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THE FREQUENCY OF OCCURRENCE OF ACTINOMYCES PYOGENES AND "ACTINOMYCES LIKE ORGANISMS" ("ALO") IN ENDOMETRIUM BIOPSY OF COWS FROM VOJVODINA AND BARANJA

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The aim of the study was to determine the prevalence of Actinomyces pyogenes and "ALO" in the endometrium of cows in this geographical region. It included 102 animals from 12 heards in the period from October 6. 1994. to may 10. 1995. Sampling was performed with Folmer-Nielsen catheters, as modified by Jović. Samples were seeded on solid media immediately after callection under field conditions. Aerobic incubation was begun on the same day at 37°C. The primary isolates, their subcultures and colonies obtained after passages in liquid media were examined for colony morphology and staining characteristics, catalase, oxidase, CAMP phenomena and biochemical activity. The biochemical identity of Actinomyces pyogenes was confirmed using the API Coryne system (Bio Merieux, Marny, L'Etoil, France). The "ALO" identity was established by excluding parameters guoted as crucial. Every coryneform organism which caused beta haemolysis on blood agar but was not designated as Actinomyces pyogenes, Arcanobacterium haemolyticum, Corynebacterium pseudotuberculosis, Corynebacterium ulcerans and beta strains of Erysipelothrix rhusiopathiae was identified as "ALO".

Key words: Actinomyces pyogenes, "Actinomyces-like organism", Arcanobacterium haemolyticum, Corynebacterium pseudotuberculosis, Corynebacterium ulcerans, Erysipelothrix rhusiopathiae, endometrial biopsy

INTRODUCTION

Actinomyces pyogenes is a clearly defined species of the genus Actinomyces, different, from the taxonomic category of "Actinomyces like organisms" (ALO) which is represented by a large group - from Corynebacteria and Mycobacteria to Actinomycetaceae, on the basis of their genetic, phylogenetic and biological similarity (Klaus 1986). Many representatives of this group, such as Actinomyces pyogenes, are ubiquitous organisms, strictly or conditionally pathogenic, mainly because of the proteinase and neuroaminidase they produce

(Lammler 1990). They do not show selectivity towards mammals nor tropism for mammalian tissues (Akkermans et al., 1992; Krech et al., 1991; Bonnet et al., 1991; Chauhan et al., 1992; Kirkbride 1993; Saini et al., 1992; Semambo et al., 1991; Steinar et al. 1991; Queen et al., 1994). Many of them ar zoonthropous species (Krech et al., 1991; Collins et al., 1986; Klaus, 1986; Clarridge, 1989; Brander et al., 1992) thus being more important in the diagnostic, epizootiologic and preventive sense. Literary data (Akkermans et al., 1992; Bonnet et al., 1991; Kirkbride, 1993; Noakes et al., 1991; Semambo et al., 1991) and our experience have obliged us to establish the frequency of their appearance in the endometrium of cows in this geographical region which was the aim of this investigation.

MATERIAL AND METHODS

Sampling. This investigation included 102 cows from Vojvodina and Baranja examined from October 10, 1994. To May 5, 1955. Specimens were taken, with sterile Folmer-Nielsen catheters using the, modification of Jović. Solid bacteriological nutrients were inoculated in the field immediately after collection and the aerobic incubation at 37^0C was started on the same day.

Cultivation. Inoculated media (blood agar with 10% sheep blood, endo agar, Mc Conkey agar and Sabouraund agar) were held ina thermostat for three days fetore fring declared negative.The suspect colonies were subcultured on blood agar and held under aerobic,anaerobic and microaerophilic conditions.

Identification of isolates. Primoisolates, their subcultures and colonies obtained after passage in a liquid medium, were examined regarding their culture, staining, morphology, catalase, oxidase and CAMP properties. Furthermore, their relation to molecular oxygen and some biochemical properties were also determined. The biochemical identity of Actinomyces pyogenes was confirmed in the API Coryne system (Bio Merieux, Marny, L 'Etiol, France). The "ALO" identity was established by excluding parameters quoted as crucial. Every coryneform organism which caused beta haemolysis on blood agar but did not belong to Actinomyces pyogenes, Arcanobacter haemolyticus, Corynebacterium pseudotuberculosis, Corynebacterium ulcerans and beta forms of Erysipelothrix rhusiopathiae was identified as "ALO".

