# Activity of disinfectants and biofilm production of Corynebacterium pseudotuberculosis<sup>1</sup>

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**ABSTRACT.-** Sá M.C.A., Veschi J.L.A., Santos G.B., Amanso E.S., Oliveira S.A.S., Mota R.A., Veneroni-Gouveia G. & Costa M.M. 2013. **Activity of disinfectants and biofilm production of** *Corynebacterium pseudotuberculosis. Pesquisa Veterinária Brasileira 33(11):1319-1324*. Laboratório de Microbiologia e Imunologia Animal, Universidade Federal do Vale do São Francisco, Petrolina, PE 56300-990, Brazil. E-mail: giseleveneroni@yahoo.com.br

To verify the occurrence of caseous lymphadenitis in sheep and goats on farms of Pernambuco, Brazil, and in animals slaughtered in two Brazilian cities (Petrolina/PE and Juazeiro/BA), and to characterize the susceptibility profile of *Corynebacterium pseudotubercu*losis to disinfectants and antimicrobials, and its relationship with biofilm production were the objectives of this study. 398 samples were tested for sensitivity to antimicrobial drugs, disinfectants, and biofilm production. Among the 108 samples collected on the properties, 75% were positive for *C. pseudotuberculosis*. Slaughterhouse samples indicated an occurrence of caseous lymphadenitis in 15.66% and 6.31% for animals slaughtered in Petrolina and Juazeiro respectively. With respect to antimicrobials, the sensitivity obtained was 100% for florfenicol and tetracycline; 99.25% for enrofloxacin, ciprofloxacin and lincomycin; 98.99% for cephalothin; 98.74% for norfloxacin and sulfazotrim; 97.74% for gentamicin; 94.22% for ampicillin; 91.71% for amoxicillin; 91.21% for penicillin G; 89.19% for neomycin and 0% for novobiocin. In analyzes with disinfectants, the efficiency for chlorhexidine was 100%, 97.20% for quaternary ammonium, 87.40% for chlorine and 84.40% for iodine. 75% of the isolates were weak or non-biofilm producers. For the consolidated biofilm, found that iodine decreased biofilm formation in 13 isolates and quaternary ammonia in 11 isolates. The reduction of the biofilm formation was observed for iodine and quaternary ammonium in consolidated biofilm formation in 33% and 28% of the isolates, respectively. The results of this study highlight the importance of establishing measures to prevent and control the disease.

INDEX TERMS: *Corynebacterium pseudotuberculosis*, disinfectants, biofilm, agribusiness, animal health evaluation, sheep and goat livestock, slaughterhouse, prevention.

**RESUMO.-** [Atividade de desinfetantes e produção de biofilme em *Corynebacterium pseudotuberculosis.*] Verificar a ocorrência de linfadenite caseosa de ovinos e ca-

prinos em fazendas de Pernambuco, no Brasil e em animais abatidos em duas cidades brasileiras (Petrolina/PE e Juazeiro/BA), caracterizar o perfil de suscetibilidade de *Corynebacgerium pseudotuberculosis* a desinfetantes e a antimicrobianos e analisar sua relação com a produção de biofilme foram os objetivos deste estudo. 398 amostras foram testadas para a sensibilidade aos medicamentos antimicrobianos, desinfetantes e produção de biofilme. Entre as 108 amostras coletadas nas propriedades, 75% foram positivas para *C. pseudotuberculosis*. Amostras do matadouro indicaram a ocorrência de linfadenite caseosa em 15,66% e 6,31% para animais abatidos em Petrolina (PE) e Juazeiro (BA), respectivamente. Com relação aos antimicrobianos, a sensibilidade

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obtida foi de 100% para florfenicol e tetraciclina, 99,25% para a enrofloxacina, ciprofloxacina e lincomicina; 98,99% para cefalotina; 98,74% para norfloxacina e sulfazotrim; 97,74% para gentamicina; 94,22% para ampicilina, 91,71% para amoxicilina, 91,21% para a penicilina G, 89,19% para a neomicina e 0% para novobiocina. Nas análises com desinfetantes, a eficiência para a clorexidina foi de 100%, 97,20% para amônia quaternária, 87,40% para o cloro e 84,40% para o iodo. 75% dos isolados foram fracos produtores de biofilme. Para o biofilme consolidado, observou-se que o iodo diminuiu a formação de biofilme em 13 isolados e a amônia quaternária em 11 isolados. A redução da formação de biofilme foi observada no biofilme consolidado, para iodo e amônia quaternária, em 33% e 28% das amostras, respectivamente. Os resultados deste estudo destacam a importância de se estabelecer medidas para prevenir e controlar a doença.

