Bayesian estimation of sensitivity and specificity of two serological tests for Hepatitis E antibodies in pigs

N. Rose[1], A. Boutrouille[2], C. Fablet[1], A. Fraisse[2], A. Lunazzi[2], M. Eloit[2] and N. Pavio[2]

[1]Unité d'Epidémiologie et Bien-Etre du Porc, AFSSA, Ploufragan.
[2]UMR 1161 Virologie AFSSA LERPAZ, ENVA, INRA, Ecole Nationale Vétérinaire d'Alfort, Maisons-Alfort

Introduction

The close genetic proximity between human and swine Hepatitis E Virus (HEV) strains in non endemic regions suggests that zoonotic transmissions occur. Direct zoonotic transmissions have been described in Japan after consumption of raw or under cooked pig liver or wild boar infected with HEV. Thus it is important to properly characterize the Hepatitis E status of pig herds and estimate the need to develop surveillance plan.

HEV is divided into four genotypes and so far, only one serotype has been described. In endemic regions, genotypes 1 and 2 are mainly found in human population and genotypes 3 and 4 in animals. In non-endemic regions, such as Europe, USA and Japan the situation is different and genotype 3 is found in both human autochthonous cases and animals (swine, wild boar or deer).

HEV serological diagnosis is based on the recognition of recombinant proteins or peptides derived from genotype 1 and 2. Considering HEV genotype 3 diversity (10 subtypes) [1] and quasi-species it is possible that serotype divergence might impair accurate serodiagnosis. Furthermore, since swine is considered as a possible reservoir for human infection, it might be necessary to develop a serological test specific of swine strains. Thus, we have produced and purified a swine genotype 3 recombinant protein using a baculovirus system and designed an Elisa test for genotype 3 HEV in swine. The sensitivity and specificity of the test were estimated and compared with the results from a commercial test (EIAgen HEV, Adaltis).

Methods

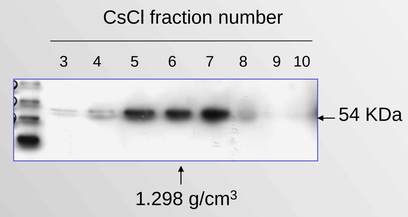
HEV ORF2 was purified from the supernatant of SF9 cells infected with a recombinant baculovirus. This protein self-assembles into "Virus Like Particles" (VLPs). VLPs were directly coated onto polystyrene ELISA plates and after incubation with swine sera, bound HEV antibodies were detected with a rabbit anti-swine IgG HRP-conjugated polyclonal antibody which was then revealed using a TMB-substrate.

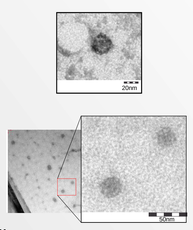
A latent-class Bayesian approach for correlated tests [2] was used to estimate the sensitivity and specificity of both tests performed on sera samples taken from 34 pig farrow-to-finish farms in France (15 sera/farm taken from fatteners at slaughter). Prior distributions for prevalence, sensitivity and specificity were determined using previous data. Analysis were carried out using WinBUGS® software to build Monte Carlo Markov Chains (MCMC) that are iteratively sampled from specified prior distributions and combined with the maximum likelihood estimate of the parameters to provide posterior distributions of sensitivities and specificities.

Results 1

Protein expression and purification from cell supernatant

ORF2 SwG3 is expressed in both Sf9 cells (C) and supernatant (Sp)



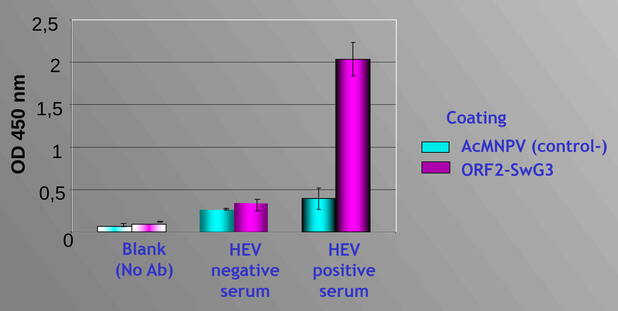


The ORF2 SwG3 has a density of 1.298 g/cm^3 corresponding to VLPs (24-27 nm of diameter in electronic microscopy)

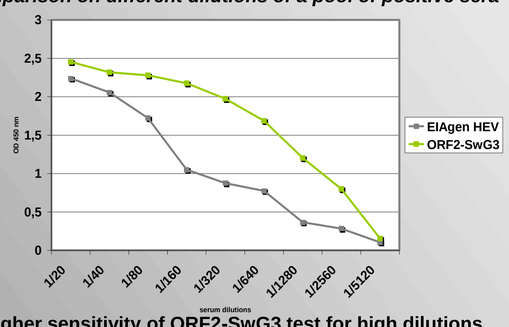
Results 2

ORF2 SwG3 is specifically recognized by anti HEV positive sera and can be used in an ELISA test. Comparison with a commercial kit