RESULTS

Colony characteristics. All examined strains were slow-growing (requiring 36-48 h for detectable colony development), beta-haemolytic and of typical S-form (bulging colonies, smooth and glossy surface, clear margins and slightly raised edges). At the beginning all colonies were translucent, but after 3 days of incubation a whitish center appeared, and afterwards the colonies became all white. On nutritive agar they grew as tiny, dustlike colonies, with a 24 hour delay compared with blood agar. Differences appeared in the size and type of haemolysis. In the strains confirmed as Actinomyces pyogenes, haemolysis was double the diameter of the colony and of type "A" (clear margin). In "ALO" isolates,

haemolysis was not double in size (the colonies were larger) and possessed characteristics of group "B" (burred haemolysis margins). (Figures 1,2)

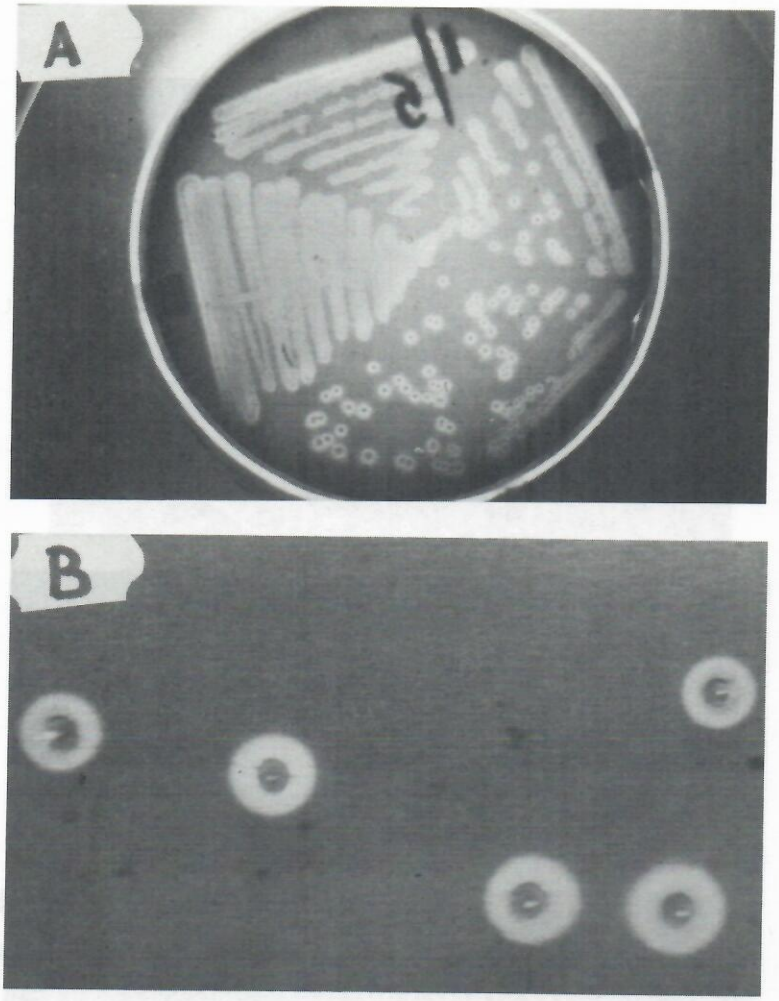

Figure 1. Actinomyces pyogenes - after 5 days of incubation on 10% sheep-blood agar. a. culture. b. colonies with hemolysis.

