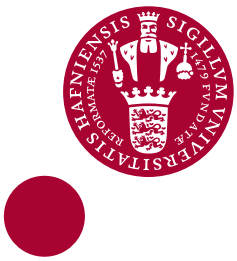

Estimation the diagnistic performance of PathProof Mastitis PCR and bacterial culturing for the detection of Streptococcus agalactiae in milk of Danish dairy cows using latent class analysis

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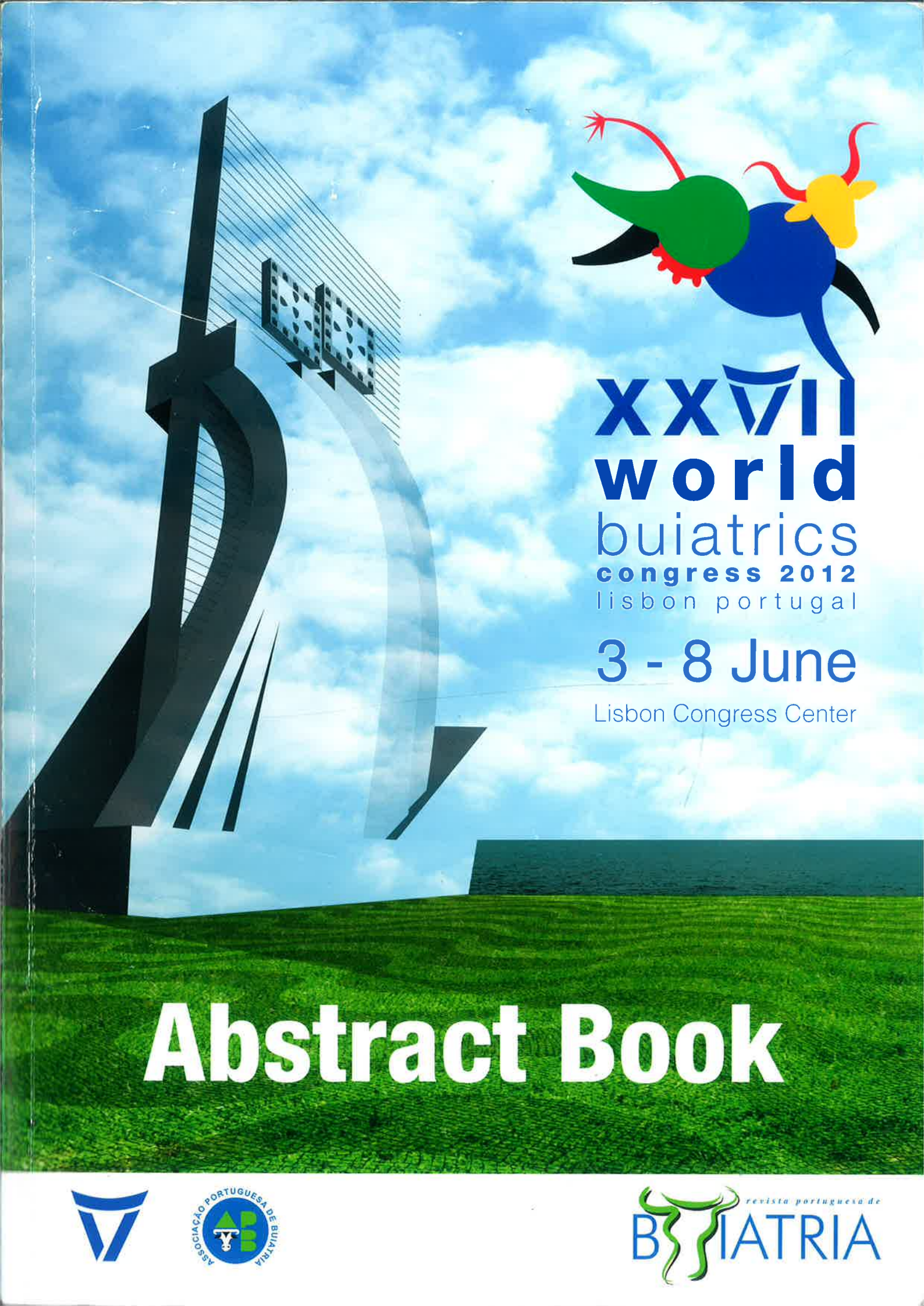

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Editorial

Dear colleagues, dear delegates and friends

In this special issue of our Journal, Revista Portuguesa de Buiatria, are available all abstracts and posters to be presented in the XXVII World Buiatrics Congress, held in Lisbon.

