https://dx.doi.org/10.22161/ijfaf.1.4.3 *ISSN: 2456-8791*

Cow's Mastitis produced by Unicellular Algae of the Genus *Prototheca*, an Emerging Disease: Treatment Evaluations (Literature Review)

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Abstract—*Mastitis induced by Prototheca have become emerging diseases over the last 10-15 years, evolving endemically in some farms, and leading to important economic losses.The diseased cows eliminate the algae by milk, with the risk of transmission to humans, which is why protothecosis is part of the zoonosis group.Many antibiotics and antifungals have been tried in the treatment of mastitis, but without result, even though the sensitivity of isolated strains was detected in vitro.In the present study we present the antibiotics and antifungals used by various authors in the treatment of mastitis with Prototheca spp, as well as the results obtained.Among antibiotics, good and consistent efficacy was obtained with: gentamicin, neomycin, kanamycin, colistin. In antifungals, good results include: amphotericin B, miconazole, mycostatin, ketoconazole, econazole, itraconazole, batrafen and clotrimazole.It has been concluded that mastitis produced by Prototheca species (especially P. zopfii) are incurable, and the slaughter of sick animals is recommended.In order to avoid spreading within the herd, monitoring will be carried out by applying a screening program and avoiding contaminated sources from the environment.*

Keywords—Mastitis, protothecosis, therapy, emerging disease.

I. INTRODUCTION

Unicellular green algae (*Chlorococcales*) are described as lower plants, which contain chlorophyll and a cellulosic cell wall. Exceptions to this rule are algae of *Prototheca* genus, which have lost their ability to synthesize chlorophyll, becoming heterotrophs (Calley et Lloyd, 1964); (Lagneau, 1996). As a consequence, prototeca are considered to be unicellular, saprophytic, chlorophyll-less, mutant algae of the *Chlorella* genus. The first description is made by Krüger who isolated them from the bark of the trees (Krüger, 1894). *Prototheca* is distinguished from bacteria and fungi by: size, shape, reproduction (formation of endospores), and the fact that the cell wall does not contain glucosamine and muramic acid (Milanov et Suvajdzic[a], 2006).

Taxonomic framework of the *Prototheca*: Domain: *Eukaryota*, Kingdom: *Viridiplantae*, Phylum: *Chlorophyta*, Class: *Trebouxiophyceae*, Order: *Chlorellales*, Family: *Chlorellaceae*, Genus:*Prototheca*. Although 12 species have been described over time, according to current taxonomic data, only the following are now recognized: *P. zopfii*, *P. wickerhamii*, *P. stagnora*, *P. ulmea*, *P. moriformis* and *P. blaschkeae*(Lass-Flürl etMayr, 2007); (Marques Sara et al., 2008) but recently, other two species were also included:*P. cutis* (Satoh et al., 2010) și *P. miyajii* (Masuda et al., 2016).

P. zopfii is more frequently involved in animal diseases and *P. wickerhamii* in human disease, without a clear differentiation from this point of view.The first case of mastitis in cows was reported in 1952 in Germany (Lerche, 1952). Subsequently, the disease was described in many other farms in Europe, Asia, America, Australia and Africa.

II. EPIDEMIOLOGY

Prototheca are ubiquitous in nature, being isolated from a wide variety of habitats, such as vegetation, river water, and even marine water (Tyler et al., 1980); (Gonzalez, 1996); (Thompson et al., 2009). Almost all authors report their isolation from mud deposited on tree bark, wet soil around trees, vegetation, floodplains, acidic water, sewage, clogged ponds(Pore et al., 1983);(Huerre et al., 1993).Isolation has also been reported from green fodder, shelters, drains, milk, milking machines, bulk milk tanks, shelter floors, barns(Gonzalez, 1996); (Moubamba[a] et al., 1997).*Prototheca* can be found in the faecesof adult cattle (Enders et Weber, 1993), but also in the faeces of other animal species (horses, pigs, rats).Various sources mention ahigh frequency isolation of *P. zopfii* (94%), and more rarely of *P. wickerhamii* (6%) (Anderson et Walker, 1988).Moisture (even the presence of wetlands) is a factor

https://dx.doi.org/10.22161/ijfaf.1.4.3 *ISSN: 2456-8791*

that favors the proliferation of algae (Pore et al., 1983); (Huerre et al., 1993); (Moubamba[a], 1997). Contamination of different aquatic systems takes place through faeces(Huerre et al., 1993). It is also mentioned the *Prototheca*'s ability to form a biofilm (correlated with a certain temperature), which makes it difficult to eliminate them by sanitation procedures (Gonçalves et al., 2015).

Unicellular algae of the genus *Prototheca*, although considered to be weak virulent, have the ability to cause disease in different species of animals and humans.Among domestic animals, they are more commonly mentioned in cows, in the form of mastitis, sometimes as endemic disease (Costa[c] et al., 1997); (Milanov[b] et al., 2006) andsporadic cases in dogs (Buyukmihci et al., 1975); (Imes et al., 1977); (Cook et al., 1984); (Gaunt et al., 1984); (Blogg et Sykes, 1995); (Salvadori et al., 2008)and cats (Kaplan, 1976); (Coloe et Allison, 1982); (Dillberger et al., 1988). Among wild animals, cases are reported in: bat (Mettler, 1975), snake (Crispens et Marion, 1975), salmon (*Atlantic salmon*) (Gentles et Bond, 1977), carp(Loupal et al., 1992); (Jagielski[a] et al., 2017), and in some other animal species (beaver, rabbit, ferret, hamster, rat, mouse)(Spalton, 1985).

Generally, illnesses occur in weakened organisms, with different immunodeficiency's, chronic diseases, or antibiotic abusive treatments.In the case of cows, repeated intramammary infusions with antibiotics and the uncontrolled administration of other drugs in the mammary gland is incriminated(Corbellini et al., 2001); (Pieper et al., 2012). In humans, illnesses are reported, especially in individuals with different forms of immunosuppression(Kaminski et al., 1992); (Woolorich et al., 1994); (Hariprasad et al., 2005); (Lass-Flörl et Mayr, 2007); (Takano et al., 2014). It often causes bursitis (olecranon), but also some different localizations: cutaneous (Satoh et al., 2010), ocular (Hariprasad et al., 2005), intestinal (Meinke et al., 2017), meningeal (Kaminski et al., 1992), systemic infections (Masuda et al., 2016).

Prototheca species (especially *P. zopfii*) seem to be common in the environment around dairy farms, with or without a history of mastitis.Among the extrinsic factors, humidity and the presence of organic matter are considered to be important factors that sustain the existence of the *Prototheca*, favoring the appearance of mastitis (Anderson et Walker,1988); (Schlenstedt et al., 1997). In this context it is shown that in some cow's farms, mainly in the last 10 years, mastitis with *Prototheca* became an issue of emerging pathology, leading to important economic losses by decreasing milk production, compromising the mammary gland and financial expenses with applied therapy(Camboim et al., 2010); (Langoni et al., 2013); (Milanov et al., 2016); [116].The elimination of *Prototheca* through milk can reach high values, which can go up to 10^3/ml (McDonald[a] et al., 1984), posing a risk to public health(Costa[a] et al., 2006); (Milanov et al., 2016); (Alves et al., 2017). In this context, protothecosis is considered a potential zoonotic disease, associated with bovine mastitis, especially as some strains show resistance to pasteurization (Melville et al., 1999); (Bozzo et al., 2014). It is noted that mastitis with *Prototheca* spp. has become a serious global problem, being currently underestimated both as incidence and severity(Lagnoni, 2013); (Sarale et al., 2013).

