SEROPREVALENCE OF *HYPODERMA* (WARBLE FLY) INFECTION IN THE BELGIAN CATTLE POPULATION

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1. INTRODUCTION

Belgium has a central position in Western Europe. It has a surface of 30,500 km^2 and the national cattle herd is around 3 millions heads. With the exception of Luxembourg, the neighbouring countries have a low prevalence of cattle hypodermosis (France, Germany) or are considered as virtually warble free (The Netherlands) (4). In the frame of the French warble fly eradication campaign there is an increasing pressure put on the Belgian Ministry of Agriculture in order to organise a co-ordinated action against this parasitic disease.

Few recent data are available in Belgium about the distribution and prevalence of *Hypoderma* infestation in cattle. The only available information goes back to 1991 (2). In this latter study an ELISA technique was used to assess the seroprevalence of *Hypoderma* infestation in two provinces (Liège and Luxembourg). In the province of Luxembourg 43% of individual serum samples were considered as positive whereas in the province of Liège 86% of pooled serum samples were over the cut-off value.

To investigate hypodermosis prevalences in the Belgian adult cattle population, a survey was conducted from December 1997 to March 1998 in all the provinces of Belgium. The goal of this survey was to provide an unbiased estimate of the national herd-level seroprevalence of *Hypoderma spp.* infected dairy, mixed and beef herds, by random selection of herds to sample.

2. MATERIAL AND METHODS

2.1 Survey design

The collect of these sera was carried out in 1997-1998 in the frame of a national infectious bovine rhinotracheitis and paratuberculosis (Johne's disease) survey. This survey design was described in detail elsewhere (1). Briefly, the survey was organized using the co-ordinates for the cattle herds registered in SANITEL-Cattle, the central computerized database for the identification and registration of the Belgian cattle population (Ministry of Small Enterprises, Traders and Agriculture, Belgium). The sampling units were defined as the cattle herds.

The survey was conducted on herds of all types from December 1997 to March 1998. A stratified random sample design was followed. The total number of herds to be sampled was set at 1% (N=594) of the total number of Belgian cattle herds. The sample was stratified by province. The number of herds to be sampled in each province was determined by proportional allocation (6). Herds were randomly selected from SANITEL-Cattle using a software random generator function of Visual Basic 3.0 (Microsoft Corp., 1993). In the selected herds, all of the adult herd, i.e. all adult cattle aged 2 years or more were blood sampled and pooled by groups of maximum 10. A herd was defined to be *Hypoderma spp.*-seropositive if it had at least one *Hypoderma spp.*-seropositive pooled serum.

2.2 Serological testing

The pooled sera were tested for antibodies to *Hypoderma spp.*, using a commercially available ELISA (Calfcheck Hypodermose ®, Vetoquinol Lure, France). All samples were tested according to the manufacturer's instructions.

3. RESULTS

In some cases there was not enough pooled serum available any more for analysis, since the collected sera were analysed in the framework of a national infectious bovine rhinotracheitis and paratuberculosis (Johne's disease) survey. The initially sampled and finally analysed numbers of serum samples are presented in Table 1.

Table 1. Sampling scheme of the national *Hypoderma spp.* serosurvey, Belgium 1998.

	Number of herds		Number of cattle (aged 2 years or more)		Number of pooled sera	
	N	%	N	%	N	%
To be sampled	594	100	15,635	100		
Sampled and pooled	515	87	14,559	93	1,814	100
Sampled* and analysed	482	81			1,596	88

* In some cases there was not enough pooled serum available any more for analysis.

The national seroprevalences for *Hypoderma spp.* is given in Table 2. Fifty-four and fifty-six percents of the herds and pooled sera were seropositive respectively. The frequency distribution of the *Hypoderma spp.* within-herd proportion of seropositive pooled sera is shown in Figure 1. Its median was 100%, whereas its first and third quartiles were 76% and 100% respectively.

Table 2. National seroprevalence to *Hypoderma spp.*, Belgium 1998.

Number of herds			Number of pooled sera		
N	N positive	% positive	N	N positive	% positive
482	262	54	1,596	901	56

The national herd seroprevalence was described by herd type (Table 3), by region and province (Table 4), and by geographical distribution (Fig. 2). There was an apparent higher herd and pooled sera seroprevalence in mixed herds, compared to dairy and beef herds. A marked difference between Northern and Southern Belgium was observed: the former region had a herd and pooled sera seroprevalence of 36% and 33% respectively, whereas the analogous figures for the latter region were 92% and 88% respectively. Although in Southern Belgium the seroprevalence was high and evenly distributed this was not the case in Northern Belgium where the herd seroprevalence was low in some areas along the Dutch border (Antwerp 12%) and higher in others (West Flanders 54%).

Table 3. National seroprevalence to *Hypoderma spp.*, per herd type, Belgium 1998.

	Number of herds			Number of pooled sera		
	N	N positive	% positive	N	N positive	% positive
Dairy herds	94	51	54	470	206	44
Mixed herds	97	73	75	468	327	70
Beef herds	236	111	47	489	269	55

Table 4. National seroprevalence to *Hypoderma spp.*, per region and per province, Belgium 1998.

	Number of herds			Number of pooled sera		
	N	N positive	% positive	N	N positive	% positive
Northern Belgium	323	116	36	928	310	33
Southern Belgium	159	146	92	668	591	88
West Flanders	92	50	54	293	153	52
East Flanders	105	33	31	282	70	25
Antwerp	57	7	12	187	23	12
Limburg	34	10	29	81	19	23
Flemish Brabant	35	16	46	85	45	53
Walloon Brabant	9	7	78	27	18	67
Hainaut	47	44	94	192	161	84
Namur	26	26	100	123	118	96
Luxembourg	26	23	88	103	96	93
Liège	51	46	90	223	198	89

4. DISCUSSION

Immunodiagnosis, a well established technique for the detection of warble fly infestation in live cattle (7), was used extensively during control or eradication campaigns throughout Europe (5). The present work was designed as a preliminary step before launching a control programme in Belgium.

This pilot survey used a stratified random sampling method. Hence, it allowed evaluating the seroprevalences to *Hypoderma spp.* nation-wide. Although the serum samples were collected during the winter 1997-1998 at a time suitable for the detection of specific antibodies to *Hypoderma* (3) the results were probably an underestimation as pools of a maximum of ten sera were used (7).

The results of this survey show that warble fly infestations were widely distributed in Belgium, although there were marked differences between and within the two main regions of the country. Climatic and environmental factors such as the presence of hilly rural areas in Southern Belgium might possibly explain this observation. Other risk factors such as the soil type, the mean temperature, the density of cattle are being currently investigated.

In conclusion Belgium must be considered as an endemic area for cattle hypodermosis. However the launching of a national eradication campaign should be facilitated for the following reasons :

1) The country is surrounded by neighbours which are warble free or have a low prevalence of the disease,
2) Luxembourg has indicated its interest for a joined campaign,
3) Belgium has a very efficient animal identification system (SANITEL) which allows the precise identification, registration and follow-up of each bovine,
4) The prophylactic treatments for hypodermosis are safe, highly efficient and some can be used safely, also in dairy cattle.

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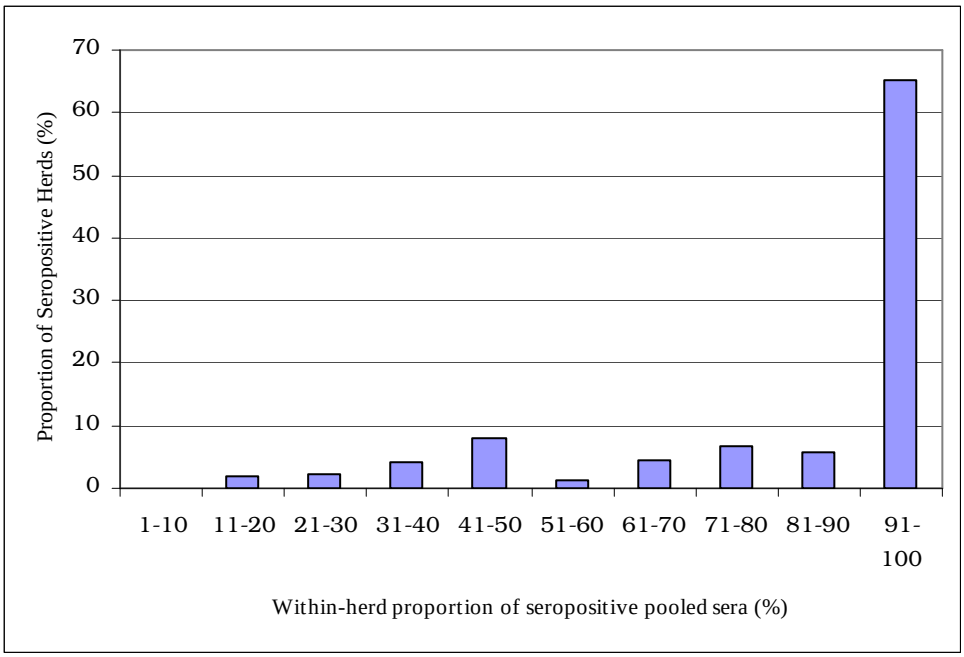

Figure 1. Frequency distribution of the within-herd proportion of seropositive *Hypoderma spp.* pooled sera, Belgium 1998.

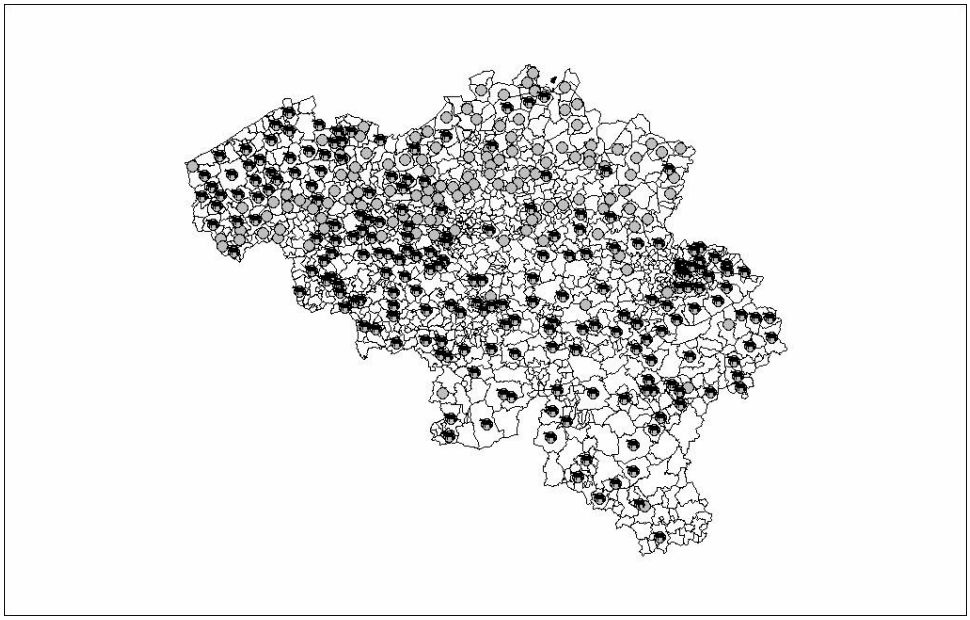

Figure 2. Geographical distribution of the herd seroprevalence of *Hypoderma spp.*, Belgium 1998 (Grey circle: seronegative herd; Grey circle + black cow: seropositive herd).

THE INFLUENCE OF BATCH-PASTEURISATION ON MAILLARD REACTION IN SWEETENED MILK PRODUCTS WITH MODIFIED CARBOHYDRATE CONTENT

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1. INTRODUCTION

The Maillard reaction is a chemical reaction generally initiated by condensation between the ε-amino group of lysine and a reducing sugar. It is a complex reaction which usually occurs during storage and processing of foods. The Maillard reaction results in physical changes, in loss of nutritional available lysine, in the formation of new compounds and leads to brown pigment or melanoidin formation, which affects the colour of the dairy products. The reaction rate is affected by the chemical nature, concentration and ratio of the reactants (8,9), water activity (7), pH (1) and heating time and temperature.

Different model systems, consisting of solutions containing certain types of sugar and protein or amino acid, have been used to investigate the intensity of the Maillard reaction. Here, a model system of lactose free milk was used, consisting of nearly all the milk proteins and which is directly applicable to dairy products. The addition of different sugars or syrups and batch-pasteurisation for increasing periods of time made it possible to compare the extent of the Maillard reaction for different carbohydrates in sweetened milk products. The brown colour (10) and the concentration of furosine (11), a product formed after conversion by acid hydrolysis of the Amadori compound and thus an indicator of the extent of the early Maillard reaction, were used as parameters. The influence of the non-protein nitrogen fraction, by adding different amounts of urea, was also investigated.

2. MATERIAL AND METHODS

2.1 Production of lactose free milk

Lactose free milk was obtained by diafiltration of raw skim milk against the milk salt solution of Jenness and Koops at pH = 6.6 (6). A Stork WFB polysulfon membrane (module type 11PEB4125) with a membrane surface of 0.183 m², a cross-section of 275 mm² and a cut-off of 50,000 Dalton was used (Stork, Brussels, Belgium). As half the original volume was removed as permeate, the same amount of milk salt solution was added. Six cycles were completed, but in the sixth cycle the milk salt solution was added to only 75% of the original volume. In this way, milk with a lactose concentration of less then 0.1% was obtained. For the determination of the lactose content a colorimetric - enzymatic Lactose / D-Glucose test (4) was used (Roche, Mannheim, Germany). Determination of the protein content was carried out using the Kjeldahl method (5) and a concentration of 2.85% was found. The casein to whey protein ratio determined according to Cartuyvels *et al.* (3) was 5.58 (w/w), indicating small losses of whey protein.

2.2 Batch-pasteurisation

Different sugars and syrups were dissolved in the lactose free milk at a ratio of carbohydrate to protein 5:1 on the basis of weight of the dry matter. All mono- and disaccharides were of analytical quality. The different syrups were of food grade quality. The different solutions (5 ml) were heated in 10 ml stoppered glass tubes (1.2 cm x 9.8 cm) in a water bath at 80°C for various periods of time. After the incubation the tubes were removed and immediately cooled in ice water. A stirring device was used to obtain a homogeneous temperature distribution in the water bath. The homogeneity of the temperature was monitored and a deviation of maximum 1°C was found.

2.3 Determination of browning

Measurements of the brown colour were carried out based on a spectrophotometric method (10) involving liberation of the brown pigments by means of pronase. Pronase from *Streptomyces griseus* (7.0 U/mg) was obtained from Roche (Mannheim, Germany).

2.4 Determination of furosine

For the determination of furosine, essentially the method described by Resmini *et al.* (11) was followed, with the exception that 50 µl of hydrolysate were injected instead of 10 µl. A standard of pure furosine was used (Neosystem Laboratoire, Strassbourg, France).

3. RESULTS AND DISCUSSION

Batch-pasteurisation of the sweetened milks with modified carbohydrate content caused browning. Results of browning index and furosine formation for all the carbohydrates investigated are shown in Table 1, where the intensity of browning is expressed as the increase in browning index for one hour of heating at 80°C (Δ browning index/h). Considerable differences can be observed indicating that brown colour formation is strongly dependent on the type of carbohydrate. The monosaccharides dextrose and fructose had the highest increase in browning index, indicating that both sugars are very sensitive to the Maillard reaction. This was also reflected by the dextrose and fructose syrups. Moderate browning could be observed for lactose and syrups with a high maltose content. A decreasing brown colour formation for an increasing percentage of polysaccharides and a decreasing fraction of di- and especially monosaccharides could be observed for the syrups. This can be explained by a lower amount of reducing groups per unit of mass and possibly by steric hindrance. As observed in earlier studies almost no browning occurred when sucrose was used as carbohydrate (2). Also some polyols were investigated and as expected a very low sensitivity for Maillard browning was found.

Table 1: Increase in browning index for 1 h of heating at 80°C and furosine content after 10 h of heating at 80°C for different carbohydrate-lactose free milk samples

Carbohydrate	Composition	Δ Browning index ($.10^{-3}$)/h	Furosine (mg/100g protein)
Monosaccharides			
dextrose	pure dextrose[1]	37.20	2898
fructose	pure fructose	36.99	112
Disaccharides			
lactose	pure lactose	11.02	1343
sucrose	pure sucrose	0.41	23
Syrups			
glucose syrups	95% dextrose	26.43	3347
and	40.5% dextr.; 31% fruct.; 13.5% malt.; 5% triose; 10% ps[3]	33.20	1691
maltodextrins[2]	30.5% dextr.; 9% fruct.; 38% malt.; 3% triose; 19.5% ps	22.52	1902
	27% dextr.; 9% fruct.; 31.5% malt.; 11.5% triose; 21% ps	23.12	1532
	32.5% dextrose; 32.5% maltose; 11% triose; 24% ps	20.20	1785
	17% glucose; 12% maltose; 71% ps	14.24	1127
	5% dextrose; 50% maltose; 20% triose; 25% ps	14.62	786
	<5% dextrose; 67.5% maltose	11.51	1027
	3% glucose; 37% maltose; 60% ps	8.20	812
	3% glucose; 12% maltose; 85% ps	7.08	558
	3% glucose; 7% maltose; 90% ps	6.40	336
	1% dextrose; 33% maltose; 23% triose; 43% ps	6.94	823
	1% dextrose; 3% maltose; 6% triose; 90% ps	4.32	286
	0.5% glucose; 1% maltose; 98.5% ps	3.72	129
fructose syrups	83% fructose; 15% glucose	37.88	637
	55% fructose; 43% glucose	34.08	1728
	42% fructose; 55% glucose	30.84	1978
	5% fructose/glucose/sucrose; 95% oligofructose	10.69	31
	8% fructose/glucose;7% sucrose; 85% oligofructose	9.22	117
Polyols			
lactitol	>89% lactitol	0.34	15
sorbitol	97% sorbitol	0.01	15
sorbitol syrup	80% sorbitol	3.04	26

[1] dextrose = D-glucose
[2] Maltodextrins are dried starch hydrolysis products with a DE of 20 or below
[3] ps = polysaccharides

Furosine determinations were carried out after 10 h of heating. The furosine contents, as shown in Table 1, are to a large extent in agreement with the browning index with the exception of fructose and oligofructose containing mixtures in which as expected very low furosine concentrations were detected. This is probably due to the fact

that with fructose as reducing sugar no precursors of furosine are formed or that they quickly degrade after formation (12).

Fig. 1 illustrates the influence of urea (one of the major components of the non-protein nitrogen fraction in milk) on the brown pigment and furosine formation. Mixtures of lactose free milk with lactose and dextrose added respectively were heated. Different amounts of urea were added to create samples with varying non-protein nitrogen fractions. Relatively low concentrations of urea reduced the furosine formation for both sugars and the brown pigment formation in the case of dextrose probably due to competition of urea with the proteins for reaction with the sugars.

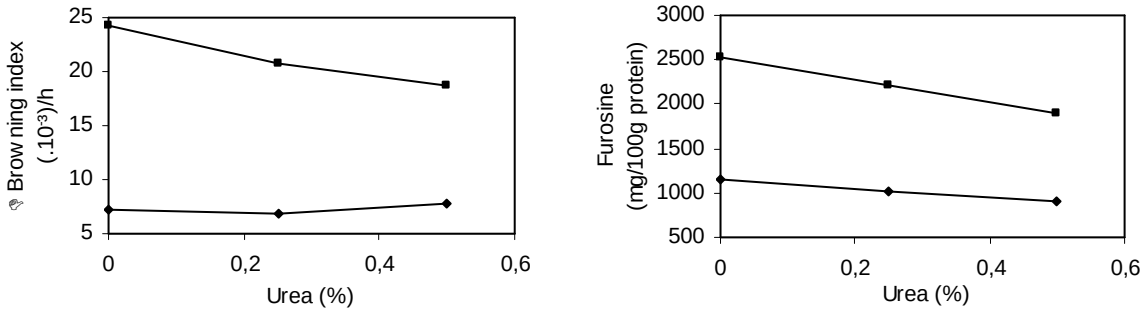

Figure 1:Increase in browning index for 1 h of heating and furosine content after 10 h of heating, both at 80°C, when different amounts af urea are added to solutions of (◆) lactose and ( ) dextrose in lactose free milk

4. CONCLUSION

Many food products contain both sugars and proteins. Since both heat treatment (during processing) and storage can deteriorate structural, functional and nutritional properties of the food system by Maillard reactions, the ability to reduce these undesired reactions may therefore be of interest. This study shows that the choice of carbohydrate and/or sweetening system is a significant factor for the extent of the Maillard reaction. As demonstrated, the commonly used monosaccharides dextrose and fructose are very sensitive to the Maillard reaction. Sucrose and syrups with a high percentage of polysaccharides especially are appropriate means of limiting these undesired reactions. Paying attention to the non-protein nitrogen fraction can also be useful.

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A FAST AND SIMPLE METHOD TO DETERMINE THE WHEY POWDER TO MILK POWDER RATIO USING SPECTROSCOPY IN ALKALI

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1. INTRODUCTION

Several methods are available to determine the whey protein and casein in total milk protein. The spectroscopic methods are based on differences in the UV spectrum between tryptophan and tyrosine, as well as on differences in the tryptophan to tyrosine ratio which is 0.19 for casein and 0.59 for whey proteins.

A new method to determine the whey powder to milk powder ratio based on spectroscopy in alkali is presented here. Whereas the zero order spectra of tryptophan and tyrosine overlap at neutral pH, the spectrum of tyrosine shifts towards a longer wavelength at alkaline pH and the absorption is duplicated as a result of the ionisation of tyrosine. Thanks to the enhanced resolution between both spectra, a method was developed based on the zero order and first order spectra. Since a formula is used for the standard curve by which results are presented as a ratio of absorption differences, the results are not influenced by the protein concentration and are affected only to a small extent by background absorption or light scattering. The method is reliable, simple and fast and even a simple spectrophotometer without scanning mode can be used.