TERMOS DE INDEXAÇÃO: *Corynebacterium pseudotuberculosis*, desinfetantes, biofilme, Agronegócio, saúde animal, caprinovinocultura, abatedouro, prevenção.

#### **INTRODUCTION**

Caseous lymphadenitis (CL) of sheep and goats is an inflammation in the lymph nodes characterized by the formation of abscesses containing caseous and yellowish content (Ribeiro 1997). This disease is prevalent among small ruminants in most countries, with a negative impact on the world economy (Fontaine & Baird 2008). The high number of skin abscesses suggests that CL is the most frequent disease in goats in Pernambuco, Brazil (Alencar et al. 2010). This demonstrates the need to implement measures to prevent and control the disease, and increase the productivity of goat and sheep herds (Alencar et al. 2010).

The removal of abscesses and the application of disinfectants are important alternatives for disease control (Santiago et al. 2010). The use of antimicrobials to treat abscesses is recommended by some authors, though with controversial results (Abreu et al. 2008, Washburn et al. 2009). These two options are important because they aim to reduce the spread of the pathogen in the environment, which occurs at the time of rupture of the abscess (Baird & Fontaine 2007).

Biofilms are associated with chronic infections, which are often resistant to treatment with antimicrobial drugs (Costerton et al. 1999). Biofilms were described in *C. pseudotuberculosis, C. renale* and *C. diphtheriae* (Olson et al. 2002, Gomes et al. 2009).

The objectives of this study were to verify the occurrence of CL on farms of the Pernambuco, Brazil, and in animals slaughtered in Petrolina (PE, Brazil) and Juazeiro (BA, Brazil), two cities in the northeastern Brazil, to characterize the susceptibility profile of *C. pseudotuberculosis* to disinfectants and antimicrobials, and relate these profiles to biofilm production.

## **MATERIALS AND METHODS**

**Sample collection.** One hundred and eight samples were collected from lymph node abscesses of sheep and goats on 20

farms located in five municipalities of the state of Pernambuco, Brazil. First antisepsis was performed with alcohol 70% and then drainage of the lymph node was performed to collect the exudate using sterile swabs. 437 samples were collected from animals slaughtered in the municipal slaughterhouse in Petrolina, PE, Brazil, and 12 samples in the slaughthouse in Juazeiro, BA, Brazil, from December 2010 to January 2011. At the Juazeiro slaughterhouse, federal inspection is performed before the slaughter.

**Bacterial isolation and identification.** The samples were cultured on agar base supplemented with 8% sheep blood and incubated for 48 hours at 37°C (Carter 1990). After the growth, gram staining, catalase and CAMP tests were performed (Christie, Atkins and Munch-Petersen) (Pelczar Jr et al. 2007). The bacterial colonies isolated were screened for macro and micromorphological aspects and dyeing (Quinn et al. 1994). The carbohydrate fermentation test (glucose and maltose), esculin test, nitrate reduction test and determinations of the presence of urease and DNAse were also performed (Holt et al. 1994).

Sensitivity to antimicrobial drugs test. The sensitivity test to antimicrobial drugs was performed by diffusion in Kirby-Bauer disk (Bauer et al. 1966, CLSI, 2007). The following antibiotics were tested: novobiocin (30mg) and florfenicol (30 mg); the aminoglycosides: neomycin (30mg) and gentamicin (10mg); beta-lactams: ampicillin (10mg), amoxicillin (10mg) and penicillin G (10mg); quinolones: enrofloxacin (05mg), ciprofloxacin (5mg) and norfloxacin (10mg); tetracycline: tetracycline (30mg); cephalosporin: cephalothin (30mg); sulfonomidas: sulfazotrim (25mg); lincosamides: lincomycin (2mg).

The multiple antibiotic resistance index (MDR) was calculated by dividing the number of antimicrobial groups to which the isolates were resistant by total number of groups tested (Kruperman 1983).

