Screening of Salmonella antibodies in pigs on serum vs meat juice : effect of muscle type, chilling of carcasses and duration of sample conservation.

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INTRODUCTION

Monitoring of Salmonella status of pig farms is performed according to various protocols including meat juice collection at slaughterhouse (1). However data about the impact of sampling procedures on the results are scarce. In this trial, we compare several protocols, in order to test the effects of various origins of sample (serum vs meat juice collected on different muscles), time of sampling (end of slaughter vs after first cooling) and conservation duration of frozen meat juice samples before analysis.

MATERIAL AND METHODS

Samples were collected on 90 individually identified pigs, from farms with high Salmonella prevalence:

- for analysis of serum (SE): blood at slaughtering,
- for analysis of meat juice: before chilling of carcasses, diaphragm muscle (DI) and sterno-mastoid muscle (SM1); after chilling of carcasses, sterno-mastoid muscle (SM2).

Analyses were performed both on serum and meat juice after 1 month of freezing, using the "HerdCheck IDEXX Swine Salmonella" serological test (2). On meat juice from sterno-mastoid muscle the tests were also repeated after 6 and 12 months of freezing.

Dilution rates were 1/20 and 1/2 respectively for serum and meat juice (2). Results are expressed as percentages of optical density (OD %) at different cut-off values ranging from 10 to 40% (OD10 to OD40).

RESULTS

The linear regressions between meat juice samples and the reference (serum) are significant ($p < 0.0001$) with R^2 values between 0.70 and 0.86. The all negative y-intercepts ($p < 0.05\%$) indicate that meat juice systematically underestimates serum OD. Moreover, slopes differ from 1 ($p < 0.0001$) showing that the differences depend on antibody levels, with largest discrepancies for low serum OD.

With cut-off OD values of 10 and 20%, positive samples occur at significantly higher rate on serum than on meat juice (except for the diaphragm muscle at OD20). Besides, there is no significant difference between the 3 types of meat juice (Table 1). With OD30 and OD40 cut-off levels, there is no difference between serum and meat juice.

Table 1- Comparison of meat juice vs serum at different cut-off levels.

N=85	**Mean OD**	**% Positive samples**			
		OD10	**OD20**	**OD30**	**OD40**
SE	20.4 a	60.0 a	32.9 a	20.0 a	12.9 a
DI	14.9 b	31.8 b	25.8 ab	17.6 a	14.1 a
SM 1	13.8 b	35.3 b	18.8 b	14.1 a	11.8 a
SM 2	12.5 b	36.5 b	20.0 b	14.1 a	9.4 a
Statistics	p=0.05	p=0.02	p=0.05	ns	ns
	values with different letters within the same column are significantly different ns : no significant difference for p<0.05				

Good agreement between methods; ie discordant pairs <10% and Kappa values >0.70 ; depends on cut-off levels and type of muscle (Table 2). Acceptable concordances occur respectively at 40% cut-off level between serum and meat juice on sterno-mastoid and at 20% between serum and meat juice on diaphragm muscle. Differences between the two types of meat juices (DI and SM) are not consistent at 30 or 40% cut-off.

Time elapsed between collection and analysis of frozen meat juice samples (1, 6 or 12 months) has no effect on the results.

With serum as reference values, accuracy of prediction of positive samples varies according to protocols. At all cut-off levels, high specificities (values > 0.90) are obtained with meat juice (Table 3: only results with OD10 and OD40 are shown). At OD10, sensibility, always remains lower than 0.60. At OD40, only acceptable values (> 0.80) are those calculated on meat juice from sterno-mastoid muscle either collected after chilling of

carcasses and analyzed after 12 months of freezing or collected before chilling and analyzed after 1 month of freezing.

Table 2- Agreement between sampling protocols at different cut-off values. (% discordant pairs and Kappa values).

% discordants - Kappa value		SE	SM1	SM2
OD10	**DI**	30%- 0.44	13%- 0.72	14%- 0.70
	SM1	26%- 0.51		13%- 0.73
	SM2	30%- 0.43		
OD20	**DI**	9%- 0.78	15%- 0.57	15%- 0.60
	SM1	23%- 0.44		15%- 0.56
	SM2	22%- 0.47		
OD30	**DI**	8%- 0.74	10%- 0.61	5%- 0.83
	SM1	14%- 0.55		8%- 0.69
	SM2	9%- 0.69		
OD40	**DI**	7%- 0.71	7%- 0.67	8%- 0.62
	SM1	3%- 0.85		3%- 0.84
	SM2	5%- 0.79		

Table 3- Sensibility (Se) and specificity (Sp) of prediction of positive samples (Serum as reference values).

	OD10		**OD40**	
	Se	**Sp**	**Se**	**Sp**
DI	0.58	0.96	0.75	0.99
SM1	0.53	0.97	0.83	0.99
SM2	0.61	0.91	0.69	0.99
SM2 after 12 months of freezing	0.53	0.92	0.87	0.97

DISCUSSION

For 10% and 20% cut-off levels, results differed between serum and meat juice. Antibody concentrations in meat juice are probably lower than in serum. Furthermore, high concentrations of proteins in muscle, as found in serological investigation on Aujeszky disease (4), can promote non-specific binding and inhibit the action of specific LPS antigens. The kit provider recommends different dilution rates for meat juice and serum samples. However this fails to reduce all the differences between serum and meat juice, especially for low antibody levels. Nielsen et al (5) obtained good correlation between serum and meat juice values, but their ELISA kit and initial rates of dilution were different.

For 30% or 40% cut-off values, concordance of results is good. Differences may be explained by the coefficient of variation of all serological methods (here 5.6%) and by possible bias linked to batch repeatability and operator effects.

Differences according to origin of meat juice sample, especially at cut-off values of 10% and 20%, suggest that in addition to the effect of analytical precision, some differences may occur in antibodies and/or proteins concentration between the diaphragm and sterno-mastoid muscle. As seen on data from sterno-mastoid muscle, chilling of carcasses also seems to bias results. Negative temperatures and high air speed, which both cause a water loss in muscle, could lead to changes in antibodies concentration and /or proteins. Long conservation of frozen meat juice samples (up to 12 months), does seem to influence results.

Sensibility calculations suggest that the best protocol would consist in using meat juice from sterno-mastoid muscle, with subsequent analysis after 1 month of freezing and a 40% OD cut-off level.

With current commercial ELISA techniques, 10% or 20% cut-off levels do not seem to be appropriate. In fact in this range, results are influenced by sampling choices with high risks of false negatives. On the contrary, at higher cut-off levels (30% or 40%), results are unaffected by the type of sample.

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