We are very pleased to inform you that we received twelve hundred abstracts all submitted to a blinded review by 3 referees, to ensure no bias occurred in the evaluation. At the end we have 289 oral communications and 787 posters.

The high quality of the selected oral and posters communications will assure a really update on several areas of expertise to the practitioners as well for Academics and we are convinced that the congress program will cover your expectations.

I would like to thank you all for the time you took in participating in the WBC 2012.

Sincerely yours

João Cannas da Silva
Chairman of the Organizing Committee

elastase, and inflammatory LDP. In the S. aureus dry-period mastitis, secretions of CXCL8, elastase, and LDP may be involved in its pathogenesis.

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Estimation the diagnostic performance of PathoProof™ Mastitis PCR and bacterial culturing for the detection of Streptococcus agalactiae in milk of Danish dairy cows using latent class analysis

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Objectives: Streptococcus agalactiae (S.agalactiae) is a contagious bovine mastitis pathogen that can rapidly spread in a herd from a single infected animal and causes immediate loss due to reduced milk yield and milk quality. The aim of this study was to evaluate the diagnostic properties of the PathoProof ™ Mastitis PCR assay at different cut-offs for cyclic threshold (Ct) values against bacterial culturing for diagnosis of S.agalactiae Intramammary infection (IMI) using latent class analysis through estimation of (SePCR, SeBC, SpPCR, and SpBC) to avoid the assumption of a perfect reference test.
Materials and Methods: A total of 614 cows were selected randomly from 6 Danish dairy herds with bulk tank PCR Ct value =34 for S.agalactiae, S.aureus and beta lactamase. At milk recording, quarter foremilk samples were taken aseptically for culturing and the routinely taken cow level milk samples were analysed with PCR assay during the period from 28th March to 28th May 2011. Diagnostic accuracy of PCR was evaluated at cut-offs =39, =37, =34, and =32 by latent class analysis which estimates the sensitivity, specificity and disease prevalence that are not affected by the selection bias from the comparison to a flawed gold standard method.
Results: Results showed 53 cows (8.6%) were positive for S.agalactiae IMI by culturing. SePCR at cut-offs; =39, =37, =34, and =32, was 96.2%, 91.9%, 87.2% and 73.9%, while SeBC 25.7%, 29.9%, 59.9% and 72.1%. SpPCR at cut-offs; =39, =37, =34, and =32, was 96.8%, 96.9%, 96.7%, and 97.22%, while SpBC 99.7%, 99.5%, 99.2%, and 98.9%. The estimated true prevalence of S.agalactaie IMI by PCR at the tested cut-offs was 2-3 percent higher than apparent prevalence, indicating the under estimation of S.agalactaie IMI and reflect its true existence in Danish dairy herds.
Conclusions: In conclusion, SePCR is always higher than SeBC at all tested PCR cut-offs. The lower the cut-off for PCR, the more comparable becomes SePCR and SeBC. The similar sensitivities at cut-off =32 may indicate a higher concentrations of viable cells of S.agalactiae at low cut-offs for PCR, which are easier to be detected with BC. Specificity remains nearly unchanged and was always above 95% for both assays regardless the cut-offs. Latent class estimation proposes a useful alternative to classic test evaluation of diagnostic tests used for detection of S.agalactiae IMI in milk.