1.Optimization of the ORF2-SwG3 ELISA test



2.Comparison on different dilutions of a pool of positive sera



Higher sensitivity of ORF2-SwG3 test for high dilutions

References

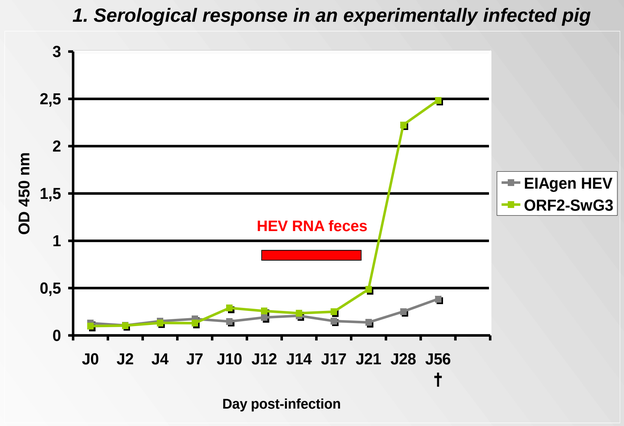
[1] Lu L, Li C, Hagedorn CH., 2006. Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. Rev Med Virol. 2006 Jan-Feb;16(1):5-36

[2] Branscum, A.J., Gardner, I.A., Johnson, W.O., 2005. Estimation of diagnostic-test sensitivity and specificity through Bayesian modelling. Prev. Vet. Med. 68, 145-163.

Results 3

A swine anti-HEV antibodies IgG detection assay (ELISA) was optimized using HEV positive and negative swine serums from experimentally infected pig or SPF facility, respectively.

1. Serological response in an experimentally infected pig



Evidence of lack of sensitivity of EIAgen HEV kit

2. Comparison on negative sera samples (SPF pigs)

	Positive	Negative	Total
ELISA EIAgen HEV	0	32	32
ELISA ORF2-SwG3	0	32	32

High specificity of the ORF2-SwG3 and EIAgen HEV kits

Results 4

1. EIAgen HEV and ORF2-SwG3 comparison on field samples (505 sera from 34 herds)

	Frequency of positive results [95% Confidence interval]		
	Farm level[1] (n=34)	Within-farm[2]	Individual level[3] (n=505 pigs)
ELISA EIAgen HEV	**0.74 [0.59-0.88]**	**0.34 [0.25-0.43]**	**0.25 [0.17-0.34]**
ELISA ORF2-SwG3	**0.77 [0.62-0.91]**	**0.63 [0.49-0.75]**	**0.48 [0.35-0.62]**

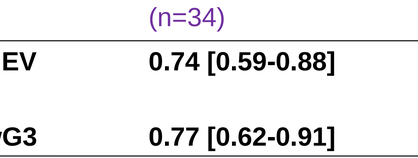
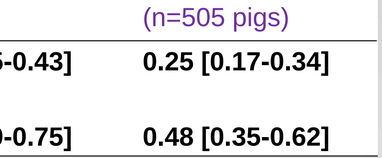
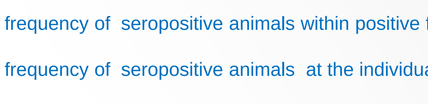
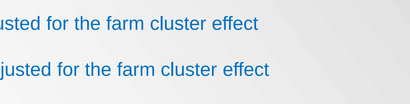
[1] a farm is considered positive if at least 1 animal tests positive

[2] frequency of seropositive animals within positive farms, adjusted for the farm cluster effect

[3] frequency of seropositive animals at the individual level, adjusted for the farm cluster effect

2. Bayesian estimation of sensitivity and specificity of EIAgen HEV and ORF2-SwG3 serological tests

Prior distributions of sensitivity of both tests were assumed to be >0.6 with a mode at 0.9 with 95% certainty. More informative priors were taken for specificity according to previous results on SPF pigs: they were assumed to be >0.95 with mode at 0.99 with 95% certainty.

	EIAgen HEV	ORF2-SwG3
Sensitivity		
	0.47 [0.39-0.55]	**0.92 [0.81-0.99]**
Specificity		
	0.98 [0.95-0.99]	**0.98 [0.93-0.99]**

Posterior distributions from the MCMC samples evidenced a significant higher sensitivity of ORF2-SwG3-based test in comparison with the commercial one.

Posterior estimates were not dramatically influenced by the choice of prior distributions

Conclusion

VLPs derived from swine HEV genotype 3 are good candidates for the improvement of hepatitis E serology in non endemic region and particularly in pigs. It is thus of major interest for a zoonotic agent with possible transmissions to human through food products.