2. MATERIALS AND METHODS

2.1. Fat extraction

The milk powder was suspended in a 2 ml Eppendorf tube at a concentration of approximately 25 mg/ml on protein basis to a final volume of 400 µl. 400 µl of water was used for the blank . A Röse-Gottlieb type fat extraction was performed (1) for fat containing milk powders by subsequent addition of 40 µl of ammonia, 200 µl of ethanol, 500 µl of diethyl ether and 500 µl of petroleum ether. Ethanol, diethyl ether and petroleum ether were added using a Hamilton syringe. After each addition the samples were mixed. Subsequently, the samples were centrifuged in a microcentrifuge for 5 min at 2500 g and the supernatant layer was carefully removed by aspiration; the lower layer had to remain intact. A second extraction was carried out in the same way after addition of 100 µl of ethanol, 300 µl of diethyl ether and 300 µl of petroleum ether, followed by centrifugation (5 min, 2500 g). After having removed the upper layer, the tubes were left open at room temperature for 1 hour to allow volatile compounds to evaporate. The tubes were mixed in order to disperse possible pellets. All reagents and solvents were of analytical or HPLC quality. For skimmed milk powder the fat extraction procedure can be omitted.

2.2. Clarification step

1.4 ml of clarifying solution (7 M urea, 0.1 M Na_2EDTA, 0.2 % (w/v) SDS and NaOH to pH ±13) and 100 µl of the pre-treated sample or blank were added and mixed in a 2 ml Eppendorf tube.

2.3. UV spectroscopy

UV spectra or absorptions were recorded 30 min after having added the clarifying solution. Using a spectrophotometer with scanning mode and derivative facilities UV spectra were recorded in micro quartz cuvets versus the blank from 300 to 265 nm with a data interval of 0.1 nm, a scan speed of 50 nm/min and a band width of 2 nm, using a Kontron Uvikon 943 double beam spectrophotometer (Kontron Instruments, Milan, Italy). The absorption spectra were derivatized with the instrument's build-in derivative mode. From the medium smoothed zero order spectra $\Delta_1/\Delta_2 = (A_{281}-A_{277})/(A_{293}-A_{295})$ and from the first order spectra (highly smoothed derivative from medium smoothed zero order spectra x 100)
$\Delta_1'/\Delta_2' = (\delta A_{279}/\delta\lambda - \delta A_{274}/\delta\lambda)/(\delta A_{288}/\delta\lambda - \delta A_{285}/\delta\lambda)$ were calculated.
The ratio $\Delta_A/\Delta_B = (A_{282}-A_{276})/(A_{292}-A_{296})$ can be calculated using a spectrophotometer without scanning mode.

3. RESULTS

Ionisation of tyrosine (pK = 10.02) by raising the pH to 12.5 improves the resolution between the absorption spectra of tyrosine and tryptophan strongly. To calculate the whey to total milk protein ratio in milk powders, absorption determinations in alkaline solutions (≥ pH 12.5) are preferred to determinations at neutral pH where resolution between tyrosine and tryptophan is minimal. Quite different absorption spectra between 250 nm and 330 nm can be observed for casein and whey proteins due to differences in the molecular ratio of tryptophan to tyrosine which is 0.19 for casein and 0.59 for whey proteins. This results in differences in slope in certain regions of the spectra. The slope in the region between 276 nm and 282 nm decreases with increasing whey protein concentrations, while the slope in the region between 290 nm and 300 nm decreases. Consequently the Δ_1/Δ_2 ratio between both slopes decreases with increasing whey protein concentration. In addition this ratio is independent of protein concentration since both slopes will diminish proportionally upon dilution. Other regions were chosen for the first derivative UV spectra. In this case the difference in $\delta A/\delta\lambda$ between the maximum at 279 nm and the point of inflection at 274 nm is divided by the difference in $\delta A/\delta\lambda$ between the maximum at 288 nm and the minimum at 285 nm, resulting in a Δ_1'/Δ_2' ratio which is high for caseins and low for whey proteins and which is also independent of protein concentration.

Zero and first order standard curves can be calculated from the absorption spectra of mixtures of whole milk powder and whey powder (0 – 25 % whey powder proteins) (Fig. 1). Standard deviations expressed as % milk protein substitution by whey protein were 1.45 % (n = 11) for the zero order ratio (Δ_1/Δ_2) and 2.69 % for the first order ratio (Δ_1'/Δ_2').

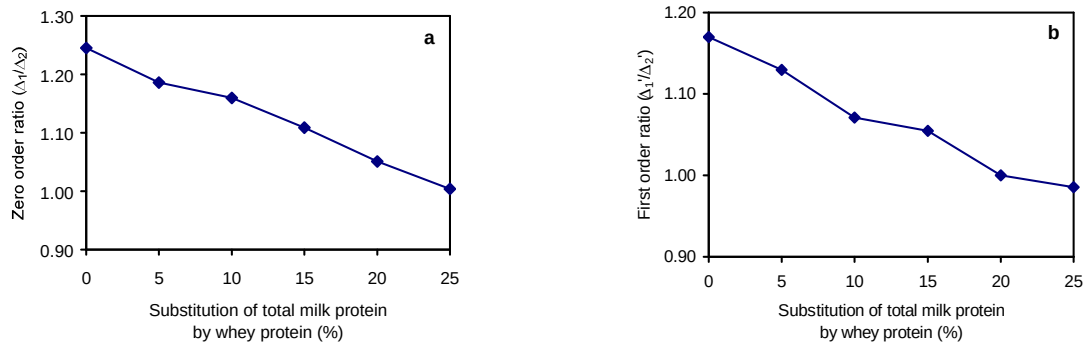

Fig. 1: Zero (a) and first (b) order standard curve calculated from the absorption spectra of whole milk powder and mixtures of whole milk powder and whey powder (0 - 25 % whey powder proteins) at pH 12.5.

For mixtures of skimmed milk powder and whey powder the extraction procedure can be omitted. A typical standard curve is shown in Fig. 2. In this case standard deviations of 0.4 % (n = 12) for Δ_1/Δ_2 and 1 % for Δ_1'/Δ_2' were calculated.

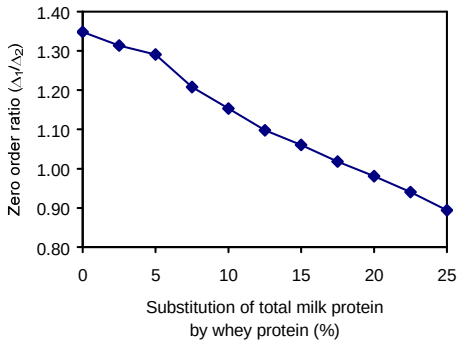

Fig. 2: Zero order standard curve calculated from the absorption spectra of skimmed milk powder and mixtures of skimmed milk powder and whey powder (0 - 25 % whey proteins) at pH 12.5. The simplified sample preparation method (without extraction procedure) was used.

Finally a method was developed for spectrophotometers without scanning mode. In this case the absorptions of all clarified samples were detected at 276 nm, 282 nm, 292 nm and 296 nm respectively. Since these wavelengths have to be adjusted manually in most cases, larger differences in wavelengths were preferred to determine Δ_A/Δ_B. Standard deviations were very low: 0.71 % expressed as % substitution by whey protein (n = 12) for whole milk powders. This means that at least 3 % whey powder in milk powder (on protein basis) can be demonstrated.

To optimise the time between fat extraction and addition of the clarifying solution, after removing the upper layer the tubes were left open for various times (0 - 180 min) in order to allow volatile compounds to evaporate. Too high concentrations of these compounds cause light diffraction at lower wavelengths (below 280 nm), which resulted in an underestimation of Δ_1 (or Δ_A) while Δ_2 (or Δ_B) remained unaffected. Consequently the ratios obtained for Δ_1/Δ_2 were too low. After 30 min relatively constant Δ_1/Δ_2 ratios were obtained. The first order ratio Δ_1'/Δ_2' was almost independent of the time between extraction and addition of the clarifying solution.

The time between addition of the clarifying solution and absorption determinations was varied as well. An important decrease of this ratio can be observed during the first 30 min for the zero order ratio. After 30 min this decrease is less pronounced, but a constant value is not obtained within the first 3 hours. The first order ratio Δ_1'/Δ_2' is more or less constant as a function of time between clarification and spectrophotometric determinations.

We can conclude that 1 hour was preferred for the time between extraction and clarification, whereas 30 min was preferred for the time between clarification and absorption determination.

4. DISCUSSION

As demonstrated earlier (2) the quantification of the whey powder to milk powder ratio using spectroscopy in alkali can be preferred to spectroscopy at neutral pH due to the enhanced resolution between the spectrum of tyrosine and tryptophan. To remove the fat globules an extraction procedure (Röse-Gottlieb type) is used, and after addition of clarifying solution a clear protein solution was obtained suited for spectrophotometric determination.

Two sample preparation methods are presented: a method for whole milk powders, and a method for skimmed milk powders without the fat extraction step. Although similar results were obtained with both methods the first method should be preferred on all samples if the series of powder to be examined consists of whole milk powders as well as skimmed powders.

Three determination methods are presented: a zero order and a first order method using the scanning mode of the spectrophotometer and a (zero order) mode with fixed wavelengths.

Using a spectrophotometer with scanning and derivative facilities two closely related wavelengths on the slopes of the absorption spectra were chosen to calculate the Δ values in order to avoid too large differences in background values. However, the blank value turned out to be relatively constant throughout the wavelength range used. On the contrary, using a simple spectrophotometer without scanning and derivative facilities maximal wavelength distances on the slopes were chosen.

The zero order ratio determination offers advantages over the first order ratio determination insofar as a simple spectrophotometer without sophisticated software can be used while a better repeatability is obtained. On the other hand the zero order ratio determination also offers specific disadvantages. If reagents are added and the pH is corrected immediately after fat extraction, the absorption ratio is relatively low, probably as a result of light scattering. If after fat extraction the tubes containing the powder solutions are left open for at least 1 hour before adding the reagents, the absorption ratio increases to a relatively constant value.

Using the manual mode with fixed wavelengths is faster than using the scanning mode (in our laboratory circumstances), and better results were obtained with the manual mode. In addition we obtained better results with the zero order spectra than with the first order spectra.

We can conclude that the manual mode is preferred to the scanning mode to determine the whey powder to milk powder ratio. This is an important advantage of the method, since it allows the use of a simple spectrophotometer without scanning mode.

5. ACKNOWLEDGEMENTS

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RAPID CONFIRMATORY ASSAY FOR THE SIMULTANEOUS DETECTION OF RONIDAZOLE, METRONIDAZOLE AND DIMETRIDAZOLE IN EGGS USING LC-MS/MS

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1. INTRODUCTION

Nitroimidazoles are antibacterial and anticoccidial drugs primarily used to prevent and treat histomoniasis and coccidiosis in poultry and game birds. Together with their metabolites they are suspected carcinogens and mutagens. Their use in veterinary practice is strictly regulated within the European Union by Council Regulation 2377/90 (1). The compounds were integrated in Annex IV, which means that any residue of these compounds found in food producing animals or products intended for human consumption has to be considered as a violation of the regulations. In this paper a simple and reliable liquid chromatographic-tandem mass spectrometric method is described for the simultaneous detection of ronidazole (RNZ), dimetridazole (DMZ) and metronidazole (MNZ) in fresh chicken eggs. The results of preliminary validation experiments and of analysis of samples taken by the Ministry of Public Health will also be presented.

2. MATERIAL AND METHODS

2.1 Reagents and Chemicals

RNZ, DMZ and MNZ were from Sigma (St. Louis, MO, USA). Deuterated ronidazole (RNZ-D3) was from RIVM (Bilthoven, The Netherlands). Acetonitrile was gradient grade Lichrosolv from Merck (Darmstadt, Germany). Formic acid was from UCB (Leuven, Belgium). Water was HPLC grade (generated by an ELGA purification system). Filters for filtration of the extract were from Millipore (Millex GV, 0.22 µm). Standard stock solutions (1 mg/ml) were prepared in water-acetonitrile (50/50, v/v) and stored at 4°C. Fresh solutions were prepared every month. A dilution of 1 ng/µl in water-acetonitrile (50/50, v/v), containing 0.1 % formic acid was used for tuning and optimisation of the MS instrument. For spiking the eggs, fresh dilutions (10, 1 and 0.1 ng/µl) were made in water. The eggs analysed for the Ministry of Public Health were gathered by officials.

2.2 Sample Preparation

After mixing the eggs with an ultra-turrax, 10 g was weighed in a tube. Spiking was performed at this stage if necessary by adding an appropriate amount of standard solution in water. The internal standard RNZ-D3 was added in a concentration of 5 µg/kg. After 5 min, 10 ml of acetonitrile was added and the sample was vortex mixed and placed in an ultrasonic bath for 2 min. The sample was then centrifuged during 10 min at 3500 rpm. The supernatant was transferred into another tube and was concentrated to a volume of 4 ml under nitrogen in a water bath at 60°C. After filtration, 40 µl of the remaining extract was injected into the LC-MS-MS system.

2.3 Liquid Chromatography-Tandem Mass Spectrometry

The HPLC system consisted of a Kontron system coupled to the Quattro LC (triple quadrupole) of Micromass equipped with the Z-spray system. The MS system was controlled by version 3.3 of the Masslynx software. Chromatography was performed on an Alltima C_{18} column (5 µm, 150 mm x 2.1 mm i.d.) protected by a guard column containing the same material. A gradient was applied with water (A) and acetonitrile (B), each containing 0.1 % formic acid. The gradient conditions were as follows: from 0-0.5 min, hold 100 % A; ramp over 0.1 min to 55 % A; ramp over 7.9 min to 35 % A; ramp over 0.1 min to 100 % B; hold for 1 min; ramp over 0.2 min to 100 % A. Hold 100 % A for 7.2 min to re-equilibrate the system. The total run time was 17 min. The flow rate was 0.25 ml/min. The Quattro LC mass spectrometer was operated in the ESI (electrospray ionisation)-MS-MS positive ion mode. High-purity nitrogen was used as drying gas and an ESI nebulising gas. Argon was used as collision gas to fragment the parent ion into daughter ions. A summary of the cone voltages, collision energies, and parent and daughter ions of the compounds is presented in Table 1. The source block and desolvation temperature were set at 100 and 250 °C respectively.

Table 1 : Optimised ESI (+) MS-MS conditions for RNZ, DMZ, MNZ and RNZ-D3

Compound	Parent ion (m/z)	daughter ions (m/z)	Collision energy (eV)	Cone Voltage (V)
RNZ	201	140	15	28
DMZ	142	81, 96	25	28
MNZ	172	128, 82	15	24
RNZ-D3	204	143	20	24

2.4 Validation Study

Recovery of the extraction procedure was determined at the 2 µg/kg level using spiked egg samples. The MS response of a compound obtained for samples spiked before clean-up was divided by the MS response obtained for a blank matrix that was spiked after clean-up, and multiplied by 100 to obtain percentage recovery. The detection limits (LODs) were those concentrations of analyte that yielded a signal-to-noise ratio (S/N) of at least 3:1 (2). A mix of blank egg samples was first analysed to control the absence of the analytes and was then spiked at different levels (from 0 to 10 µg/kg) to determine the LODs. Linearity was checked by injecting extracts of samples spiked with increasing amounts of the different standards ranging from 0 to 10 µg/kg. Repeatability experiments were carried out at the 2 µg/kg level. Specificity was tested by spiking blank samples with one compound and analysing them for the other two compounds to check for interferences. The samples analysed for the Ministry of Public Health were gathered in different regions of Belgium.

3. RESULTS AND DISCUSSION

Because the instrument was used in electrospray for other types of analysis, prior attempts were made in this mode, contrary to what was published by other authors, who used APCI (atmospheric pressure chemical ionisation) (3,5,6). The best results were obtained in the positive ionisation mode.

For the recovery of the compounds, a simple extraction with acetonitrile was performed. Some authors (6) used solid phase extraction but it was our intention to develop a very simple method with a minimum of manipulations. Therefore we omitted the solid phase clean-up step and concentrated the acetonitrile extract before injection into the LC-MS-MS instrument.

Complete chromatographic separation was not accomplished but this was not necessary due to the very specific detection of multiple reaction monitoring (MRM). No interferences were detected when testing for specificity.

As stated in the final version of revision of Commission Decision 93/256/EC a minimum of 4 identification points are required for the confirmation of substances. For LC-MS-MS in which the transition of 1 precursor ion to 2 daughter ions is followed, 4 points can be earned. In the method we developed this criterion is accomplished for DMZ and MNZ. For RNZ there is only one daughter ion, namely 140 that is abundant enough to generate a signal in the low µg/kg range. In eggs spiked at 2 µg/kg of each analyte (n=5), recovery percentages were 76 % for DMZ, 79 % for MNZ and 83 % of RNZ with RSDs of respectively 3, 3 and 9 %.

The LOD was 0.5 µg/kg for all three compounds. Matusik *et al.* Reported a thermospray MS method which could detect 2 µg/kg of DMZ in turkey tissues (4). Sams *et al.* reported a MS method using atmospheric pressure chemical ionisation and obtained LOD values in the order of 0.1 µg/kg in eggs (6). Govaert *et al.* reported LOD values of 2 µg/kg for MNZ and DMZ and 5 µg/kg for RNZ in porcine tissue samples (3). Van Rhijn *et al.* reported an electrospray LC-MS-MS method for DMZ with a LOD value of 1 µg/kg in muscle tissues (7). However, for confirmation according to the EU criteria amounts of 5 µg/kg had to be present due to the very weak abundance of one daughter ion. In summary, the LODs obtained with the method developed in this study are similar to, or, in some cases, better than those reported in the literature.

The correlation coefficients (R^2) for the calibration curves in the matrix were between 0.9886 and 0.9977 for all compounds.

Out of the 67 egg samples analysed for the Ministry of Public Health, 8 were found positive on the presence of dimetridazole. The concentrations found ranged from 2 to 5 µg/kg. A chromatogram of a sample found positive on dimetridazole at a concentration of 2 µg/kg is shown in figure 1. The residues were probably caused by contamination of the feed in the feeding mill because dimetridazole is allowed in feed for turkeys. When other feed was given to the animals the residues of dimetridazole disappeared.

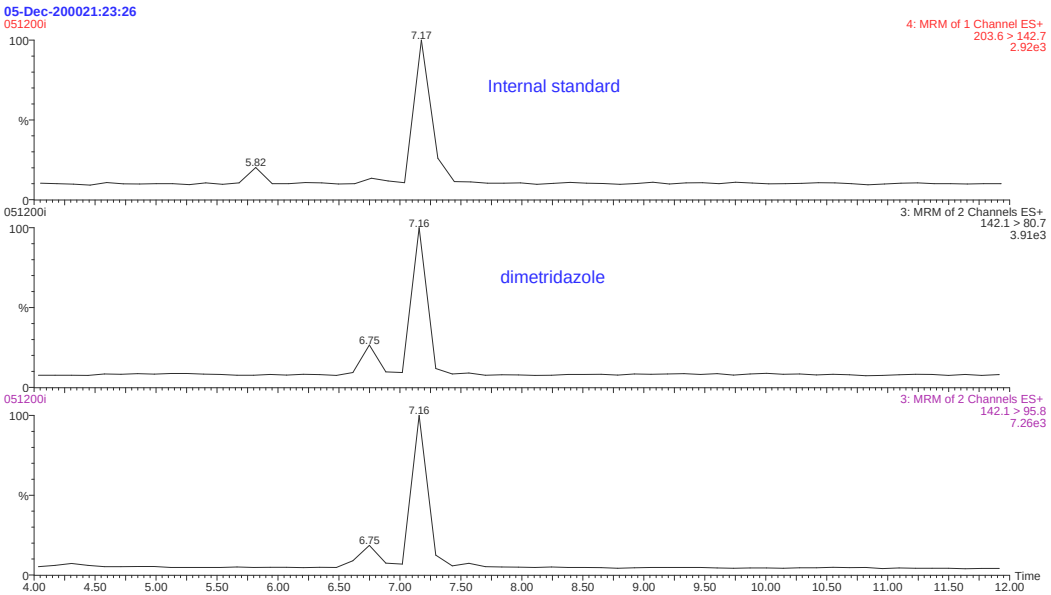

Figure 1 : MRM chromatogram of a sample positive on dimetridazole at a concentration of 2 µg/kg

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DETERMINATION OF FLUBENDAZOLE AND METABOLITES IN EGGS AND POULTRY MEAT WITH LC-MS/MS

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1. INTRODUCTION

Flubendazole (FLUB) is a broad-spectrum benzimidazole anthelmintic, effective against endoparasites such as gastro-intestinal roundworms, gapeworms and tapeworms (2). This drug is widely used in the veterinary medicine of poultry. An administration of flubendazole can result in the presence of residues of the parent compound FLUB and its hydrolysed metabolite (HMET) and/or its reduced metabolite (RMET) in eggs and meat. Some chromatographic methods have been published to determine flubendazole in eggs and/or muscle (1, 8, 9, 11). Only a few residue depletion data in eggs of laying hens are available in the literature (1, 8). By EEC Council Regulation No 2377/90 and updates (3), the EU sets the MRLs for FLUB in eggs at 400 μg kg^{-1} and for FLUB + HMET in poultry muscle at 50 μg kg^{-1}.
This poster shows the optimization, the validation and the application of a quantitative and sensitive LC-MS/MS analytical method for the determination of FLUB, HMET and RMET in eggs and poultry muscle. In this study, the excretion of flubendazole and its metabolites in turkey muscle and liver after oral administration of flubendazole at two concentration levels was examined.

2. MATERIALS AND METHODS

Because of good results in former research work on benzimidazole drugs in milk (4), the benzimidazole analytes were extracted with ethyl acetate after the sample mixture had been made alkaline (7). The HPLC separation was performed on a reversed phase C_{18} column using a mobile phase consisting of 0.04 M ammonium acetate (1, 8 10) adjusted to pH 5.2 (A) and acetonitrile (B). Gradient elution was applied and the programme consisted of 50A:50B (0 min), 50A:50B to 25A:75B (0-3 min), 25A:75B (4-5 min), 25A:75B to 50A:50B (6-7 min) and 50A:50B (8-15 min). The flow rate was 0.25 mL min^{-1} and the injection volume was 10 μL. The analytes were detected and identified with a tandem quadrupole mass spectrometer. Atmospheric pressure electro spray ionization in the positive mode (ESI^+) was applied. FLUB, HMET, RMET and the IS were determined with MS/MS by the multiple reaction monitoring function of the transition of the molecular, parent ion to the most abundant daughter ion.

The method is completely validated conform the EU criteria of decision 93/256/EC (6) for determination of drug residues. The validation parameters are linearity of response, matrix calibration curve, extraction recovery, limit of detection (LOD) and limit of quantification (LOQ), trueness, repeatability and specificity.