In the production of mastitis in cows, *P. zopfii* occurs more frequently(Hodges et al., 1985); (Spalton, 1985); (Pore et al., 1987); (Anderson et Walker, 1988); (Higgins et Larouche, 1989); (Furuoka et al., 1989); (Almeraya, 1994); (Langoni et al., 1995); (Lagneau, 1996); (Aalbaek et al., 1998); (Jensen et al., 1998); (Filippsen et al., 1999); (Jánosi et al., 2001); (Corbellini et al., 2001); (Ahrhold et al., 2012); (Sobukawa et al., 2012)and, more rarely, other species are involved: *P. wickerhamii*, *P. blaschkeae*(Marques et al., 2008); (Thompson et al., 2009); (Ahrholdt et al., 2012); (Ricchi et al., 2013). Within the species *P. zopfii*, three biotypes were described, which were reclassified into 2 genotypes (1 and 2) and a new species renamed as *P. blaschkeae*(Roesler et al., 2006). Genotype 2 is thought to be pathogenic, has greater adhesion capacity to mammary epithelium and is considered to be the main cause of mastitis in cows(Moller et al., 2007); (Pieper et al., 2012); (Ahrholdt et al., 2012); (Shahid[a] et al., 2017).Some data mention the isolation from milk samples taken from the same farm, both *P. zopfii* genotype 2 (predominantly) and genotype 1 (uncommon) and *P. blaschkeae*(Bozzo et al., 2014). The ability of *P. zopfii* to produce mastitis in cow was also demonstrated by experimental infections(Dion, 1982); (McDonald[a] et al., 1984); (Bergmann[b], 1993).

Epidemiological study of clinical mastitis in dairy cattle reveal the endemic character of these infections in the case of favoring factors, recalling in particular: repeated intramammary infusions, antibiotic pretreatment, antibiotic abusive treatments and the action of various immunosuppressive factors (often difficult to identify), associated with inadequate hygiene conditions, both at farm and milking levels. All these factors increase the risk of mastitis with *Prototheca*(Tenhagen et al., 1999);(Corbellini et al., 2001); (Pieper et al., 2012).The incidence of infection in dairy farms is variable, with values ranging from 4-47% (Anderson et Walker, 1988); (Costa[b] et al., 1996); (Kirk et Mellenberger, 2011). The isolation of *P. zopfii* is reported at higher values, from lactating cows, and lower from dry cows (Costa[a] et al.,

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1996); (Kirk et Mellenberger, 2011). Some data indicate that mastitis caused by *Prototheca* have a peak in the 2nd lactation month (Tenhagen et al., 1999). Cows with and without mastitis can shelter and eliminate *Prototheca* species in a permanent or intermittent manner (Gonzalez, 1996). Algae can persist in the mammary gland for 45 to 100 days, or even more, and as a consequence they pose a real danger to other cows in the flock.Algal persistence is mentioned for up to 13 months in the dry period(Spalton, 1985)and serum antibodies are absent 6 months after lactation but will reappear following parturition(Dion, 1982).

III. PATHOGENESIS

Prototheca is considered as germ with moderate pathogenicity and virulence (Huerre et al., 1993); (Răpuntean et al., 2004).However, once they enter the body, they produce local granulomatous lesions, but they can spread lymphatically, causing a systemic or even generalized infection, with fatal evolution.Such evolutionary forms have been described in cows (Taniyama et al., 1994),dogs (Tyler et al., 1980); (Gaunt et al., 1984); (Imes et al., 1977); (Souza et al., 2009), and in humans after immunosuppressive therapy (Wolfe, 1976) or immunocompromised hosts (Wirth et al., 1999).In the case of cows, penetration into the mammary gland occurs at the level of the streak canal, frequently after milking, when the teat meatus remains partially open and the nipples come into contact with a heavily contaminated environment (Costa[a] et al., 2006); (Gonzalez, 1996); (Langoni et al., 3013). Spreading during milking is not significant, but may be important for poor milking hygiene (Kirk et Mellenberger, 2011).

Protothecahas a tendency to invade the connective tissue between the nipple and the abdominal region, as well as the lymph nodes. It is noted that, often,the algae remain restrictedin the mammary gland and regional lymph nodes, causing inflammatory granulomas (Jensen et al., 1998). The microorganisms are phagocytosed, by macrophages and neutrophils, where they can proliferate, resisting the host's defense mechanisms, generating an infection that cannot be defeated by therapy.Mastitis caused by *Prototheca* differs from those produced by other microorganisms by the fact that the inflammatory reaction is poorly expressed or does not occur at all.There are cases where neither congestion nor edema occurs, and the decrease in milk production is due to the destruction of the secretory tissue (Moubamba[b], 1997).At the mammary tissue level, *P. zopfii* genotype 2 induces oxidative stress, ultrastructural changes associated with apoptosis of bovine epithelial cells (Shahid et al., 2017[a]); (Shahid et al., 2017[b]).

IV. CLINICAL AND PATHOLOGICAL ASPECTS

Mastitis with *Prototheca* may develop as clinical and subclinical forms (Langoni et al., 1995); (Schlenstedt et al., 1997). Most often, it evolves chronic, subclinical, with decreased milk production.Milk secretion becomes watery, sometimes with the presence of gray flocks (Wawron[a] et al., 2013). The mastitis may also develop acutely (frequently after parturition), comprising one or more quarters, with severe hardness, accompanied by fever, inappetence, drastic reduction of milk production, also affecting retro-mammary lymph nodes, which are hypertrophic (Furuoka et al., 1989); (Schelenstedt et al., 1997);(Spalton, 1985); (Wawron[a] et al., 2013). In the majority of cases, infected cows stays undetected, until the affected quarter is almost dry[115].Many infected cows have only a decrease in milk production, in which, along with *Prototheca* cells, there is a high number of somatic cells(Tenhagen et al., 1999); (Kirk et Mallenberger, 2011);[116].

Anatomo-pathologically, in the affected quarters there are milliar nodules (0.5-3 mm), yellow-cream-colored papules (Wawron[a] et al., 2013), granulomas, regional lymphangitis, thrombosis and hemorrhages, even massive necrotic lesions (Taniyama et al., 1994). Histopathological, fibrosis lesions and granulomas, are characterized by numerous algae at various stages of development, infiltrations with numerous macrophages, lymphocytes, plasmocites and rare neutrophils, in the alveoli and interstitial spaces(Corbellini et al., 2001); (Milanov[b] et al., 2006); (Wawron[a] et al., 2013), rare epithelial cells and even giant cells (Hodges et al., 1985).

V. DATA ON THERAPY

Diseases caused by unicellular algae of the genus *Prototheca* generally respond poorly or not at all to treatments. Numerous antibiotics and antifungals were used in the treatment, but the results were often contradictory, depending on how the disease evolved (localized or systemic infection), the species of affected animal and the age of the disease.*Protothecai*s considered resistant to most antimicrobials (Baumgartner, 1997).

In the case of *Prototheca* mastitis in cows, although many therapeutic trials have been made, the results were, in most cases, unsatisfactory.Although treatments have often been established based on the antibiogram results, which demonstrate *in vitro* susceptibility of *Prototheca*, this has not been correlated with *in vivo* efficacy.For this reason, many authors consider that the treatment of mastitis caused by *Prototheca* is difficult, inefficient and totally non-economic (Almeraya, 1994); (Moubamba[b], 1997); (Kirk, 1999).

https://dx.doi.org/10.22161/ijfaf.1.4.3 *ISSN: 2456-8791*

Through numerous *in vitro* research, these algae have been shown to be sensitive to some antibiotics, such as ”polyene and aminoglycoside”, as well as some ”azole” antifungals.The percentage of susceptible strains is higher for antifungals, which is why they were more commonly used in therapeutic trials.