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Immunological response to an experimental intramammary inoculation with a killed staphylococcus aureus strain in vaccinated and non-vaccinated lactating dairy cows

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Objectives: The objective of this study was to unravel the immunological response after administration of a new mastitis vaccine containing the inactivated Escherichia coli J5 and Staphylococcus aureus SP 140 strain (Startvac®, HIPRA, S.A.).
Materials and Methods: In a challenge trial, the effect of vaccination on the quarter milk somatic cell count (qSCC) as well as on the milk neutrophil concentration and viability was evaluated. In addition, the production of antigen-specific antibodies (IgG1, IgG2, anti-SAAC and anti-E.coli J5), interleukines (IL-4 and IL-17) and IFN? were determined in both serum and whey. The vaccine induced antibody-mediated phagocytosis was studied as well. Eight animals were included in the study of which four were immunized at 45 days before the expected calving date followed by a second vaccination 35 days later. The other four cows served as non-vaccinated controls. Fifteen days after calving, two contralateral quarters of each cow were infused with a formaldehyde killed virulent S. aureus C195 strain. The two control quarters were inoculated with phosphate buffered saline. Blood samples were collected at 45 and 10 days before calving as well as at 15 days after calving just before the infection was induced. Milk samples were collected at 2 hours before, and at 4, 12, 24 and 48 hours after challenge. Milk neutrophil concentration and viability were determined using flow cytometry.
Results: In both groups of animals, the qSCC of the challenged quarters increased over time. The difference in qSCC between the control and inoculated quarters was substantially higher in the non-vaccinated animals compared with the difference in vaccinated animals ($P < 0.001$). Interestingly, in the vaccinated group the increase of the qSCC in the infected quarters was not significantly different from the qSCC in the control quarters. Similar results were obtained for the milk neutrophil concentration. The difference in neutrophil viability between inoculated and control quarters during the trial period did not depend on the vaccination status of the animal.
Conclusions: Based on these preliminary results, vaccinated cows seem to develop a less severe inflammatory reaction after inoculation compared to non-vaccinated animals. Analyses of the cytokine and antibody production in the upcoming months will allow us to further unravel the cows' (innate) immune response after inoculation with S. aureus in both vaccinated and non-vaccinated cows.

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MRSA detection in bulk milk from Italian dairy farms

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Objectives: Methicillin-Resistant variant of Staph. aureus (MRSA) is an important issue in human infection and has been recently becoming a veterinary matter since it has been isolated in pets and livestock as well. Poultry and swine holdings seem to be important MRSA reservoir but also lactating cows can be positive. As a mastitis pathogen it is an issue in the regards of antimicrobial treatment and zoonotic potential.
Materials and Methods: In order to investigate MRSA pressure and prevalence in an area characterized by a high livestock density, bulk milk samples from 270 Staph. aureus positive dairy farms were collected in Brescia area. All samples underwent search for MRSA both by molecular and cultural methods. An aliquot from each sample was incubated overnight before DNA extraction. After a milk sample pre-treatment, in order to remove protein and fat content, the DNA extraction was done following a protocol described in literature. PCRs amplifying Staph. aureus 23S specific region and mecA gene were performed in two independent reactions. One hundred microliters of milk were plated onto Baird Parker agar, in order to confirm Staph. aureus positivity, and onto MRSA Chromogenic Agar (Pronadisa) after incubation firstly in Mueller Hinton broth added with 6,5 % NaCl and then in Tryptic Soy broth added with cefoxitin and aztreonam. Both cultural media were incubated for 48 hours at 37°C. Suspected colonies were isolated and re-cultured onto Mueller Hinton agar with 6 g/l of oxacillin. Isolates able to grow underwent DNA extraction and a duplex-PCR targeted to nuc gene and mecA gene in order to confirm the results obtained on bulk milk samples.
Results: To our knowledge this is the first survey in Italy searching for MRSA in bulk milk both by PCR and classic bacteriological methods. Up to now 110 samples have been processed and 5 MRSA positive farms have been recognized both by PCR and culturing. Beside them, other 33 farms resulted mecA gene-positive through total milk PCR but no MRSA was isolated. In these cases only mecA gene but not nuc gene has been detected by PCR on isolates.
Conclusions: That justifies total milk PCR results and identifies the presence of coagulase-negative Staphylococci (CNS) harboring the same DNA resistance determinant. A provisional MRSA prevalence of 4,7 % has so been found, that is a relevant one in consideration of the threat to public and animal health.