Sensitivity test to disinfectants. To determine *in vitro* antimicrobial activity of *C. pseudotuberculosis*, the disinfectants with the following active ingredients were used: iodine, chlorhexidine, chlorine and quaternary ammonia, following descriptions of the M7-A7 protocol of the Clinical and Laboratory Standards Institute(CLSI, 2007). Disinfectants were diluted in BHI (Brain Heart Infusion Broth) to achieve concentrations of 20%, 10%, 5%, 2.5%, 1.25% and 0.625% of chlorine, quaternary ammonium, iodine and clorhexidine. Inhibition was considered as lack of observable microorganism growth. As a negative control, one well with only BHI broth was used, and as a positive control, one well containing BHI broth and the bacterial inoculum was used. Assays were performed in triplicate.

**Biofilm production.** The gentian violet test was used for phenotypic characterization of biofilm production. As a negative control for this test an *Escherichia coli DH5-alpha* strain (does not form biofilm) was used and also wells containing only sterile tryptone soy broth (TSB) (Merino et al. 2009). The optical density (OD) of each well was measured at 570 nm using an ELISA plate reader (ELISA ASYS EXPERT PLUS BioChrom). After the reading, the samples were classified as positive or negative for biofilm formation. From the arithmetic mean of triplicate of isolates, it was obtained of produced by each isolate (DOI). Based on the average of the negative control (DOC), the micro-organisms were classified as not forming biofilm (DOI  $\leq$  DOC), weak (DOC  $\leq$  2.doc DOI), moderate (DOC <DOI  $\leq$  4. doc) or strong (DOI <4.DOc) biofilm forming (Stepanović et al. 2000). All assays were performed in triplicate.

Isolates classified as strong biofilm producers were selected for testing interference of iodine, chlorine, chlorhexidine, and quaternary ammonia disinfectants on the formation of biofilm and biofilm consolidated.

To assess the interference of disinfectants on the consolidated biofilm, microplates containing  $100~\mu L$  of the bacterial suspensions were incubated at  $37^{\circ}C$  for 48 hours to the prior formation of biofilm. After incubation, the microplates were washed three times with  $200~\mu L$  of distilled water, and disinfectants were added at concentration of 0.625%. The OD was determined after addition of the disinfectant at the times 00 (D00h) and 0.24 h (D024hs). The reading was performed in ELISA plate reader (ELISA ASYS EXPERT PLUS BioChrom) at 0.25% m, and the interference of disinfectants in the biofilm consolidated defined by the equation: D00h average/D024h average x 0.25% (Nostro et al. 0.2007).

In the evaluation of the effect of disinfectants on biofilm formation, it was placed in microplates  $100~\mu L$  of bacterial suspensions, added  $100~\mu L$  of disinfectants and incubated at  $37^{\circ}C$  for 24 hours. After, the microplates were subjected to staining with Gentian Violet. All tests were performed in triplicate using one negative control and one positive control. Reading of the OD was measured in ELISA plate reader (ELISA ASYS EXPERT PLUS BioChrom) at 595 nm. Interference of the disinfectant on the biofilm was evaluated by comparing the initial formation of biofilm with biofilm formation after to use the disinfectant (Nostro et al. 2007).

This study was authorized by the Ethics Committee for Research in Human and Animal Studies at Univasf under number 27091054 of October 6, 2010.

#### **RESULTS**

Altered lymph nodes with suspicion of caseous lymphadenitis (CL) were found in sheep and goats on all farms, as shown in the Table 1. After culturing, characteristic colonies of the *Corynebacterium pseudotuberculosis* agent were observed. *C. pseudotuberculosis* was isolated in 75% of samples analyzed, whereas in other strains other microorganisms were isolated, such as *Bacillus* spp., *Staphylococcus* spp. and *Proteus* spp.

Table 1. Samples collected on the farms of Pernambuco, Brazil, and isolates of *Corynebacterium pseudotuberculosis* 

Location in Brazilian farms (n=20)	Samples collected from goats (n=48)	Samples collected from sheep (n=60)	Isolates from Goat (n=29)	Isolates from sheep (n=52)
Farm (n=11) (Petrolina/PE)	26	43	18	37
Farm (n=1) (Afrânio/PE)	0	02	0	02
Farm (n=1) (Floresta/PE)	04	03	04	03
Farm (n= 05) (Petrolândia/P	14 E)	09	04	07
Farm (n=02) (Jatobá/PE)	04	03	03	03

Most breeders reported not properly disposing of the material removed from the abscesses, and, in most cases, abscesses are suppurated in the field, thereby facilitating environmental contamination and infection to other animals.