All strains survived at 4^0C, but the so called "cold enrichement" did not occur. Moreover, all strains grew in aerobic and anaerobic atmospheres, but the

optimum was reached under microaerophilic conditions with 5% CO2. All strains caused the CAMP phenomenon of the "match-head" and a poor, postponed phenomenon of beta haemolysis restriction (7-10 days). All strains of Actinomyces pyogenes were catalase and oxidase negative, and all the four "ALO" strains had a weakly positive test to catalase (3% H2O2).

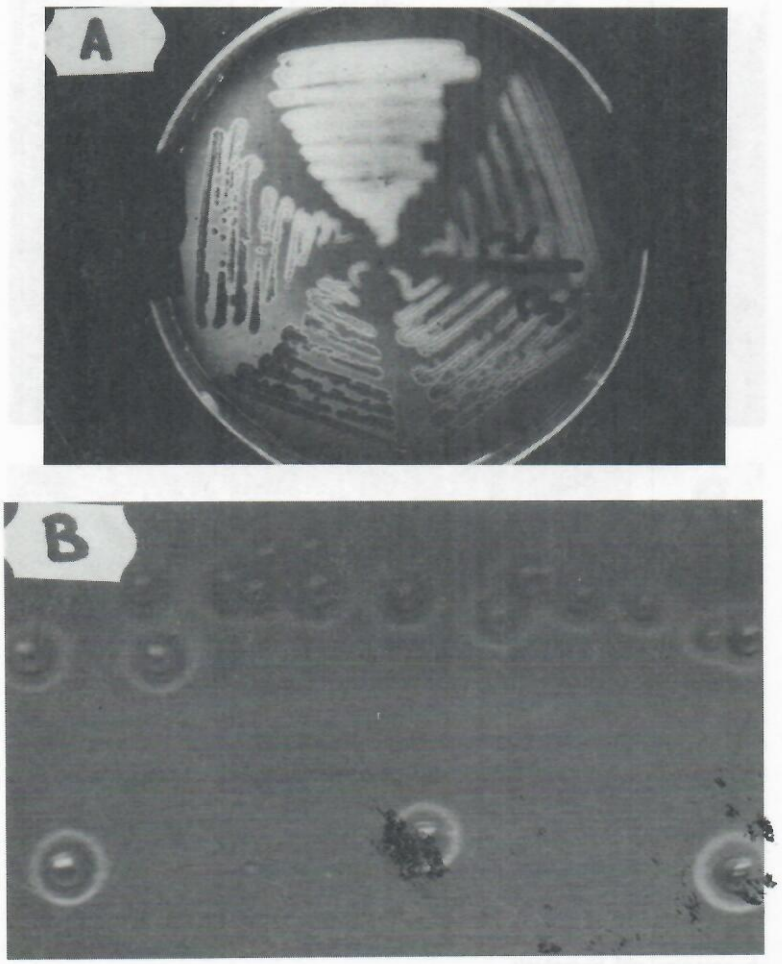

Figure 2. "Actinomyces like organism" (ALO) after 5 days of incubation on 10% sheep blood agar. a. culture. b. colonies with hemolysis.

Morphologic and staining characteristics. All strains were pleomorphic and polychromatic with irregular distribution and Gram instability which increased in old cultures. In all strains metachromatic granulae seemed to be present, but their existence was excluded by Neisser's staining.

Some biochemical propertied of the examined isolates are shown in Table 1.

Table 1. Biochemical characteristics of the examined isolates and identification table % of positive reactions after 24 hrs at 35-37°C