Sensitivity was recorded more frequently for aminoglycosides (gentamicin, neomycin, kanamycin, spectinomycin), but various authors report variability in the percentage of susceptible strains.McDonald[b] et al., (1984) by testing 48 strains of *Prototheca* spp., reports the following results (in mm) on susceptibility of the examined strains: 18 to gentamicin, 21 to polymyxin B, 22 to amphotericin B;at 21 other antibiotics resistance was observed; all strains have been shown to be susceptible to myxin and nystatin. Bodenhoff et Madsen (1978) by testing a strain of *P. zopfii* isolated from mastitis cows reports that it showed moderate sensitivity to streptomycin, polymyxin gentamicin, and resistance to other antibiotics or antifungals currently used in clinical therapy. Shahan et Pore (1991), by testing 100 *Prototheca* strains of different species and different isolation sources, found susceptibility to gentamicin at concentrations ranging from 0,3-0,9 µg/ml. Lagneau (1996) was examining the susceptibility of *P. zopfii* strains isolated from mastitis cows versus 14 antibiotics (micro-tablets, agar gel diffusion technique) and 7 antifungals (on the casein medium) mentions that they were sensitive to gentamicin - 150 µg/disc (20 mm), polymyxin sulfate - 150 µg/disc (25 mm), but resistant to all 12 other antibiotics; of the antifungals, sensitivity was observed for amphotericin B - 10 µg/disc (25 mm) and nystatin - 50 µg/disc (24 mm).The antibiotics and antifungals tested were: streptomycin, tetracycline, cefuroxime, penicillin, erythromycin, gentamycin, polymyxin B sulfate, ampicillin, cefoperazone, novobiocin, cephalosporin, cloxacillin, lincomycin, trimethoprim (antibiotics); and amphotericin B, nistatin, natamycin, ketoconazole, 5-florocytosine, miconazole and isoconazole (antifungals).A strain of *P. zopfii* isolated from mastitis cows was found to be sensitive to natamycin and nystatin but they only have local action (Moubamba[b] et al., 1997). *In vitro* resistance to various antimicrobial agents of *Prototheca* isolates, from mastitis cases, has been reported by other authors(Costa[b] et al., 1996); (Lagneau, 1996); (Filippsen et al., 1999); (Wawron[b] et al., 2013).

Among the antifungal agents, *in vitro* inhibitory action, and some more or less satisfactory results in clinical cases, have been demonstrated to amphotericin B (0.15 µg/ml) on both human and animal isolates.It is a polyclonal macrolide antibiotic, recommended in invasive fungal infections, with diverse etiology, with fungistatic or fungicidal effect.*In vitro* testing by broth dilution of 5 *Prototheca* spp. Strains, against amphotericin B or a combination with flucytosine (5-FC) and rifampicin revealed that amphotericin B combined with rifampicin produced the best effect at the most decreased CMI, and flucytosine combined with amphotericin B demonstrated the lowest effect (Srimuang et al., 2000).

Following the exposure of the *P. zopfii* and *P. wickerhamii* to sub-inhibitory concentrations of amphotericin B, it was found that ultrastructural changes of the treated cells occurred, including mitochondrial swelling, cell organ degradation, accumulation of lipid droplets and starch granules in the cytoplasm, as well as charges of the inner layer of the cell walls(Segal et Socher, 1981). The study on the correlation of the lipid composition in *P. wickerhamii* (16529 ATCC) and sensitivity to some antimicrobial agents: polyenes, polymyxins and imidazoles, showed that the presence of ergosterol in the neutral lipid fraction of the membrane is probably responsible for increased susceptibility to amphotericin B; and the presence of a large amount of free fatty acids in the membranes, creates susceptibility to imidazoles. The membrane determinants of susceptibility to polymyxin B were less defined (Sud etFeingole,1979).*P. wickerhamii* and *P. zofii* strains were resistant to fluconazole and itraconazole, demonstrated by E-test and cultivation on the casitone yeast extract agar(Blaschke-Hellmessen, 1996). Treatment with nistatin has also been shown to be ineffective, although the *Prototheca* has been shown to be susceptible *in vitro* (Goudswaard, 1977).

Casal et Gutierrez[a](1981) have investigated, *in vitro*, the action of several antibiotics against *P. wickerhamii* strains, and found inhibitory effect on amikacin, colistin, dibekacin, framichetin, gentamicin, kanamycin, lividomycin, neomycin, polymyxin, paromomycin, ribostamycin, sisomicin and tobramycin, being recommended for treating protothecosis in humans with *P. wickerhamii* infection.Casal et Gutierrez[c](1983) by examining the *in vitro* susceptibility of *Prototheca* strains to ribostamycin, specified that concentrations of 4 mcg/ml were required to inhibit 100% of *P. zopfii* strains; 16 µg/ml to inhibit 100% of *P. stagnora* strains and 95% of *P. wickerhamii* strains.The determined values were inferior to plasma ones, obtained after the ribostamycin injection, and this antibiotic was recommended for the treatment of human protothecosis in case of*P. wickerhamii* infection.The same authors have found that susceptibility to clotrimazole (50 µg/disc) allows the differentiation of *P. zopfii* (which is resistant) from *P. wickerhamii* (which is susceptible).Casal et Gutierrez[e](1995), recommends the testing of the susceptibility to neomycin (30 µg /disc) which allows the differentiation of *P. wickerhamii* and *P. zopfii* from *P.*

https://dx.doi.org/10.22161/ijfaf.1.4.3 *ISSN: 2456-8791*

stagnora.On the other hand, the sensitivity to ribostamycin (60 mcg/disc) is a criterion for differentiation between *Candida* (resistant) and *Prototheca* (inhibited) (Casal et Gutierrez[d], 1986).Costa[b] et al.,(1996) as a result of the treatments carried out in cows with subclinical mastitis caused by *Prototheca* spp, conclude that these algae do not respond to antimicrobial treatment, requiring the elimination of the affected animals as the best way of controlling the disease.Also Lagneau (1996),states that the treatment of protothecosis is a real problem caused by the lack of response to common anti-mastitis medications and that treated cows are not recovered.Linares et al., (1998) following the examination of 11 *Prototheca* strains by E-test, against 5 antifungal agents (flucytosine, ketoconazole, itraconazole, fluconazole and amphotericin B), showed only susceptibility to amphotericin B.It is stated that the E test is easy to perform and provides MIC values similar to those obtained using other techniques.Aalbaek et al., (1998) by testing a number of 16 strains of *P. zopfii* isolated from cows with mastitis, showed that they all proved to be resistant to clotrimazole.

Răpuntean Gh.,et al.,(2004) have tested the *in vitro* susceptibility of 16 strains of *Prototheca zopfii*, isolated from cattle mastitis (agar gel diffusion technique) against 26 antibiotics and 10 antifungals.Of the antibiotics, full sensitivity (100%) wasrecorded at: lincocin-forte (16-32 mm), neomycin (24-30 mm), gentamicin (14-20 mm), kanamycin (14-30 mm) and colistin (22-28 mm).All strains were resistant to lincomycin-spectinomycin, tylosin, novobiocin, spiramycin, penicillin, ampicillin, tetracycline, clindamycin, ofloxacin, ciprofloxacin, cephalothin, ceftazidime, amoxicillin, erythromycin, flumequin, trimethoprim, and chloramphenicol.Of the antifungals, total sensitivity (100%) was recorded to mycostatin (16-22 mm), econazole (18-24 mm), batrafen (cyclopiroxolamine) (20-30 mm), amphotericin B (10-12 mm).In some antifungals, resistance was also recorded: 2 strains showed resistance to ketoconazole and clotrimazole, and to itraconazole 3 strains showed resistance.Linares et al., (2005),following testing of 104 strains of *P. wickerhamii* and 2 control strains, all strains were shown to be susceptible to voriconazole, the inhibition being produced at $\leq$ 0.5 µg/ml, indicating that this substance may be an option for treating infections with *Prototheca* spp.Marques et al., (2006) mentions the susceptibility of *P. zopfii* and *P. wickerhamii* strains isolated from mastitis cows to amphotericin B and nystatin, the latter being more active.Milanov[b] et al., (2006) have pointed out that *Prototheca* isolates from mastitis cows were susceptible *in vitro* to nystatin and amphotericin B, intermittently sensitive to polymyxin B, gentamicin and neomycin and resistant to kanamycin, enrofloxacin, ceftriaxsone, streptomycin, amoxicillin, tetracycline, penicillin, lincomycin and novobiocin. Lassa et Malinowski (2007) by testing the *in vitro* susceptibility of 168 strains of algae isolated from cow's mastitis versus 10 antifungals and 10 antibiotics (disc diffusion method), showed only sensitivity to aminoglycosides and gentamicin (75.5%), kanamycin (71.7%) and neomycin (30.6%), and from antifungal only to nistatin, amphotericin B and pimaricin (natamycin).Buzzini et al., (2008) conducted a screening on the sensitivity of 105 strains of *P. zopfii* isolated from different locations of dairy cows farms versus a panel of polyene antibiotics, find a good *in vitro* efficacy of amphotericin B and pimaricin, followed by nystatin and filipin, mentioning that two strains were resistant to all 4 tested polyenes.Bouari et al., (2011) finds, by the E-test, an *in vitro* inhibitory effect on ketoconazole and amphotericin B against *P. zopfii* strains and one*P. wickerhamii* reference strain. Sobukawa et al., (2012) shows by E-test that *P. zopfii* genotype 1 strains have been shown to be more susceptible to amphotericin, gentamicin and kanamycin than the genotype 2 and resistant to itraconazole. Wawron[b] et al., (2013) by examining 27 strains of *P. zopfii* isolated from cow's milk, mentions the 100% resistance of strains to several antifungals and antibiotics: clotrimazole, fluconazole, econazole, flucytosine, cefoperazone, cephalexin, enrofloxacin, lincomycin, oxytetracycline and 92.6% to miconazole; nistatin, ketoconazole and amphotericin B shows the best inhibitory activity; sensitivity was also recorded to gentamicin (96.3%), kanamycin (59.3%) and polymyxin (59.3%).

