DEVELOPMENT AND VALIDATION OF A NEW DIAGNOSTIC SYSTEM FOR RAPID MYCOPLASMA SPP. DETECTION AND ANTIBIOTIC SUSCEPTIBILITY EVALUATION IN VETERINARY MEDICINE

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Introduction and Purposes

- Mycoplasmas are recognized as a cause of relevant infections in veterinary medicine, resulting in different clinical signs in domestic, wild and exotic animals.[5]
- The long and difficult in vitro growth, the growing antibiotic resistance and the ability to produce biofilms[3,4,6], have hampered the study of their epidemiology.
- A timely diagnosis and an appropriate choice of the antibacterial agent are necessary for a correct clinical and therapeutic approach.

AIMS OF THE STUDY

- to develop a new diagnostic system for the rapid and semi-quantitative detection of Mycoplasma species in veterinary medicine;
- to validate the performances of the new system, named MYCOPLASMA SYSTEM VET (Liofilchem®, Italy);
- to optimize the antibiotic susceptibility testing through MIC evaluation of nine antibiotics used in veterinary medicine.

Materials and Methods

- In collaboration with the Liofilchem® LTD Company (Italy), it was manufactured and validated a 24-well system containing dried biochemical substrates and antibiotics for search, semi-quantitative determination and susceptibility testing of Mycoplasma spp. animal strains.
- From February 2015 to May 2016, 131 biological samples, collected from different clinically animal species, were examined at the Laboratory of Microbiology and Infectious Diseases (UniCam) for the diagnosis (Fig. 1 and 2).

Results

- Mycoplasma System VET (Liofilchem®, Italy) allowed the highlighting and the semi-quantitative determination of Mycoplasmas of veterinary interest, recording the following performances (Tab. 1, Fig. 3):

<table>
<thead>
<tr>
<th>Mycoplasma System VET</th>
<th>Culture and/or PCR positive</th>
<th>Culture and PCR negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Tp 33</td>
<td>Fp 1</td>
<td>Tp 34</td>
</tr>
<tr>
<td>Negative</td>
<td>Fn 12</td>
<td>Tn 65</td>
<td>Tn 77</td>
</tr>
<tr>
<td>Total</td>
<td>Tp 45</td>
<td>Tp 86</td>
<td>N 131</td>
</tr>
</tbody>
</table>

Legend: Tp = True positives; Fp = False positives; Fn = False negatives; Tn = True negatives; TP = Total Positives; TN = Total Negatives; TP = Total Ill; TD = Total healthy; N = Total population.

Fig. 1: Animal distribution of clinical samples

Fig. 2: Distribution (%) of anatomical districts

TEST PROCEDURE of Mycoplasma System VET

- dip the clinical swab or biological material (0.2mL) or Mycoplasma Transport Broth (1mL) (Liofilchem, Italy) into the vial of sterile physiological solution (7mL);
- wait 5 minutes and dispense 0.2mL of clinical sample suspension into each well;
- cover all the wells with 1 drop of vaselin oil;
- incubate at 36±1°C for 24-48 hrs;
- record the color change of wells (yellow-negative, red-positive);
- interpret the red color of well 4-4-ADC test (arginine) as positivity for Mycoplasma spp.;
- evaluate the bacterial load: red color in well 1-GR+ (≥10⁵CFU/mL<10⁶) and 2-GR++ (10⁶≤CFU/mL<10⁷), and 3-GR+++ (>10⁷CFU/mL); for MIC evaluation, record the color change from yellow (sensitive) to orange-red (resistant) in the wells containing 9 veterinary antibiotics tested at two concentrations (mg/mL): tetracycline (4-8), enrofloxacin (5-10), marbofloxacin (10-20), doxycycline (4-8), erythromycin (8-16), chloramphenicol (8-16), tetracycline (30-60), clindamycin (4-8) and azithromycin (4-6).

VALIDATION PROCEDURE

- PPLO broth (Liofilchem®, Italy), with antibiotic supplement, was cultured at 4°C for 24 hrs, then transferred into enrichment PPLO broth and incubated for three days at 36±1°C in aerobic conditions. After sowing on Mycoplasma Agar (Liofilchem®, Italy) and incubation at 36±1°C with 5-10% CO₂, colonies were observed under the optical microscope 10x every 3 days up to 21 days;
- 1mL of enrichment PPLO broth, as above, was used for DNA extraction and PCR. In particular, after centrifugation, the pellet was re-suspended in digestion buffer and subjected to DNA extraction with a commercial kit (Norgen Biotek, CA). The primers F1 and R1 were used[1].

Conclusions

- MYCOPLASMA SYSTEM VET was a rapid test, simple to perform directly from different animal species and samples: secretions, washes, biological fluids, swabs and tissues.
- The system proved to be a valuable support for the complete diagnosis.
- The reactions were defined after 48 hrs with clear and well defined color changes, allowing a semi-quantitative infection assessment.
- The system proved a great tool for rapid reporting of the antibiotic susceptibility profile.
- The system provided an easy interpretation of the MIC, allowing the clinician a prompt and appropriate therapeutic intervention.
- MYCOPLASMA SYSTEM VET is the first system developed for veterinary medicine for the rapid diagnosis and targeted therapy of Mycoplasma spp. infections.

Tab. 1: Results (frequency) by Mycoplasma System VET, bacterial culture and PCR.

References