For the samples collected in the slaughterhouses, it was observed that 54.1% of them were from the pre-scapular lymph nodes, and other abscesses were found in several organs, as shown in the Table 2. 398 strains were isolated in all.

Table 2. Occurrence and location of the samples collected from goats and sheep at slaughter in Petrolina/PE and Juazeiro/BA, Brazil

Organ	Petrolina		Juazeiro		Total	
		%		%		%
Prescapular	237	54.24	6	50.02	243	54.1
Submandibular	64	14.64	0	0.00	64	14.3
Inguinal	0	0.00	1	8.33	1	0.2
Precural	0	0.00	1	8.33	1	0.2
Mesenteric	0	0.00	1	8.33	1	0.2
Liver	83	18.99	1	8.33	84	18.7
Lung	21	4.81	1	8.33	22	4.90
Large intestine	9	2.06	1	8.33	10	2.2
Preparotid	20	4.57	0	0.00	20	4.5
Kidney	1	0.23	0	0.00	1	0.2
Esophagus	2	0.46	0	0.00	2	0.5
Total abscesses	437	100	12	100	449	100
Total of animals slaughtered	2790		190		2980	-

For antimicrobial susceptibility testing, the results are shown in the Figure 1. Among the antibiotics tested, sensitivity was obtained for the following drugs: 100% for florfenicol (n=398/398) and tetracycline (n=398/398); 99.25% for lincomycin (n=395/398), enrofloxacin (n=395/398) and ciprofloxacin (n=395/398); 98.99% for cephalothin (n=394/398); 98.74% for norfloxacin (n=393/398) and sulfazotrim (n=393/398); 97.74% for gentamicin (n=389/398); 94.22% for ampicillin (n=375/398); 98.71% for amoxicillin (n=365/398); 91.21% for penicillin G (n=363/398); 89.92% for neomycin (n=355/398) and resistance in all isolates to novobiocin (n=0/398).

The results of the sensitivity test to disinfectants are shown in the Table 3. These tests showed that chlorhexidine was effective in all isolates tested in lower concentra-

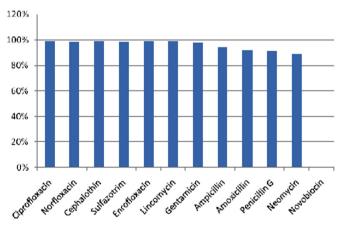


Fig.1. Profile of antimicrobial susceptibility of Corynebacterium pseudotuberculosis isolates strains obtained from caseous lymphadenitis.

tion (<0.625%). For quaternary ammonia the result was 97.20% in the concentration of 1.25%, the chlorine was effective in 87.4% in lower concentration (<0.625%), and iodine was effective in 84.4% (<0.625%) isolates.

In the test for determining the capacity of isolates for producing biofilm, it was observed that 32 samples were negative for the formation of the structure, but there was a 1322 Maria da C.A. Sá et al.

Table 3. Minimum bactericidal concentration of disinfectants commonly used in animal production against isolates of *Corynebacterium pseudotuberculosis*, isolates from sheep and goats

Disinfectant	Minimum bacterial concentration						
	Concentrations of disinfectants						
	20%	10%	5%	2.5%	1.25%	0.625%	<0.625%
Iodine	10	33	8	5	4	2	336
Chlorine	0	9	7	11	9	14	348
Quaternary ammonia	a 0	0	3	0	8	387	0
Chlorhexidine	0	0	0	0	0	0	398

weak formation of biofilm in 252 samples, moderate in 75, and strong in 39 samples.

The mean values of MDR and resistance to disinfectants in regard to biofilm production are shown in the Table 4.

Table 4. Mean values of indices of multiple resistances to antimicrobials and disinfectants according to biofilm production in isolates of *Corynebacterium pseudotuberculosis* obtained from goats and sheep

Biofilm production	MDR disinfectants	MDR antibiotics
Negative (n= 32)	0.131	0.156
Weak (n= 252)	0.061	0.160
Moderate (n= 75)	0.045	0.164
Strong (n=39)	0.041	0.163

Where: n = number of isolates; MDR = multiple resistance index, calculated according to descriptions from Krumpermam (1983).

Among the isolated strong biofilm producers, when disinfectants were added in biofilm formation, only six isolates had reduced biofilm production, which three isolates for chlorine, two isolates to chlorhexidine and one isolated for iodine, as demonstrated in Table 5.

