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CA causative agent, although other species of mycoplasmas such as *M. capricolum* subsp. *capricolum*, *M. mycoides* subsp. *capri* and *M. putrefaciens* may be involved. Infection can be diagnosed by bacterial isolation in rich sterols medium or by serological tests such as ELISA, which has been widely used with consistent results. In this study, antibodies anti-*M. agalactiae* were surveyed from serum of 20 goats located in São Gonçalo city, Rio de Janeiro state, Brazil. Animals did not show any clinical signs of agalactia, mastitis, polyarthritis or pneumonia. Surveys were conducted using an indirect ELISA with sonicated antigen of *M. agalactiae* and G-protein conjugate. Moreover, due to the genotypic and phenotypic similarity between *M. agalactiae* and *M. bovis*, an indirect ELISA with whole cell antigen of *M. bovis* was used. Of all analyzed samples, 85% (17 /20) showed anti-*M. agalactiae* antibodies and all goats were negative for *M. bovis*. The presence of *M. agalactiae* in this herd was confirmed by milk culture on modified Hayflick medium and colonies were identified using indirect immunoperoxidase test. To our knowledge, this is the first description of *M. agalactiae* infecting goats in Rio de Janeiro state. This agent has only been previously isolated from goats in the Brazilian states of Paraíba, Rio Grande do Norte and Pernambuco, which are located in the northeastern region. This finding corroborates the need for research on *M. agalactiae* in goat herds located in the southeastern region of Brazil.

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Mollicutes found in pinnipeds from Deception Island (Antarctica)

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Background: During January and February 2010, 46 Antarctic fur seals (*Arctocephalus gazella*) (AFS), 14 Weddell seals (*Leptonychotes weddellii*) (WS), and one Southern elephant seal (*Mirounga leonina*) (SES) were captured at Deception Island, Antarctica, and the presence of *Mycoplasma* was studied in nasal, oral and genital samples. *Methods*: Samples were collected by direct insertion of sterile cotton wool swabs into the mouth, nose and prepuce or vagina of the marine mammals. The swabs were placed in FBP medium with 0.5% active charcoal and were kept at -20°C until culture in the lab. The samples were thawed at room temperature, vortex and inoculated into standard mollicutes broth and agar media. Positive cultures were kept in FBD without active charcoal at -80°C until mycoplasma identification. For that positive samples were subcultured into liquid SP4-II medium. After the incubation, the cultures were filtered through 0.45 µm pore size sterile membranes into the same medium. When color change of the medium was observed, culture DNA extraction was done and plated culture onto SP4-II medium agar. The plates were incubated at 37°C under aerobic conditions. PCR of the 16S-23S Intergenic Spacer Region, and 16S rDNA were performed and the products sent for sequencing. Nucleotide sequences were compared with the GeneBank database. *Results*: *Mycoplasma (M.) zalophidermidis* (genital), and M. sp. CSL7529-conjunctiva (genital and mouth) were found in AFS; M. sp. *Mirounga* ES2805-ORL (genital), *M.* sp. *Mirounga* ES2774-NASSP4 (91% similarity) (mouth), and *M.* sp *Phocoena* C-269 (mouth) in WS; *M. lipofasciens* ATCC 35015 (78%) (mouth) in WS and SES; and *M. zalophi* (genital and mouth) were found in all the species. *Conclusions*: This is the first report of *Mollicutes* in pinnipeds from Antarctica and the information can be useful to know more about the ecology of this species.

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A putative spiroplasma isolated from jellyfish (*Pelagia noctiluca*)

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Background: There are not previously records of mollicutes in jellyfish. *Methods*: Specimens of jellyfish *Pelagia noctiluca* (Scyphozoa: Pelagiidae) stranded massively in the Canary Islands were studied for the presence of mollicutes. Nine batches (10-40 animals/batch) were homogenates and 1 ml was inoculated in triplicates in liquid SP4-II medium supplemented with 1.5% NaCl and incubated at 18°C for 24 h. After incubation, cultures were filtered through 0.45 µm pore size sterile membranes into the same medium and incubated with the same conditions but anaerobically. DNA was extracted two months later. For sequencing, PCR targeting partial 16S rDNA was performed. *Results*: Four samples were positive for mollicutes real time PCR. All samples grew in broth medium, but no colonies were seen in agar medium. The sequences of a partial 16S rDNA PCR products show complete similarity among them and corresponds with *Spiroplasma* sp. *Conclusions*: A possible new putative *Spiroplasma* species is presented.