In addition to antibiotics and antifungals, the sensitivity of *Prototheca* to other chemical substances was also tested.Bodenhoff et Madsen (1978) have tried to treat *Prototheca* mastitis, with ethidium bromide, that was found to be active *in vitro*, but unsuccessfully *in vivo*. It is shown that the number of excreted algae decreases, but complete healing is not obtained. In the histological sections made from the udder, after the slaughter of the animal, microorganisms were present both intracellularly and extracellularly.Bergmann[a] (1993)reports the use of tetramisole and levamisole hydrochloride by intramammary administration in mastitis cows produced by *P. zopfii* using 40 ml/quarter (4 mg/kg mcg) in 6 administrations simultaneously with milking. Algae elimination is suppressed for at least 6 hours. Administration of 200 ml/quarter, results in suppression of elimination for 36 hours. The same author mentions that oral administration of nilverm (overdose) does not lead to the suppression of the elimination of microorganisms through milk.

https://dx.doi.org/10.22161/ijfaf.1.4.3 *ISSN: 2456-8791*

Bergmann[b](1993), through experimental infections, showed that the administration of tetramisole hydrochloride (4 mg/kg mc) resulted in symptom relief within 3-24 hours after first application and a significant reduction of milk algae elimination. The greatest reduction occurs after the administration of levamisole hydrochloride.Melville et al., 2002,reports the susceptibility of *P. zopfii* strains to 0.01% chlorexidine (chlorhexidine), 0.1% copper sulfate and 0.3% silver nitrate.Lee et al., (2004) mentions the *in vitro* susceptibility of *Prototheca* strains isolated from mastitis milk to lactoferrin(a multifunctional protein) that completely inhibited this microorganism, even at a low concentration of 7 mug/ml.Krukowski et al., (2013),notes the sensitivity of *P. zopfii* strains to chlorhexidine and iodine.

Răpuntean S., et al., (2015), following the *in vitro* susceptibility testing (by diffusion method), of 22 strains of *P. zopfii* (isolated from cows with mastitis) and one strain of *P. wickerhamii* (ATCC 16529), showed a good inhibitory activity of the products with iodine (iodine tincture, betadine, videne). The inhibition zones had variable sizes in correlation with the composition of the respective products and the iodine release capacity."For the22*P. zopfii* tested strains, the size of inhibition areas was within the following values (average diameter): iodine tincture 25.71 mm, betadine 26.4 mm, videne 25.61 mm, Lugol's solution 11.33 mm and potassium iodide 11.14 mm.For *P. wickerhamii* strain inhibitionareas had thefollowing values: betadine 26 mm, iodine tincture 24 mm, videne 24 mm, Lugol's solution 10 mm, potassium iodine 14 mm". The induced effect on both species was algicidal.

Jagielski[b] et al., 2017, mentions the anti- *P. zopfii* inhibitory effect of a preparation containing iodine (iodopropynyl butylcarbamate). A pronounced *in vitro* sensitivity was also found for hydroxyquinoline (HQ) based products in combination with copper (Cu^{2+}) deposited on hydroxyapatite (HAP), the inhibition zones ranging from 26-30 mm to *P. zopfii* and of 30-36 mm to *P. wickerhamii*. The most effective combination, with the largest inhibition zones, contained nitro-hydroxyquinoline (NHQ-Cu^2 + -HAP_2). The inhibitory effect was algicidal(Răpuntean S., et al., 2016).*P. zopfii* genotype 2 isolated from cows with clinical and subclinical mastitis has been shown to be sensitive *in vitro* at low guanidine concentrations with algicidal effect, this substance being recommended as an antiseptic for pre- and postdiping, and in intramammary infusion therapy (Alves et al., 2017).

Some data mention the sensitivity of these algae to some plant extracts and apiculture products. Lagnoniet al., (1995) treats cows with algal mastitis with propolis mixed in dimethyl-sulfoxide. Răpuntean Gh, et al., (2007) report the *in vitro*efficacy of the Polioel preparation (apiculture product.), plant oil extract (*Artemisia annua*, *Hyssopus officinalis*, *Pimpinella anisum*), which inhibited the growth of *P. zopfii* strains and *P. wickerhamii* strain (ATCC 16529), the inhibition areas having the size of 24 mm, with algicidal effect.

Treatment of *Prototheca* infections in animals often encounters difficulties or failures, especially in the disseminated forms, and it is noted that even if some improvements are made, they are passive and recurrent, and it is justified to state that no effective treatment is known (Alves et al., 2017). The presence of inflammatory substances can diminish the effectiveness of antifungals and most often microorganisms are housed in macrophages, by their action. Therapy does not deliver results due to granulomatous lesions in different tissues/organs, as their architecture and normal functioning are seriously altered, and the tissues suffer irreversible changes(Kirk et Mellenberger, 2011); (Krukowski et al., 2013).

Mastitis produced by *Prototheca* species are considered incurable(Schlenstedt et al., 1997); (Pieper et al., 2012); [115]; [116]. In all cases, treatments have been ineffective, and algae persist in tissues, even during dry periods. No spontaneous healing of *Prototheca* mastitis has been reported. (Lassa et Malinowski, 2007).

VI. PREVENTION AND CONTROL

Infections of the mammary gland with *Prototheca* have grown in importance, being more and more frequently reported on some farms, with an emerging character(Lagneau, 1995); (Bozzo et al., 2014); (Milanov[b] et al., 2016); (Alves et al., 2017). It is concluded that the treatment of mastitis caused by *Prototheca* is totally unprofitable, and the slaughter of the infected animals is recommended, thus eliminating the polluting sources (Baumgartner, 1997); (Costa[b] et al., 1996); (Schlenstedt et al., 1997). (Migaki, 1988); (Kirk et Mellenberger., 2011): [115]; [116].

