a dose of 0.02 mg/kg,
- the last two groups were a positive control. Before treatment the manual separation of retained placenta was tried. Metrisan in combination with propranolol (Uterotonic) *i.m.* in a dose of 50 mg/cow was used in the fourth group (10 cows), or with alphaprostalol (Gabbrostim) intramuscularly in a dose of 1.5 mg/100 kg in the fifth group (15 cows).

Smears from the uterus for bacteriological tests were taken on the first day of the treatment and then three weeks later. The samples were cooled and immediately transported to the laboratory. Bacteriological examinations were performed according to commonly accepted rules (17). Microorganisms that grew on agar with blood were examined in light microscopy, and then analytical profile index (API) tests, (BioMerieux) were applied.

Results

The results of bacteriological examination of smears from the uterus of sick and healthy cows are presented in Table 1. As seen from the Table the same species were found in sick and healthy cows. In a few cases identification was impossible because of the presence of numerous different species. Most often *E. coli* and other species from *Enterobacteriaceae* family were isolated. These species were found in 67.4% of sick cows and about 47.6% of healthy, control cows. The degree of *A. pyogenes* infection was slightly lower in sick than in healthy cows (8.4% versus 9.5%). *Streptococcus* sp. was isolated from 15.8% of FMR cows and from 19% of controls ones. Staphylococci were isolated from 12.6% of FMR and from 9.5% of control cows. Other bacterial species were isolated only from less than 10% of the examined cows.

The highest rate of infection with *A. pyogenes* in 21 day after treatment was found in the IV-th group (30%), and the lowest in the V-th group (13.3%). Infections with this pathogen increased in third week after parturition in comparison to the first day in each experimental group. The cows infected with *A. pyogenes* on day 21 after the start of therapy showed worse reproduction indicators and often were culled because of infertility. Altogether 12 cows from experimental groups were culled. Ten from them (83.3%) were infected with *A. pyogenes*.

Table 1

Numbers (%) of cows with the different species of bacteria in the uterus before and after treatment of FMR

Group (n)		*Arc. pyog.*		*Coliforms*		Streptoc.		Staphyloc.		Others		Negative	
		„0”	21	„0”	21	„0”	21	„0”	21	„0”	21	„0”	21
I Antibiotics	n	3	5	21	6	4	2	2	0	2	0	3	12
(30)	%	10	16.7	70	20	13.3	6.7	6.7	0	6.7	0	10	40
II Lys. dimer	n	2	4	12	2	2	0	3	1	1	0	3	10
(20)	%	10	20	60	10	10	0	20	5	5	0	15	50
III Ant. + lys. dimer	n	1	4	14	0	3	0	3	0	1	0	1	13
(20)	%	5	20	70	0	15	0	15	0	5	0	5	65
IV Ant. + β-blocker	n	1	3	7	2	3	0	3	1	0	0	0	4
(10)	%	10	30	70	20	30	0	30	10	0	0	0	40
V Ant. + PGF2α	n	1	2	10	2	3	1	1	0	1	0	1	8
(15)	%	6.7	13.3	66.7	13.3	20	6.7	6.7	0	6.7	0	6.7	53.3
Control	n	2	0	10	2	4	2	2	0	2	0	2	17
(21)	%	9.5	0	47.6	9.5	19.0	9.5	9.5	0	9.5	0	9.5	81.0

Explanation: *Arc. pyog. – Arcanobacterium pyogenes; Coliforms - Escherichia coli, Proteus vulgaris, Enterobacter agglomerans, Enterobacter aerogenes, Klebsiella oxytoca, Klebsiella* sp.*, Hafnia alvei, Citrobacter freundi;* Streptococci – *Str. equisimilis, Str. suis, Str. equi, Str. acidominimes, Lactococcus lactis, Lactococcus acidophilus Aerococcus viridens Enterococcus foecalis, Enterococcus durans, Peptostreptococcus* sp.*;* Staphylococci – *Staph. chromogenes, Staph. epidermidis, Staph. lentus, Staph. capitis, Staph. intermedius, Staph. xylosus, Micrococcus* sp.*;* others - *Diplococcus* sp.*, Sphiringomonas paucimobibis, Pasteurella multocida, Provotella melaninogenes.*

The infections caused by bacteria from *Enterobacteriaceae* family were found in 60-90% of cows. These microorganisms dominated during the first few days after parturition. Streptococci were mainly isolated in the first week after parturition, mostly in group IV. Staphylococci were mainly isolated in groups IV and II and were present rarely in the whole postpartum period. Other bacteria were isolated in 0 – 7% during both examinations.

The negative bacteriological results of smears from the uterus on days 21-22 after parturition were noted in the highest percentage in cows from group III (65%), and in the lowest percentage in the I-st and IV-th group (40%). The efficacy of the treatment is presented in another paper (11).