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Histological and immunohistochemical findings in mastitis caused by *Mycoplasma agalactiae* in sheep

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Contagious agalactia can be caused by different mycoplasmas which share mammary, articular or ocular tropism. This study describes histological and immunohistochemical findings in mastitis caused by *Mycoplasma agalactiae* in sheep. Laboratory investigations were previously carried out on milk samples from all slaughtered sheep to confirm the disease. Udders and supra-mammary lymph-nodes were collected and selected tissue samples were taken for histological and immunohistochemical examination. Cells in tissue expressing *M.agalactiae* antigen, MHC-II, CD3 and CD79 lymphocytes were also investigated by immunohistochemistry. Cultures test on milk showed the presence of typical «fried egg» colonies. Further biochemical and molecular biological tests confirmed tissue infection by *M.agalactiae*. Histological findings showed an interstitial monocytic infiltrate in the acute phase, while in advanced stages, we found a persistence of monocytic infiltration with a slight reduction of secretory tissue; in severe cases, a marked atrophy of secretory tissue associated with monocyte infiltration and interstitial fibrosis was observed. The lymph nodes showed a reactive hyperplastic lymphadenitis. Interestingly, we detected, in spite of negative culture, rare focal monocytic infiltration within tissues with recovered secretory activity in the udder of some convalescent sheep. Immunohistochemistry for *M.agalactiae* was strongly positive in the lumen of the acini and ducts, while interstitial monocytic cells were positive for MHC-II and CD3 antigen. CD3 positive cells, in particular, were located in the interstitial tissue around alveoli and ducts, while MHC-II was more reactive in the central areas of interstitial infiltrates. In general, our histological study describes different histopathological patterns relating to *Mycoplasma agalactiae* infection in sheep. In the acute phase, the main histological lesion is an interstitial mastitis characterised by lymphocytes, macrophages and dendritic cells. In the chronic phase, we observed a reduction of secretory tissue (accompanied by a reduction in milk production) associated with fibrosis; sometimes the disease had a benign course with slight reduction of secretory tissue associated with a moderate fibrosis. In some cases, after an initial drop of lactation (acute phase), there was a recovery of the daily amount of milk which in two weeks came back to normality. In these cases *Mycoplasma agalactiae* was not recovered although histology showed the presence of rare lymphocytic infiltrates that might represent a latent phase of disease, where the immune system can not completely eliminate the pathogen.

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Isolation of *M. agalactiae* from the brains of experimentally infected sheep

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In Italy the presence of mycoplasma in the nervous system of animals was studied for the first time in 1950 (Valenti/Zavagli, 1951) at the Istituto Zooprofilattico Sperimentale of Latium and Tuscany. In those years the difficulties to obtain sufficient amounts of antigen for vaccine preparation and the lack of appropriate media induced laboratories to utilize tissue homogenates from infected animals such as the "Mirri vaccine" made from formolized infected milk and the "Zavagli vaccine" made from udder and brain; these early vaccines were used by farmers and vets to effectively control CA. The brain, together with the udder, as target tissue from infected sheep for vaccine preparation was chosen because they contained the highest levels of pathogen. The mycoplasma affinity for the nervous system

was not further investigated but today vaccines from such tissues are strictly forbidden. Nevertheless mycoplasma isolation from nervous system continues to be occasionally reported in livestock in different countries as occasional "case reports": *M.bovis* seems to be the most involved in neuropathology because of the frequent infection of the ear duct of calves which often evolves in purulent encephalitis/meningitis. We report here the presence of *M agalactiae* in the brain of sheep experimentally infected via the mammary gland during a vaccine efficacy trial. After euthanization and slaughtering at different stages of the infection some animals were investigated for mycoplasma. Out of 4 sheep slaughtered on days 15, 30, 45, and 60, all four were found to be positive for mycoplasma antigen in brain tissues. Other organs, udder and kidney but not liver, lung or spleen, from the same sheep were also found to be positive. The presence of the pathogen in the brain of these sheep underlines the potential role of mycoplasmas as causes of encephalitis and other CNS diseases.