The management and conditions in the shelter where the animals are kept, greatly influence the occurrence of mastitis with *Prototheca* species, the outer environment being considered the main epidemic source.Other factors influencing the occurrence of algal mammary infections are poor hygiene of milking equipment, milk collection and storage containers, and poor sanitary hygiene in shelters.It is advisable to avoid leaving the cows to adopt the lying position immediately after milking because the sphincter of the teat canal is relaxed, it does not close instantly, allowing easy access of various microorganisms that can penetrate to the level of glandular acins (Costa[a] et al., 1996); (Jánosi et al., 2001); (Langoni et al., 2013).

https://dx.doi.org/10.22161/ijfaf.1.4.3 *ISSN: 2456-8791*

Avoid contact of nipples with drainage water, which can be highly contaminated.The effort of a strictest sanitation should be followed and achieved throughout the milking process to avoid any contamination. (Costa[b] et al., 1996).Intensive and prolonged antibiotic treatments should be avoided, which often lead to the increase and spread of antibiotic resistance and therapeutic inefficiency(Lagneau,1996).

In the cow mastitis control programs, in several countries, unicellular algae of the genus *Prototheca* are also included among potential pathogens(Schlenstedt et al., 1997);(Kirk, 1999); (Kirk et Mellenberger, 2011). Detection of infected cows could be done by bacteriological (cultivation) and serological (ELISAi) exams for the detection of IgA and IgG1 (Roesler et Hensel, 2003), but also through other techniques (fluorescence, PCR, MALDI-TOF mass spectrometry etc). Control measures are preferable (with all the stress the animals are subjected to) to identify the eliminators, and epidemiological investigations should be systematically applied even if the disease is sporadic.

Due to the spontaneous emergence and lack of efficacy of the medication used, the slaughter of infected cows seems to be the only method to be used to eradicate the infection(Schlenstedt et al., 1997).At the same time, it is necessary to improve farm management and milking hygiene(Costa[a] et al., 2006); (Baumgartner, 1997).

VII. CONCLUSIONS

Prototheca mastitis have become emerging, and leading to important economic losses.

Treatment of mastitis with *Prototheca* in cattle, often established on the basis of antibiogram results, which demonstrate *in vitro* susceptibility to various medicinal products, has been shown to be ineffective *in vivo*.

For this reason, mastitis produced by *Prototheca* species are believed to be incurable, treatment is doomed to failure and totally non-economic.

It is advisable to remove diseased or carrier animals from the stock, thereby eliminating the sources of contamination.

REFERENCES

[1] B. Aalbaek, H.E. Jensen, A. Huda,"Identification of *Prototheca*from bovine mastitis in Denmark," APMIS, 1998, 106(4): 483-488.

[2] J. Ahrholdt, J. Murugaiyan, R.K. Straubinger, T. Jagielski, U. Roesler,"Epidemiological analysis of worldwide bovine, canine and human clinical *Prototheca*isolates by PCR genotyping and MALDI-TOF mass spectrometry proteomic phenoptyping," Med. Mycol., 2012, 50(3): 234-243.

[3] A.P Almeraya, "Isolation of *Prototheca spp.*, in a case of bovine mastitis," Vet.Mexico, 1994, 25(1): 65-67.

[4] A.C Alves, E. Capra, S. Morandi, P. Cremonesi, J.F.C. Pantoja, H. Langoni, A.P.C. de Vargas, M.M. da Costa, T. Jagielski, C.A.D. Bolaños, S.T. Guerra, M.G. Ribeiro, "*In vitro* algicidal effect of guanidine on *Prototheca zopfii* genotype 2 strains isolated from clinical and subclinical bovine mastitis," Lett. Applic. Microbiol., 2017, 64(6): 419-423.

[5] K.L. Anderson, R.L. Walker, "Sources of *Prototheca spp.*, in a dairy herd environment," J. Am. Vet. Med. Assoc., 1988, 193(5): 553-556.

[6] B. Baumgartner, "Occurance and control of bovine mastitis caused by *Prototheca*in the Region of Veterinary Institute Posdam," Praktische Tierarzt., 1997, 78(5): 406.

[7] A. Bergmann[a], "Udder compatibility of tetramisole and levamisole hydrochloride and suggestion for the prescription of their intramammary use in cattle against *Prototheca zopfii*,"Berl. Munch. Tierarztl. Wochenschr., 1993, 106(8): 253-256.

[8] A. Bergmann[b],"Experimental*Prototheca* mastitis in cattle and therapywith tetramisole hydrochloride," Berl. Munch. Tierarztl. Wochenschr, 1993, 106(8): 257-260.

[9] J.R. Blogg, J.E. Sykes, "Sudden blindness associated with protothecosis in a dog," Aust. Vet. J., 1995, 72(4): 147-149.

[10] R. Blaschke-Hellmessen,"Fluconazole and itraconazole susceptibility testing with clinical yeast isolates and algae of genus *Prototheca*by means of the E test.", 1996, Mycoses, 39(Suppl 2): 39-43.

[11] J. Bodenhoff, P.S. Madsen, "Bovine protothecosis. A brief report of ten cases," Acta Pathol. Microbiol. Scand. (B), 1978, 86(1): 51-52.

[12] C. (Cuc) Bouari, C. Cătoi, N. Fiț, S. Răpuntean, P. Bolfă, M. Tăulescu, A. Gal, F. Tăbăran, A. Nagy, G. Borza, R. Moussa, G. Nadăș, "*In vitro* susceptibility of *Prototheca*species to antifungal agents," Bull. USAMV, Vet. Med., 2011, 68(1): 92-96.

[13] G. Bozzo, E. Bonerba, A. Di Pinto, G. Bolzoni, E. Ceci, A. Mottola, G. Tantillo, V. Terio,"Occurance of *Prototheca spp.*, in cow milk samples." New Microbiologica, 2014, 37: 459-464.

[14] N. Buyukmihci, L.F. Rubin, A. DePaoli,"Protothecosis with ocular invovement in a dog,"J. Am. Vet. Med. Assoc., 1975, 167(2): 158-161.

[15] Prosenjit Talukdar, Mst. Mahfuja Siddiqa, Md. Mahidul Islam Masum, A.B.M. Habibullah, Md. Khurshed Alam Bhuiyan, P.(2017).Effect of Trichoderma Fortified Compost on Disease

https://dx.doi.org/10.22161/ijfaf.1.4.3 *ISSN: 2456-8791*

Suppression, Growth and Yield of Chickpea. International Journal of Environment Agriculture and Biotechnology(ISSN: 2456-1878).2(2), 0831-0839.10.22161/ijeab/2.2.34

[16] P. Buzzini, B. Turchetti, E. Branda, M. Goretti, M. Amici, P.E. Lagneau, L. Scaccabarozzi, V. Bronzo, P. Moroni, "Large-scale screening of *in vitro* susceptibility of *Prototheca zopfii* towards polyene antibiotics," Med. Mycol., 2008, 46: 511-514.

[17] A.G. Calley, D. Lloyd,"The metabolism of acetate in the colorless alga, *Prototheca zopfii*,"Biochem. J., 1964, 99: 483-489.

[18] K.A. Camboim Expedito, B. Neves Patricia,F. Junior Garino, M. Mendeiros Josemar, F. Riet-Correa,"Protothecosis: an emergent disease," Pesq. Vet. Bras. 2010, 30(1).

[19] M. Casal[a], J. Gutierrez,"Preliminary investigation of the *in vitro* inhibitory effect of antibiotics on algae of the genus *Prototheca*,"Mycopathologia, 1981, 75(1): 45-49.

[20] M. Casal[b], A.J. Gutierrez, "In vitro actvity of ribostamycin against *Prototheca sp*"Mycopathologia, 1983, 83(1): 21-23.

[21] M. Casal[c], J. Gutierrez,"Simple new test rapid differentiation of *Prototheca wickerhamii* from *Prtotheca zopfii*,"J. Clin. Microbiol., 1983, 18(4): 992-993.

[22] M. Casal[d], J. Gutierrez,"Simple new test for presumptive differentiation between genus *Candida* and genus *Prototheca*,"Mycopathologia, 1986, 94(1): 3-5.

[23] M. Casal[f], J. Gutierrez,"Simple new test for rapid differentiation of *P. stagnora* from *P. wickerhamii* and *P. zopfii*,"Mycopathologia, 1995, 130(2): 93-94.

