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Point of care diagnostics for rapid and cheap companion animals' pathogen detection (POC4PETS)

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Background: Diagnostics is a key tool in the prevention and control of infectious diseases in animals. Funded within the European 7th Framework Program, POC4PETS aims at delivering an innovative technologies to identify pathogens via different nucleic acid detection technologies: on site PCR, isothermal amplification and mini-array probing. The consortium involves RTD performers with expertise in virology, nanotechnology & biosensors and molecular biology and SMEs specialized in veterinary diagnostics manufacturing, laser technology and veterinary services. POC4PETS will provide veterinarians and physicians with new effective tools and detection systems for rapid point of care diagnosis of selected diseases.

Methods & Materials: The project has enabled to develop innovative technologies for the identification of pathogens based on nanobiosensing systems and nucleic acid amplification that can be performed in the field. For Leishmania detection, nanobiosensing systems consisting in optical/electrical detection systems combined with the use of microfluidics and nanoparticles as plasmonics/electrocatalytic signalling tools have been developed. The first platform uses screen-printed electrodes as detectors of the amplified DNA strand hybridized in a sandwich format using complementary DNA strands with one modified with gold nanoparticles. Hydrogen evolution reaction (HER) induced by AuNP is used as analytical signal. In addition a lateral flow paper based platform is used to detect the same DNA through AuNP plasmonics evaluation. The Mini-Array detection method, combining target amplification by PCR and specific hybridization on probes followed by a colorimetric detection on the mini-array membrane, has been applied to identify zoonotic poxviruses from scab lesions of diseased animals and humans.

Results: The developed DNA nanobiosensors shown interesting results in terms of reproducibility and sensitivity. Efforts in designing of a label-free DNA detection impedimetric nanobiosensor are still in the way. The first mini array kit allowed the detection of a number of field strains belonging to *Parapoxvirus* (PPV) and *Orhopoxvirus* (OPXV) genera, even without any preliminary DNA purification step. Furthermore this method permitted to identify OPXV/PPV co-infected animals.

Conclusion: The first prototypes are ready to be validated and the consortium is available to collect pathological material taken from suspected cases. All information on how to send samples is available on the site http://www.poc4pets-fp7.eu/project/default.aspx.

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Clinical usefulness of pathology in diagnosis of tuberculosis

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Background: While chronic granulomatous inflammation with concomitant caseous necrosis is a typical histological finding for tuberculosis (TB), few studies have investigated the frequency of histopathological findings in actual clinical settings or identified other possible TB pathological findings in patients without caseating granuloma.

Methods & Materials: A retrospective study was conducted of the medical records of 231 human immunodeficiency virus-negative, culture-positive TB patients who presented at Chosun University Hospital and underwent biopsy from January 2002 to December 2011. The interpretation sheets of histological examination were analyzed twice: the first analysis determined the frequency of TB-specific pathological findings, while a pathologist performed a reanalysis to reclassify the histopathological findings of suspected TB into "possible TB pathologic findings."

Results: The first pathologic interpretation revealed the following: 63 (34.8%) of 181 pulmonary TB patients were confirmed to have caseating granuloma, 36 (19.9%) had only chronic granulomatous inflammation, and 6 (3.3%) had only caseous necrosis. Among the 46 patients with extrapulmonary TB, only 16 (34.8%) had caseating granuloma and 14 (30.4%) had chronic granulomatous inflammation only. Of the pulmonary and extrapulmonary TB patients, 58% and 65.2% had either caseous necrosis or chronic granulomatous inflammation, respectively. Depending on the examination method for lung biopsy, granuloma or caseous necrosis accounted for 76.3% of patients undergoing percutaneous needle biopsy, which is higher than the 53.6% of patients undergoing transbronchial lung biopsy. The secondary analysis confirmed all caseous necrosis cases interpreted as such at the primary analysis. We also reanalyzed 20 (95.2%) of 21 cases that had been interpreted as other necrosis cases and found that they had a high probability of being caseous necrosis cases. The secondary analysis yielded 11 cases (7 pulmonary; 4 extrapulmonary) of changed interpretation from other necrosis cases to "possible TB pathologic findings."

Conclusion: The analysis of histological findings in TB-diagnosed patients revealed that caseating granuloma cases accounted for only about one-third of cases of both pulmonary and extrapulmonary TB. And it is important for clinicians to remember that some cases reported as other types of necrosis can be in fact caseous necrosis cases

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