The discussed method was applied to a pharmacokinetic study with turkeys (5). In two pens, the turkeys were fed medicated feed containing 19.9 and 29.6 mg kg^{-1} flubendazole (Flubenol 5%, Janssen-Cilag, Beerse, Belgium) (State Analysis Laboratory, Tervuren, Belgium) for seven consecutive days during week 13 and week 15 of age for females and males respectively. Three male and three female turkeys were weighed and slaughtered at different ages according to the flubendazole feeding schedule, just before the start, daily during the administration and 2, 4 and 6h, 1, 2, 5 and 7 days post administration. At each time the same muscle group of breast and thigh and the liver were removed, frozen and stored at –18°C until investigation.

3. RESULTS AND DISCUSSION

The proposed MS detection method operating in the MS/MS mode is very selective and very sensitive. The limits of detection are around or lower than 1 μg kg^{-1}. A representative view of the separation and the detection

of FLUB, the metabolites and the IS of a blank egg sample spiked at 10 µg kg^{-1} is shown in the chromatogram in figure 1.

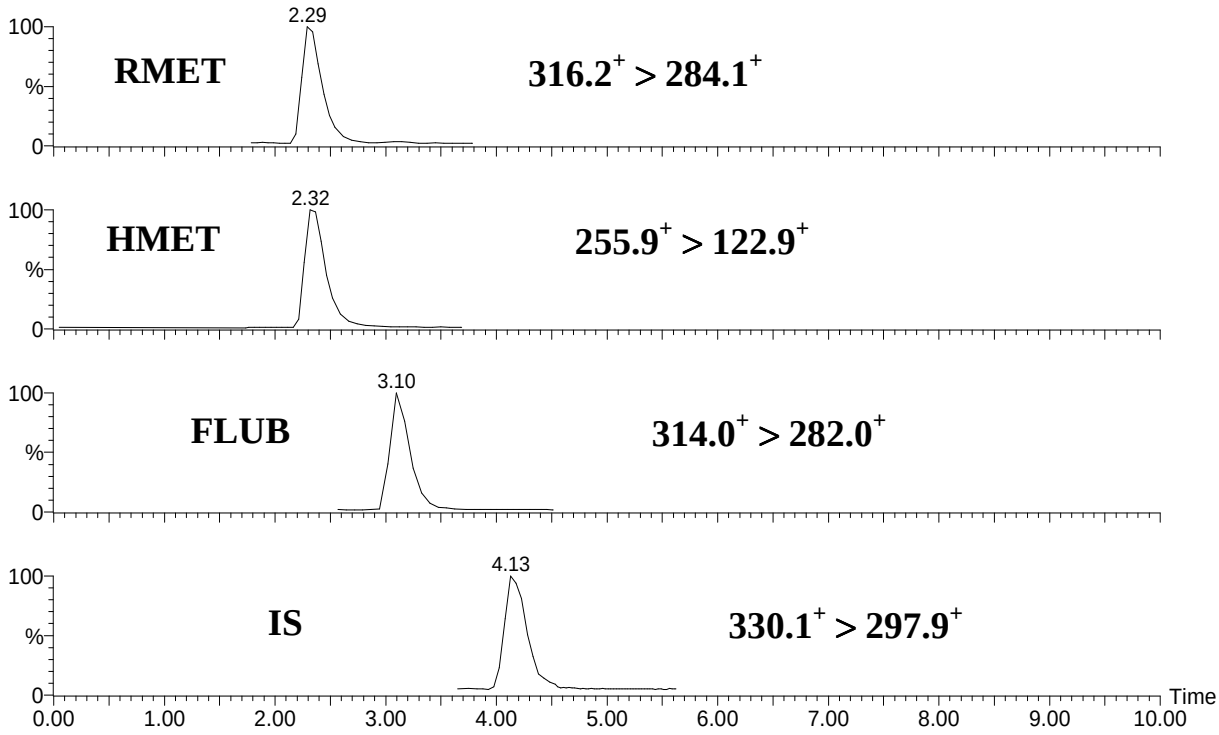

Figure 1. Chromatogram of a spiked egg sample at 10 µg kg^{-1} with a mixture of FLUB, HMET, RMET and IS

The validation parameters were completely in accordance with the criteria of the European rules (6). The over-all extraction recovery values for FLUB, HMET and RMET in eggs (fortification levels 200, 400 and 800 µg kg^{-1}) and muscle (fortification levels 25, 50 and 100 µg kg^{-1}) were respectively 77, 78, 80 and 92, 95 and 90%. The trueness (fortification levels 400 and 50 µg kg^{-1} respectively for eggs and muscle), expressed as percentage of the added values for these analytes were respectively 89, 100, 86 and 110, 110 and 98%. The LC-MS/MS confirmatory method operating in the MS/MS mode is very sensitive. The LODs for FLUB, HMET and RMET in egg and muscle were respectively 0.19, 0.29, 1.14 and 0.14, 0.75 and 0.31 µg kg^{-1}. The LOQs were respectively 1, 1, 2 and 1, 1 and 1 µg kg^{-1}.

One day after the end of the animal treatment, the mean sum of the FLUB + HMET residue values in thigh and breast muscle declined to around or below the MRL (50 µg kg^{-1}) and were respectively 36.6 and 54.1 µg kg^{-1}. The corresponding values with the higher dose of 29.6 mg kg^{-1} were respectively 101.7 and 119.7 µg kg^{-1} (5). Figure 2 represents the mean FLUB and HMET residue values in breast and thigh muscle during and after the administration of flubendazole medicated feed at the recommended dose of 19.9 mg kg^{-1}.

4. ACKNOWLEDGEMENTS

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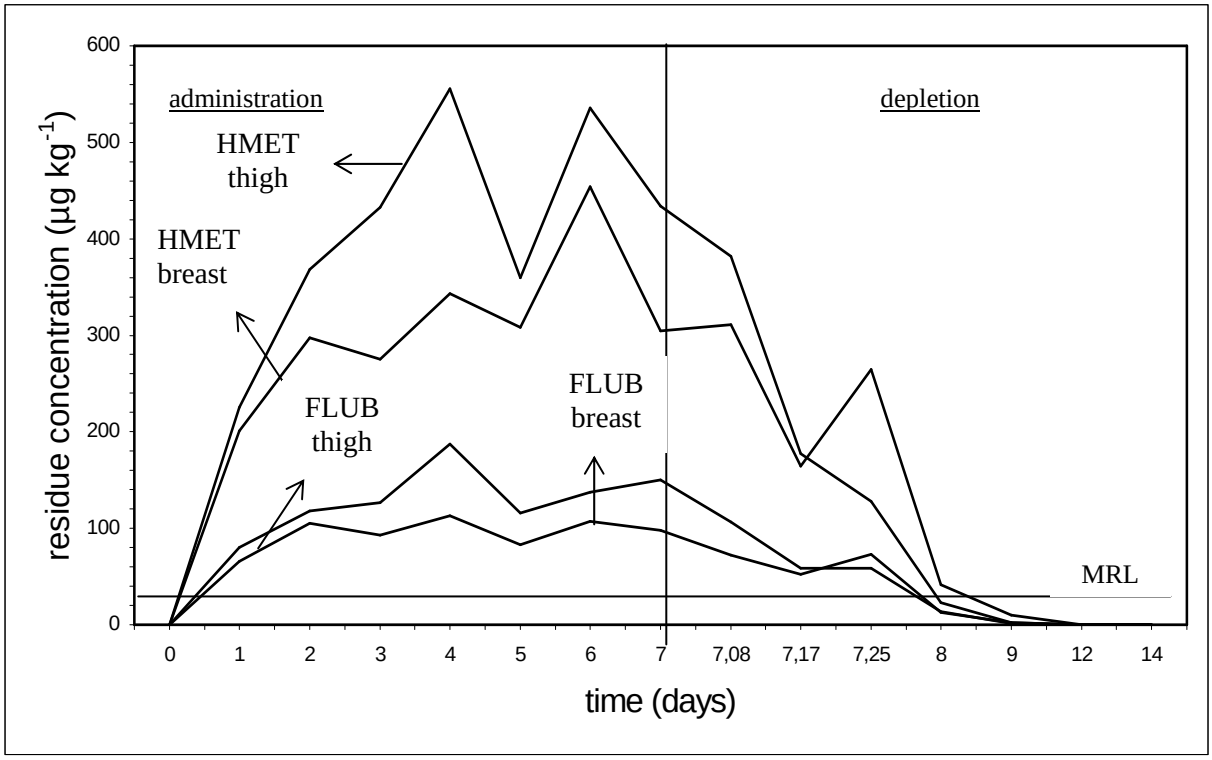

Figure 2. FLUB and HMET residues in turkey breast and thigh muscle during and after oral administration of 19.9 mg flubendazole kg^{-1} of feed

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INTEGRALE KWALITEITSZORGMELK: MOGELIJKHEDEN EN MOEILIJKHEDEN

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1. UITGANGSSITUATIE

De landbouwproductie wordt afgeremd. De rekken puilen uit van goedkope producten uit de ganse wereld. Kwaliteit is binnen een **overschottenmarkt** de meest bepalende factor geworden voor het landbouwinkomen. De producent biedt op de markt meer aan dan de verbruiker nodig heeft. De keuze naar kwantiteit en naar kwaliteit is groot. De keuze van voedingsmiddelen is overweldigend en van gans de wereld afkomstig. De wereld ons dorp. De Westerse consument is veeleisend, hoge kwaliteit tegen lage prijzen maar daarbij wordt kwaliteit als vanzelfsprekend beschouwd.

De grootdistributie bepaalt de behoeften. Afwijkende kwaliteit brengt niet alleen grote economische schade toe aan de producent en de verwerking maar tevens aan de distributie, die uit recente crisissen zijn lessen heeft getrokken. Voorkomen is beter dan genezen. Daarom stelt de grootdistributie zelf een aantal eisen aan zijn leveraars. Die eisen hebben niet alleen betrekking op de basiskwaliteit van de landbouwproducten maar evenzeer op de goede landbouwpraktijken van de producenten. Lastenboeken voor rundvlees, voor groenten en fruit, en voor tal van sectoren worden opgesteld, sommigen over de landsgrenzen heen door de Europese vereniging van grootdistributeurs.

De moderne verbruiker staat ver af van de huidige landbouwactiviteiten en van de hedendaagse voedselproductie. De **voedselketen** is complex en ondoorzichtig voor de consument. Wat vroeger geproduceerd werd vond meestal rechtstreeks of via plaatselijke handelaars zijn weg naar de verbruikers, waarvan trouwens een groot aantal in de landbouw of de aanverwante sectoren was te werk gesteld. Meer produceren betekende meer inkomen. Kwantiteit was gelijk aan verdienste en ondernemen gestoeld op massale maar goedkope inzet van arbeidskrachten.

De huidige, intensieve veeteelt wordt vastgelegd in eerder afschuwelijke beelden, voorbeelden zijn er bij de vleet: de massale afslachtingen van dioxinekippen, het stuntelig geschuifel van gekke koeien, de traditionele drijfmesttank en de bijhorende berichten over nitraatvervuiling. De spreekwoordelijke naald in de inmiddels verdwenen hooiberg wordt snel gevonden met methoden uit de spitstechnologie en de resterende sporen worden uitgedrukt in nanogrammen en picogrammen, in miljardsten en biljoensten van een gram.

De consument treft in dit alles geen enkele blaam, hij oordeelt op basis van de gegevens die hem voorgeschoteld worden. De **media** bepalen de werkelijkheid. De sector, in casus de melkveehouderij, is in dit verband aan een mentaliteitsverandering toe. De zuivelsector krijgt vandaag nog altijd veel krediet en vertrouwen van de moderne verbruiker, de biologische kwaliteit van de grondstof melk en van de afgeleide zuivelproducten staan buiten kijf. Het gevaar bestaat echter dat het product goed wordt bevonden maar de producent en zijn methoden eerder verdacht.

Beter zelf te sturen dan gestuurd te worden zal dus de leuze voor de toekomst moeten zijn. Beantwoord de vraag vooraleer ze wordt gesteld. Zeg wat je doet en handel ernaar, maar zorg er ook voor dat je geloofd wordt. De bewijsvoering en de betrouwbaarheid is een essentieel onderdeel van een doorgedreven **kwaliteitsmanagement**. Een klantgerichte benadering van kwaliteit met als doel datgene aan te bieden waar de klant recht meent op te hebben krijgt in de toekomst meer aandacht dan de productgerichte benadering waarvan het eindresultaat als vanzelfsprekend wordt beschouwd.

2. KWALITEIT

De inhoud van de vraag naar kwaliteit is nogal verschillend van de invalshoek van de betrokken partijen. De zorg voor de basiskwaliteit, het opstellen van de minimale kwaliteitseisen en de controle op de naleving ervan is een taak van de **overheid**. Deze basiskwaliteit moet voornamelijk gericht zijn op sanitaire aspecten zoals residuen, microbiologische normen, e.d. en gebaseerd zijn op objectieve, meetbare en wetenschappelijk gedocumenteerde normen.

Voor de **consument** blijkt kwaliteit nog altijd uitzicht en smaak te zijn. Enkel bij crisissen, wanneer emotionele argumenten het aankoopgedrag bepalen, worden labels en certificaten als een garantie ervaren. Het is belangrijk dat deze producten zijn geproduceerd op een manier zoals de consument en de distributie het wil: diervriendelijk, duurzaam, traceerbaar, gezond, zonder residuen. Met kwaliteitsborging probeert **de producent en de verwerker** het hele imago van de sector op te krikken en het vertrouwen van de consument te herstellen of te behouden.

Algemeen wordt gesteld dat traceerbaarheid, bewaking van de ganse keten, publieke toegankelijkheid, externe borging, sanctiebeheer en meldingsplicht minimaal moeten aanwezig zijn in een kwaliteitssysteem dat verder moet gaan dan enkel de minimale wettelijke normen.

3. KWALITEITSBELEID

Kwaliteitsbeleid is het sturen van de processen om de kwaliteit te beheersen. Kwaliteit is de som van de eigenschappen en de kenmerken van een product waarmee het aan een aantal specifieke behoeften voldoet. Kwaliteit is de som van een basiskwaliteit plus een onderhandelde kwaliteit plus een specifieke kwaliteit.

Basiskwaliteit bepaalt de minimale normen waaraan iedere participant binnen een kwaliteitsproject moet voldoen. Die normen worden gesteld door de overheid en kunnen zowel europees, federaal als gewestelijk bepaald worden. Basiskwaliteit omvat normen gericht op veiligheid en traceerbaarheid, op volksgezondheid en residupolitiek, op biodiversiteit en dierenwelzijn, op duurzaam ondernemen en milieu en op een aantal sociale aspecten van tewerkstelling. De controle gebeurt door de overheid zelf, rechtstreeks of via geaccrediteerde lastenboeken. Er bestaat zowel bij de overheid als bij de sectoren een behoefte aan overleg over deze minimumnormen, zowel op federaal als op gewestelijk niveau.

Onderhandelde kwaliteit bepaalt de bovenwettelijke normen die overeengekomen worden tussen personen en/of organisaties uit de distributie en de toeleveraars. Deze eisen hebben betrekking op smaak, uitzicht, voedingswaarde, hygiëne en veiligheid, service en gebruiksgemak maar toch ook op thema's zoals dierenwelzijn en milieu. Ook hier bestaat de behoefte om overleg te plegen over de haalbare normen. De controle gebeurt door de distributeurs zelf of via een geaccrediteerde autocontrole door de toelevering.

Specifieke kwaliteit wordt meestal vastgelegd in lastenboeken. Het doel van specifieke kwaliteitsborging is, inspelend op de vraag van de sector, het imago van bepaalde producten verbeteren om zo de verkoop te stimuleren en een sterke positie op te bouwen t.o.v. de distributie en de overheid. Het resultaat van deze lastenboeken zijn certificaten of labels al dan niet met herkomstbenamingen, productgaranties, biologische productie, ketenbeveiliging, e.d. Deze autocontrole-systemen behoeven omwille van geloofwaardigheid een externe borging en een overheidscontrole via accreditatie van de deelnemende labo's, inspecties en de certificering zelf. De initiatiefnemer voor de specifieke kwaliteit is de producent en de verwerker die in samenspraak met de ganse keten (interprofessioneel) een kwaliteitsvereniging opzet.

De sector bepaalt de normen van het lastenboek rekening houdend met de basisnormen, de met de distributie onderhandelde normen en de basisvoorwaarden zoals beschreven in de definitie van kwaliteit. De interprofessionele kwaliteitsverenigingen, per sector opgericht, certificeren op basis van een erkend lastenboek en inspecties die uitgevoerd worden door een geaccrediteerd labo.

4. INTEGRALE KWALITEITSZORG MELK (IKM): EEN KWALITEITSBORGINGSSYSTEEM

De producenten zijn zelf verantwoordelijk voor de kwaliteit en de veiligheid van de producten die ze voortbrengen. De producenten zelf zijn dan ook verantwoordelijk voor de controle op die kwaliteit en veiligheid. **Zelfcontrole** wordt dan ook de toegangscode tot de markt. De overheid moet hierbij instaan voor normering en voldoende inspecties.

De melk is tot nu toe waarschijnlijk de best overwaakte grondstof binnen de landbouwactiviteit. De globale kwaliteitscontrole gekoppeld aan een systeem van vergoedingen en strafpunten en de systematische controle op kritische punten binnen de totale productie zijn echter gericht op het product. De zelfcontrole hoort echter eveneens de productieomstandigheden te borgen. De moderne, geschoolde verbruiker wil immers ook weten hoe zijn product tot stand is gekomen. Termen uit de wereld van milieubeheer, dierenbescherming, biotechnologie, e.a. zijn in de zuivelsector binnengeslopen en hebben er zich onomkeerbaar gevestigd. Trouwens ook buiten de landbouw doen deze veranderingen zich voor. Werd enkele jaren geleden een bepaald merk van sportschoenen niet geboycot omdat ze geproduceerd werden in landen met kinderarbeid.

Aandacht voor de productieomstandigheden is minstens even belangrijk geworden als de zorg voor de intrinsieke productkenmerken. De nood om naast de huidige topkwaliteit van de melk tevens de productieomstandigheden en de voedselveiligheid te garanderen is perturbant. De goede productiemethode, de bezorgdheid voor dierenwelzijn en milieu, de zuiverheid en de veiligheid van het eindproduct, het vermijden van insleep van bedrijfsvreemde stoffen, dat zijn de onderwerpen van IKM, Integrale Kwaliteitszorg Melk. Naast de voedselveiligheid is er in IKM dus ook aandacht voor andere aspecten van een **duurzame landbouwproductie**. Een groot deel van deze borgingspunten gaan dus verder dan wat door de wetgever geregeld is. Hierna volgt een overzicht van deze **bovenwettelijke normen**.

Het IKM-certificaat waarborgt de goede landbouwpraktijk op het melkveebedrijf. Het lastenboek beschrijft meer dan honderd borgingspunten in vijf essentiële onderdelen van de melkproductiemethode. Dierengezondheid, dierenwelzijn, melkwinning, reiniging en milieu zijn de vijf modules waarin bestaande reglementering onderschreven wordt en aangevuld met een aantal punten van goede landbouwpraktijk.

De module **dierengezondheid** heeft als doelstelling om met ruime preventieve maatregelen en gerichte ziektebestrijding de gezondheid van de koeien te bevorderen. Een bijkomende algemene inspectie van de gezondheidstoestand van de koeien, de invoering van een geneesmiddelenregister, de vereiste van een GVP-erkenning (Good Veterinary Practice) voor de bedrijfsdierenarts en het instellen van een procedure voor het herkennen van met medicijnen behandelde dieren lopen een op til zijnde wetgeving vooruit. Verder wordt een beperking van het geneesmiddelengebruik nagestreefd om de veiligheid van de geleverde melk op elk ogenblik te garanderen o.a. door het jaarlijks verplicht nameten van de melkinstallatie.

In de tweede module **dierenwelzijn** zijn de huisvesting, de voeding en verzorging van het melkvee de beoogde borgingspunten. Voor de huisvesting zijn tal van normen ingevoerd i.v.m. bindstelsels, afmetingen van ligboxen en eetstanden, de veiligheid in de stal, de verlichting en de verluchting van de stal. Tevens wordt de beschikbaarheid en de kwaliteit van het drinkwater omschreven en wordt weidebeloop en grasvoorziening geregeld. Van de leveranciers van mengvoeders, natte bijproducten en droge enkelvoudige voeders wordt geëist dat zij een GMP-erkenning halen; verkopen tussen landbouwers onderling worden geregistreerd. Gescheiden opslag en respect voor de alfatoxine-convenant moeten de risico’s beperken.

De module **melkwinning** borgt het hygiënisch melken door een gemotiveerde melker met een goed functionerende melkinstallatie in een hygiënische omgeving. Ook de voorbehandeling van de uiers wordt een belangrijk punt in het IKM-kwaliteitsstreven. De goede bewaring van de melk in een snel en diep koelende melktank met voldoende capaciteit, een goed en regelmatig roerwerk, voorzien van een gemakkelijk afleesbare thermometer, een melkwacht en een instroombeveiliging (voor nieuwe tanks), geplaatst in een net, goed verlicht en verlucht, voldoende ruim en gemakkelijk bereikbaar lokaal wordt extra in de aandacht gebracht om mogelijke besmetting te vermijden. Zowel de melkinstallatie als de koeltank worden respectievelijk jaarlijks en tweejaarlijks uitgemeten door een erkend specialist die de goede werking nagaat volgens een ISO-normering.

Een doeltreffende **reiniging** van de melkveestal, van de melkinstallatie, van de koeltank en van het melk- en tanklokaal staan in de module reiniging centraal. Ook worden de gebruikte reinigings- en ontsmettingsmiddelen en hun dosering gecontroleerd evenals de reinigingstemperatuur en het gebruikte water dat van drinkwaterkwaliteit moet zijn.

Uiteindelijk als laatste maar niet als minste de module **milieu**. Borgingspunten over de veilige opslag van reinigingsmiddelen en over de verzorging van de bedrijfsomgeving moeten de optimale bedrijfsomstandigheden creëren voor een gezonde, veilige en verantwoorde melkproductie.