Table 5. Interference of disinfectants on biofilm formation in isolates of *C. pseudotuberculosis* previously classified as strong biofilm producers

Biofilm	Isolates	Iodine	Chlorine	Ammonia	Chlorhexidine
		Quantity	Quantity	Quantity	Quantity
Non-producing	0	0	0	6	01
Weak	0	25	33	30	25
Moderate	0	13	3	3	11
Strong	39	01	3	0	02

In the analysis of consolidated biofilm, the interference test at 24 hours relative to average consolidated formation at 0h, found that iodine decreased biofilm formation in 13 isolates and quaternary ammonia in 11 isolates. While chlorine and chlorhexidine showed no interference with the established biofilm. The percentage of reduction of the biofilm formation is shown in Table 6, where it is observed that for iodine and quaternary ammonium a reduction in consolidated biofilm formation in 33% and 28% of the isolates, respectively.

#### **DISCUSSION**

Our results show the widespread distribution of *Coryne-bacterium pseudotuberculosis* in sheep and goats on the

Table 6. Reduction of consolidated biofilm in isolates of C. pseudotuberculosis by disinfecting agents tested

Range of reduction of	Disinfectants					
the consolidated biofilm	Iodine	Ammonia	Chlorine	Chlorhexidine		
10 to 12.99%	2	0	0	0		
8 to 9.99%	4	1	0	0		
6 to 7.99%	1	1	0	0		
4 to 5.99%	3	2	0	0		
2 to 3.99%	1	1	0	0		
0.12 to 1.99%	2	6	0	0		
Total of isolates with	13	11	0	0		
reduced biofilm						
Total of isolates tested	39	39	39	39		
% reduction of the total isolates	33%	28%	0%	0%		

farms studied. A study conducted in 2008 in the Pernambuco (PE), Brazil, showed the same incidence of the disease (Abreu et al. 2008a). Caseous lymphadenitis (CL) was found in 15.66% (437/2790) of the animals slaughtered in Petrolina/PE, and 6.31% (12/190) of animals slaughtered in Juazeiro/BA, Brazil, a cause of concern in the local economy. The results of this study are similar to those observed in the Paraiba, also located in the northeast of Brazil (Souza et al. 2011). However, the values obtained in this study were lower than those reported in Canada in 2003 (Arsenault et al. 2003). The lower rate observed in the animals slaughtered in Juazeiro/BA is due to the pre-slaughter inspection carried out, which does not allow animals with clinical disease to be slaughtered. The inspection of animals at the slaughterhouse can be an important measure for monitoring the CL situation in herds, yet this practice is not carried out in the slaughterhouse at Petrolina/PE, Brazil (Baird & Malone 2010).

Most of the abscesses were located in pre-scapular lymph nodes (54.10%). In other studies, it was observed a frequency of 15.9% of macroscopic lesions similar to CL in the state of Paraiba, Brazil, with the greatest occurrence in the pre-scapular lymph nodes (Souza et al. 2011). Results of studies in Egypt agree with our paper, where the pre-scapular and parotidos abscesses are the most common, as well as lesions in the lymph nodes of the liver (Al-Gaabary et al. 2010). This higher incidence can be explained by the fact that this type of lymph node is located closer to surface and is thus exposed to fomites, livestock facilities and vegetation, especially in the caatinga biome where cactus plants have spines, which increases the skin wounds of animals contributing to a higher prevalence of the disease (Riet-Correia et al. 2001).

Abscesses in other organs were also observed in this study, but at a lower frequency, which is in agreement with other studies (Arsenalt et al. 2003). Abscesses in the lungs were observed in 4.9% of slaughtered animals. Lesions in this organ are described in previous studies and indicate an important source of infection in animals in herds, especially when the lung injury is the only one observed in the animals (Abreu et al. 2008a). Thus, in accordance with what was observed in this work, the greater the animal density, higher is prevalence of the disease, animals reared even extensively, usually are at night together, which

allows exchange of aerosols, increasing contamination of the disease (Riet-Correia et al. 2007, Fontaine & Baird 2008).

The high frequency of CL observed in this study may be associated with handling procedures that favor the spread of the agent between animals, such as tattooing, marking and castration, using unsterilized tools. Moreover, the region in which the animals are raised has vegetation composed of cactus plants, causing injury to the skin of the animals. Along with these factors is the ability of the micro-organism to survive in the environment for several months, and the lack of technical assistance on several properties (Pinheiro et al. 2000).