TESTS	REACTIONS	REACTION OF ISOLATES						
		EXAMINED STRAINS		IDENTIFICATION TABLE				
		1	2	3	4	5	6	7
NIT	NITrate reduction	–	–	0	1	1	1	0
PYZ	PYraZinamidase	–	–	10	98	0	0	20
PyrA	Pyrrolidonyl Arylamidase	–	–	100	70	0	0	50
PAL	ALkaline Phosphatase	–	–	65	85	96	54	4
β - GUR	beta GlucURonidase	+	–	100	36	0	0	0
β - GAL	beta GALactosidase	+	–	91	89	0	0	12
α - GLU	alpha GLUcosidase	+	–	94	92	98	25	0
β - NAG	N-Acetyl-β Glucosaminidase	–	–	41	89	0	0	95
ESC	ESCulin (β Glucosidase)	–	–	0	0	0	0	0
URE	UREase	–	–	0	0	100	100	25
GEL	GELatine (hydrolysis)	+	+	88	0	1	0	0
O	Oxidase	–	–	0	0	0	0	0
GLU	GLUcose	+	–	100	100	100	100	75
RIB	RIBose	+	–	100	91	100	100	37
XYL	XYLose	+	+	100	0	0	0	0
MAN	MANnitol	–	–	0	0	0	0	0
MAL	MALtose	–	+	97	94	98	75	0
LAC	LACtose	–	+	91	100	0	0	99
SAC	Sucrose	–	–	50	44	6	0	0
GLYG	GLYcoGen	–	–	11	0	95	0	0
CAT	CATalase	–	–	0	0	100	100	0

Legend: 1-Strains identified as Actinomyces pyogenes; 2-Strains dentified as "Actinomyces-like" organism; 3- Actinomyces pyogenes; 4- Arcanobacterium haemolticum; 5- Corynebacterium ulcerans; 6-Corynebacterium pseudotuberculosis; 7-Erysipelothrix rhusiopathiae

The prevalence of Actinomyces pyogenes and ALO is shown in Table 2.

Actinomyces pyogenes was found in 4 out of 12 examined herds "ALO" was found in 2 out of 12 examined herds. Actinomyces pyogenes was found in 9 cows and "ALO" in 4 cows out of 102, respectively. In 1 out of 12 examined herds Actinomyces pyogenes was found in only 1 cow.In the other herds with positive cases, Actinomyces pyogenes was found in 4 out of 10,2 out of 10 and 2 out of 10 and 2 out of 2 cows. "ALO" was found in 2 out of 12 examined herds, in both cases in 2 out of 10 cows. All Actinomyces pyogenes and "ALO" positive cows had cytological and/or clinical signs of endometritis.

Table 2. The frequency of detection of Actinomyces pyogenes and ALO

Stock No.	Possibility of isolation		Total
	Actinomyces Pyogenes	"Actinomyces like" organism (ALO)	
1	1/10	0/10	1/10
2	4/10	0/10	4/10
3	2/10	0/10	2/10
4	2/2	0/2	2/2
5	0/10	2/10	2/10
6	0/10	2/10	2/10
7–12	0/50	0/50	0/10
TOTAL	9/102	4/102	13/102

DISCUSSION

These findings of Actinomyces pyogenes are similiar to those of other authors (Akkermans et. al., 1992; Bonnet et al., 1991; Kirkbride, 1993). There are numerous reports about the detection of "ALO" in the female uterus and vagina (Hermet, 1984; Kozuh et al., 1984; Keith et al., 1986; Mao, 1984; Ortner, 1985; Pan, 1995; Petitti et al., 1983). However, no information could be found so far in the available literature about the appearance of "ALO" organisms in endometrial biopsies of cows. Homez et al. 1990. detected "ALO" in the vagina and pyogenic processes in swine, but they used broader criteria for identification including, besides beta, both alpha and gamma haemolytic coryneform organisms. Collins et al., 1993 examined only one "ALO" strain and have suggested it to be registered as a separte species named Corynebacterium hyovaginalis.

However, the causative agent found in a relatively small number of cases might indicate the existence of a herd infection. The percentage of positive cases (12,74) requires further examination, the elaboration the elaboration of more precise diagnostic methods and permanent collaboration of allprofessionals - the veterinary practitioner, bacteriologist and epizootiologist.

REFERENCES

1. *Akkermans, J. P., Kreeft, H. J.* 1992. Results of studies with aborted cattle foetuses. *Tijdschr-Diergenegskd. 117, 13,* 375-379.

2. *Barbour, E. K., Brinton, Caput, A., Johnson J. B.,Poss, P. E.* 1991. Characteristics of Actinomyces pyogenes involved in lameness of male turkeys in north-central United States. *Avian-Dis. 35, 1,* 192-196.