Voor het eerst in België is zo een alles omvattend kwaliteitssysteem op het spoor gezet. De zuivelsector wil met deze kwaliteitsbenadering via autocontrole aan de spits blijven staan van de integrale ketenbewaking. De uiteindelijke doelstelling is de bijna totale Belgische productie van rauwe melk binnen een tweetal jaar in het

IKM-label te borgen. Verder wordt in diezelfde periode de ganse keten van producent tot consument in een duurzaam systeem vastgelegd om het kwaliteitsimago van de melkveehouderij naar de verbruiker toe te versterken.

De start werd gegeven met de productie van de melk op de melkveebedrijven, maar in de loop van dit jaar volgt tevens de melkophaling, het transport en de aflevering. Het lastenboek IKM-transport omvat 48 borgingspunten over de melkophaling, het transport, de melkontvangst, de reiniging, de chauffeurs en de identificatie van de leveraars. De verwerking door de melkerijen, die momenteel gebeurt op basis van ISO- en HACCP-lastenboeken, en de distributie van de afgewerkte producten zijn onderwerp voor een voorbereidende studie vanaf volgend jaar. Het eerste luik van de keten is volop in uitvoering. Momenteel, na één jaar werking, heeft ongeveer 60 % van de melkveehouders in België een aanvraag voor de certificering van zijn bedrijfsvoering lopen en zijn er IKM-certificaten uitgereikt aan ongeveer 50 % van de producenten.

5. MOGELIJKHEDEN EN MOEILIJKHEDEN

Aangezien melk een bulkproduct is en de traceerbaarheid niet tot de individuele producent en de individuele koe kan gewaarborgd worden, kan en wil IKM dus niet aan productcertificatie doen. IKM kan geen label zijn. Dit betekent dat er in de rekken bij de distributie geen keuze kan zijn tussen IKM-gelabelde producten en dezelfde producten zonder IKM-label. IKM wil aan systeemcertificatie doen voor de ganse keten en voor de totale productie. Dit betekent dat alle schakels binnen de keten bereid moeten zijn om mee te werken en dat binnen elke schakel alle deelnemers zich moeten engageren. Hiermee wil de zuivelsector tevens een versnippering naar verschillende kwaliteitssystemen en het ontstaan van verschillende, concurrerende labels voorkomen.

Het kwaliteitsstreven en de zorg voor veiligheid in de productie kan maar naar zijn waarde geborgd worden als ook de **toelevering** naar het productiebedrijf zichzelf een borgingsysteem oplegt. Wat de wetgever soms met moeite kan opleggen komt onder druk van de melkveehouder, die klant is bij de toelevering, in een stroomversnelling terecht. Voor de voedingssector is daarover geen twijfel meer. Recente crisissen zoals de dioxinecrisis in de mengvoederindustrie en de gipscrisis in de pulpindustrie hebben tot schade en schande van de hele sector de vinger op de wonde gelegd. Nochtans blijven vandaag nog altijd tal van industriële afvalproducten een weg vinden naar de melkveevoeding. GMP (good manufacturing practice) voor de veevoederindustrie en voor de producenten van droge enkelvoudige voeders en van natte bijproducten is een noodzaak als toegang tot de markt van de melkveehouderij. Of hoe de brouwers zich verantwoordelijk moeten achten voor de afgeleverde draf, de oliebereiders voor de schroten en schilfers, de chipsindustrie voor de aardappelafval, de suikerfabrieken voor de pulpen, de mengvoederfabrikanten voor de melkveevoeders, de kernvoederfabrieken voor de mineralen en premixen, en zo voort.

Meer problemen zijn er bij de reinigingsmiddelen en de dip- en sprayproducten. Het ontbreken van erkenningen voor ontsmettingsmiddelen is opvallend maar de dubbelzinnige regelgeving voor dezelfde producten verkocht als reinigingsmiddelen is nog meer verbazend. Zowel de dip- en spraymiddelen als de reinigings- en ontsmettingsmiddelen komen rechtstreeks met het voedingsmiddel melk in aanraking en kunnen dus aanleiding geven tot insleep van bedrijfsvreemde stoffen. Vandaar dat ook in deze tak van de toelevering een strikte opvolging van de wetgeving, inclusief traceerbaarheid, en een strikte borging van de kwaliteit en de veiligheid van het grootse belang zijn in een integrale ketenbewaking.

Een derde belangrijke contaminatiebron kan het gebruik van diergeneesmiddelen en de veterinaire behandeling zijn. Residuen van antibiotica zijn niet alleen een probleem in de verwerking van melk en de bereiding van bepaalde zuivelproducten, ze kunnen aanleiding geven tot resistentie van bepaalde bacteriën waardoor het gebruik van dezelfde producten in de humane geneeskunde dreigt ondoelmatig te worden. Hieromtrent is een nieuwe wetgeving van toepassing die echter nog in de gepaste uitvoeringsmodaliteiten moet gegoten worden. IKM heeft echter zelfs een medicamentenregister ingevoerd om dit probleem op te lossen.

De reactie bij de **producenten** is nogal uiteenlopend. Een hele reeks bedrijven zijn onmiddellijk en zonder extra inspanningen klaar voor certificering. Deze bedrijfsleiders waren reeds van vroeger begeesterd door kwaliteitsstreven. Het zijn tevens deze mensen die actief zijn in de beroepsorganisaties en dus medespelers zijn in de ontwikkeling van het IKM-project. Hun aanvragen voor certificering zijn dan ook snel binnengekomen. Een tweede groep melkveehouders die kleinere investeringen moeten doen om in orde te zijn met het lastenboek, volgden in een tweede peloton. Bij deze groep werden overwegingen gemaakt over de kosten voor certificering en de mogelijke meeropbrengst van die certificering zowel korte als op lange termijn. Een groep melkveebedrijven die onvoldoende aangepast zijn aan de toenemende eisen van de moderne melkproductie zullen in versneld tempo de achterstand moeten inlopen. De verschillen in prijs en de separate behandeling van

IKM-gecertificeerde en niet IKM-gecertificeerde melk zullen bepalend zijn voor de gevraagde aanpassingen. Een deel van deze groep zal waarschijnlijk niet in aanmerking komen voor certificering. Verwacht wordt dat deze op termijn onmogelijk kunnen blijven produceren en dat hun melkhoeveelheden overgelaten worden aan veehouders die wel IKM-geborgd zijn.

IKM heeft een positief effect op de kwaliteit van de productie van melk en op de kwaliteit van de melk, maar biedt onvoldoende waarborgen voor maximale voedselveiligheid. Invoering van HACCP op de primaire bedrijven vermindert de risico's en verhoogt de garanties op veilig voedsel. Verwacht wordt dan ook dat HACCP op korte termijn op de melkveebedrijven wordt verplicht. Studies zijn momenteel bezig om de mogelijkheid te onderzoeken om het IKM-lastenboek bij te sturen naar aangepaste HACCP-normen. De eigen ruwvoederwinning op de producerende bedrijven is niet echt opgenomen in de kwaliteitsborging. Hier zijn nochtans een aantal risico's voor insleep van bedrijfsvreemde stoffen met name via het zaaizaad, de bemesting, de plantenbeschermingsmiddelen, het loonwerk, e.a. Over ruwvoeder zal in het lastenboek een aparte module worden toegevoegd.

De verwerkende **zuivelindustrie** is veelal de stuwende kracht voor de implementatie van het IKM-project naar de de producenten toe. Alle melkerijen die in Vlaanderen actief zijn, onderschrijven deze kwaliteitsbenadering via autocontrole. Vooral de eigen organisatie BCZ (Belgische Confederatie van de Zuivelindustrie) heeft hier het voortouw genomen. De verwerkende industrie heeft zich tevens voorgenomen de melkophaling, het transport en de ontvangst van melk in de ophaalcentra tegen het einde van dit jaar te certificeren. De verdere verwerking van de melk binnen de zuivelindustrie en de behandeling van de afgewerkte producten gebeurt volgens HACCP-methoden en ISO-procedures. Er zal nagegaan worden in hoever deze kwaliteitsborging binnen een IKM-ketenbewaking kan ingepast worden.

Voornamelijk de dialoog met de **distributie** en de **consument** blijft momenteel problematisch. Enerzijds heeft IKM nog weinig te garanderen omdat slechts de helft van de productie gecertificeerd is en nog niets van de transporten en de verwerking. Anderzijds is het vertrouwen van de distributie en de consument in de zuivelproducten opvallend hoog en wordt aan de zuivelketen veel krediet gegeven. Alhoewel een aantal verenigingen van distributeurs gemeenschappelijke normen en standaards uitschrijven zoals BRC en EurepGAP, zijn verschillende, grote distributeurs afzonderlijk met eigen kwaliteitssystemen bezig. Hierin schuilt voor een uniek systeem als IKM een gevaar om zijn uiteindelijk doel, de verbruiker, niet te bereiken. Uiteindelijk is het ook deze groep die moet bereid gevonden worden om de inspanningen van de keten op een redelijke wijze te vergoeden. Aan steeds stijgende kwaliteitseisen hangt immers een prijskaartje. Indien dit niet kan verwezenlijkt worden komt de rendabiliteit van de keten in gedrang. Voornamelijk de producenten krijgen het dan het zwaarste te verduren.

De Vlaamse **overheid** wil in verband met kwaliteitszorg een nieuw en sturend beleid voeren en wil daar ook de organisatorische en financiële consequenties van dragen en de afzonderlijke financiering daarvoor onderzoeken. De thema's van de Vlaamse overheid zijn voornamelijk de duurzaamheid van de landbouw, de biodiversiteit, het milieu, e.a. De federale overheid ontwikkelt binnen het Federaal Agentschap voor Veiligheid in de Voedselketen de thema' voedselveiligheid en traceerbaarheid en binnen het Ministerie van Volksgezondheid o.a. de thema' volksgezondheid en dierenwelzijn. Voor beide niveau's wil het IKM-project als bewaker van deze items in de zuivelketen een voordeelstatus bereiken en op die manier dubbele controles en dubbele kosten vermijden.

Vooral de benadering van kwaliteit door samenwerking van **de volledige keten** van producent tot consument blijkt dus een medaille met een keerzijde. De moeilijkheden situeren zich voornamelijk bij de organisatie van alle deelnemers binnen alle schakels van de keten. De voordelen zijn de mogelijkheid om de kwaliteit te sturen, de duidelijkheid naar de consument en de overheid, de synergie van het kwaliteitsstreven in de verschillende schakels en de kracht van het kwaliteitssysteem naar de buitenwereld.

AN EXPERIMENTAL INFECTION TO INVESTIGATE THE SPREAD OF THE CLASSICAL SWINE FEVER VIRUS BY EXCRETIONS OF INFECTED PIGS.

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1. INTRODUCTION

Different routes for between-herd transmission of the Classical Swine Fever (CSF) virus have been described. The most cited are direct animal contact, mechanical vectors such as vehicles, equipment and persons, artificial insemination with semen from infected boars, and neighbourhood contacts (7, 10, 11). Most of these virus transmission routes were identified based on epidemiological research and expert opinions (3, 4, 9, 12). Only a limited number of these types of virus transmission have been identified under experimental conditions (1, 2, 5). Hughes and Gustafson (1960) found that only 2 out of 10 susceptible pigs that were brought in close contact with excretions of infectious pigs became infected. In preliminary experiments by our group (Koenen: unpublished data) inoculation of susceptible pigs with faeces from infectious pigs did not succeed.
Because of the above-mentioned contradiction an experiment was designed to evaluate the importance of virus spread by mechanical vectors contaminated with excretions of infected pigs. More specific a field situation where susceptible pigs are transported with a vehicle that previously transported infectious pigs was mimicked in an experimental setting.

2. MATERIAL AND METHODS

2.1 Animals

Twenty conventional weaner pigs of 12-15 kg, originating from an isolated pig herd and controlled for the absence of bovine viral diarrhoea virus (BVDV) and CSFV antigen and antibodies, were used.

2.2 Virus

The isolate used for the experimental inoculation was originally obtained from the first CSF-infected herd of the 1993-1994 Belgian epizootic. The isolate was verified to be free of African swine fever virus and BVD virus. By using monoclonal antibodies, it was characterized to be similar to an isolate known as the 'souche Lorraine' (6). Virus infectiousness was 10^3 median tissue culture infective dose ($TCID_{50}$ / ml).

2.3 Experimental design

Upon arrival, 10 pigs were randomly allocated to 5 pens (2 pigs per pen). After an acclimatisation period of 6 days all pigs were individually inoculated with CSF virus by deep intramuscular injection (2 ml) plus intranasal inoculation (2 ml). During an eight days period (incubation period), the infection status of the inoculated pigs was checked by clinical examination, rectal temperature monitoring, and blood sampling. After this incubation period the pens were depopulated and the pigs were euthanized.
Twenty hours after depopulation the pens were restocked with 10 susceptible weaner pigs (random allocation, 2 pigs per pen). The susceptible pigs stayed in the pens during the next 35 days (observation period). After the observation period all pigs were euthanized. During the incubation period, in the period between depopulation and restocking, and during the first two weeks of the observation period, the pens were neither cleaned nor disinfected. During the whole experiment the temperature in the pens was around 20°C.

2.4 Sample collection and analyses

During the incubation period and the first two weeks of the observation period clotted and heparinized blood samples were collected every two days. During the last three weeks of the observation period blood samples were taken weekly. During the whole experiment rectal temperature was monitored daily. From every pig that died or was euthanized, tissue samples, blood, faeces and nasal swabs were collected. Both at the moment of depopulation and at the moment of restocking 2 faeces samples were collected in each pen.

For virus isolation (VI) 100µl whole blood, serum or buffy coat was inoculated in duplicate onto a non-confluent monolayer of PK15 cells cultured in multiwell plates (24 wells / plate). For VI in tissue samples one cm^3 of each organ was put into 9ml minimal essential medium (MEM) and grounded with an ultra-Turrax (Janke and Kunkel). After centrifugation for 10 min at 4000g, 300µl of the supernatant was inoculated in duplicate onto a non-confluent monolayer of PK_{15} cells cultured in multiwell plates (24 wells / plate). After 48 hours, the cells were fixed with isopropanol and stained with a polyclonal fluorescein-conjugated anti-CSF immunoglobulin. Virus titration was done using ten fold dilutions. For antigen detection an Ag ELISA (CHEKIT, Dr. Bommeli AG, Libefeld-Bern) on serum was used. Additionally, a single tube RT-nPCR test (8) was used to detect virus in blood samples. For antibody detection in serum, the virus neutralisation (VN) test was performed.

3. RESULTS

The experimental inoculation succeeded in all pigs (Table 1). The average time between experimental inoculation and onset of viraemia, based on the VI in whole blood, was estimated to be 3.2 days. On the day of depopulation virus titres in whole blood varied between 10^{-3} and 10^{-4}. Moreover, blood samples of all pigs were positive in all tests used to indicate viraemia. At the end of the incubation period clinical symptoms such as erythema, conjunctivitis, diarrhoea and ataxia were present. Furthermore the rectal temperature of all pigs rose above 40°C (Figure 1).

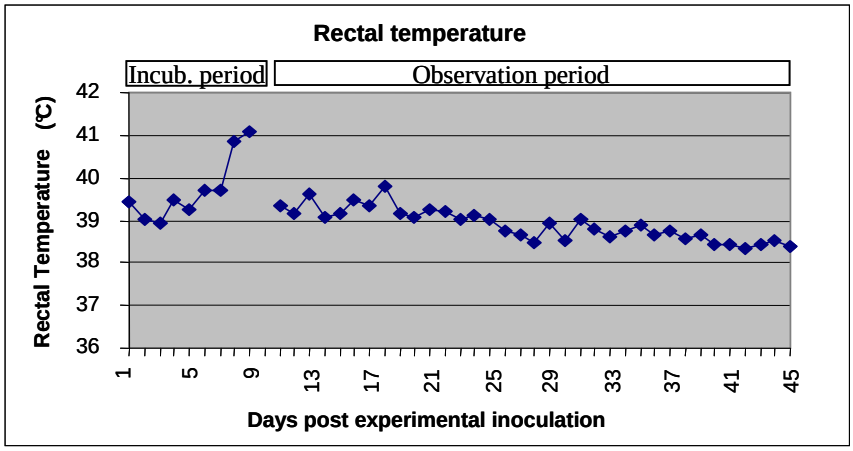
Table 1: Virus detection

Virus Isolation in whole blood

	Incub. period				Observation period										
	0	2	4	6	8	9	11	13	15	17	19	21	23	30	37	44
1.1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	
1.2	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	
2.1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	
2.2	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	
3.1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	
3.2	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	
4.1	0	1	1	1	0	0	0	0	0	0	0	0	nd	nd	nd	
4.2	0	1	1	1	0	0	0	0	0	0	0	0	0	0	nd	
5.1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	
5.2	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	

Days post experimental inoculation

Figure 1: Average rectal temperature curve



In only one pig virus could be isolated (titre 10^{-1}) from the faeces collected directly from the colon (Table 2). On the other hand virus could be isolated from most of the tissue samples, and from the nasal swabs of four of the

inoculated pigs (Table 2). The virus titres in the nasal swabs varied between 10^{-1} and 10^{-2}. From the faeces samples collected in the pens at the moment of depopulation and at the moment of restocking no virus could be isolated.

Table 2: Virus Isolation in different tissues and blood, faeces, and saliva samples after slaughter

	Virus Isolation in experimentally inoculated pigs							
	tonsil	kidney	spleen	hart	liver	faeces	nas. fl.	blood
1.1	1	1	1	1	1	1	0	1
1.2	1	1	1	1	0	0	0	1
2.1	1	1	1	1	1	0	1	1
2.2	1	1	1	1	1	0	1	1
3.1	1	1	1	1	1	0	1	1
3.2	1	1	1	1	0	0	1	1
4.1	1	1	1	1	0	0	0	1
4.2	1	1	1	1	1	0	0	1
5.1	1	1	1	1	0	0	0	1
5.2	1	1	1	1	0	0	0	1

During the observation period all blood samples of all pigs remained negative for VI in whole blood, leucocytes and serum, as well as for RT-nPCR and Ag-ELISA, and no antibodies could be detected. Additionally all tissue samples were negative for virus isolation. Also no clinical signs or fever could be detected during the observation period (Figure 1).

Two pigs (nrs 4.1 and 4.2) died 17 and 32 days after restocking, respectively. The death of pig nr 4.1 was due to stress during the blood sampling, whereas for pig nr 4.2 the cause of death remained unknown. However, since all blood as well as tissue samples of both pigs were negative during the whole observation period and after death it was concluded that they did not die as a result of a CSF infection.

4. DISCUSSION

The experiment was designed to correspond as much as possible to the field situation. Therefore the incubation period was deliberately limited to 8 days to allow all pigs to become viraemic but to avoid the pigs to become undeniably clinically diseased since visibly diseased animals are unlikely to be transported during a CSF epidemic. The time interval between depopulation and restocking was set to be 20 hours mimicking a vehicle transporting infectious pigs on one day and susceptible pigs the next day. However this time interval is believed to be of minor importance since CSF virus can remain infective up to 2 weeks in liquid manure kept at 20°C (13). The fact that the pens were neither cleaned nor disinfected between depopulation and restocking mimics a worst-case scenario where the mandatory hygienic procedures of cleaning and disinfection between subsequent animal transports were totally ignored. On the other hand, it should be stressed that the risk of virus spread via contaminated vehicles includes more then the vehicle itself. It also includes the driver who can wear contaminated clothing or footwear or uses contaminated equipment. These modes of transmission were not examined in this experiment.

Although most of the pigs were already viraemic 5 days prior to depopulation, the incubation period was most likely too short for the infected pigs to shed enough virus to contaminate the environment and subsequently to infect susceptible pigs. This conclusion is based on the fact that no virus could be isolated from the faecal samples in the different pens at depopulation and restocking. Regarding the fact that all pigs were already viraemic during five days, with high virus titres on the day of depopulation, this failed transmission is rather unexpected.

The result of this experiment indicates that virus spread via excretions is of minor importance in the early stages of the infection. Based on these results, there are arguments to assume that the importance of virus spread via mechanical vectors has been overestimated in studies on the epidemiology of CSF. This may imply that the importance of other known virus transmission routes, e.g. illegal animal contacts or visits by screening teams, have been underestimated. Furthermore, this could also mean that the number of herds, preventively emptied because of a so-called high-risk contact during a CSF epidemic, could possibly be reduced. To be able to evaluate in more detail the spread of CSF virus by excretions of infectious pigs, more experiments are needed to examine different incubation periods, virus strains or housing conditions.

Finally it should be stressed that the results of this experiment may in no means be interpreted as that hygienic procedures should be neglected during the control of a CSF outbreak.

5. ACKNOWLEDGEMENTS

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A CONSUMER SURVEY ON THE USE OF EGGS IN THE HOUSEHOLD WITH FOCUS ON MICROBIOLOGICAL FOOD SAFETY

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1. INTRODUCTION

A survey of parents of students of a higher education college (KHBO campus HTI, Brugge) was carried out to assess the purchasing behaviour, the storage conditions and the consumption of eggs in the household, aimed at quantifying microbiological unsafe handling.

2. MATERIALS AND METHODS

A questionnaire with 23 questions was distributed among 964 students of KHBO campus HTI Brugge for their parents to fill in. All questions were of the categorical or multiple response type. The questions can be subdivided in 3 categories: sociologic information, purchase and storage conditions of the eggs and type of egg dishes.

3. RESULTS AND DISCUSSION

537 valid questionnaires were returned (55.7% of total distributed questionnaires). The average consumption of eggs is 6.3 per person per month with a standard deviation (s.d.) of 4.8. The majority of these eggs are bought at a super market (43 %), but also a rather high share of own chickens is reported (18 %). Only 39 % pays attention to the price, but 75 % watches the shelf life. According to the survey, 37 % of the eggs bought in shops are from free-range chickens. This number contrasts sharply with the official number of 3 % free-range eggs in the total egg supply (1). This large percentage is in agreement with the relatively small amount of people who claim not to give attention to the price. A maximum likelihood chi-square (MLC2) test showed that the relationship between these two items is significant. The eggs are mainly kept in a refrigerator (59%) and the mean temperature of storage, as estimated by the interviewees, is 10°C (s.d. 5.6). It was verified with a MLC2 test that there is a significant relation between keeping temperature and place of storage. An additional spot check was done on 10 families where the temperature of egg storage was measured with a thermometer. This revealed that people tend to underestimate the real temperature in the refrigerator by more than 2°C. The interviewees were asked to quantify the frequency with which they prepared certain egg dishes on a 5-point scale. The dishes were afterwards combined into 3 categories: safe, middle and more hazardous types. A random resampling of the 3 distributions allowed constructing a hazard behaviour distribution (2). 0.4 % of the interviewees handle their eggs in hazardous way, while 7.8 % fall in a medium hazardous category.