[24] P.J. Coloe, J.F. Allison,"Protothecosis in a cat," J. Am. Vet. Med. Assoc., 1982, 180: 78-79.

[25] J.R. Cook Jr., D.E. Tyler, D.B. Coulter, F.W. Chandler, "Disseminated protothecosis causing acute blindness and deafness in a dog," J. Am. Vet. Med. Assoc., 1984, 184(10): 1266-1272.

[26] L.G. Corbellini, D. Driemeier, C. Cruz, M.M. Dias, L. Ferreiro,"Bovine mastitis due to *Prototheca zopfii*: clinical, epidemiological and pathological aspects in a Brazilian dairy herd," Trop. Anim. Health Prod., 2001, 33(6): 463-470.

[27] E.O. Costa[a], A.R. Ribeiro, E.T. Watanabe, R.B. Pardo, J.B. Silva, R.B. Sanches, "An increased incidence of mastitis caused by*Prototheca*speciesand *Nocardia* on a farm in Sao Paulo, Brazil,"Veterinary Research Communications,1996, 20(3): 237-241.

[28] E.O. Costa[b], A.R. Ribeiro, P.A. Melville, M.S. Prada, A.C. Carciofi, E.T. Watanabe, "Bovine mastitis due to algae of the genus *Prototheca*," Mycopathologia, 1996, 133(2): 85-88.

[29] E.O. Costa[c], P.A. Melville, A.R. Ribeiro, E.T. Watanabe, M.C. Parolari,"Epidemiological study of environmental sources in a *Prototheca zopfii* outbreak of bovine mastitis," Mycopathologia, 1997, 137(1): 33-36.

[30] C.G. Crispens Jr., K.R. Marion,"Algal infection in a corn snake (*Elaphe gutata gutata*)," Lab. Anim. Sci., 1975, 25(6): 788-789.

[31] J.E. Dillberger, B. Homer, D. Daubert, N.H. Altman,"Protothecosis in two cats," J. Am. Vet. Med. Assoc., 1988, 192(11): 1557-1559.

[32] W.M. Dion, "Bovine mastitis due to *Prototheca zopfii*,"II. Can.Vet.J., 1982, 23(9): 272-275.

[33] F. Enders, A. Weber,"Investigation on the occurence of *Prototheca*in fecal samples of cattle," Berl. Munch. Tierarztl. Wochenschr., 1993, 106 (8): 165-169.

[34] L.F. Filippsen, F.B. Moreira, A.T. Sakashita,D.R. Bittencourt,"Prevalence of bovine mastitis due to *Prototheca zopfii* in dairy cattle in North Parana, Brasil,"Cienc. Rural., 1999, 29(1): 87-89.

[35] H. Furuoka, A. Auri,Y. Arita, N. Tuzuki, H. Satoh, C. Itakura, "*Prototheca*l mastitis in a cow," Jap. J. Vet. Sci., 1989, 51(1): 197-199.

[36] S.D. Gaunt, K.R. McGrath, H.U. Cox, "Disseminatedprotothecosis in a dog," J. Am. Vet. Med. Assoc., 1984, 185: 906-907.

[37] J.C. Gentles, P.M. Bond, "Protothecosis of Atlantic salmon," Sabourandia, 1977, 15(2): 133-139;

[38] J.L. Gonçalves, S.H. Lee, E. de Paula Arruda, D. Pedroso Galles, V. Camargo Caetano, C.A. Fernandes de Oliviera, A.M. Fernandes, M. Veiga dos Santos, "Biofilm-producing ability and efficiency of sanitizing agents against *Prototheca zopfii* isolates from bovine subclinical mastitis," J. Dairy Sci., 2015, 98(6): 3613-3621.

[39] R.N. Gonzalez, "*Prototheca*, yeast and *Bacillus* as a cause of mastitis," National Mastitis Council, Annual Meeting, Nashville, T.N., 1996, pag. 82.

[40] J. Goudswaard,"Protothecosis in cattle. Report of a case," Tijdschr. Diergeneeskd., 1977, 102(9): 567-570.

[41] S.M. Hariprasad, A. Prasad, M. Smith, G.K. Shah, M.G. Grand, J.B. Shepherd, J. Wickers, S. Apter,R.S. Liao,R. Van Gelder,"Bilateral choroiditis from *Prototheca wickerhamii* algaemia," Arch. Ophtalmol., 2005, 123(8): 1138-1141.

[42] R. Higgins, Y. Larouche,"Isolationand identification de *Prototheca*, as agent of bovine mastitis," Med. Vet. Quebec, 1989, 19(3): 140-141.

https://dx.doi.org/10.22161/ijfaf.1.4.3 *ISSN: 2456-8791*

[43] R.T. Hodges, J.T.S. Holland, F.J.A. Neilson, N.M. Wallace,"*Prototheca zopfii* mastitis in a herd of dairy cows," NZ Vet. J., 1985, 33: 108-111.

[44] M. Huerre, P. Ravisse, H. Solomon, P. Ave, N. Briquelet, S. Maurin, N. Wuscher,"Human protothecosis and environment," Bull. Soc. Pathol. Exot., 1993, 85, (5 Pt 2), 484-488.

[45] G.D. Imes, J.C. Lloyd, M.P. Brightman,"Disseminated protothecosis in a dog," Onderstepoort J. Vet. Res., 1977, 44(1): 1-6.

[46] T. Jagielski[a], M. Dylag, U. Roesler, J. Murugalyan, "Isolation of infectious microalga *Prototheca wickerhamii* from a carp (*Ciprinus carpio*) – a first confirmed case report of protothecosis in a fish," J. Fish. Dis., 2017, DOI: 10.1111/jfd.12614.

[47] T. Jagielski[b], Z. Bakula, S. Di Mauro, C. Casciari, V. Cambiotti, H. Krukowski, B. Turchetti, M. Ricchi, E. Manuali, P. Buzzini, "A comparative study of the *in vitro* activity of iodopropynyl butylcarbamate and amphotericin B against *Prototheca spp.*, isolates from European dairy herds," J. Dairy Sci., 2017, 100(9): 7435-7445.

[48] S. Jánosi, F. Rácz, G. Szigeti, M. Kulceár, J. Kerényi, T. Laukó, F. Katona, G. Huszenicza,"Review of the microbiological, pathological and clinical aspects of bovine mastitis caused by the alga *Prototheca zopfii*," Vet. Q., 2001, 23(2): 58-61.

[49] H.E. Jensen, B. Aalbaek, B. Bloch, A. Huda,"Bovine mammary protothecosis due to *Prototheca zopfii*," Med. Mycol., 1998, 36(2): 89-95.

[50] Z.C. Kaminski, R. Kapila, L.R. Sharer,P. Kloser, L. Kaufman,"Meningitis due to *Prototheca wickerhamii*in a patient with AIDS," Clin. Infec. Dis., 1992, 15(4): 704-706.

[51] W. Kaplan, F.W. Chandler, E.A. Holzinger, R.E. Plue, R.O. Dickinson,"Protothecosis in a cat: first recorded case," Sabourandia, 1976, 14(3): 281-286.

[52] J.H. Kirk,"Diagnosis and treatment of difficult mastitis cases," Agripractice, 1999, 8, 15-20;

[53] J.H. Kirk, R. Mellenberger,"Mastitiscontrol program for *Prototheca*mastitis in dairy cows,"2011, p. 1-5. www.milkquality.wisc.edu/wp-content/uploads/2011/09/

[54] W. Krüger, "Über einen neuen pilztypus reprasentier durch die gattung*Prototheca*. Zopf's Beitr. Physiol. Morphol., 1894, 4: 69.

[55] H. Krukowski, A. Lisowski, B. Nowakowicz-Debek, L. Wlazlo, "Susceptibility of *Prototheca zopfii* strains isolated from cow's mastitis to chlorhexidine and iodine," Turk. J. Vet. Anim. Sci., 2013, 37: 106-108.