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Contagious agalactia: a preliminary study on its impact on milk production of Sicilian sheep

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Economic losses due to contagious agalactia (CA) in small ruminants herds are mainly associated with significant reductions or complete loss of dairy production, mortality, abortions, early culling and costs of control. With the aim of estimating milk production losses caused by CA, 46 primiparous lactating Valle del Belice ewes were monitored after experimental infection. Sixty days after lambing, 2 ewes were each experimentally infected with a single dose of 10^8 CFU/ml of a live *Mycoplasma agalactiae* strain in both teats by intracanalicular route. Three days after inoculation, the infection was spread naturally by manual milking of the infected sheep before the rest of the flock. The milk yield (morning and evening) was recorded daily for 12 weeks: 5 weeks before the experimental infections and 7 weeks post-challenge. Daily milk data collected from each ewe before experimental infections were used to design individual lactation curves in order to estimate the impact of CA infection. Individual milk samples were also screened for the presence of *M. agalactiae* as well as other pathogenic causes of mastitis including *Staphylococcus aureus*. No pathogens were detected in the milk of 10 (22%) of the 46 ewes kept with the experimentally infected sheep. The quality of the milk of 6 (13%) ewes also remained normal despite the isolation of *M. agalactiae*. There was a reduction of 17% in milk output of 13 (28%) ewes from which *M. agalactiae* was isolated; 17 (37%) other ewes also had a similar drop in milk production but quickly recovered and, after 2 - 3 weeks, the final losses were estimated to be 3%. In conclusion, the loss of milk following the infection by CA is variable and probably related to the degree of exposure and capacity of the individual ewe to resist the pathogen.

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Association between diversity of *Mycoplasma hyopneumoniae* strains in pig herds and lung lesions at slaughter

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Background-The clinical importance of infections with different *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) strains in pig herds is not known. The present study investigated whether the presence of different *M. hyopneumoniae* strains was associated with more severe lung lesions in slaughter pigs. Methods-Ten pig herds infected with and vaccinating the piglets against *M. hyopneumoniae* were selected. One batch of slaughter pigs from each herd was investigated. Lung lesions were scored (0-100) from at least 65 pigs per herd (1190 lungs in total), presence of *M. hyopneumoniae* DNA in bronchoalveolar lavage fluid was tested using nPCR on 20 pigs per herd. Blood samples were taken from 20 pigs and tested for presence of *M. hyopneumoniae* antibodies using ELISA. All nPCR positive samples were tested using a Multiple-Locus Variable number tandem repeat Analysis (MLVA). Based on the MLVA results, herds were classified into 3 categories, namely presence of 1, 2 to 6 or ≥ 7 strains in the herd. Kruskal-Wallis test was used to assess the association between the MLVA results and the extent of pneumonia. Logistic regression analyses assessed associations between MLVA results and prevalence of pneumonia, fissures, pleurisy and percentage of seropositive pigs. Results-In herds with 1, 2 to 6 or ≥ 7 different *M. hyopneumoniae* strains, pneumonia scores were 1.14, 2.85 and 3.88 ($P<0.05$). The percentage pigs with pneumonia, fissures, pleurisy and positive ELISA results in herds with 1, 2 to 6 or ≥ 7 different strains were: pneumonia 16.9%, 20.2% and 27.3% ($P<0.05$), fissures 25.3%, 35.6% and 32.4% ($P>0.05$), pleurisy 2.6%, 13.4% and 27.6% ($P<0.001$), and ELISA 5%, 55% and 86% ($P<0.001$), respectively. Conclusion-In herds where more different *M. hyopneumoniae* strains were found, measured using MLVA, pneumonia lesions were more severe and there was a higher percentage of pigs with pneumonia, fissures and pleurisy.

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Immunogenic profile of inactivated vaccines against contagious agalactia in sheep

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The contagious agalactia (CA) has reported in Brazil in 2002 and has since been constituted as a serious health problem for small ruminants, especially the Northeast that concentrates 90.7 % and 55.5 % of the goats and sheep, respectively. Microbiological and serological diagnosis of infection has been done but the production of vaccines with local samples was only recently developed. This study aimed to evaluate the immunogenic profile of three inactivated vaccines against AC samples prepared with *Mycoplasma agalactiae* containing different adjuvants. Forty sheep divided into four groups of ten animals each were used. The group 1 (G1) was immunized with a preparation with aluminum hydroxide as adjuvant vaccine, group 2 (G2) with a preparation with Montanide IMS 2215 VG vaccine, group 3 (G3) with a vaccine prepared with montanide gel 01 and group 4 (G4) was not immunized (control). Each animal received two doses of 2.0ml administered subcutaneously on days zero and 21 days and reinforcements on 180 and 360 days. Collection of sera were performed on zero, 21 and 35 days and every 30 days during 12 months. For analysis of protein, Western blotting was prepared with proteins produced from *M. agalactiae* separated by SDS-PAGE and transferred to nitrocellulose membrane. The G1 pool of the ten sera showed temporary immunogenic proteins in the first 15-30 days after each vaccination while the G3 showed immunogenic proteins only 35 days after the primary vaccination. The G2 showed immunogenic proteins from 21 days staying for approximately 90-120 days each booster. Proteins with 48, 55, 80 and 82 kD were the most striking, and the P48 remained present in all periods. It is concluded that the vaccines induced immune response and the vaccine prepared with montanide IMS 2215 VG gave more consistent and promising results, allowing control of AC from periodic vaccinations of animals.