3. *Bonnet, B. N., Martin, S. W., Gannon,V. P., Miller, R. B., Etherington, W. G.* 1991. Endometrial biopsy in Holstein-Friesian dairy cows III. Bacteriological analysis and correlations with histological findings. *Can-J-Vet-Res., 55, 2,* 168-173.

4. *Brander, M. A., Jousimies-Somer, H. R.* 1992. Evaluation of rapid ANA II and API ZYM systems for identification of Actionomyces species from clinical specimens. *J.Clin-Microbiol., 30, 12,* 3112-3116.

5. *Brinton, M. K., Schellberg, L. C., Johnson, J. B., Frank, R. K., Halworson, D. A., Newman, J. A.* 1993. Description of osteomyelitis lesions associated with Actionomyces pyogenes infections in the proxymal tibia of adult male turkyes. *Avian-Dis., 3, 71,* 259-262.

6. *Chauhan, R. S., Kaushik, R. K.,* 1992. Pyometria in camels: case report. *Br-Vet-J. 148, 1,* 84-85.

7. *Collins M. D. et al.* 1986. Genus Corynebacteriuym, Section 15 in Bergey s Manual of Systematic Bacteriology, *Vol 2, Williams and Wilkins, Baltimore, MD, U.S.A. pp:* 1266 - 1276 and 1287 - 1288.

8. *Hermet R.* 1984. Type of intrauterine device and Actinomyces-like organisms: is there a corelation? *Acta=Cytol. 28, 6,* 774-775.

9. *Keith, L. G., Bockoff, C. A., Hidvegi, D. F., Method, M.* 1986. Persistent cervicovaginal actinomyces-like organisms following antibotic therapy in an IUD user.*Adv - Contracept. 2, 2,* 123-130.

10. *Kirkbride. C. A.* 1993. Bacterial agent detected in a 10 year study of bovine abortions and stillbirth *J-Vet-Diagn-Invest. 5, 1,* 64-68.

11. *Klaus P. Schaal.* 1986. Genus Actinomyces in Bergey s Manual of Systematic Bacteriology, Williams and Wilkins Baltimore, MD, U. S. A., 1383 - 1418.

12. *Kozuh, M., Bonta, S., Kovačić, J., Levicnik, A., Lushi, A.* 1984. Actinomyces-like organisms in users of intrauterine centraception; *Jugosl.-Ginekol - Opstet. 24,* 5-6, 87-91.

13. *Krech T. et al.* 1991. Corynebacterium and Related Organisms, chapter 31 in *Manual of Clinical Microbiology, fifth edition, Washington,* 277-286.

14. *Lammler, Von C. H.* 1990. Untersuchungen zu moglichen Pathogenitatsfaktoren von Actinomyces pyogenes, *Ubersichtsreferat Berl. Munch Tierarztl. Wschr., 103,* 121-125.

15. *Mao, K., Guillebaund., J.* 1984. Influence of removal of intrauterine contraceptive devices an colonisation of the cervix by Actinomyces-like organism. *Contraception. 30,6,* 535-544.

16. *Madsen M.* 1989. Studies on microaerophilic Cocci, (Stuart-Schwan Cocci) isolated from Summer mastitis and other Pyogenic infections of cattle. *Acta Vet. Scand., 30,* 165-174.

17. *Madsen M., Sorens G. H. Aalbeak B.* 1990. Summer mastitis in heifers:a bacterial examination of secretions from clinical cases of summer mastitis *in Denmark. Veterinary Microbiology, 22,* 319-328.

18. *Madsen M., Sorens G. H. Aalbeak B., Hansen J., Bjorn H.* 1992. Summer mastitis in heifers: studies on the seasonal occurrence of Actinomyces pyogenes, Peptostreptococcusindolicus and Bacteroidaceaee in clinically healthy cattle in *Denmark. Veterinary Microbiology, 30,* 243-255.