[56] H. Lassa, E. Malinowski,"Resistanceof yeasts and algae isolated from cow mastitic milk to antimicrobial agents," Bull. Vet. Inst. Pulawy, 2007, 51: 575-578.

[57] C. Lass-Flörl, A. Mayr, "Human prototechosis," Clin. Microbiol. Rev., 2007, 20(2): 230-242.

[58] H. Langoni, P.F. Domingues, S.R.C. Funari, H.L.T. Dias, R.A. Mota, N.S. Rocha, A. Sforcin,"*P. zopfii* as agent of bovine mastitis: clinic and therapeutics," Arquivo Brasiliero de Medicina Veterinaria E Zootehnia, 1995, 47(5): 727-731.

[59] H. Langoni, M.Z. Troncarelli, G.G. Wanderley, A. Salina, "Mammaryprotothecosis, a serious problem in a dairy cattle," Veterinária e Zootecnia, 2013, 20(4): 522-556.

[60] P.E. Lagneau,"Emergence of *Prototheca zopfii*: a cause of bovine mastitis," 2nd Congress of European Confederation of Medical Bruxelles, 1995, Abstr. 26.

[61] P.E. Lagneau,"Firstisolation of *Prototheca zopfii* in bovine mastitis in Belgium," J. Mycol. Med., 1996, 6(3): 145-148.

[62] M. Lerche,"Eine durch algen (*Prototheca*) hervorgerufene mastitis der kuh," Berl. Munch. Tierarztl. Wochenschr., 1952, 4: 64-69.

[63] N.Y. Lee, K. Kawai, I. Nakamura, T. Tanaka, H. Kumura, K. Shimazaki, "Susceptibilities against bovine lactoferrin with microorganisms isolated from mastitic milk,"J. Vet. Med. Sci., 2004, 66(10): 1267-1269.

[64] M.J. Linares, J.F. Munoz, F. Solis, C. Rodriguez, A. Valero, M. Casal,"Study of the susceptibility of yeast isolates of clinical interest to five antifungal agents using the E test," Rev. Esp. Quimioter., 1998, 11(1): 64-69.

[65] M.J. Linares, F. Solis, M. Casal,"*In vitro*activity of voriconazole against*Prototheca wickerhamii*: comparative of sensititre and NCCLS M27-A2 method of detection," J. Clin. Microbiol., 2005, 43(5): 2520-2522.

[66] G. Loupal,E.S. Kuttin, O. Kölbl,"*Prototheca* as the cause of swim bladder inflamation in carp (*Cyprinus carpio*)," Tierarztliche Umschau, 1992, 47(11): 850-854.

[67] S. Marques, E. Silva, J. Carvalheria, G. Thompson, "Short communication: In vitro susceptibility of *Prototheca wickerhamii* and *Prototheca zopfii* isolated from bovine mastitis," J. Dairy Sci., 2006, 89: 4202-4204.

[68] S. Marques, E. Silva, C. Kraft, J. Carvalheria, A. Videira, V.A.R. Huss, G. Thompson,"Bovine mastitis associated with *Prototheca blaschkae*," J. Clin. Microbiol., 2008, 46(6): 1941-1945.

[69] M. Masuda, N. Hirose, T. Ishikawa, Y. Ikawa, K. Nishimura,"*Prototheca miyajii* sp. nov., isolated

https://dx.doi.org/10.22161/ijfaf.1.4.3 *ISSN: 2456-8791*

from a patient with systemic protothecosis," Int. J. Syst. Evol. Microbiol., 2016, 66: 1510-1520.

[70] J.S. McDonald[a], J.L. Richard, N.F. Cheville,"Natural and experimental bovine intramammary infections with *P. zopfii*," Am. J. Vet. Res., 1984, 45(3): 592-595.

[71] J.S. McDonald[b], J.L. Richard, A.J. Anderson,"Antimicrobial susceptibility of *P. zopfii* isolated from bovine intramammary infections," Am. J. Vet. Res., 1984, 45(6): 1079-1080.

[72] K.W. Meinke, F. Hamedani, S. Wu, A. Balla, G. Guzman, "*Prototheca zopfii* associated diverculitis in an immunosuppressed host, a case presentation and literature review," Human Pathology: Case Report, 2017, 10: 43-45.

[73] P.A. Melville, E.T. Watanabe, N.R. Benites, A.R. Ribeiro, J.A. Silva, F. Garino Jr., E.O. Costa, "Evaluation of the susceptibility of *Prototheca zopfii* to milk pasteurization," Mycopathologia, 1999, 146(2): 79-82.

[74] P.A. Melville, N.R. Benites, I.L. Sinhorini, E.O. Costa,"Susceptibility and features of the ultrastructure of *Prototheca zopfii* following exposure to copper sulphate, silver nitrate and chlorhexidine," Mycopathologia, 2002, 156: 1-7.

[75] F. Mettler, "Generalizedprotothecosis on a fruit bat," Vet. Pathol., 1975, 12(2): 118-124.

[76] G. Migaki, "Protothecosis. Manual of small animal diseases," (Edited by Jeffrey E. Baxlouch), 1988, 337-341.

[77] D. Milanov[a], L. Suvajdžić,"Characteristics and importance of the genus *Prototheca*in human and veterinary medicine," Proc. Nat. Sci. Matica Srpska Novi Sad, 2006, 110: 15-27.

[78] D. Milanov[b], L. Suvajdžić, I. Pušić, B. Vadić, V. Dordević-Milić, "Outbreak of endemic from of *Protothec*al mastitis on a dairy farm," Acta Veterinaria (Beograd), 2006, 56(2-3): 259-265.

[79] D. Milanov, T. Petrović, V. Polačec, L. Suvajdžić, J. Bojkovski, "Mastitis associated with *Prototheca zopfii* – an emerging health and economic problem on dairy farms," J. Vet. Res., 2016, 60: 373-378.

[80] A. Moller, U. Truyen, U. Roesler,"*Prototheca zopffi* gentype 2 – the causative agent of bovine *Protothec*al mastitis?" Vet. Microbiol., 2007, 120: 370-374.

[81] D. Moubamba[a],"Mammites à*Prototheca* dans la province du Hainant," Thèse pour obtenir du grad de master of science (m. sc) en santé animale tropicale Institut de Mèdecine Tropicale, Antwerpen, Belgique, 1997.

[82] D. Moubamba[b], P. Desmet, P.E. Lagneau, Danielle Swinne, "La protothecose bovine en Belgique," Inst. De Med. Tropic. Lab. Mycologie, Nationalstraat 155-B-2000, Antwerpen, 1997.

[83] L. Pieper, A. Godkin, U. Roesler, A. Polleichtner, D. Slavic, K.E. Leslie, D.F. Kelton, "Herd characteristics and cow-level factors associated with *Prototheca*mastitis on dairy farms in Ontario, Canada," J. Dairy Sci., 2012, 95(10): 5635-5644.

[84] R.S. Pore, E.A. Barnett, W.C. Barnes Jr., J.D. Walker, "*Prototheca* ecology," Mycopathologia, 1983, 81(1): 49-62.

[85] R.S. Pore, T.A. Shahan, M.D. Pore, R. Blauwiekel, "Occurance of *Prototheca zopfii*,a mastitis pathogen, in milk," Vet. Microbiol., 1987, 15(4): 315-323.

[86] Gh. Răpuntean, I. Groza, S. Răpuntean, E. Boldizsar, "The sensitiveness of *Prototheca zopfii* stems isolated from cows with mastitis to some antibiotics and chemotherapics," The 5th Middle European Buiatrics Congress, 2-5 June, Hajduszoboszlo, Hungary, 2004, vol. 2, p. 504-509.

[87] Gh. Răpuntean, L. Mărghitaș, S. Răpuntean, D. Dezmirean, N. Fiț, C. Cuc,"The effect of honey and some plant extract on unicellular algae from *Prototheca*genus," Buletin USAMV-CN, 2007, 64(1-2): 277-282.