The results of this study indicate a high sensitivity of isolates to antimicrobial agents, and 13 of the drugs tested showed high efficacy (89%). Similar results are described by other researchers (Muckle & Gyles 1982). None of the isolates analyzed in this study were sensitive to novobiocin, results discrepant with those described in a previous study who reported a resistance of 87.09% (Abreu et al. 2008a). The high sensitivity to other antibiotics is justifiable, since the breeders of the study area do not make regular use of antibiotics in goat and sheep raising (Abreu et al. 2008). Nevertheless, antimicrobial sensitivity in vivo is less, because this bacterium induces the formation of a fibrous capsule, which prevents contact between the drug used and the pathogen (Baird & Fontaine 2007, Washburn et al. 2009). A study held at UK and Bursa/Turkey, tested three protocols of antimicrobial therapy for caseous lymphadenitis in sheep and goats, using penicillin G and tulathromycin, but they were unsuccessful. In another study, a treatment based on rifampicin and oxytetracycline applied intramuscularly demonstrated results in reducing the size of abscesses, but without evidence of bacteriological cure (Baird 2006, Senturk & Temizel 2006).

Disinfectants are also used in the control of caseous lymphadenitis (Santiago et al. 2010). In this study, the disinfectants tested showed a sensitivity exceeding 80%, the highest activity being found for formaldehyde and chlorhexidine, effective in all isolates. A sensitivity of 84.40% was found for iodine. One can justify this lower sensitivity by the constant use of iodine in various concentrations by breeders at the time of removal of the caseous abscess. In another, surgical removal and antimicrobial therapy alone were not effective for long-term eradication of CL. The use of serologic testing and disposal of animals may provide better results (Baird & Malone 2010).

Most isolates (71.35%) were negative or weak for the production of biofilms. This fact may be associated with the high sensitivity to antimicrobials and disinfectants observed. Comparing the MDR average obtained for these isolates, it may be seen that the index was lower for antimicrobials and greater for disinfectants, indicating a possible association between no/weak biofilm production and low resistance to antimicrobial drugs. Biofilm-producing bacteria show increased synthesis of exopolysaccharides, which contributes to resistance to antibiotics (Costerton et al. 1999). A study in Calgary, Alberta, Canada, tested the

sensitivity of *C. pseudotuberculosis* to seven antimicrobial agents, including tetracycline and ampicillin, and found high resistance when the bacteria produced biofilm (Olson et al. 2002).

In tests of interference of the disinfectants on biofilm formation can observe a reduction in the formation of this structure by all the active principles evaluated. This decrease adhesion can be justified by the increase of secondary metabolites, which can reduce biofilm formation causing death bacteriana (Freitas et al. 2010). Isolated studies of the constituents of disinfectants are needed to better explain this interaction, because several reactions can happen to promote the reduction of sensitivity of bacteria in biofilm to disinfectants, as well as the chemical interaction between the disinfectant and the biofilm itself, modulating the microenvironment, production of enzymes, among other reations (Augustini et al. 2004).

In analyzes with consolidated biofilm, disinfectants did not obtain the same efficiency; however iodine reduced biofilm formation in 33% of the isolates and quaternary ammonia prevented 28% of the isolates to form biofilm. For chlorine and chlorhexidine, there was not change in the adherence of the consolidated biofilm. This variation can be explained by the inhibition mechanism of action of each individual sample, although this mechanism requires further studies to elucidate the factors that influence the growth and development of biofilm, particularly the interactions with the material extracelular (Costerton et al. 1999). A study using chlorhexidine to reduce biofilm on Streptococcus mutans, observed that consolidation of the biofilm decreased in 21.38% of the isolates, result different from that obtained in the present study, since the chlorhexidine did not inhibit any isolated with consolidated biofilm (Liu et al. 2012).

In the present study, disinfectants were more effective when the biofilm was in formation, decreasing effectiveness when biofilm was already consolidated. This can be explained by the lower penetration of these drugs when the matrix of the biofilm is already consolidated. Studies suggest that resistance caused by biofilm formation is explained by the presence of polysaccharides that coat the biofilm and protect the micro-organism from external agents and, their stability depends on its concentration in the biofilm (Cluterbuck et al. 2007, Smith 2005).

### **CONCLUSIONS**

The high incidence of lesions characteristic of caseous lymphadenitis (CL) and the isolation of *Corynebacterium pseudotuberculosis* from sheep and goats on farms and in slaughterhouses of Petrolina/PE and Juazeiro/BA, Brazil, should be noted.