Quantitative risk analysis of microbiological safety of eggs and egg products is only possible when sufficient data is available on the consumer link. Such information can only be obtained with surveys as the one presented here. They provide valuable information on risky conduct and can help to identify critical points where more measures and increased consumer awareness is necessary.

PREVALENCE AND CONTROL OF BACTERIAL PATHOGENS IN THE PIG PRODUCTION CHAIN

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ABSTRACT

Safety has become a very important aspect of meat quality, including the absence of pathogens (mainly *Salmonella*, *Campylobacter* and *Listeria monocytogenes*). For *Salmonella* and *Campylobacter* the upward trend in human reports is mirrored by that in farm animals, including pigs. The most prevalent *Salmonella* serotype isolated from pork samples is *S.* Typhimurium. Of particular concern is the emergence of the multiple antibiotic resistant *S.* Typhimurium DT104 in pig herds. Animals are considered to be the natural reservoirs of *Campylobacter* with a predominance of *Campylobacter coli* in pigs. In most cases the animals are symptomless carriers of these pathogens. As a consequence, apparently healthy animals arriving at the slaughterhouse can be shedding pathogenic bacteria so that other animals and carcasses are quickly cross-contaminated. For *L. monocytogenes*, various studies have demonstrated that the contamination of pork products is mainly derived from cross contamination between pork meat and the environment of chilling and cutting rooms or further processing steps. For efficient control of these pathogens, efficient and accurate detection methods (bacteriological or serological) and intervention strategies to reduce carriage of *Salmonella* in pigs are necessary, and the contamination cycles both at the farm level and at the slaughterhouse have to be broken.

1. INTRODUCTION

Pig production in Flanders is confronted with a variety of new responsibilities in the field of environment, animal welfare, food safety (zoonotic bacteria, residues) and meat quality. The consumer is more critical about the quality of meat and its related products. Recent scandals (hormones, dioxin), the detection of antibiotic residues and the outbreaks of several infectious diseases carried over to humans (BSE) have raised the consumer concerns about safety and meat quality issues. This implies that the classical production systems, mainly focusing on performance and carcass quality traits, have to take into account new aspects directed towards consumer and society demands. Safety has become a very important aspect of meat quality, including the absence of pathogens (mainly *Salmonella*, *Campylobacter* and *Listeria monocytogenes*) and of residues (antibiotics and tranquillisers). In all developed countries, an increase in food infections is noticed during the last 10 years. *Campylobacter* and *Salmonella* are now the most common bacteria, giving rise to food poisoning. For *Salmonella* and *Campylobacter* the upward trend in human reports is mirrored by that in farm animals, including pigs. A serious problem is that the mechanisms of infection, colonisation and shedding of these pathogens are poorly understood so that control measures on farms (e.g. vaccins) are not available or ineffective. The removal of antibiotics in feed as growth promoter might also increase the continuous colonisation and shedding of these pathogens. In this paper, data are presented from own research as well as from literature on the prevalence of the different pathogens mentioned in the pork production chain. In the own research, molecular identification, detection and typing techniques were used. It will be evaluated how these data can help to elaborate more efficient control measures.

2. MATERIALS AND METHODS

2.1. Sample collection

Four pig farms were sampled: two closed farms, one closed farm where also cows and calves were kept and one fattening farm. In a first screening, different age groups of pigs were tested for the presence of *Salmonella* and *Campylobacter*. In a second screening, individual pigs of the same age group (20 to 30 pigs) were examined by

taking a rectal swab (± 0.1g of faeces) and additionally pooled ground faecal samples of different pens. For testing the environment of the farm, an overshoe sample and a swab sample of the footwear of the farmer were taken. The feed and drinking water in the pens were tested by taking a swab sample in every pen. If available, faecal material of other animals from the farm was taken. On farm 1, two groups of pigs with the same age were followed. At the other farms only one group was followed.

Samples were collected in five commercial slaughterhouses named A, B, C, D, and E, located in the western part of Belgium, where a dense pig area is situated. The slaughter pigs sampled were marked at the beginning of the slaughter line (in A2 and D1, each 25^{th} and 50^{th} pig was marked, respectively). From these pigs, different samples were taken along the slaughter line: rectal contents, mesenteric lymph nodes, a piece of the diaphragm, and carcass swabs. One half of the carcasses were swabbed according to the procedure described by Korsak *et al.* (18). The inside and the outside of the ham were swabbed together with the sternum along the incision line. Sterile gloves were used and changed after every sampling. In the "clean zone", the environment was sampled by taking overshoe-samples during slaughter. The knife blade was swabbed from its tip to its base twice. The blade from the splitting machine was swabbed on both sites.

2.2. Bacteriological methods

For isolation of *Salmonella*, rectal swabs were incubated in 50 ml of buffered peptone water (BPW, Oxoid Ltd., Basingstoke, England), environmental swabs were incubated in 100 ml BPW, overshoes in 225 ml BPW, carcass swabs and 5 g of the faeces were incubated in 50 ml of BPW. The lymph nodes were trimmed of any attached fat, meat or other tissue in order to keep the lymph node intact. Ten gram was homogenised in a stomacher bag with 50 ml BPW for two minutes. All samples were incubated at 37°C overnight. After this non-selective enrichment, selective enrichment was done in Rappaport Vassiliadis broth (RV, Oxoid) and on diagnostic semi-solid *Salmonella* agar (Diassalm, LabM, Bury, UK) at 42°C for 24h. A 10 µl loop of the RV broth and a purple migration zone on Diassalm was streaked on xylose lysine desoxycholate agar (XLD, Oxoid) with. 24 h incubation at 42°C. Presumptive *Salmonella* colonies on XLD were confirmed by PCR (see below).

For isolation of *Campylobacter*, the rectal swabs and the swabs of the feed and drinking water, 1 g of faeces and carcass swabs were incubated in 40 ml of Preston broth (Oxoid) supplemented with 5% blood under microaerophilic atmosphere (O_2 5%; CO_2 7.5%; H_2 7.5%; N_2 80%) for 24 and 48 h at 42°C. From the BPW homogenate (see *Salmonella*) of the overshoe samples and the feed samples, 10 ml was taken before incubation, and incubated in 40 ml Preston broth. After enrichment, a 5 µl loop was streaked on *Campylobacter* blood free selective agar base (CCDA, Oxoid). The glazy presumptive *Campylobacter* colonies were first examined microscopically for the typical spiral morphology and further identified by PCR (see below).

2.3. Identification and typing methods

For the preparation of crude cell lysate, a bacterial cell pellet was lysed in 100 µl of 0.05M NaOH, 0.125% SDS and heating for 17 min at 90°C. For *Salmonella* confirmation by PCR, *Salmonella* specific primers described by Aabo *et al*. (1) were used. The *Campylobacter* isolates were identified to the species level by a multiplex PCR that differentiates *C. coli* and *C. jejuni* (22).

For REP-PCR, the primers described by Versalovic *et al*. (35) were used. All the *Salmonella* isolates were initially typed to the serotype level with REP-PCR (14). From each different REP type, one strain was sent to the Veterinary and Agrochemical Research Centre (Ukkel, Belgium) for conventional serotyping.

For determination of the antibiotic resistance profiles of the *Salmonella* isolates, 6 different antibiotics were tested: ampicillin, tetracycline, streptomycin, nalidixic acid, chloramphenicol and sulfadiazine (Sigma Aldrich, Belgium). The minimal inhibitory concentration (MIC) was determined in Mueller Hinton broth (Oxoid) at 37°C by making a two fold serial dilution of the antibiotic in a microtiter plate. From this MIC values we classified the *Salmonella* fenotype as resistant (R), sensitive (S) or intermediary (I).

2.4 Serological method

To measure the immunological response for *Salmonella* in swine meat juice, the German serological test kit Salmotype®-ELISA (Labor Diagnostik, Liepzig, Germany) was used following the supplier 's instructions. The test result is presented as the percentage optical density of the sample, relative to the optical density of positive reference samples (%OD). As cut-off value both %OD >40 (recommended by the kit) and %OD >10 (under discussion) are presented.

3. RESULTS

Table 1 presents the results about the prevalence of *Salmonella* on four different farms. On farm 3, *Salmonella* was not isolated. In total fifteen rectal samples were positive for *Salmonella* Typhimurium $O5^+$ and these were all taken from pigs at farm 1. In the environment of farm 1, *S.* Schwarzengrund was isolated from the feed trough and *S.* Typhimurium $O5^-$ and *S.* Typhimurium $O5^+$ from an overshoe sample. In the other two farms, *Salmonella* was isolated only in the environment (*S.* London in farm 2 and *S.* Typhimurium in farm 4). Most of the *Salmonella* strains were sensitive for the 6 antibiotics tested. Two strains isolated from an overshoe sample and one from a swab sample of the drinking water in the pens were resistant to ampicillin. One *S.* Typhimurium strain isolated from an overshoe at farm 4 was resistant to the 4 antibiotics ampicillin, tetracyclin, chloramphenicol and sulfadiazine.

From the 150 rectal swabs taken at the four farms, 51 were positive for *Campylobacter*. *Campylobacter* was isolated at all the pig farms with about the same frequency, namely from 30 to 40 % of the rectal swabs (Table 1). Also in the environment (overshoe sample), *Campylobacter* was isolated on all farms. All the *Campylobacter* strains were identified using PCR as *Campylobacter coli*.

Table 1: The prevalence of *Salmonella* and *Campylobacter* on 4 different pig farms.

Sample	Farm 1	Farm 2	Farm 3	Farm 4
Overshoes				
Salmonella	5/7°	1/6	0/3	2/14
Campylobacter	2/4	2/5	1/7	1/6
Faeces other domestic animals and farm environment				
Salmonella	1/4	0/1	0/4	ND
Rectal swabs				
Salmonella	15/101	0/26	0/50	0/60
Campylobacter	17/52	7/18	14/50	13/30
Feed				
Salmonella	1/8	0/2	0/8	0/3
Campylobacter	0/4	ND	0/4	ND
Swab of the feed and drinking water in the pens				
Salmonella	7/41	0/8	0/28	0/10
Campylobacter	0/25	1/9	4/8	0/10
Pooled faecal sample				
Salmonella	0/3	1/8	0/12	ND
Campylobacter	ND	ND	1/12	ND

°Expressed as total samples positive for *Salmonella* or *Campylobacter* on total samples taken
ND: not done

Five different slaughterhouses were visited of which slaughterhouse A, C and D were visited twice. Table 2 shows the prevalence of *Salmonella* and *Campylobacter* at different sampling points taken at the different slaughterhouses. In total, 37% and 29% of the carcasses turned out to be positive for *Salmonella* and *Campylobacter*, respectively. High variations were observed between the different abattoirs and the different sampling days at the same slaughterhouse. The prevalence of *Salmonella* in the environmental samples of the slaughterhouses was excessive, while *Campylobacter* was present in most of the slaughterhouses but at a much lower level. The knives and the carcass splitter were found *Salmonella* positive during 3 slaughterhouses visits. In total, from 19% of the faeces samples, taken from the colon after evisceration, *Salmonella* could be isolated and 21% of the mesenteric lymph nodes turned out to be positive. At the herd level, 27 from the 68 different herds sampled (41.2%) were positive for *Salmonella.* A herd was considered positive when a faeces sample from one pig of the herd turned out positive. From each herd an average of 4.85 pigs were sampled and in a positive herd an average of 2.44 animals were positive.

The most prevalent serotypes isolated at the slaughterhouse environment were *S.* Typhimurium (11%), *S.* Livingstone (6%) and *S.* Derby (5.5%). Nine different serotypes (*S.* Typhimurium, *S.* Brandenburg, *S.* Derby, *S.* Infantis, *S.* Virchow, *S.* Livingstone, *S.* Ohio, *S.* London, non-typeable) were isolated from the carcasses. *S.* Typhimurium was isolated in 71% of the cases. At D1, where the carcass contamination was 70%, almost only *S.* Typhimurium was found (97%). Thirty-five *S.* Typhimurium isolates, all from slaughterhouse D1, showed a resistance against five antibiotics: ampicillin, tetracycline, streptomycin, chloramphenicol and sulfadiazine. This

multiresistance profile is suggestive for the phage type DT104 and this was indeed confirmed for several isolates which were sent to the Institut Pasteur-Brussels for phage typing. *S.* Typhimurium was isolated from 38.4% of the *Salmonella* positive pigs (colon faeces), 30.7% *S.* Livingstone and 16.9% *S.* Derby. *Salmonella* Livingstone was isolated in 34.7% of the mesenteric lymph nodes, 25% *S.* Typhimurium and 11.1 % *S.* Derby. The knives and the splitting machine became contaminated during activity and could be a source of cross-contamination of the carcasses. At A2, C1, D1 and D2 the same serotypes were isolated from the carcasses as well as from knifes or the splitting machine. However, not all the *Salmonella* serotypes found on the equipment were transferred to the carcasses. At slaughterhouse B, *S.* Bovismorbificans was only isolated from the knife and not from the carcasses or the environment. From these data, it is clear that carcass contamination is a result of the status of the animal shipment and the slaughterhouse hygiene. A strong correlation is found between the *Salmonella* contamination of the animals (positive faeces and/or mesenteric lymph nodes) and the number of contaminated carcasses at the end of the slaughter line. At B and D1 a high carcass contamination was noticed whereas the delivery of *Salmonella* positive pigs was not as high; here the impact of the cross-contamination of the slaughterhouse on the carcasses was calculated to be 20% and 57%, respectively.

Table 2: Isolation of *Salmonella* and *Campylobacter* in 5 commercial slaughterhouses in different samples.

Slaughterhouse	A1[b]	A2[a]	B[b]	C1[b]	C2[b]	D1[a]	D2[c]	E[b,d]	Total %
Carcasses									
Salmonella	0/14[e]	32/120	5/26	13/25	1/30	76/108	2/30	9/17	37
Campylobacter	6/14	ND	1/26	4/25	18/30	ND	ND	3/17	29
Faeces									
Salmonella	0/15	26/120	0/28	17/25	2/30	14/110	ND	7/17	19
Campylobacter	ND	ND	10/29	3/25	10/30	ND	ND	4/17	27
Mesenteric lymph nodes									
Salmonella	0/15	27/120	0/29	22/25	6/30	14/110	ND	3/17	21
Knives									
Salmonella	0/5	1/5	1/2	3/5	1/3	0/13	5/8	ND	27
Splitting machine									
Salmonella	ND	ND	0/2	1/1	0/1	1/1	1/6	ND	27
Overshoes slaughter line									
Salmonella	3/4	10/12	7/7	4/5	8/8	14/14	2/4	7/10	86
Campylobacter	6/21	ND	1/6	2/16	1/6	ND	ND	0/10	17

[a]: Sampling during a whole day; [b]: Sampling of one herd; [c]: At random sampling during the morning; [d]: Herd sampled at the start of slaughtering; [e]: Positive / total samples examined; ND, not determined.

A positive *Salmonella*-ELISA result was measured in 182 (56.3%) of the 323 meat juice samples (103 samples with an "OD% >10" and 79 samples with an "OD% >40"). When cut-off OD% >40 was used, 24.6% of the samples were found positive, which correlates better with 19% of the pigs found bacteriological positive for *Salmonella* (Table 2). Thirteen of the 66 bacteriological-positive pigs were serological negative (19.6%). Forty-three of the 257 bacteriological-negative pigs (17%) were serological positive (cut-off OD% >40) at the moment of sampling. At the herd level, where a fraction of the ELISA results was used, it was seen that it is possible that a herd is bacteriological positive but that all the pigs sampled are serological negative.

4. DISCUSSION

4.1 Salmonella

The last decade there has been a dramatic increase in the human cases of salmonellosis. In 1999, 15774 infections with *Salmonella* spp. were registered in Belgium (Belgian National Reference Centre for *Salmonella* and *Shigella*), which means an increase with 33 % compared with 1995. It is assumed that the prevalence figure is even underestimated because of incomplete reporting and sporadic cases. *S.* Enteritidis and *S.* Typhimurium together represent 80% of the human cases in Belgium. In Denmark, The Netherlands and in Germany it is estimated that 15 to 20 % of all human cases of salmonellosis are associated with the consumption of pork (7, 5, 34, 29). In this study, we found that 19% of the pigs were *Salmonella* positive (positive colon faeces in the slaughterhouse). *Salmonella* prevalence figures for slaughter pigs can however be substantially different in different countries due to different sampling methods and isolation methods. In Germany and The Netherlands, respectively, 3.7% and 25.6% of the faeces samples were positive (17, 30). The most prevalent serotype isolated from swine faeces samples is *S.* Typhimurium. A study in The Netherlands found 88% *S.* Typhimurium (33).

Belgian studies indicate a lower proportion of *S.* Typhimurium with figures of 26% (8) and 38.4% in the faeces (this study).

The serotype *S.* Typhimurium belongs to the *Salmonella* group that mostly produces no severe disease in pigs. However, *Salmonella* has the potential to colonize the gut of pigs. The pathogen is excreted in faeces and this leads to contamination of the farm environment and other animals. In this study, we found that the environment of 3 of the 4 farms visited was *Salmonella* positive. During the slaughtering process, the carcasses get contaminated with faecal material and this forms a source for *Salmonella* food poisoning. Also during slaughtering contaminated tonsils and lymph nodes may be cut, so that slaughter equipment becomes a source of *Salmonella* contamination (30). In this study, we found that 37 % of the carcasses at the end of the slaughter line were contaminated with *Salmonella*, with a predominance of *S*. Typhimurium (71% of positive carcasses). In comparison with similar studies in Belgium and other Western countries, this level is high. Korsak *et al*. (18) found about 27% of the pork carcasses positive for *Salmonella* in four different Belgian slaughterhouses. In the Netherlands, only 1.4% of the carcasses were positive at the end of the slaughter line (30), in the UK between 7 and 26% of the carcasses were contaminated (9), in Germany about 10% of the carcass swabs collected in seven different abattoirs were positive (17)**.** In the slaughterhouse not only carcasses of infected animals get contaminated (estimated to be 70% of the contaminated carcasses) but also cross-contamination occurs by which carcasses of *Salmonella* negative pigs get contaminated (estimated to be the case for 30 % of the contaminated carcasses) (3, 4; this study). The cross-contamination is evidenced by the appearance on carcasses of other *Salmonella* serotypes, which are also found in the slaughterhouse environment and on cutting material.

An additional specific problem associated with the endemic infection of pigs with *S.* Typhimurium, is the recent emergence of the multiple antibiotic-resistant *S.* Typhimurium DT104 in several European countries, e.g. Denmark, the UK and Germany. This multiple-resistant type has been found in clinical human cases as well as in pig herds in Denmark (2) and in pig farms and on carcasses in Belgium (this study). This type is much feared in hospitals because of its multiple-resistance against antimicrobial agents used in both human and veterinary medicine (ampicillin, chloramphenicol, streptomycin, sulphonamide, tetracyclin).

The Danish *Salmonella* Control program stands as the type example in Europe for control of *Salmonella* in the pork production chain (24). In this control program, farms are classified in 3 different levels of *Salmonella* prevalence based on serological (detection of antibodies against *Salmonella*) examination of blood samples. This information on *Salmonella* prevalence is used to organize a logistic slaughtering in which *Salmonella* negative herds are slaughtered separate from *Salmonella* positive herds. This system is based on a good correlation on herd prevalence between bacteriological and serological examination (28)**.** However, our data collected on individual animals did not confirm this tight correlation. Both situations, a positive serological test with a negative bacteriological result and vice versa were encountered and could be explained as follows: a positive serological result does not necessarily imply that the animal is still a *Salmonella* shedder because the animal has become a silent carrier of *Salmonella* in the gut and/or in different organs, on the other hand, recent *Salmonella* infections in pigs can not be detected by serological examination. It is also not clear which cut-off value has to be used in the serological test: in the Danish monitoring program a cut-off value OD% >40 is used, while other authors suggest to use the lower cut-off value OD% >10 (33).

Our data indicate the presence of a significant number of infected animals at the farm level and therefore it will be important to work towards a reduction of *Salmonella* in the pigs delivered to the slaughterhouse and to organize a logistic slaughtering. Different strategies can be used to reduce carriage of *Salmonella* in pigs such as probiotics, prebiotics (fructooligosaccharides or FOS), vaccination with a *S.* Choleraesuis avirulent live strain, and acidification of drinking water (21).

4.2 Campylobacter

Together with *Salmonella*, *Campylobacter* is responsible for most of the human foodborne infections. In Belgium, the amount of reported cases in the period from 1987 till 1999 has more than doubled to 6514 cases in 1999, while in the UK (England and Wales) 58.000 infections were reported in 1998 (Public Health Laboratory Service Communicable Surveillance Centre). Because of the more sporadic nature of *Campylobacter* infections compared to *Salmonella* infections, the exact amount of campylobacteriosis cases is surely underestimated. At present, *Campylobacter* is even regarded as a more important foodborne pathogen than *Salmonella*. Most of the *Campylobacter* induced infections are considered to be caused by the thermotolerant species *Campylobacter jejuni* and *Campylobacter* coli. In Belgium, 66% of the human isolates were *C. jejuni* and 21.5% *C. coli* (Scientific Institute for Public Health, Brussels, 1999), which means that the prevalence of *C. coli* in human cases seems to be at least twice as high than in other European countries. While *C. jejuni* infections are generally attributed to poultry, there is a lack of epidemiological information to know the origin of *C. coli* infections.