Discussion

According to Zerbe *et al*. (30) *E. coli* and *A. pyogenes* are the most prominent bacteria present in uterine lochial secretions of cows that developed endometritis after parturition. *A. pyogenes* is rarely isolated alone from the uterus in the course of a disturbed *puerperium*. This was confirmed in our study, that average and high-grade uterine contaminations were always associated with the presence of both bacteria. The contamination grade was positively correlated with uterine polymorphonuclear granulocyte (PMN) number and negatively correlated with blood PMN number (13). In our study, *A. pyogenes* was found about twice more often in the third week after parturition in comparison to the first days in the cows from each experimental group. The same results were published by other authors (3, 27). The cause of this fact can be suppressed growth of *A. pyogenes* by foetal fluids in the first days after calving. Infections with *E. coli* decreased during a few weeks after calving.

This fact proves, that a correlation between *A. pyogenes* and *E. coli* may exist. Other authors (9) write about the correlation between *A. pyogenes* and *Bac. melaninogenicus* in infected uterus. *A. pyogenes* is isolated very often after manual separation of foetal membranes (8). In our experiment this difference was not statistically significant, but results of the treatment were better in cases of conservative methods in comparison to methods involving manual separation (11). The infection indicates that antibiotic preparation which was used did not eliminate *A. pyogenes* infection. In case of infections with this bacterium the use of systematic therapy is recommended (8).

Postpartum disturbances are connected with the decrease in the activity of phagocytes (7, 24). In our investigations a decrease in spontaneous chemiluminescence level in the first week after parturition was noted in cows with FMR in comparison to healthy cows. This finding indicates lower phagocytic activity of granulocytes (15). The increase in chemiluminescence level after lysozyme dimer injection proved the activation of phagocytosis. It is interesting that after using this drug the antioxidant activity also increased (16).

The decreased immunity, atonia of the uterus, open uterus cervix, presence of fluid and fragments of foetal membranes in uterus lumen are good conditions to generate bacterial infection, mostly by opportune and pathogenic species. Infections are often caused by: *A. pyogenes*, *E. coli* and other Gram-negative *Enterobacteriaceae*, streptococci and staphylococci (5, 21, 22, 29). In this experiment the same bacterial species were noted in healthy and sick (FMR) cows, but in the 4th week after calving, the pathogenic microorganisms were isolated only in FMR cows. From cows without FMR *A. pyogenes* was isolated only in the first week after parturition. This fact let us to conclude that *A. pyogenes* is very often present in the lumen of the uterus after parturition. The results of our experiment also demonstrate that the conservative therapy method of FMR in cows with the use of antibiotics and additional lysozyme dimer injection is safe and efficacious. The combination of intrauterine inlocation of antibiotic product, the stimulation of immunity and limitation of free oxygenic radix activity (18) can be widely used. This method lets us to avoid the alteration of mucous lamina and infections and leads to natural separation of retained foetal membranes.

In conclusion, it can be stated that the same species of bacteria can be isolated from the uterus of healthy and sick (*retentio secundinarum*) cows. The presence of *A. pyogenes* in smears from the uterus after clinical cure of FMR negatively prognoses the future fertility of cows.

References

1. Anderson T.D., Meador V.P., Cheville N.F.: Pathogenesis of placentitis in the goat inoculated with *Brucella abortus*: Part I. Gross and histologic lesions. Vet. Pathol., 1986, **23**, 219-226.
2. Callaghan C.J., Horstman L.A.: Treatment of early postpartum metritis in a dairy herd: Response and subsequent fertility. Bov. Pract., 1987, **22**, 124-128.
3. Callahan C.J., Horstman L.A.: Treatment of *postpartum metritis* in dairy cows caused by *Actinomyces pyogenes*. Bov. Pract., 1993, **21**, 162-165.
4. Dohmen M.J.W., Nagy P., Gacs M.: The relationship between bacteriological and clinical findings in cows with subacute/chronic endometritis. Theriogenology, 1995, **43**, 1379-1388.
5. Dohmen M.J.W., Huszenicza G., Fogor M., Kulcsar M., Vamos M., Porkolab L., Szilagyi N., Lohuis J.A.C.M.: Bacteriology and fertility in health postpartum cows and cows with acute endometritis. Proc. XIX World Buiatrics Congress., Edinbourgh, 1996, pp. 238-241.
6. Foley G.L., Schlafer D.H.: Candida abortion in cattle. Vet. Pathol., 1987, **24**, 532-536.
7. Gilbert R.O., Gröhn Y.T., Miller P.M., Hoffman D.J.: Effect of parity on periparturient neutrophil function in dairy cows. Vet. Immunol. Immunopath., 1993, **36**, 75-82.
8. Gnemmi G.: Therapeutic protocol of reproductive pathologies of the postpartum cow. Konferencja Naukowa Sekcji Fizjopatologii Rozrodu PTNW, Wenecja, 2002, ed. PIWet, Puławy, pp. 50-72.
9. Gröhn Y.T., Eicker S.W., Hertl J.A.: The association between previous 305-day milk yield and disease in New