This study highlights the widespread occurrence of lesions characteristic of CL and isolation of *C. pseudotuberculosis* from sheep and goats on farms and in slaughterhouses from Petrolina/PE and Juazeiro/BA.

High sensitivity of isolates to antimicrobial agents and disinfectants was also observed, which may be related to low production of biofilm by the *C. pseudotuberculosis strains* evaluated.

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#### REFERENCES

- Abreu S.R.O., Mota R.A., Pinheiro Júnior J.W., Rosinha G.M.S. & Castro R.S. 2008a. Perfil de sensibilidade antimicrobiana *in vitro* de isolados de *Corynebacterium pseudotuberculosis* de caprinos e ovinos com linfadenite caseosa no sertão de Pernambuco, Brasil. Vet. Zootec. 15:3:502-509.
- Abreu S.R.O., Mota R.A., Rosinha G.M.S., Forner O., Pinheiro Júnior J.W., Pereira R.B.R., Castro R.S., Elisei C., Soares C.O., Araújo F.R. & Madureira R.C. 2008. Comparação genotípica de isolados de Corynebacterium pseudotuberculosis de caprinos e ovinos do sertão de Pernambuco. Pesq. Vet. Bras. 28:10:481-487.
- Alencar S.P., Mota R.A., Coelho M.C.O.C., Nascimento S.A., Abreu S.R.O. & Castro R.S. 2010. Perfil sanitário dos rebanhos caprinos e ovinos no sertão de Pernambuco. Ciênc. Anim. Bras. 11(1):131-140.
- Al-Gaabary M.H., Osman S.A., Ahmed, M.S. & Oreiby A.F. 2010. Abattoir survey on caseous lymphadenitis in sheep and goats in Tanta, Egypt. Small Rumin. Res. 94:117-124.
- Arsenault J., Girard C., Dubreuil P., Daignaultb D., Galarneau. J.R., Boisclair J., Simard C. & Bélanger D. 2003. Prevalence of and carcass condemnation from maedi-visna, paratuberculosis and caseous lymphadenitis in culled sheep from Quebec, Canada. Prev. Vet. Med. 59:67-81.
- Augustini M., Ali-Vehmas T. & Atroshi F. 2004. Assessment of enzymatic cleaning agents against bacterial biofilms. J. Pharmaceut. Sci. 7:55-64.
- Baird G. 2006. Treatment of ovine caseous lymphadenitis. Vet. Rec. 159:500.
- Baird G.J. & Malone F.E. 2010. Control of caseous lymphadenitis in six sheep flocks using clinical examination and regular ELISA testing. Vet Rec. 166:358-362.
- Bauer A.W., Kirby W.M.M., Sherris J.C. & Turck M. 1966. Antibiotic susceptibility testins by a standardized single disk method. Am J. Clin Pathol. 45:493-496.
- Carter G.R. 1990. Diagnostic procedures in veterinary bacteriology and mycology. 5th ed. Academic Press Inc., California. 620p.
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; Seventeenth Informational Supplement. CLSI document M100-S17 [ISBN 1-56238-625-5]. 2007. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA. 177p.
- Costerton J.W., Stewart P.S. & Greenberg E.P. 1999. Bacterial Biofilms: a common cause of persistent infections. Science 284:1318-1322.
- Fontaine M.C. & Baird G.J. 2008. Caseous lymphadenitis. Small. Rumin. Res. 76:42-48.
- Freitas V.R., Van der Sand S.T. & Simonetti A.B. 2010. Formação *in vitro* de biofilme por *Pseudomonas aeruginosa* e *Staphylococcus aureus* na superfície de canetas odontológicas de alta rotação. Rev. Odontol. Unesp 39.4.193-200
- Gomes D.L.R., Martins C.A.S., Faria L.M.D., Santos L.S., Santos C.S., Sabbadini P.S., Souza M.C., Alves G.B., Rosa A.C.P., Nagao P.E., Pereira G.A., Hirata R. & Mattos-Guaraldi A.L. 2009. *Corynebacterium diphtheriae* as an emerging pathogen in nephrostomy catheter-related infection: evaluation of traits associated with bacterial virulence. J. Med. Microbiol. 58:1419-1427.