Animals such as poultry and pigs are considered to be the natural reservoirs of campylobacters. The prevalence of *Campylobacter* normally found in the intestinal tract of pigs is high. *Campylobacter* was isolated from 96% of the pig faeces in Denmark and in the Netherlands (7). We isolated *Campylobacter* from 25 to 30% of the pigs using rectal swabs and all four farms visited were *Campylobacter* positive both in the animals and in the environment. In several countries, *C. coli* is the predominating *Campylobacter* species in pigs (19, 7). In our study, exclusively *C. coli* was found in the rectal samples taken at the pig farms, and in the slaughterhouses 29% of the carcasses were contaminated with *C. coli*. In The Netherlands, the percentage of contaminated carcasses was 3 times higher at 66% (7). This large variation in contamination on slaughterhouse level even in the same country can be caused by differences in isolation methods and/or in slaughter practices. Although *Campylobacter* was found in the environment of most of the slaughterhouses visited in this study, this was at a much reduced level (17% of the samples positive) compared to *Salmonella*. Our data indicate a clear correlation between positive animals and carcass contamination, while cross-contamination at the slaughterhouse seems to be very limited.

At the farm level, it seems that the infectious pressure of *Campylobacter* on piglets is high from the moment of birth onwards. Piglets can become infected during the first few days of life. It has been shown in a study of two multiplier farms in the Netherlands that half of the piglets became infected with *Campylobacter* during the first week of life and 85% after four weeks (37). On the two farms investigated, 9 of the 10 sows monitored were infected with *Campylobacter*. The fact that piglets become infected at such a young age limits the opportunity for intervention in the infection routes at least at the level of multiplier or fattening farms. A more realistic approach would be to provide multiplier farms with *Campylobacter*-free sows from *Campylobacter*-free top-breeding farms (top-down approach). More attention should thus be given to hygienic measures during the breeding of the sows to reduce the level of contamination. In addition, it has to be investigated whether the intestinal colonisation of the sows by *Campylobacter*s can be reduced or inhibited by supplementing the feed with pre- or probiotics.

Of particular concern is the fact that in several countries (UK, Spain) nearly all or all *C. coli* isolates from pigs are resistant to antibiotics with a very high incidence of resistance to erythromycin (belonging to the macrolides) and quinolones (10). The high erythromycin resistance in pig isolates may be related to the extensive veterinary use of macrolides in growing pigs. This multidrug resistance is considered as highly undesirable in *Campylobacter* as these two classes of antibiotics are generally advocated as first- and second-line drugs for antimicrobial treatment of *Campylobacter* enteritis.

4.3 Listeria monocytogenes

Listeria monocytogenes is a Gram-positive, food-borne pathogenic bacterium. The incidence of *L. monocytogenes* infections is low (0.4/100.000 inhabitants in Belgium), but mortality ranges may be as high as 30%. Immunosuppressed individuals, pregnant women, fetuses and neonates are most susceptible to *Listeria* infection but a minority of patients has no predisposing conditions. Clinical features include meningitis or meningo-encephalitis, septicemia, in some cases gastroenteritis, abortion, and perinatal infections. Two important features regarding food safety are its ubiquitous nature (e.g. farm surroundings) and ability to grow at refrigerator temperatures. The organism has been found in a wide variety of foods including poultry, lamb, meats, chopped beef, patés, dry sausages and sausage meats. The incidence of *L. monocytogenes* in foods sold on the Belgian retail market varies from 5-22% in ready-to-eat foods to 13-61% in raw foods. Uyttendaele *et al.* (31) found 14.92% of Belgian raw ham samples contaminated with *L. monocytogenes*. Recent events in Europe have shown that pork meat and processed pork products, such as delicatessen, can be sources of listeriosis, as occurred in France in 1992 and 1993 (15, 12) and in other European countries (16, 23). *L. monocytogenes* has been found at all levels of the pig production chain and the pork processing industry.

There is still no consensus which sources are the most important for the contamination of the end product. In a German study, *L. monocytogenes* was found in 5.9% of faecal pig samples (36). Various studies have demonstrated that the contamination of pork products is mainly derived from cross-contaminations between pork meat and the environment of chilling (13) and cutting rooms (32, 7, 25) or the further processing steps (27). Colonization of processing surfaces is therefore recognized to be an important source of contamination for any food material in contact with these surfaces. Also, the consistent finding of strains of one genotype in environmental samples, which are not in direct contact with pork carcasses, over long periods, even after cleaning and disinfection, support the hypothesis that persistence of strains in the pork processing environments is a very important factor (11, 6). However, several studies have also shown identical epidemiological *L. monocytogenes* types on live animals and in meat processing environments (20, 26, 11).

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RISK ANALYSIS AND ECONOMICS

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ABTRACT

Risk analysis is an area of growing interest for the veterinary profession, especially with respect to international trade of livestock and livestock products. The major outcome of such analyses is a certain probability with respect to the occurrence of a specific event. The decision maker(s) then must decide whether a chance of virus import of one in so many thousands of tons or hundreds of years of imports is acceptable or not. In this paper it is argued that for most decision makers such an outcome is too difficult to interpret and decide upon, if possible at all. Therefore, it is suggested to bring the current approach of risk analysis one step further, by combining it with economic analysis. That would make it possible to convert the concept of risk into some sort of money value. The basic framework for such an integrated approach -- including issues of welfare theory and demand/supply analysis -- is presented and discussed, and illustrated with a simplified example.

SAMENVATTING

Risicoanalyse is een gebied dat snel aan belang en aandacht wint binnen de veterinaire wereld. Dit geldt in het bijzonder als het gaat om internationale handel van vee en veeproducten. Het belangrijkste resultaat van een (standaard) risicoanalyse is een kansverdeling van een bepaalde uitkomst. De beslisser moet aan de hand hiervan beslissen of de kans op insleep van bijvoorbeeld een bepaald virus (1 op de zoveel duizend) acceptabel is of niet. In deze bijdrage wordt duidelijk gemaakt dat dit onmogelijk is voor de meeste beslissers en beleidsmakers. Derhalve wordt voorgesteld om de (standaard) risicoanalyse een stap verder door te voeren door het te combineren met een economische analyse waarin de uitkomst in een bepaalde economische waarde (meestal in geld) wordt uitgedrukt. Het basisraamwerk voor een dergelijke economische stap wordt in deze bijdrage verder uitgewerkt en bediscussieerd aan de hand van een voorbeeld.

1. INTRODUCTION

Decisions in real life have to be made under conditions of uncertainty, which means that there is imperfect knowledge about the various input factors included and/or about the outcome of possible actions. This is also the case for decisions with respect to animal health.

Traditional economic analyses of decision making have distinguished two types of imperfect knowledge: risk, when the probabilities of the uncertain outcomes are known, and uncertainty, when they are not. However, this distinction is of little practical use and is discarded by most economists today. Probabilities can be "known" only for the so-called stationary stochastic processes, i.e., for events where there is variability but where the sources and nature of the variability remain constant through time. Such processes are rare in practical decision making. In modern economic analyses, therefore, the terms risk and uncertainty are used interchangeably.

In the area of Animal Health Economics increasing efforts are being made to quantify the costs and benefits of measures to control disease and reduce the risk of occurrence. Various techniques are available to help perform this kind of analysis, ranging from simple partial budgeting, to decision-tree analysis and stochastic computer simulation and optimization [5,6,8]. These techniques differ considerably in complexity, but have in common that they all can convert risks for and consequences of disease into costs and benefits, and hence into money values. Money values are easy to interpret by decision makers, including farmers and government officials.

The major outcome of most risk analyses, at least those with respect to trade issues, is a certain probability with respect to the occurrence of a specific event. The decision maker(s) then must decide whether a chance of virus import of one in so many thousands of tons of animal products or hundreds of years of imports is acceptable or not [13]. Such an outcome is for most decision makers too difficult to interpret and decide upon, if possible at all. In our view, therefore, the current approach of risk analyses should be brought one step further, and combined with economic analyses. That would make it possible to convert the concept of risk into some sort of money value. Economic effects to be included are, on the one hand, the benefits (i.e., utility) to consumers who actually buy the product and the profit (if any) made by those who import and trade the product under consideration. On the other hand, there are losses involved when the virus introduction causes an outbreak of the disease. These losses include direct costs (e.g. affected animals and control measures) -- but may also include indirect losses through export bans (at least for major exporting countries).

The type of economic analysis that is able to quantify these benefits and costs is based on welfare theory and demand/supply analysis. Moreover, specific choice criteria (such as a utility function and stochastic efficiency criteria) are needed to discriminate between the (uncertain) outcomes. In this paper, the basic principles of such an approach will be presented and illustrated with a simplified example.

2. THE CONCEPT OF DEMAND AND SUPPLY TO MEASURE WELFARE EFFECTS

2.1. Producer and consumer surplus

It is common practice (and an invaluable aid to comprehension) to express demand and supply schedules in graphical form, with prices on the vertical axis and quantity on the other (see Figure 1). Such a graph is often called the "scissors graph" because of its shape; most demand curves slope downwards from left to right -- more of the commodity is demanded as price falls -- whereas supply curves slope upwards from left to right -- more is supplied as price rises. Where the two curves cross is the equilibrium price at which the quantities demanded and supplied are in exact balance.

A measure of the responsiveness of the quantity demanded or supplied to changes in the market price of that good is referred to as the price elasticity of demand or supply respectively. Specifically, it is the percentage change in quantity divided by the percentage change in price. If the percentage change in price "causes" a larger percentage change in quantity, the demand or supply curve is called "elastic" (i.e., price sensitive). "Inelastic response" refers to a smaller percentage change in quantity resulting from a given change in price. Agricultural products are characterized by rather steep (i.e., inelastic) demand and supply curves. In other words, relatively small changes in quantities may have considerable price effects.

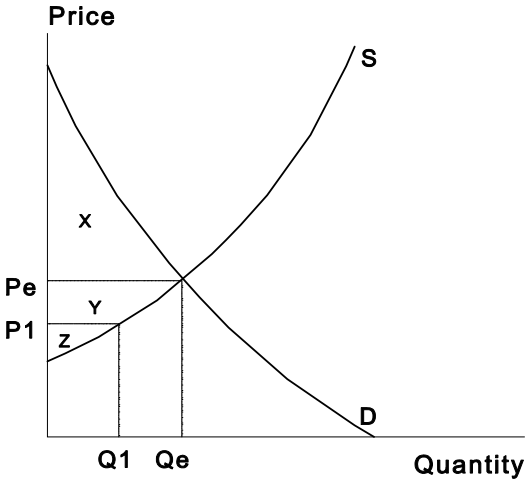

Figure 1. Graphical representation of demand and supply functions

The area between the supply and demand curves to the left of their point of intersection provides basic information on the welfare effects for producers, consumers and the society as a whole. For instance, the supply curve tells us that some producers would have been willing to produce in return for prices below P_e. To give an example, in Figure 1 the production of Q_1 units of output would have been realized at a price as low as P_1. In practice, all of those units of output which comprise the total of Q_1 sell at price P_e. Because the market determines a unit price of any commodity as a valuation, some producers actually obtain more value (or benefit) from the sale of their products than they might have sought or expected. In other words, they obtain a kind of economic surplus. To be precise, this surplus equals P_e-P_1 -- not for the total production Q_1, but for the last unit of output at Q_1. When adding up the surpluses associated with all other units of output between the origin and the equilibrium output Q_e, the total economic surplus is given by the area Y+Z (see Figure 1). This total area measures what, for fairly obvious reasons, is called the producer surplus. By analogy, consumer surplus is equal to area X. All consumers pay P_e for each unit of the product, but some would be willing to pay more if supply were less abundant. They need not do so in the circumstances described, and so they benefit from getting their product cheaper than they otherwise would.

2.2. Losses due to export bans

The concept of producer and consumer surplus can also be used to quantify the losses from export bans, in case the import of a risky product causes an outbreak of a contagious disease. This is illustrated in Figure 2. Figure 2 shows the supply curve (S) and the demand curve (D) for a country exporting a certain product. At the basic price level P, producers supply amount Q_s, while consumers demand amount Q_d, with the difference (Q_s-Q_d) being exported. When export bans are in effect, a new equilibrium will arise at a lower price level and influencing the welfare of both producers and consumers. The losses to the producers due to a drop in price from P to P' is the reduction in producer surplus (area PFCP'). In the short term, a large part of the costs is fixed and the supply curve will be steep. With disease outbreaks that do not last long, therefore, the vertical supply curve (S') can be used to quantify the losses in producers' income. Actual losses to the producers are reduced by any compensation paid by the government. Consumers gain from the drop in price; their gain is indicated by the increase in consumer surplus (area PGBP'). From the alternative demand curve (D') it can be concluded that the slope of the curve (i.e., the price elasticity of demand) influences the increase in consumer surplus.

Not only is it possible to identify the net effects on producers and consumers respectively, but also to summarize the consequences for a society as a whole (i.e., for people irrespective of whether they are producers, consumers or both). Within the theory of welfare economics, however, there is discussion about the aggregation of benefits and costs at the national level [10]. Simple aggregation of these effects presumes an equal weight of benefits and costs for each group and individual -- which is usually not the case. From an investigation of EU dairy policy over the years 1980 to 1987, for instance, it emerged that one dollar of producer income was considered twice the weight of one dollar of consumer income [14]. It is, therefore, recommended to report both the separate effects for producers and consumers, and their equally-weighed total -- leaving policy makers the opportunity to apply their own weights.

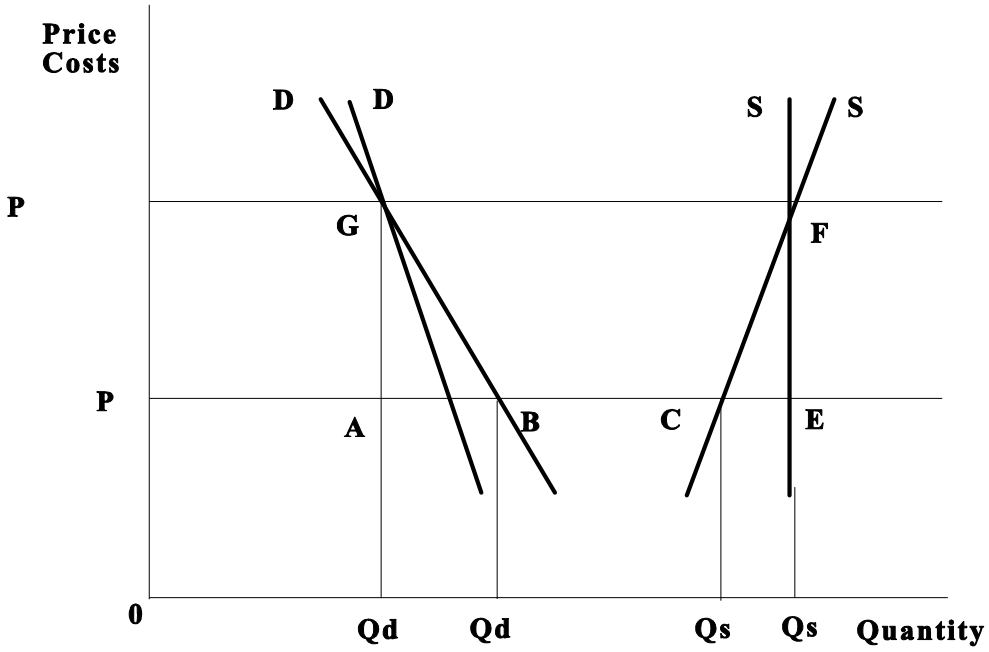

Figure 2. Supply and demand curves in case of an exporting country

For the Netherlands, a modeling approach has been developed to quantify these losses related to outbreaks of Foot-and-Mouth Disease [3, 9]. This approach is general and could also be applied to other countries and disease conditions.

3. CHOICE CRITERIA TO DISCRIMINATE AMONG UNCERTAIN OUTCOMES

3.1. Components of a risky decision problem

Any risky decision involves five components: acts, states, probabilities, consequences and a choice criterion [1, 8]. Acts (a_i) are the relevant actions available to the decision maker. They constitute the relevant set of mutually exclusive alternatives among which a choice has to be made. Examples of acts in animal health management are "treat" versus "do not treat" an animal, and "import" versus "do not import" animal products from a specific country. The possible events or states of nature (t_i) must also be defined by a mutually exclusive and exhaustive listing. Examples of states of nature are "good", "average" or "poor" rainfall, or "severe", "normal", "minor" or "no" outbreaks of a certain disease. The essence of a risky decision problem is that the decision maker does not know for certain which state will prevail. Some state variables are intrinsically continuous (e.g., herd-health status), but generally a discrete representation (such as good, average or bad) will prove adequate. Prior probabilities (P_i) reflect the degrees of belief held by the decision maker about the chance of occurrence of each of the possible states. Such probabilities are considered subjective or personal in nature. They can be based on outcome from experiments or field research, but -- when not available -- also on expert opinion or one's own experiences. Example prior probabilities for a disease problem can be as follows: a probability of 0.2 for a "severe" outbreak, 0.3 for a "normal", 0.25 for a "minor" and 0.25 for "no" outbreak of a certain disease. Depending on which of the uncertain states occurs, choice of an act leads to some particular consequence, outcome or payoff. Finally, some criterion of choice is necessary to compare the possible consequences of any act with those of any other act. One such criterion is the expected monetary value, defined as the summation of the possible money outcomes multiplied by their probabilities.

Consider a simplified case in which a choice has to be made between two acts, i.e., to import (a_1) versus not to import (a_2) a product from a specific country. "No import" is the current situation and defined to have a zero payoff. The payoffs of the import options are expected to differ according to whether or not an outbreak of the disease under consideration will occur. These "states of nature" can be no outbreak, minor outbreak or severe outbreak for a specified time frame, with an estimated prior (i.e., subjective) probability of 0.80, 0.15, and 0.05

respectively. Benefits and losses are calculated according to the producer and consumer surplus approach (explained before). Results are summarized in Table 1.

Table 1. Payoff matrix for two import options (1000 US$)

States of nature (t_i)	$P(t_i)$	Import (a_1)	No import (a_2)
No outbreak (t_1)	0.80	750	0
Minor outbreak (t_2)	0.15	-100	0
Severe outbreak (t_3)	0.05	-5000	0
Expected monetary value		335	0

When taking into account the mean outcome (i.e., expected monetary value) to compare the alternatives, import (a_1) is the preferred option. This choice holds for so-called risk-neutral decision makers (i.e., decision makers who implicitly put an equal weight on one dollar above or below the expected outcome). Most people, however, tend to be risk averse (i.e., decision makers who consider a relatively big loss as a more-than-proportional threat). With respect to the example in Table 1, this means that they put a higher weight on each dollar loss with a severe outbreak than on each dollar involved with no outbreaks. That may lead to a different choice than one based strictly on the expected-monetary-value criterion.

3.2. Subjective expected utility model

One of the most-widely applied conceptual models for studying decision making under risk is the subjective expected utility (SEU) model [8]. Using the model, actions are ordered according to the beliefs and risk attitude of the decision maker. Each outcome is assigned a utility value (i.e., preference), according to a personalized, arbitrarily scaled utility function. The utility values for each possible outcome of an action are weighed by their (subjective) probability and summed across outcomes. The resulting expected utility is a preference index for that action. Actions are ranked according to their levels of expected utility with the highest value being preferred.

The implementation of the SEU model requires the risk preferences of decision makers (i.e., the utility function) to be known. The notion of certainty equivalent is central to the measurement of these preferences, and hence to the elicitation of the utility function. When given a choice between (a) payment of US$1000 for sure versus (b) a chance of winning US$5000 with a probability of 0.25, for instance, most people will opt for (a) -- even though (b) has a higher expected monetary value. The certainty equivalent (CE) of a risky prospect then is the value which the decision maker is just willing to accept in lieu of the risky prospect. So, the relationship between the CE and the expected monetary value of the outcomes tells something about the decision maker's attitude towards risk. If the person is averse to risk (which is usually the case) he or she will assign a CE less than the expected monetary value. For people that have a preference for risk CE will be greater than the expected monetary value -- while in the case of risk indifference CE equals the expected monetary value.

Methods of eliciting utility functions involve asking people to specify their CEs for specified risky prospects. According to Anderson et al. [1], the simplest recommended method is based on considering an Equally Likely risky prospect and finding its Certainty Equivalent. In using this so-called ELCE-method, the first step is to find the CE for a hypothetical 50/50 lottery with the best and worst possible outcomes of the decision problem as the two risky consequences. The next step is to find the CE for each of the two 50/50 lotteries involving the first-established CE and the best and worst possible outcomes. This process of establishing utility points is continued until sufficient CEs are elicited to plot the utility function. In order to obtain meaningful values, it is important to provide enough realism for this type of game setting [15]. Moreover, reliable outcomes require utility functions to be described in a mathematically sound way, thus making the choice of the function form very important.

Suppose that for a risk-averse decision maker, the utility function for gains and losses is adequately represented by:

$$U(x) = 1 - e^{-0.0005\ X}$$

where X denotes thousands of US dollars.

This function makes it possible to convert the money values in Table 1 (with a probability of occurrence of 0.80, 0.15 and 0.05 respectively) for each of the alternatives (a_1 and a_2) to utility values (U). The utility of 750

thousand US dollars (in case of import (a_1) and no outbreak), for instance, is $1 - e^{-0.0005x750} = 1 - e^{-0.0375} = 1 - 0.687 = 0.313$. In this way, the total utility (TU) can be obtained for each of the alternatives, taking into account also the probabilities of occurrence of 0.8, 0.15 and 0.05 respectively:

$TU(a_1)$ = 0.8U(US$750) + 0.15U(US$-100) + 0.05U(US$-5000)
= 0.8(0.313) + 0.15(-0.0053) + 0.05(-11.183) = -0.317

$TU(a_2)$ = 0.8U(US$0) + 0.15U(US$0) + 0.05U(US$0) = 0.00

So, taking into account the risk-averse attitude of the decision maker makes option a_2 the preferred one (i.e., the one yielding the highest subjective expected utility).

3.3. Stochastic-efficiency criteria

Utility functions may not always be easy to elicit (if possible at all). Moreover, the model of risky choice as outlined above, relates primarily to a situation where there is one decision maker whose preferences are to be used in the analysis and who also bears the consequences of the choice. Often, however, more than one person will be involved in any decision and/or affected by the consequences, as is the case with trade issues. Unfortunately, the extension of the methods of decision analysis to multi-person decision problems is not a simple matter.

Policy makers often tend to react in a risk-averse fashion, fearing the personal consequences of being seen to have made decisions that turned out bad. The uncertainties of particular public projects or programs, however, are often rather insignificant when measured against the total performance of the economy. That is why economic theory teaches that governments make the best economic choice among risky projects by using risk-neutral decision rules such as the expected monetary value criterion [12]. There are two major reasons to consider risk-related (rather than risk-neutral) decision rules to be appropriate for the choice among projects: (1) when the projects are unusually large (e.g., affecting 10% or more of national income), or (2) when the project's consequences are not spread widely -- and fairly evenly -- among the population. The latter will often apply to contagious-disease outbreaks, since losses primarily affect producers' income (especially on farms and in areas that are actually affected by the disease) [2].