York State dairy cows. J. Dairy Sci., 1995, **78**, 1693-1702.
10. Jędraszczyk J.: Ochrona epizootiologiczna buhajów w stacjach produkcji nasienia. Życie Wet., 2001, **7**, 370-373.
11. Kaczmarowski M., Markiewicz H., Kuźma K., Malinowski E.: The efficacy of some therapy methods of the retained placenta and the puerperal metritis in cows. III Middle-European Congress for Buiatrics Health: problems in ruminants, Milowy, 2001, p. 270.
12. Katkiewicz M., Wierzchoń M.: Occurrence of *Neospora caninum* in domestic animals and its role in the etiology of reproduction disturbances. Medycyna Wet., 2002, **58**, 332-336.
13. Kimura K., Goff J.J., Kehrli M.E., Reinhardt T.A.: Decreased neutrophil function as a cause of retained placenta in dairy cattle. J. Dairy Sci., 2002, **85**, 544 - 550.
14. Kossaibati M.A., Esslemont R.J.: The costs of production diseases in dairy herds in England. Vet. J., 1997, **154**, 41-51.
15. Malinowski E., Kuźma K., Sobolewska S., Kłossowska A.: Metabolic activity of milk and blood phagocytic cells in samples from healthy and mastitis cows. Medycyna Wet., 1998, **54**, 321-324.
16. Malinowski E., Smulski S., Kaczmarowski M., Markiewicz H.: Wpływ iniekcji wybranych leków na status antyoksydacyjny krwi cielnych jałówek. Praktyczne aspekty badań doświadczalnych układu rozrodczego i gruczołu mlekowego zwierząt. Konferencja Naukowa Sekcji Fizjopatologii Rozrodu PTNW, Wenecja 2002, ed. PIWet, Puławy, p. 136.
17. Malinowski E., Kłossowska A.: Diagnostyka zakażeń i zapaleń gruczołu mlekowego krów. ed. PIWet, Puławy, 2002.
18. Malinowski E.: Lysozyme dimer in therapy and prophylaxis of animal disease. Princeton – Poznań, 2001, pp. 8-9.
19. Marciszewska M.: Occurrence of mycoplasma in the infections in cows. Medycyna Wet., 1979, **35**, 141-143.
20. Markiewicz H., Malinowski E.: Poporodowe zapalenie macicy u krów – problem ciągle aktualny. Życie Wet., 2000, **5**, 260-262.
21. Markiewicz H., Kuźma K., Kaczmarowski M., Malinowski E.: Pathogenesis of puerperal metritis in cows. Proc. II Middle-European Congress for Buiatrics, Slovak Republic, 2000, pp. 258-260.
22. Messier S., Higgins R., Couture Y., Morin M.: Comparison of swabbing and biopsy for studing the flora of the bovine uterus. Can. vet. J., 1984, **25**, 383-386.
23. Petter A.T., Bosu W.K.T., Gilbert R.O.: Absorption of *Escherichia coli* endotoxin (lipopolysacharide) from the uteri of postpartum dairy cows. Theriogenology, 1990, **33**, 1011-1014.
24. Pfützner H., Sachse K.: *Mycoplasma bovis* as an agent of mastitis, pneumomia, arthritis and genital disorders in cattle. Rev. Sci. Tech. Off. Int. Epiz., 1996, **15**, 1477-1494.
25. Seguin B.E., Morrow D.A., Oxender W.O.: Intrauterine therapy in the cow. JAVMA, 1984, **164**, 609-614.
26. Silva N., Lobato F.C.: Isolation and antimicrobial susceptibility of bacteria recovered from uteri of dairy cows with postpartum endometritis. Rev. Bras. Reprod. Anim., 1999, **23**, 410-411.
27. Subandrio A.J., Noakes D.E.: Neutrophil migration into the uterine lumen of the cow: the influence of endogenous and exogenous sex steroid hormones using two intrauterine chemoattractans. Theriogenology, 1997, **47**, 825-835.
28. Varadin M., Borjanović S., Jalšoweć A., Urošev D., Ugarak M.: Behandlung der Nachgeburstsverhaltung bei Kuhen. Schweiz. Arch. Tierheilk, 1985, **127**, 279-283.
29. Zerbe H., Schuberth H.J., Ossadnik C., Leibold W.: Influence of *Escherichia coli* and *Arcanobacterium pyogenes* from contaminated lochial secretion on phenotypic and functional properties of bovine neutrophils. Esdar Newsletter, 2000, **5**, 67.
30. Zerbe H., Ossadnik C., Leibold W., Shubert H. J.: Influence of *Escherichia coli* and *Arcanobacterium pyogenes* isolated from bovine puerperal uteri on phenotypic and functional properties of neutrophils. Vet. Microbiol., 2001, **79**, 351-365.