19th meeting of the International Organization for Mycoplasmology, 15-19th July 2012, Toulouse, France

Contagious Caprine Pleuropneumonia cELISA validation

Vaccine quality assurance
Prevalence studies

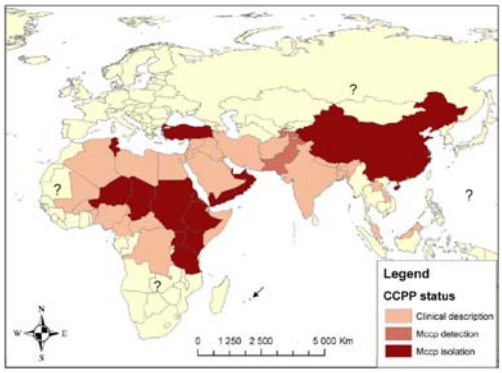
Peyraud A. [1], Bodjo C. [2], Wesonga H. [3], Shiferaw J. [4], Poumarat F. [5], Manso-Silván L. [1], Thiaucourt F.[1]
CIRAD UMR 15, Montpellier, France, CCPP and CBPP World Reference Lab. OIE FAO

1) CIRAD, Montpellier, FR 2) AU-PANVAC, Debre-Zeit, ET 3) KARI, Muguga, KE 4) NVI, Debre-Zeit, ET 5) ANSES, Lyon, FR

Introduction

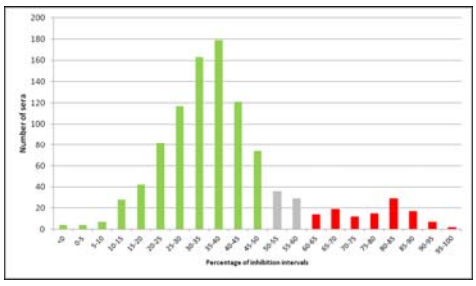
Contagious caprine pleuropneumonia (CCPP) is a severe infectious disease affecting goats and wildlife, characterized by acute unilateral lesions of pleurisy and pneumonia. The distribution of CCPP is still not well known although its presence in China and Tajikistan has recently been demonstrated. However there are very few studies concerning its real prevalence and economic impact. In countries where it exists, control strategies can rely on vaccination which is performed with inactivated adjuvanted vaccines. Although these vaccines have been shown to be effective, their production is very costly as the CCPP causative agent, *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) is highly fastidious to grow *in vitro*. In addition, the procedures to control the quality of these vaccines are not entirely satisfactory as there was no tool to specifically evaluate the immunogenicity of these products. Within the framework of the VACNADA project, it was decided to re-formulate and validate a specific competition ELISA with two main goals: allowing a quality control of CCPP vaccine batches and detecting specifically CCPP outbreaks in countries where vaccination is not implemented.

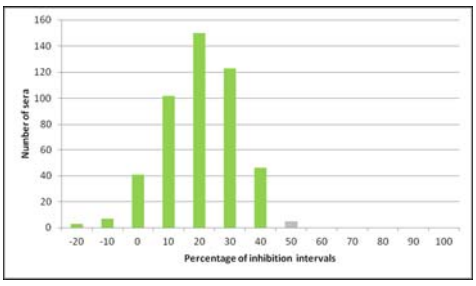
Material and Methods



The cELISA technique developped at CIRAD (1) was adapted to a new format in collaboration with IDEXX Montpellier SA. This allowed the production of a kit batch with precoated plates whose thermostability was evaluated. A reference CCPP vaccine batch was produced at CIRAD. It adhered to OIE guidelines and contained 0.15mg of purified Mccp antigen and 3mg of saponin. The sero-conversion ellicited by this vaccine was first tested at CIRAD and then at the AU-PANVAC. Goat sera were collected in France, Kenya and Ethiopia. In France, which is CCPP free, they originated from goat herds that had been affected by mycoplasmas belonging to the « mycoides cluster » but not by Mccp. In Kenya and Ethiopia, sera originated from regions where CCPP was known to be enzootic but where no vaccination had been implemented.

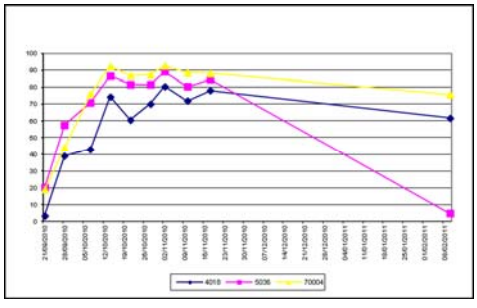
Results and Discussion





Detectability: Out of 1000 sera collected in Ethiopia, 104 were positive with titers ranging from 63% to 97% of inhibition . In addtion, 43 sera had titers above 55% but were considered as dubious because of the uncertainty of measurement. These results establish the CCPP sero-prevalence in this Ethiopian region between 10 and 15% and confirm that CCPP has a real impact for the livehood of nomadic herdsmen in the Afar region.

Specificity of the test: 478 sera from French origin had titers ranging from -20 to 57% of inhibition. The distribution of these titers was Gaussian . The cut-off value was established at 55% of inhibition to ensure a very high specificity (99%) for the test. As CIRAD is adhering to ISO17025 guidelines, an internal reference serum with a titer around this cut-off was produced . This serum was used to evaluate the uncertainty of measurement for this test at CIRAD around the cut-off: +/- 8%.



Post-vaccinal sero-conversion: Goats that were vaccinated with a reference CCPP vaccine batch presented a rapid sero-conversion already noticeable one week post vaccination. The titers reached their maximum three weeks post-vaccination, ranging from 73 to 93% of inhibition. These titers remained stable for at least 5 weeks. By comparison, animals vaccinated with vaccines containing lower quantities of antigen or adjuvant had either lower titers or titers that were waning more rapidly.

Conclusions

-This cELISA CCPP kit has now been validated.
-As it detects a post-vaccinal sero-conversion it can be used either to evaluate the quality of CCPP vaccine batches or the quality of a vaccination campaigns.
-As it is strictly specific it can be used in countries that do not vaccinate against CCPP to measure the prevalence of the disease.
-The test should also be very helpful for the surveillance of the disease in regions or countries that are supposedly free but which border CCPP infected zones.

Acknowledgements

This study was financed by the European Union, Grant: ***DCI-FOOD/2009/226-469***
Vaccines for neglected animal diseases in Africa: *« VACNADA »*
Coordinated by AU-IBAR Nairobi Kenya

cirad UMR 15 INRA

Centre de coopération internationale en recherche agronomique pour le développement

Département BIOS

Contrôle des maladies animales exotiques et émergentes

TA A15/G
34398 MONTPELLIER CEDEX 5, France
Tel: + 33 467 59 37 24

Contact: F. Thiaucourt
fthiaucourt@cirad.fr