19. *Morrison, J. R. A., Tillotson, G. S.* 1988. Identification of Actinomyces (Corynebacterium) pyogenes with the API 20 Strep. system. *Journal of Clinical Microbiology, Vol* 1865-1866.

20. *Noakes, D. E., Wallace, L., Smith, G. R.* 1991. Bacterial flora of the uterus of cows after calving on two hygienically contrasting farms. *Veterynary Record., 128,* 440-442.

21. *Otner, A., Huber, A., Pertoll, J., Tabarelli, M., Mikuz, G.* 1985. Morphologic changes in cervix smears by intrauterine pessary: *Geburtshilfe-Frauenheikkd, 45,* 244-246.

22. *Pan, L.* 1993. Actinomyces-like organisms. Infection in intrauterine devices wearers. *Chung-Hua-Fu-Chan-Ko-Tsa-Chih., 28,* 292-294.

23. *Petitti, D. B., Yamamoto, D., Morgenstern, N:* 1983. Factors associated with Actinomyces-like organisms and Papanicolau smear in users of intrauterine contraceptive devices. *Am- J-Obstet - Gynecol. 1, 145, 3,* 338-341.

24. *Quenn, C., Ward, A. C., Hunter, D. L.* 1994. Bacteria isolated from nasal and tonsillar samples of clinically healthy Rocky Mountain bighorn and domestic sheep. *J-Wild-Dis, 30,* 1-7.

25. *Saini, S. S., Sharma, J. K., Kwatra, M. S.* 1992. Actinomyces pyogenes mastitis among lactating cows fallowing foot-and-mauth disease. *Veterinary Record. 15, 131, 7,* 152.

26. *Semambo, D. K. N., Ayliffe, T. R., Boyd, J. S., Taylor, D. J.* 1991. Early abortion in cattle induced by experimental intrauterine infection with pure cultures of Actinomyces pyogenes. *Veterinary Record, 129,* 12-16.

27. *Steinar, Hoie., Knut, Falk., and Bjorn M.Lium.* 1991. An abattoir survey of pneumonia and pleuritis in slaughter weight swine from 9 selected herds. IV. Bacteriological findings in chronic pneumonic lesions. *Acta Vet. Scand., 32,* 395-402.

UČESTALOST POJAVLJIVANJA ACTINOMYCES PYOGENES I "ACTIONOMYCES LIKE ORGANIZAMA" ("ALO") U UZORCIMA ENDOMETRIJUMA KRAVA SA TERITORIJE VOJVODINE I BARANJE

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SADRŽAJ

Cilj ovog istraživanja je utvrđivanje frekvence prisutnosti Actinomyces pyogenes i "ALO" u endometrijumu krava ovog geografskog područja. U periodu od 6. 10. 1994. do 10. 5. 1995. godine istraživanjem je obuhvaćeno 102 grla iz 12 zapata. Uzorkovanje je vršeno sterilnim kataterima po Folmer-Nielsenu, modifikacija po Jovičnu. Zasejavanje čvrstih medijuma obavljano je ne terenu, odmah nakon uzorkovanja, a inkubisanje je vršeno na 37 ^{0}C, u aerobnim uslovima. Primoiizolati, njihove subkulture i kolonije dobijene presejavanjem u tečnim podlogama, ispitani su kulturelno, morfološki i tinktorijelno. U okviru njihove biohemijske aktivnosti ispitani su aktivnost katalaze, oksidaze i CAMP fenomena. Biohemijski identitet Actinomyces pyogenes-a potvrđivan je API CORYNE sistemom (Bio Merieux, Marny, L "Etoil, France). Identitet "ALO" utvrđivan je putem isključivanja karakterističnih parametara za tipične predstavnike. Svaki korineformni organizam koji je na krvnom agaru uzrokovao β hemolizu, a nije pripadao vrstama Actinomyces pyogenes, Arcanobacterium haemolyticum, Corynebacterium ulcerans, Corynebacterium pseudotuberculosis i β formi Erysipelothrix rhusiopathiae, identifikovan je kao "ALO".