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Antibodies anti-*Mycoplasma agalactiae* in goats in the São Paulo State, Brazil

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Contagious agalactia (CA) is an acute or chronic disease characterized by mastitis followed by agalactia, polyarthritis and keratoconjunctivitis caused by *Mycoplasma agalactiae*, *M. capricolum* subsp. *capricolum*, *M. mycoides* subsp. *capri* or *M. putrefaciens*. At the moment, only *M. agalactiae* has been associated with CA in small ruminants in Brazil. This study aimed to report the presence of antibodies against *M. agalactiae* in dairy goats from the Jacarel, São Paulo State, Southeastern Brazil. The herd had no history of clinical signs of contagious agalactia (CA) and the examination was requested for the purpose of marketing the animals to the Northeast of the country, where the disease has caused significant damage to the farmers. 55 goat sera of which 15 (27.27%) were positive in ELISA and of these 6 (40%) had high optical density values. The results indicate that infection by *M. agalactiae* is spreading to other regions of the country, although there were no clinical reports of disease outside the Northeast. The probable reasons for this may

be related to indiscriminate treatment of common mastitis with antibiotic therapy performed by the farmers, the chronic phase of infection and the small number of specialized laboratories in the diagnosis of mycoplasmosis animals. With these results, we can conclude that the infection is spreading to other regions of the country and sanitary measures, such as requiring tests for the movement of animals between states and vaccination should be adopted to prevent further damage.

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Macrophages activity test: a practical "*In vitro*" approach for evaluation of immunostimulating efficacy of mycoplasma vaccine in sheep

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In endemic areas, control of contagious agalactia (CA) is mainly based on vaccination in spite of antibiotic treatments which are considered too expensive. Some vaccine formulations (saponin, betapropiolattone) seem to be more effective than others (OIE, 2012) but there is a lack of markers of effectiveness. Clinical trials through the use of alive animals, involving a significant number of heads, are rarely carried out because of difficulties to support economically these studies and also related to the very strict EU regulations (particularly for animal welfare). All these factors discourage scientists, keep the current vaccines against CA at the traditionally broth-inactivated approach and doesn't stimulate new possibility for control strategies. The introduction of an alternative "*in vitro*" laboratory procedures which investigate small ruminant immune response, suitable for use as a marker of the efficacy of the host cell mediated immune response and of the protection conferred by the vaccine, may help to limit the use of laboratory animals. Little is known about the immunity acquired during CA infection or vaccination: the data on seroconversion utilizing ELISA against *M.agalactiae* (MA) (Buonavoglia et al., 2008; Castro-Alonso et al., 1996) don't clarify the problem; recently, specific stimulation *in vitro* was detected during an experiment where authors demonstrated that the expansion of antigen-specific IFN-γ positive lymphocytes in infected sheep was initially sustained by $CD4^+$ T cells at day 15 after infection, followed by CD8/IFN-γ double positive cells (La Manna et al., 2011). Also this experiment showed a transient initial response, which weren't linked to the whole period of disease clinical course. We propose a different method to evaluate the small ruminant response to MA vaccination, based on primary cultures of macrophages, both isolated from milk and differentiated from peripheral blood monocytes, collected from control (group I) and vaccinated (group II) ewes. After phorbol 12-myristate 13-acetate (PMA) stimulation, assessment of reactive oxygen species and phagocytosis was performed; then, for each group, it was highlighted macrophages nitric oxide (NO) level production and their ability to phagocytize MA, in co-culture with fluorescinated MA suspension. At the same time, immunoblotting analysis for the detection of IgG subclasses was performed, in particular, in order to assess IgG2a immunoglobulin levels as type Th1 immune response marker. Nitric oxide production level, both in blood and in milk macrophages, had a boost in group II, whereas was low in group I. Compared to standard values obtained from healthy ewes, representing the cut-off for the test, the phagocytic activity in group II was much greater, both in monocytes isolated from blood and in macrophages from milk. Contrarily, in group I it was quite comparable to the standard average of healthy ewes, with no significant change. IgG2a detection went hand in hand with macrophages activity tests, resulting high in group II, and low/negative in group I. In conclusion, the results of this preliminary study showed a significantly enhancement of Th1 immune response in vaccinated ewes; moreover, this is an useful and inexpensively method to demonstrate that for evaluation of some vaccine preparations, an apparent failure of the humoral response is not indicative of an anergic status of the host.