A better insight into the potential consequences of the various decision rules and risk attitudes may be helpful in these situations, anyway, to provide useful information for a better-thought-out and more-rational decision-making approach. Stochastic efficiency criteria are proposed as a useful alternative for this type of situations. Stochastic-efficiency rules satisfy the axioms of the expected-utility model but do not require precise measurement of risk preferences. However, as opposed to the complete ordering achieved when risk preferences are known, they provide only a partial ordering [11]. Stochastic-efficiency criteria are implemented by pair-wise comparisons of cumulative distribution functions of outcomes (y) resulting from different actions [8].

First-degree stochastic dominance (FSD) holds for all decision makers who prefer more to less (i.e., whose first derivative of the utility function is positive). No assumptions are made about risk preferences of the decision maker -- which widens the possibilities of application but limits its discriminatory power. Graphically, these conditions mean that the cumulative probability of the dominant (i.e., preferred) distribution must never lie above the cumulative probability of the dominated distribution. In Figure 3, for example, F(y) dominates G(y) by FSD, but neither F(y) nor G(y) can be ordered by H(y), in which y are outcomes in money values (i.e., milk returns, pig sales, etc.).

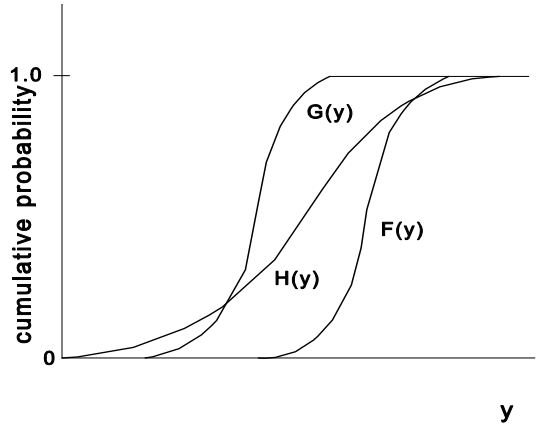

Figure 3. Graphical representation of stochastic efficiency criteria

Second-degree stochastic dominance (SSD) assumes that decision makers (in addition to preferring more to less) are risk averse, with utility functions having positive, non-increasing slopes at all outcome levels. Under SSD, an alternative with the cumulative distribution F(y) is preferred to a second alternative with cumulative distribution function G(y) if

$$\int F(y)\,dy \leq \int G(y)\,dy$$

for all possible values of y, and if the inequality is strict for some value of y. SSD has more discriminatory power than FSD, but still may not effectively reduce the number of alternatives. Graphically (because the accumulated area under F(y) in Figure 3 is always less than or equal to that under either G(y) or H(y)) only F(y) is in the so-called SSD-efficient set of these three alternatives. When only G(y) and H(y) are considered, neither one dominates the other by SSD, since the accumulated area under G(y) is less than the area under H(y) for low values of y, while the opposite occurs at high values of y.

Stochastic dominance with respect to a function (SDRF) is a more-discriminating efficiency criterion that allows for greater flexibility in reflecting preferences -- but also requires more-detailed information on those preferences. Formally stated, SDRF establishes necessary and sufficient conditions under which the cumulative function F(y) is preferred to the cumulative function G(y) by all decision makers whose risk attitude lies anywhere between specified lower and upper bounds. The method is flexible enough to include and investigate the impact of any specified value [8, 11].

PC-software has become available to perform the stochastic efficiency analyses [7]. This type of software was also used to carry out the analyses for the example given in Table 1. Results are summarized in Table 2.

Table 2. Outcome according to the various decision criteria (US$) (The preferred options are underlined or indicated with an *; ? means no ordering)

Criteria	Import options	
	import	no import
Expected monetary value	<u>335</u>	0
Utility function	-.317	<u>0</u>
FSD	?	?
SSD	?	?
SDRF (with risk aversion assumed to be):		
- low	*	
- considerable	?	?
- high		*

Table 2 shows that choices appear to vary according to the criteria. The expected-monetary-value criterion (assuming risk neutrality) leads to the choice of option 1 (import), while with the more-risk-averse type of criteria (e.g., utility function) option 2 (no import) is preferred. The latter is also the case with the SDRF-criterion -- at least with higher boundaries for the risk-aversion interval.

4. FINAL REMARKS

Risk and uncertainty are undoubtedly important factors in animal health management. Advice and modelling that are to support decisions in this area, therefore, should include appropriate probability estimates for the relevant variables under consideration. Decision analysis is considered a worthwhile approach for ensuring that farmers get advice and make decisions which are consistent with (a) their personal beliefs about the risks and uncertainties surrounding the decision, and (b) their preferences for the possible outcomes. It can also help to provide a rational basis for decision making in the public domain, and to determine the economic value of additional information to reduce and/or predict the risks and uncertainties. A good risky decision, however, does not guarantee a good outcome. That would only be possible with perfect foresight (i.e., in the absence of uncertainty). It does assure, however, that the decision made is the best possible one given the available information.

Appropriate decision criteria are considered a major component of a risky decision problem [4]. The most-widely used expected-monetary-value criterion does not always tell the whole story, as shown in the -- simplified -- example in this paper. Utility functions make it possible to provide the most-comprehensive approach (including a trade-off between the average outcome and variance) but will not always be easy to carry out and apply in actual field advice. Stochastic-dominance criteria are commonly considered promising tools in this type of analysis. User-friendly software has become available to make the application of this type of advanced criteria much easier and accessible [7]. In this way it becomes possible to transform the current outcome of risk analyses (ie, a certain probability) into values that are easier to interpret and to compare.

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AN INTERLABORATORY TRIAL TO ASSESS REPEATABILITY AND REPRODUCIBILITY OF BACTERIOLOGICAL PROCEDURES FOR DIAGNOSING INTRAMAMMARY INFECTIONS IN DAIRY COWS

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1. INTRODUCTION

In Flanders (Belgium), mastitis control is an important part of the dairy herd health program and is organised by three Flemish organisations. Bulk milk quality control is done by the Milk Quality Associations. Cow somatic cell count (CSCC) and milk production parameters are recorded by the Flemish Cattle Breeding Organisation and milk samples from subclinically infected cows and from clinically affected quarters are examined bacteriologically in four labs of the Animal Health Care Organisation.
To assess the effect of bacteriological procedures on the repeatability and reproducibility of diagnosing intramammary infections in dairy cows, an interlaboratory trial was organised.

2. MATERIAL AND METHODS

2.1 Milk sample collection

Twenty-five foremilk samples (max: 2 milk samples per cow) were collected aseptically on a farm that had a history of an elevated bulk milk somatic cell count (BMSCC) (> 250,000 cells / ml). Initial cow selection was based on an elevated CSCC (> 200,000 cells / ml). During sample collection quarters within a cow were selected based on the California Mastitis Test (CMT). Quarters were sampled when CMT score was trace, 1, 2, or 3.

2.2 Preparation and distribution of milk samples

Each of the 25 milk samples was divided into 6. Additionally out of the 25 milk samples, 15 milk samples were selected randomly and divided into 6. As a result, 40 milk samples were transported under cooled conditions (5°C) to each of the participating laboratories. Upon arrival of the milk samples in the laboratories the exact time of plating was communicated in order to avoid different time intervals between sample collection and plating of the milk samples per laboratory.

2.3 Participating laboratories

In total 6 laboratories participated in the interlaboratory trial. These were the four regional laboratories of the Animal Health Care Association in Alken, Drongen, Lier, and Torhout, and the laboratories of the Bacteriology and the Herd Health department of the School of Veterinary Medicine in Merelbeke.

2.4 Plating and bacteriological procedure

The guidelines only mentioned a volume of 0.01 ml of milk to be plated on a a-selective blood agar (5% bovine blood) with a standardised loop. Reading was done after 24 and 48 h of incubation at 37°C. Micro-organisms were reported using the following codes:

1. β-hemolysin producing staphylococci,
2. Other staphylococci,
3. Streptococci, enterococci, aerococci, lactococci,
4. Gram negatives,
5. *Corynebacterium* species,
6. Others,
7. Contaminant,
8. Contaminated (more then 2 different species), and
9. Negative.

When the number of colony forming units (CFU) was 20 or less, the exact number was reported. The number of CFU was reported as '+' if more than 20 CFU were isolated.

2.5 Data analyses

The repeatability (intra-lab agreement) of the bacteriological procedures was calculated on the 15 duplicate milk samples. The observed and kappa agreement was used to assess species identification repeatability. The colony count repeatability was assessed with the coefficient of variation.

The reproducibility (inter-lab agreement) of the bacteriological procedures was calculated on the 25 single milk samples. Pairwise kappa agreements between laboratories were calculated to assess species identification reproducibility. The geometric mean colony count (GMCC) was calculated per laboratory on these samples for which all laboratories reported the same species identification code (including the negative samples) and for which all laboratories reported an exact number of CFU.

3. RESULTS

The distribution of the micro-organisms that were isolated per laboratory was shown in Table 1. These results indicate that Lab C as well as Lab D was not able to read the β-hemolysin production of *S. aureus*. Also, Lab B did not recognise the *Corynebacterium* species (Code 5) and most probably reported this as 'others' (code 6). The species identification and the colony count repeatability per laboratory were shown in Table 2. The observed and kappa agreement was high to very high. However, the colony count repeatability was very poor as indicated by the very high coefficients of variation. The pairwise species identification kappa agreement varied from poor to very high and GMCC varied a lot between laboratories (Table 3). Moreover, the pairwise species identification kappa agreement was poor when the difference in GMCC between laboratories was large. Whereas, this agreement was high to very high when the difference in GMCC was small. Additionally, as shown in Figure 1, the proportion of negative samples increased when GMCC was small. Whereas the proportion of samples with 2 species and contaminated samples increased when GMCC was large.

Table 1: Distribution (%) of micro-organism isolated per laboratory

Identification code	Lab A	Lab B	Lab C	Lab D	Lab E	Lab F
1	10.3	3.6	0.0	0.0	3.4	3.6
2	6.9	3.6	12.5	7.1	17.2	3.6
3	24.1	28.6	28.1	25.0	31.0	28.6
4	3.4	7.1	6.3	7.1	3.4	7.1
5	24.1	0.0	31.3	35.7	24.1	25.0
6	3.4	10.7	0.0	0.0	0.0	0.0
7	6.9	10.7	9.4	3.6	0.0	0.0
8	10.3	3.6	0.0	3.6	0.0	3.6
9	10.3	32.1	12.5	17.9	20.7	28.6

Table 2: Species identification and colony count repeatability per laboratory

Lab	Species identification			Colony count	
	n	Observed (%)	Kappa (%)	n	C.V. (%)
A	18	83	80	6	50
B	16	88	83	12	29
C	16	75	68	11	37
D	18	78	71	10	58
E	21	76	70	13	94
F	19	79	73	15	27

≥ 80 % : Very high kappa agreement; 60 – 80 % : High kappa agreement
C.V.: Coefficient of variation (< 5% is good)

Table 3: Species identification reproducibility and geometric mean colony count (GMCC) per laboratory

	Species identification agreement						GMCC
Lab	A	B	C	D	E	F	
A		29	30	49	43	57	1.54
B	29		36	28	33	49	0.14
C	30	36		53	60	62	1.27
D	49	28	53		52	75	1.11
E	43	33	60	52		73	0.96
F	57	49	62	75	73		0.69
Mean	43	35	48	51	52	63	

≥ 60 % : Very high kappa agreement; 50 – 60 % : High kappa agreement; 40 – 50 % : Moderate kappa agreement

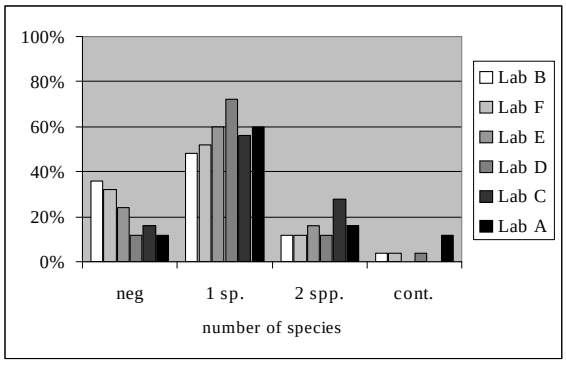

Figure 1: Distribution (%) of number of micro-organisms isolated per sample per laboratory

Geometric mean colony count (GMCC) was used to rank the laboratories (Lab B: smallest GMCC vs. Lab A: highest GMCC).

4. DISCUSSION AND CONCLUSION

Although the species identification repeatability was satisfactory, the colony count repeatability was very poor. Also the species identification reproducibility and the GMCC between laboratories varied a lot. The poor colony count repeatability and the large variation of the species identification reproducibility could be explained by the large differences in GMCC between laboratories. Because the number of CFU is associated with the plating volume, colony count repeatability and species identification reproducibility could be improved by improving standardisation of plating volume procedures.

AGREEMENT OF COMMUNITIES WITH DENSE PIG POPULATIONS AND COMMUNITIES WITH HIGH RISK FOR NEIGHBOURHOOD SPREAD OF CLASSICAL SWINE FEVER IN BELGIUM

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1. INTRODUCTION

Outbreaks of classical swine fever (CSF) in the European Union (EU) have been associated with areas of dense pig populations, a high herd density and a high average herd size (1, 2, 8). In the frame of the FAIR-project 'Development of Prevention and Control Strategies to Address Animal Health and Related Problems in Densely Populated Livestock Areas of the Community', densely populated pig areas in Belgium, France, Germany, Italy, and the Netherlands were identified as the upper 5% of the communities with the most dense pig populations (> 300 pigs / km^2) (3). These communities are assumed to contain a high risk for the introduction and spread of CSF. Mintiens et al. (4) examined factors associated with the risk of neighbourhood spread of CSF. The only risk factor that was associated with the risk of neighbourhood spread of CSF was the bivariate kernel estimate of intensity of neighbouring herds within a 1-km radius of a primary infected herd. This risk factor was used to calculate the likelihood for 'neighbourhood infections' in the neighbourhood of each pig herd in Belgium. The purpose of this study was to look whether an agreement existed between communities with high risk for neighbourhood spread of CSF and communities with dense pig populations.

2. MATERIALS AND METHODS

2.1 Data

The initial dataset was made available by Mintiens et al. (4). In this dataset, geographical co-ordinates for each pig herd in Belgium in 1997 was available. Data on the postal codes and on the number of sow and pig places per pig herd in Belgium was obtained from the identification & registration database (SANITEL-V) of the Central Animal Health Organization of Belgium. After merging these two datasets 1,486 records were deleted because no data were available on the number of sow and pig places. To calculate the actual number of pigs on the herds, a conversion was used (Table 1). With this reduced dataset (number of records = 11,629) the risk for neighbourhood spread of CSF was recalculated for each pig herd as described by Mintiens et al. (4) and the pig density per community was calculated as the number of pigs / km^2 total land area.

Table 1. Conversion from the number of sow, and pig places to the actual number of pigs per herd

Pig category	Actual number
Sows	# sow places x 0.9[1]
Piglets	# sow places x 0.9 x 4.2[2]
Pigs	# pig places
Total	(# sow places x 0.9) + (# sow places x 0.9 x 4.2) + # pig places

[1] 0.9: number of sows actually present; [2] 4.2: number of piglets with the sow during the whole year (J.M. Robijns, Central Animal Health Organization, Belgium)

2.2 Data analyses

For varying cut-off values of risk for neighbourhood spread of CSF (ranging from 5, 10, 15, … to 60%), communities were identified as communities with high risk for neighbourhood spread of CSF when the risk of at least 1 neighbourhood in the community was above the cut-off value. Similarly, communities with dense pig populations were identified. A community was classified as a community with a dense pig population when the pig density in the community was larger than the minimal pig density. For each cut-off value, the minimum pig density of a community was calculated by varying the density of a community until the agreement (kappa statistic) of the selection of communities with high risk for neighbourhood spread, on the one hand, and the selection of communities with dense pig populations, on the other hand, was maximal. The kappa statistic calculates a chance-corrected measure of agreement of two raters, i.e. the proportion of communities with high

risk for neighbourhood spread and the proportion of communities with dense pig populations. The interpretation of the kappa statistic is given in Table 2.

Table 2. Strength of agreement of kappa statistic

Kappa value	Strength of agreement
0.00	Poor
0.01-0.20	Slight
0.21-0.40	Fair
0.41-0.60	Moderate
0.61-0.80	Substantial
0.81-1.00	Almost perfect

Given the cut-off value and the minimum pig density corresponding with the maximum kappa coefficient, the observed proportion of overall agreement was calculated. The observed proportion of overall agreement calculates the fraction of communities both fulfilling and not fulfilling both inclusion criteria, i.e. the preset cut-off value and the minimum pig density.

Additionally, for each cut-off value, a receiver operating characteristic (ROC) curve for pig density was calculated. The ROC curve displays, for all possible pig densities and a given cut-off value for neighbourhood spread, the corresponding sensitivity and '1-specificity' of the identification of communities with high risk for neighbourhood spread of CSF based on the pig density of the community.

3. RESULTS

For the reduced dataset, the risk for neighbourhood spread of CSF varied from 0 to 61.4% (median = 7.5%). Of the 590 communities in Belgium, 91 had no pig herds. The pig density in the remaining communities varied from 0.5 to 3,785.6 pigs / km^2 (median = 67.8 pigs / km^2). The remainder of the analyses was performed only on the 499 communities with pig herds.

The number of communities with high risk for neighbourhood spread of CSF and the description of the selected communities, given the cut-off value, is shown in Table 3. Table 4 shows the minimum pig density for varying cut-off values for which the agreement (kappa statistic) of the selection of communities with high risk for neighbourhood spread of CSF, and the selection of communities with dense pig populations, was maximal. The ROC curves for pig density and for cut-off values ranging from 5 to 20% are given in Figure 1. Additionally for each cut-off value, the sensitivity and specificity associated with the identification of communities with high risk for neighbourhood spread is given for varying minimum pig densities.

4. DISCUSSION

This paper only deals with the risk for neighbourhood spread of CSF, which is only one aspect of disease spread during CSF-epidemics. However, the importance of 'neighbourhood infections' during a CSF outbreak (5, 6, 7) resulted in the adaptation of control strategies (pre-emptive slaughtering) in order to limit its consequences. The risk assessment for 'neighbourhood infections', as described by Mintiens et al. (4), may be used by decision makers as a decision support system (DSS) to set priorities during the control of a CSF outbreak. However, the implementation of such a DSS requires detailed geographical co-ordinates of every pig herd in the affected region (country) and these are not always available. Therefore, the pig density of a community may be an alternative tool to assess the risk of 'neighbourhood infections' in a community.

In this study, a community was considered to incorporate high risk for neighbourhood spread of CSF when the risk of 'neighbourhood infections' of at least one neighbourhood was higher than a preset cut-off value. This criterion implies that not all neighbourhoods in a community have a risk that is higher than the cut-off value. Moreover, the proportion of neighbourhoods having a risk for 'neighbourhood infections' higher than the cut-off value decreases when the cut-off value increases.

When the cut-off value is 25% or higher, the kappa agreement is only moderate or less than moderate (Table 4). Therefore, the use of the pig density of a community to select communities for which at least one neighbourhood in the community has a risk of neighbourhood spread of CSF of 25% or higher will not be appropriate. The high observed proportion of overall agreement in these classes (>0.95: Table 4) could be obtained because of the high number of communities that did not satisfy both the inclusion criteria (cut-off value and minimum pig density).

For the other cut-off values (5 to 20%), a substantial kappa agreement was found. However, the identification of communities with high risk for neighbourhood spread of CSF based on the selection of communities with dense pig populations, results in a number of misclassifications. When, in Belgium, a minimum pig density of 300 pigs / km^2 is used for the identification of communities with high risk for neighbourhood spread of CSF, the positive predictive value is 100, 74.0, 48.1, and 29.2% when the cut-off value is 5, 10, 15, and 20%, respectively. Moreover, the positive predictive value for a given cut-off value will differ from country to country because its value will change when the prevalence of communities with high risk for neighbourhood spread of CSF changes, i.e. the smaller this prevalence the smaller the positive predictive value. On the other hand, when decision makers are risk averse and they want to identify all communities in which at least one neighbourhood has a risk for neighbourhood spread of CSF of at least 5%, the minimum pig density has even to be smaller than 100 pigs / km^2 because at this minimum pig density the sensitivity is not yet 100% (Figure 1). This means also that communities will be selected that fulfill the minimum pig density but with no neighbourhoods with a risk for neighbourhood spread of CSF of at least 5% (specifity less than 100%).

In conclusion, the choice of the minimum pig density of a community to optimize the selection of communities with high risk for neighbourhood spread of CSF is not straightforward. The choice depends on the agreement of the selection of communities with dense pig populations and with high risk for neighbourhood spread of CSF, and on the risk decision makers want to take.

