Project title:

Implementation and harmonization of a molecular diagnostic tool for Equine Infectious Anemia

Partners:

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Update of the project (may 2012):

Equine Infectious Anemia Virus is responsible of the Disease Equine infectious Anemia Virus (EIAV), the etiologic agent of the disease, is an enveloped virus, classified into the R*etroviridae* family, genus *Lentivirus*. Following infection the viral genome will integrate the host genome for the life span of the animal. EIAV infection can present three distinct phases: an acute phase with high fever, high viral load as well as a drop of platelet, a chronic phase with recurrent clinical signs, horses that survive the acute and chronic episodes often progress to an inapparent stage in which no overt signs of disease are evident. Despite the lack of clinical signs, these horses still represent a permanent source of infection to other horses. Control of EIA is based on identification of inapparent carriers by detection of antibodies to EIAV in serologic tests, the Agar Gel Immundiffusion (AGID) test and Enzyme-Linked Immuno Sorbent Assay (ELISA). The main drawback of AGID test is that evaluate presence of host immune response and not specifically the presence of plasma viremia. Moreover, antibodies response takes up to 45 days to be detectable. Direct detection of EIAV is needed to identify equids who are infected but are tested before seroconversion and detection of neonatal infection would be simplified, since maternal antibodies can persist beyond 6 months of age, and passive maternal and active immunity cannot be differentiated by AGID and or ELISA tests.

During the last decade EIA cases have considerably increased in Europe with several premises in France, Italy, Germany, Hungary. The most dramatic situation is encounter in Romania where the disease is endemic. Indeed, in 2010 more than 30 000 horses have been euthanized due to EIAV infection. With the increase of horse trading around Europe the risk of exchanging negatives horses in AGID but potentially positive for the presence of EIAV is increasing too. In 2010, several EIA cases have been diagnosed in United Kingdom and in Belgium on horses coming from Romania.

This project is related to the one entitled “Harmonization and implementation of RT-PCR assay(s) for Equine Arteritis Virus” presented by our colleague Ruth Bouwstra from CVI, Netherlands. Indeed, both projects regrouped the same collaborators. In order to save money and time, we have decided to organize joint meetings. The first one was held at the Centraal Veterinary Institute, Lelystad, Netherlands on October 3rd, 2011. In this document, information and data related to the EIA project is presented. Data from EVA project will be presented in a separate document made by Ruth Bouwstra from CVI.

The first meeting held at CVI, Netherlands on October 3rd, 2011, was the opportunity to better know each other and to elaborate the project plan for the next 18 months. EIA did not have been identified recently in Netherlands nor in Sweden. In 2009, cases were identified in UK on horses that were coming from Romania. However, the last 5 years sevral EIA premises have been declared in south of France. Blood samples as well as organs have been collected on positive equids in order to caracterize viral strains responsible of those infections. Among the 4 partners, Anses and AHVLA are the ones which possess the most important tissue and blood collections. Since 2007, the laboratory has characterized 15 strains coming from 4 distincts premises that affected 25 equids. Molecular characterization have been performed by sequencing the *Gag* gene region with a length of 1400 nucleotides. In accordance with others patners and due to a lack of EIA samples in others laboratories, all participants agreed that Anses laboratory will develop, test and valdiate a quantitative PCR and/or quantitative RT-PCR to diagnose EIA infection. Once this method will be developped and validate by Anses a proficiency method for method validation will be organized with collaborators from CoVetLab.

Postmortem tissues were received from 25 equids that were seropositive for EIA. Viral RNA and DNA were extracted from all the tissue samples received from each animal. Approximately, 1 g of tissue was homogenised in 5 ml of media. DNA were extracted from 200 µl of homogenate supernatant. In order to include a broad range of samples types, RNA extraction from 3 ml plasma were performed and DNA extraction from spleen, liver and lung were performed. One hundred (100) samples were selected for the amplification of the entire *gag* gene region for this study (table 1). The PCR products were then purified and cloned into a plasmid and clones containing an insert of the entire EIAV *gag* gene were sequenced. Finally, the entire EIAV *gag* gene sequences from 15 distinct equids have been obtained during the first semester of 2012. Currently we are working on sequencing and sequences alignment using *gag* gene sequences obtained from French cases and some from GenBank. For the following year we are planning to caracterize some conserved regions in the *gag* gene sequences that will be targeted in order to develop and validate a quantitative PCR and/or RT-PCR that could be used for the diagnostic of EIAV in horses.

It is expected that some preliminary data will be presented at our next joint meeting in September 2012 that will take place in Dozulé, Normandy, France.

Table 1: DNA and RNA from different equids and from different tissues samples were extracted and tested before cloning and *gag* gene sequencing

		Tissue				
	Samples name	Spleen	Liver	Lung	Plasma	Clones
2007	R09591	x	x	x	x	x
	R09593	x	x	x	x	x
	R09595	x	x	x	x	x
2008	R09587	x	x	x	x	x
	R09589	x	x	x	x	
2009	R09831	x	x	x	x	x
	R09834	x	x	x	x	
	R09837	x	x	x	x	x
	R09840	x	x	x	x	x
	R09843	x	x	x	x	
	R09846	x	x	x	x	x
	R09849	x	x	x	x	x
	R09852	x	x	x	x	
	R09855	x	x	x	x	x
	R09858	x	x	x	x	x
	R09861	x	x	x	x	
	R09882	x	x	x	x	x
	R09886	x	x	x	x	x
	R09891	x	x	x	x	x
	R09896	x	x	x	x	
	R091186	x	x	x	x	x
2010	R10064	x	x	x	x	
	R10207	x	x	x	x	
	R10214	x	x	x	x	
	R10459	x	x	x	x	