- Holt J.G., Krieg N.R., Sneath P.H.A. & Staley J.T. 1994. Irregular, nonsporing Gram-positive rods. In: Holt J.G., Krieg N.R., Sneath P.H.A., Staley J.T. (Eds), Bergeys's Manual of Determinative Bacteriology. Williams and Wilkins, Baltimore. 593p.
- Kruperman P.H. 1983. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high-risk sources of fecal contamination of foods. Appl. Environ. Microbiol. 46:165-170.
- Liu J., Ling J., Zhang K., Huo L. & Ning Y. 2012. Effect of sodium fluoride, ampicilin, and chlorhexidine on Streptococcus mutans biofilm detachment. Antimicrob. Agents Chemother. 56:4532-4535.
- Merino N., Tolena-Arana A., Vergara-Irigaray M., Valle J., Solano C., Calvo E., Lopez J.A., Foster T.J., Penadés J.R. & Lasa I. 2009. Proteina A- mediated multicellular behavior in *Staphylococcus aureus*. J. Bacteriol. 191:832-843.
- Muckle C.A. & Gyles C.L. 1982. Characterization of strains of *Corynebacterium pseudotuberculosis*. Can. J. Comp. Med. 46:206-208.
- Nostro A., Roccaro A.S., Bisignano G., Marino A., Cannatelli M.A., Pizzimentti F.C., Cioni P.L., Procopio F. & Blanco A.R. 2007. Effects of oregano, carvacrol and thymol on *Staphylococcus aureus* and *Staphylococcus epidermidis* biofilms. J. Med. Microbiol. 56:519-523.
- Olson M.E., Ceri H., Morck D.W., Buret A.G. & Read R.R. 2002. Biofilm bacteria: formation and comparative susceptibility to antibiotics. Can. J. Vet. Res. 66:86-92.
- Pelczar Jr M.J., Chan E.C.S. & Krieg N.R. 2007. Microbiologia: Conceitos e Aplicações. 2ª ed. Macron, São Paulo. 77p.
- Pinheiro R.R., Gouveia A.M.G., Alves F.S.F. & Haddad J.P.A. 2000. Aspectos epidemiológicos da caprinocultura cearense. Arq. Bras. Med. Vet. Zootec. 52:534-543.
- Quinn P.J., Carter M.E., Markey B. & Carter G.R. 1994. Clinical Veterinary Microbiology. Wolfe, London. 648p.
- Ribeiro S.D.A. 1997. Caprinocultura: Criação Racional de Caprinos. Nobelm, São Paulo. 318 p.
- Riet-Correia F., Schild A.L., Mendez M.D.C. & Lemos R.A.A. 2001. Doenças de Ruminantes e Equinos. Vol.1. Livraria Varela, São Paulo. 426p.
- Riet-Correia F., Schild A.L., Mendez M.D.C., Lemos R.A.A. & Borges J.R. 2007. Doenças de Ruminantes e Equídeos, 3ª ed. Palloti, Santa Maria, 694p.
- Santiago L.B., Alves F.S.F., Pinheiro R.R., Santos V.W.S., Rodrigues A.S., Chapaval L., Brito I.F. & Sousa F.G.C. 2010. Avaliação in vitro da sensibilidade da *Corynebacterium pseudotuberculosis* frente a diferentes tipos de antissépticos e desinfetantes e determinação de sua curva de crescimento. Arq. Inst. Biológico, São Paulo, 77:593-600.
- Senturk S. & Temizel M. 2006. Clinical efficacy of rifamycin SV combined with oxytetracline in the treatment of caseous lymphadenitis in sheep. Vet. Rec. 159:216-217.
- Smith A.W. 2005. Biofilms and antibiotic therapy: is there a role for combating bacterial resistance by the use of novel drug delivery systems? Adv. Drug Delivery Rev. 57:1539-1550.
- Souza M.F., Carvalho A.Q., Garino Júnior F. & Riet-Correa F. 2011. Linfadenite caseosa em ovinos deslanados abatidos em um frigorífico da Paraíba. Pesq Vet Bras. 31:224-230.
- Stepanović S., Vuković D., Dakić I., Savić B. & Svabić-Vlahovic M. 2000. A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J. Microbiol. Methods 40:175-179.
- Washburn K.E., Bisset W.T., Fajt V.R., Libal M.C., Fosgate G.T., Miga J.A. & Rockey K.M. 2009. Comparison of three treatment regimens for sheep and goats with caseous lymphadenitis. J. Am. Vet. Assoc. 234:9:1162-1166.