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Mycoplasma-Associated Otitis Media of Harbor Seals in a Marine Animal Rehabilitation Center

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Current reports of mycoplasmosis of harbor seals include respiratory and central nervous system infections. Here we report primary otitis media of three rehabilitated harbor seals associated with a novel *Mycoplasma* species. Animals entered rehabilitation as pre-weaned pups without overt clinical signs. Otitis media apparently developed in captivity, and failed to respond to standard antimicrobial therapy. Post-mortem findings included mucoperiosteal inflammation with caseous exudate and heterotropic ossification. A single seal was unilaterally superinfected with *Pseudomonas aeruginosa* in the external canal, and another was bilaterally coinfected with *Brevundimonas diminuta*. Characterization of the novel *Mycoplasma* isolate is ongoing.

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Immune responses and efficacy of a chimeric protein vaccine containing *Mycoplasma hyopneumoniae* antigens and LTB against experimental *M. hyopneumoniae* infection in pigs

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A recombinant chimeric protein containing three *Mycoplasma hyopneumoniae* antigens (C-terminal portion of P97, heat shock protein P42, and NrdF) fused to an adjuvant, the B subunit of heat-labile enterotoxin of *Escherichia coli* (LTB), was used to immunize pigs against enzootic pneumonia. The systemic and local immune responses, as well as the efficacy of the chimeric protein in inducing protection against experimental *M. hyopneumoniae* infection were evaluated. In total, 60 male piglets of 4 weeks of age were randomly allocated to six different experimental groups of 10 animals each: recombinant chimeric protein by intramuscular (IM) (1) or intranasal (IN) (2) administration, commercial bacterin by IM administration (3), and the adjuvant LTB by IM (4, control group A) or IN (5, control group B) administration. All groups were immunized at 24 and 38 days of age and challenged at 52 days of age. One group that was not challenged was used as the negative control (IN [n = 5] or IM [n = 5] administration of the LTB adjuvant). Compared with the negative control group, administration of the chimeric protein induced significant ($P < 0.05$) IgG and IgA responses against all individual antigens present in the chimera, but it could not confer a significant protection against *M. hyopneumoniae* infection in pigs. This lack of effectiveness points toward the need for further studies to improve the efficacy of subunit-based vaccine approach.

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Ureaplasma diversum in pneumonic lungs of swine

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The genus *Ureaplasma* has seven recognized species which have been isolated from humans, cats, dogs, birds and cattle. Although isolations have been reported from swine, not any species has been identified in this host. To confirm previous reports of *Ureaplasma spp.* in the lungs of swine in Cuba, and to identify the species, respiratory samples collected from pneumonic and healthy Cuban pigs were studied. DNA from 106 samples, including lung and nasal swabs, bronchial mucus and tracheobronchiolar lavage was extracted and a fragment of the rRNA 16S was amplified by PCR for *Ureaplasma* genus detection. Positive samples were following tested using species-specific PCR assays for the identification of *U. urealyticum*, *U. parvum* and *U. diversum*. PCR results were confirmed by sequencing *Ureaplasma spp* positive samples and performing a phylogenetic analysis. The phylogenetic signal of the sequence dataset used in this study was researched by means of the likelihood mapping analysis of 10,000 random quartets generated using TreePuzzle. From the samples analyzed, the 6.6 % was positive by PCR detecting *Ureaplasma spp.* and *U. diversum* was identified in all these samples. The rRNA 16S partial sequences obtained in the present study showed a high nucleotide similarity (99.8%). The likelihood mapping analysis indicated that the rRNA16S gene fragment used contains sufficient phylogenetic signal since the noise observed was