[88] S. Răpuntean, Gh. Răpuntean, F. Chirilă, N. Fiț, G. Nadăș, "The Effect of Iodine Products on Unicellular Algae from genus *Prototheca*," Bulletin USAMV, Veterinary Medicine, 2015, 72(2): 306-313.

[89] S. Răpuntean, A. Pop, V. Miclăuș, C. Garbo, F. Chirilă, Gh. Răpuntean, N. Fiț, H. Farcău, M. Tomoaia-Cotișel,"*In vitro*sensitivity research concerning some microorganisms at hydroxyquinoline and cupric derivates deposited onto hydroxyapatite," Bulletin USAMV, Veterinary Medicine, 2016, 73(2): 339-350.

[90] M. Ricchi, C. DeCicco, P. Buzzini,"First outbreak of bovine mastitis caused by *Prototheca blaschkeae*," Vet. Microbiol., 2013, 162: 997-999.

[91] U. Roesler, A. Hensel, "Eradication of *Prototheca zopfii* infection in a dairy cattle herd," Dtsch. Tierarztl. Wochenschr., 2003, 110(9): 374-377.

[92] U. Roesler, A. Moller, A. Hensel, D. Baumann, U. Truyen, "Diversity within the current algal species *Prototheca zopffi*: a proposal for two *Prototheca zopfii* genotypes and description of a novel species *Prototheca blaschkeae* sp. Nov," Int. J. Syst. Evol. Microbiol., 2006, 56: 1419-1425.

[93] C. Salvadori, G. Gandini, A. Ballarini, C. Cantile,"*Protothec*al granulomatous meningoencephalitis in a dog," J. Small Anim., 2008, 49: 531-535.

https://dx.doi.org/10.22161/ijfaf.1.4.3 *ISSN: 2456-8791*

[94] A. Sarale, L. Midulla, R. Colombero, M. Franchi,"*Prototheca*, un underestimated mastitis agent," La Senttimana Veterinaria,2013, www.agrilab.com/wp-content/uploads/2015/03/SETTIMANA_VETERINARIA_2013_eng.pdf.

[95] K. Satoh, K. Ooe, H. Nagayama, K. Makimura,"*Prototheca cutis* sp. nov., a newly discovered pathogen of protothecosis isolated from inflamed human skin," Int. J. Syst. Evol. Microbiol., 2010, 60(Pt 5): 1236-1240.

[96] R. Schlenstedt, M. Zschöck, B. Kloppert, W. Wolter, "Occurance of *Prototheca* mastitis in dairy farms in Hesse," Tierarztl. Prax. Ausg. G. Grosstiere Nutztiere, 1997, 25(5): 407-412.

[97] E. Segal, R. Socher, "Theeffect of Amphotericin B on the ultrastructure of *Prototheca spp*," Mycopathologia, 1981, 76(2): 73-77.

[98] T.A. Shahan, R.S. Pore, "*In vitro* susceptibility of *Prototheca spp.*,to gentamicin," Antimicrob. Agents Chemoter., 1991, 35(11): 2434-2435.

[99] M. Shahid[a], J. Wang, X. Gu, W. Chen, T. Ali, J. Gao, D. Han, R. Yang, S. Fanning, B. Han, "*Prototheca zopfii* induced ultrastructural features associated with apoptosis in bovine mammary epithelial cells," Front Cell Infect. Microbiol., 2017, 7: 299. Doi. 10.3389/fcimb.

[100] M. Shahid[b], J. Gao, Y. Zhou, G. Liu, T. Ali, Y. Deng, N. Sabir, J. Su, B. Han,"*Prototheca zopfii* isolates from bovine mastitis induced oxidative stress and apoptosis in bovine mammary epithelial cells," Oncotarget, 2017, 8(19): 31938-31947.

[101] H. Sobukawa, R. Kano, T. Ito, M. Onozaki, K. Makimura, A. Hasegawa, H. Kamata, "*In vitro* susceptibility of *Prototheca zopfii* genotypes 1 and 2," Med. Mycol., 2012, 49(2): 222-224.

[102] N. LizaSouza, Alessandra Lima-Estrela, L.T.E.Moreira, G.R. Lorena Ribeiro, N.M. Xavier, M.A.T. Silua, A.E. Costa, L.R. Santos,"Systemic canine protothecosis," Case report. Braz. J. Vet. Pathol., 2009, 2(2): 102-106.

[103] D.E. Spalton,"Bovine mastitis caused by *Prototheca zopfii*: a cause study," Vet. Rec., 1985, 116(13): 347-349.

[104] S. Srimuang, C.Prariyachatigul, A. Chaiprasert, W. Rungsipanurant, D. Tanphaichitra,"Antifungal drug combinations for *Cryptococcus neoformans* and *Prototheca spp.*" J. Med. Assoc. Thai., 2000, 83(1): 57-60.

[105] I.J. Sud, D.S. Feingold, "Lipid composition and sensitivity of *P. wickerhamii* to membrane active antimicrobial agents," Antimicrob. Agents Chemother., 1979, 16(4): 486-490.

[106] M. Takano, S. Hoshi, K. Nagai, H. Ishidaira, M. Onozaki, K. Satoh, K. Makimura, "The first case of human protothecosis caused by *Prototheca zopfii* in Japan," J. Infect. Chemother., 2014, 20(10): 647-649.

[107] H. Taniyama,F. Okamoto, T. Kurosawa, H. Furuoka, Y. Kaji, H. Okada, K. Matsukawa, "Disseminatedprotothecosis caused by *Prototheca zopfii* in a cow,"Vet. Path., 1994, 31(1): 123-125.

[108] B.A. Tenhagen, P. Kalbe, G. Klunder, W. Heuwieser, B. Baumgartner, "Individual animal risk factors for*Prototheca* mastitis in cattle," (DTW) Dtsch. Tierarztl. Wochenschr., 1999, 106(9): 376-380.

[109] G.Thompson, E. Silva, S. Marques, A. Muller, J. Carvalheira,"Algaemia in a dairy cow by *Prototheca blaschkeae*," Med. Mycol., 2009, 47(5): 527-531.

[110] D.E. Tyler, D.E.Lorenz, J.L. Blue, J.F. Munnell, F.W. Chandler,"Diseminatedprotothecosis with central nervous system involvment in a dog," J. Am.Vet. Med. Assoc., 1980, 176(10 Pt. 1): 987-993.

[111] W. Wawron[a], M. Bochniarz, T. Piech, W. Lopuszíński, J. Wysocki,"Outbreak of *Protothecal* mastitis in a herd of dairy cows in Poland," Bull. Vet. Inst. Pulawy, 2013, 57: 335-339.

[112] W. Wawron[b], M. Bochniarz, T. Piech, J. Wysocki, M. Kocik, "Antimicrobial susceptibility of *Prototheca zopfii* isolated from bovine mastitis," Bull. Vet. Inst. Pulawy, 201357: 458-488.

[113] F.A. Wirth, J.A. Passalacqua, G. Kao, "Disseminated cutaneous protothecosis in an immunocompromised host: a case report and literature review," Cutis, 1999, 63(3): 185-188.

[114] I.D. Wolfe, H.G.Sacks, C.S. Samorodin, H.M. Robinson, "Cutaneous protothecosis in a patient receving immunosuppresive therapy," Arch. Dermatol., 1976, 112(6): 829-832.

[115] A. Woolorich, E. Koestenblatt, P. Don, W. Szaniawski, "Cutaneous protothecosis and AIDS," J. Amer. Acad. Dermatol., 1994, 31(5/II): 920-924.

[116] *** *Prototheca* mastitis. Mastitis pathogen. Factsheet#9. University of Minnesota College of Veterinary Medicine. https://www.vdl.umn.edu/sites/vdl.umn.edu/files/*Prototheca*-mastitis.pdf

[117] *** Mastitis 3 is now Mastitis 4. http://www.canwestdhi.com/pdf_files/*Prototheca*%20M4%20PCR%20factsheet%20for%20producers%20-%20Jan%202014.pdf.