5. ACKNOWLEDGEMENTS

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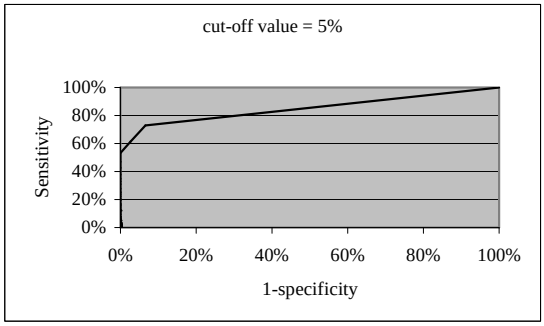
Table 3. Number of communities with high risk for neighbourhood spread of CSF[1] and description of the selected communities

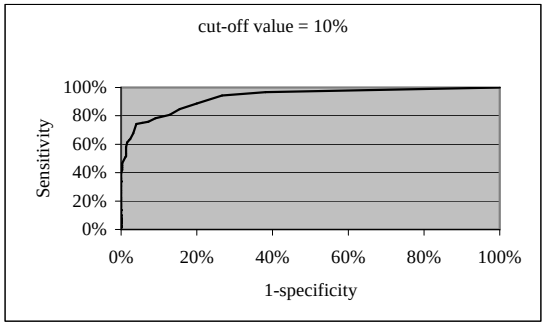
Cut-off value (%)	Number of communities	Mean number of neighbourhoods within the communities (range)	Mean total area (km^2)	Mean of median risk for neighbourhood spread of CSF (%)	Mean percentage of neighbourhoods above the cut-off value in the communities (range)
5	347	33 (2 – 253)	53.1	5.9 (0 - 16.8)	72.7 (10.0 – 100)
10	124	71 (6 – 253)	56.4	8.5 (2.5 - 16.8)	31.5 (2.1 – 82.9)
15	69	94 (17 – 253)	59.7	9.8 (6.0 - 16.8)	18.5 (0.5 – 57.3)
20	41	104 (20 –253)	61.5	10.8 (6.1 - 16.8)	13.2 (0.9 – 42.3)
25	24	120 (42 – 253)	65.5	11.7 (7.2 - 16.8)	11.5 (1.3 – 30.0)
30	17	109 (42 – 253)	51.7	12.7 (7.2 - 16.8)	9.0 (1.5 – 23.0)
35	12	111 (42 – 253)	59.6	12.5 (7.2 - 16.8)	8.5 (0.7 – 19.0)
40	9	108 (42 – 253)	59.1	12.7 (7.2 - 16.8)	5.9 (1.5 – 16.7)
45	4	153 (42 – 253)	69.6	13.8 (7.2 - 16.8)	5.8 (1.2 – 9.5)
50	2	159 (87 – 253)	78.3	12.9 (12.4 - 13.5)	2.3 (1.1 – 3.5)
55	1	230 (230 – 230)	68.9	13.5	0.4 (0.4 – 0.4)
60	1	230 (230 – 230)	68.9	13.5	0.4 (0.4 – 0.4)

[1] A community was considered a community with high risk for neighbourhood spread of CSF when the predicted risk for neighbourhood spread of CSF of at least one neighbourhood in the community was above the cut-off value

Table 4. Minimum pig density for varying cut-off values for which the agreement (kappa statistic) of the selection of communities with high risk for neighbourhood spread of CSF, on the one hand, and the selection of communities with dense pig populations, on the other hand, was maximal

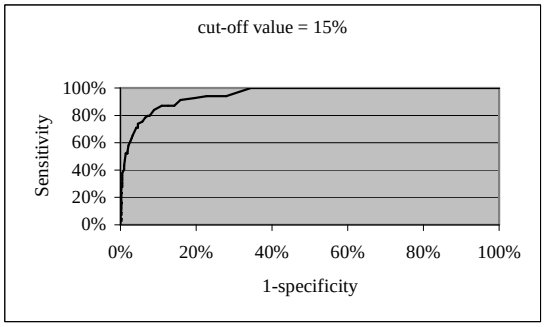
Communities with high risk for neighbourhood spread of CSF		Communities with dense pig populations		Kappa agreement (95% CI)	Observed proportion of overall agreement
Cut-off value (%)	Number of communities	Minimum pig density (# pigs / km^2)	Number of communities		
5	347	28	296	0.63 (0.56 – 0.70)	0.83
10	124	465	106	0.74 (0.67 – 0.81)	0.91
15	69	743	72	0.69 (0.60 – 0.79)	0.92
20	41	1538	28	0.64 (0.51 – 0.78)	0.95
25	24	1538	28	0.51 (0.34 – 0.68)	0.95
30	17	1853	20	0.52 (0.32 – 0.72)	0.96
35	12	1853	20	0.36 (0.14 – 0.57)	0.96
40	9	1853	20	0.33 (0.10 – 0.55)	0.96
45	4	2781	8	0.33 (-0.02 – 0.67)	0.98
50	2	2781	8	0.19 (-0.14 – 0.53)	0.98
55	1	2781	8	0.22 (-0.14 – 0.58)	0.98
60	1	2781	8	0.22 (-0.14 – 0.58)	0.98

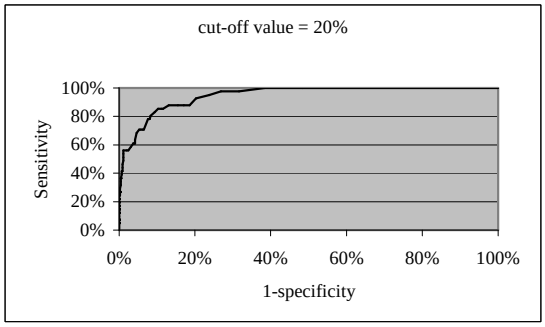




Pig density (pigs / km^2)	Sensitivity (%)	Specificity (%)
100	61.4	97.4
300	37.8	100
500	27.7	100
1000	15.8	100
1500	8.1	100
2000	4.9	100
2500	2.6	100
3000	1.7	100
3500	0.9	100

Pig density (pigs / km^2)	Sensitivity (%)	Specificity (%)
100	94.4	73.3
300	78.2	90.9
500	67.7	96.8
1000	43.5	99.7
1500	22.6	100
2000	13.7	100
2500	7.2	100
3000	4.8	100
3500	2.4	100





Pig density (pigs / km^2)	Sensitivity (%)	Specificity (%)
100	100	65.6
300	91.3	84.2
500	84.0	91.2
1000	62.3	97.2
1500	37.7	99.5
2000	23.2	99.8
2500	13.0	100
3000	8.7	100
3500	4.3	100

Pig density (pigs / km^2)	Sensitivity (%)	Specificity (%)
100	100	61.6
300	92.7	79.9
500	87.8	86.9
1000	70.7	94.3
1500	56.1	98.9
2000	36.6	99.6
2500	22.0	100
3000	14.6	100
3500	7.3	100

Figure 1. ROC curves for pig density and for varying cut-off values of risk for neighbourhood spread of CSF

INTRINSIC INDICATORS FOR MONITORING HEAT DAMAGE OF CONSUMPTION MILK

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1. INTRODUCTION

To increase the shelf life of consumption milk and to guarantee its microbiological safety, the product is heat treated. This heat treatment is the final and correcting step in the production of consumption milk and can therefore lead to overprocessing (due to a lack of care on preceding steps). To avoid this and to obligate producers to apply the HACCP-principles to all steps in the production process, the European Union is currently in a process of defining criteria for consumption milk. Conformity with such future criteria (product conformity in terms of processing as indicated by labelling) will guarantee product quality. Therefore, heat treatment conditions must be chosen in such a way that the desired results (hygienic safety and prolonged shelf life) can be achieved while undesirable changes (reduction of nutritional value, undesired reactions and changed organoleptic properties) are minimised. Therefore, heat treatment conditions must be defined by assuming a range with lower and upper limits. For the definition of heat treatment, as well as for the control of end products, indicators of heat treatment and suitable analytical methods are very important.

2. GENERAL PRINCIPLES

Heat treatment can be evaluated if irreversible changes are induced in the product. The most interesting are (bio)-chemical reactions. These reactions can be divided into two types (4).

2.1. 'Type 1'-indicators

These are components that can be denatured or inactivated by heating. Two important categories are enzymes and whey proteins. Enzymes are heat labile and loss of activity is in most cases easily to detect by a simple colour reaction. Alkaline phosphatase and lactoperoxidase are two important intrinsic indicators for monitoring heat damage of pasteurised milk. Since alkaline phosphatase is stable to temperatures slightly higher than those required to destroy milk pathogens, the control of the activity of this enzyme is the most important indicator for evaluating the hygienic safety of pasteurised milk. This means that pasteurised milk must be negative for the phosphatase test. Determination of the activity of lactoperoxidase, which is a rather stable endogenous enzyme, can be used as a simple test for the determination of the upper limit of pasteurisation. Pasteurised milk must show a positive lactoperoxidase reaction and must be labelled as "high pasteurised" when a negative result is obtained.

The individual whey proteins show distinct differences in thermal stability; the order of heat stability of the principal proteins is: α-lactalbumin > β-lactoglobulin > bovine serum albumin > immunoglobulins. The whole whey protein fraction, as well as its individual components, may be used as indicators of thermal treatment. Monitoring consumption milk in order to make the distinction between pasteurised milk and UHT milk is often carried out by determination of acid soluble β-lactoglobulin. Chromatographic techniques allow determinations with high precision and accuracy but variations in the absolute and relative concentrations of β-lactoglobulin in the milk may be a drawback.

2.2. 'Type 2'-indicators

Type 2 indicators are based on the formation of substances that are not present in the unprocessed product. During heat treatment of milk, lactose is involved both in the Maillard reaction and in isomerisation and subsequent degradation reactions.

Among the sugars derived from lactose, lactulose undoubtedly represents the most widely studied index for differentiating heated milks and for evaluating the heat load to which milk was subjected. Lactulose is a very interesting indicator for the study of heating of milk and milk products: the determination method is accurate and precise and can be carried out by column chromatography, gas chromatography or by an enzymatic method. Determination of lactulose allows distinction between pasteurised milk, UHT milk and sterilised milk.

Also components formed by the Maillard reaction can be used as intrinsic indicators for monitoring heat damage. Furosine is formed during acid hydrolysis of ε-N-deoxylactulosyl-L-lysine, the most stable Amadori-compound which derives from the Shiffs bases formed in the early stages of the Maillard reaction between lactose and side chains of milk proteins (mainly lysine). Furosine can be used for monitoring heat treatment of consumption milk. Moreover, high concentrations of furosine are formed during the production of milk powder due to favourable reaction condition during this process. This results in a considerable higher ratio of furosine to lactulose for milk powder than for consumption milk. Determination of this ratio allows demonstrating improper additions of reconstituted milk powder in consumption milk (pasteurised milk and UHT milk). Addition of reconstituted milk powder during the production of consumption milk will lead to abnormally high furosine values for pasteurised milk and to an abnormally high ratio of furosine to lactulose for UHT milk (8).

2.3. Limitations

However, these indicators have also many limitations. Upon heating of milk heat damage depends on both duration as well as on intensity (temperature) of heating. The assessment of only one indicator does not allow to distinct between a prolonged heat treatment at relatively low temperature and a short heat treatment at a higher temperature. In most cases more than one heat indicator will be used. Also pH as well as concentration of the constituents of the products will influence the chemical and biochemical heating reactions. Moreover, the range of possible heat treatments, from pasteurisation to in bottle sterilisation is far to broad for the use of just one intrinsic indicator. Finally, a good intrinsic indicator requires a relative simple analytical method.

3. MATERIAL AND METHODS

3.1. Milk Samples

All recognised Belgian consumption milk producers were invited to co-operate in the study. This co-operation included the delivery of milk samples of all process lines and the acquisition of information about these process lines. This information related to the process type (as far as UHT-milk is concerned, distinction was made between direct and indirect heating systems) and to the temperature time combinations used in heat treatments. Eight Belgian milk-producing plants, which produce more than 90% of the Belgian consumption milk, took part.

3.2. Methods

The formation of furosine was measured by reversed phase HPLC (5). The lactulose content was determined using the BM Test-Combination D-glucose / D-fructose in combination with β-galactosidase, triethanolamine hydrochloride, glucose-oxidase and catalase (6). The acid soluble β-lactoglobulin content was quantified by HPLC according to the method of the International Dairy Federation (3). The activity of alkaline phosphatase was determined spectrophotometrically at the absorption maximum using p-nitrophenyl disodiumphosphate as substrate following the procedure of the IDF (2). The lactoperoxidase activity was determined on the basis of an adopted procedure of Hernández *et al.* (1). Before measuring the activity spectrophotometrically at 412 nm with ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)) and H_2O_2 as substrate, milk proteins were precipitated by adding 1.75 M acetic acid and 1 M sodium acetate.

4. RESULTS AND DISCUSSION

The project intended to map the existing situation in the Belgian consumption milk industry with respect to conformity between label of the product and heating indictators in detail. Therefore, two inquiries, with an interval of 1 year, were organised. These inquiries resulted in 154 different consumption milk samples in which the following parameters were determined: furosine, lactulose, β-lactoglobulin, alkaline phosphatase and lactoperoxidase. As expected, the different heat treatments were clearly reflected by the different parameters. As well the lactulose content as the furosine content can be used to make a distinction between pasteurised, UHT and sterilised milk. Significant differences can also be observed between direct and indirect UHT systems. Heat treatments by direct UHT systems result in considerably less heat damage. It is also clear that the soluble β-lactoglobulin content can be used to make a distinction between the different types of heat treatment. In both inquiries there was also one sample that was labelled as 'high pasteurised'. Compared to the pasteurised samples, these samples had a lower β-lactoglobulin content. This can be expected, due to the more severe heat treatment, but was not reflected by the lactulose or furosine content. This can possibly be explained by the different reaction kinetics for the formation of lactulose (isomerisation) and furosine (Maillard reaction) on one hand and the denaturation of β-lactoglobulin on the other hand.

In both inquiries there was a good linear relation between lactulose and furosine content, which means that there is no indication for improper addition of reconstituted milk powder during the production of the consumption milk.

The second inquiry was organised to avoid that the results of just one inquiry would give a rather random indication and wouldn't be representative. The results of both inquiries were very similar, so it could be concluded that by organising two inquiries, the situation in the Belgian consumption milk industry with respect to the applied heat treatment was profoundly characterised.

Table 1: results of the determinations of the parameters in both inquiries: mean values (n= number of samples analysed)

parameter	heat treatment	1st inquiry	2nd inquiry
lactulose (mg/l)	thermisation	7.75 (n=4)	10.82 (n=5)
	(high)pasteurisation	6.52 (n=21)	19.59 (n=14)
	UHT-direct	245.67 (n=6)	414.22 (n=8)
	UHT-indirect	569.25 (n=8)	620.13 (n=5)
	sterilisation	1062.00 (n=6)	1064.37 (n=7)
furosine (mg/100g protein)	thermisation	7.00 (n=4)	6.60 (n=5)
	(high)pasteurisation	8.32 (n=21)	9.61 (n=14)
	UHT-direct	95.28 (n=6)	116.18 (n=8)
	UHT-indirect	217.34 (n=8)	196.38 (n=5)
	sterilisation	367.77 (n=6)	336.94 (n=7)
β-lactoglobulin (mg/l)	thermisation	3798.25 (n=4)	3896.00 (n=5)
	(high)pasteurisation	3460.05 (n=21)	3311.50 (n=14)
	UHT-direct	522.83 (n=6)	414.75 (n=8)
	UHT-indirect	144.88 (n=8)	134.00 (n=5)
	sterilisation	19.67 (n=6)	15.00 (n=7)
alkaline phosphatase (mg p-nitrofenol/l)	raw milk	\	1774.30 (n=20)
	thermisation	\	345.80 (n=5)
	(high)pasteurisation	\	8.29 (n=14)
lactoperoxidase (U/ml)	thermisation	\	6.26 (n=14)
	(high)pasteurisation)	\	1.28 (n=5)

5. ACKNOWLEDGEMENTS

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APPLICATION OF RISK ANALYSIS IN ANIMAL PRODUCTION

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1. INTRODUCTION

A failure such as diseased animals occurring during the establishment of a new production process can cause high costs of correction. They may often lead to losses of market share and be detrimental to the image of the supply chain. It is therefore necessary to pay attention to potential failures in the early stage of the establishment and process planning.
Failure Mode and Effect Analysis (FMEA) is a risk and weak point analysis system and therefore appropriate for a systematic and early identification of potential failures. This method is similar to HACCP concepts (Hazard Analysis Critical Control Point approach). The early search for potential failure sources allows a failure prevention strategy to be implemented to avoid costly corrections of failures. Potential causes and effects of failures are listed and assessed, wherewith controlling activities can be developed.
The establishment of both new chain oriented identification and traceability systems, and health program systems may be an operational field for FMEA in the agriculture and food industry.
Experiences collected in meat production to date have shown that in procedures that are systematic and team-coordinated unnecessary failures can be avoided by labelling action specifications.
This quality technique is particularly helpful for advisory teams that plan to implement targeted corrective and preventive actions within a comprehensive quality system such as the EN ISO 9000 standard series in addition to legal claims.

2. USE OF FMEA

FMEA is a systematic, team-oriented, preventative quality method. FMEA can help with the implementation of chain oriented actions in the meat branch – for example: the translation into action of the food hygiene decree like HACCP-concepts, the translation into action of a labelling system, or the facilitation of a new data processing systems. Should a mistake occur it can be easily corrected using FMEA (1, 6).
Risk assessment in the planning phase allows early identification of weak points and priorities for an action catalogue for failure prevention can be set. Improvement of process quality resulting from the implementation of FMEA reduces the chance that failures will occur when the product is used by the customer, which could result in extra costs due to customer claims and image damage (3, 4).
FMEA has already established itself as a practical tool within industrial production processes. In the food and nutrition industries the use of the HACCP concept has been in the forefront. Both methods have however a similar structure and basis and can therefore be easily combined together. We must however state that to date there are no practice-oriented examples where the primary producer has used these two methods for quality control.

This year a German-Dutch cooperation project began. Within the scope of this project we will also test FMEA as a planning instrument in BIQC – Borderless Integrated Quality Control - for meat production chains. It is important to consider whether or not FMEA – oriented towards the customer - will be incorporated into the system from the very first production steps. With respect to pig production this means considering whether the determination of risks will be considered also for the customer of the process as well as within the process.

This means that there must be an additional, customer-oriented, focusing of the risk determination and therefore not only within the product (piglet, pig, slaughter-ready animal, meat) but also within the product related process (piglet rearing, fattening farm, slaughter) and upon the effect of failures on the following processes or customers in the chain (for example the fattening farm, the abattoir, the butcher and the end-consumer).
Therefore there are three basic developments for the establishment of an effective FMEA:

- The exact definition and limit establishment of the area of applicability of the FMEA
- An FMEA team composed of experts with broad knowledge within the processes who can, based upon their experience, decide upon the relation of factors
- and, a production-wide based data bank must be available for all steps of the chain, and it must be of a continuous controlling nature for all steps within the production chain.

3. PERFORMANCE OF A FMEA SYSTEM

A FMEA control circuit showing the most important steps of a FMEA system is described (Figure 1) (2, 5). The general performance can be explained and illustrated best by means of a working example.

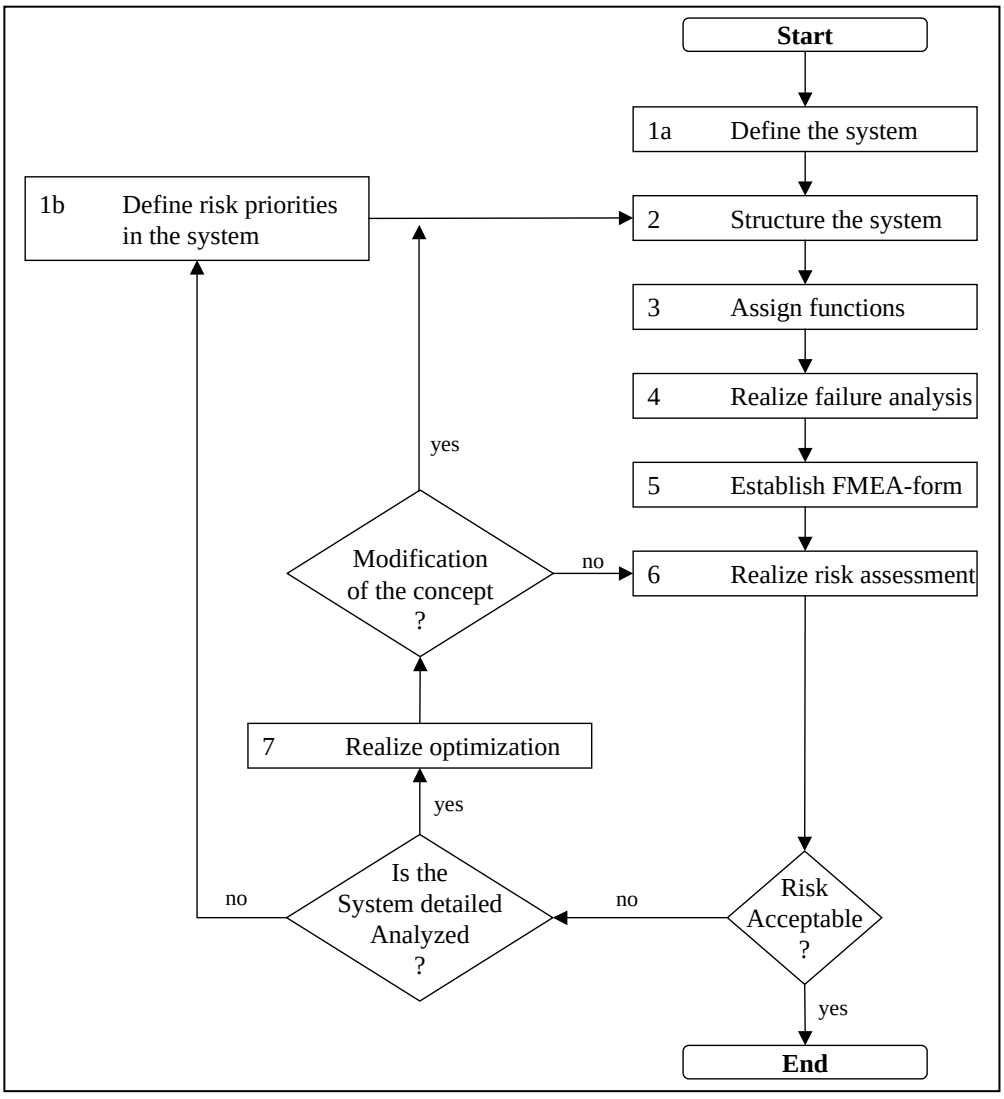

Figure 1: FMEA control circuit

4. THE FMEA METHOD IN A WORKING EXAMPLE

This example shall limit itself to the major aspects of FMEA:

- The structural error analysis including the cause and effect analysis (failure analysis)
- The determination of risk (risk assessment)
- and, the optimisation of the concept (optimisation)

5. FAILURE ANALYSIS

Dependent upon the health care desired different diseases are chosen as failures for the FMEA analysis. Health status disruptions can be divided into three large groups based upon their aetiology: primarily infectious, secondarily infectious, and non-infectious disease. Therefore at the beginning of the failure analysis the failure type (the disease) that will be used in the analysis is set. Thereafter the results of the disease are determined. The FMEA is a customer-based system, therefore the results of a disease are considered not only in the production stage where they occur but also for later stages in the production chain. Of course the potential and actual causes of the disease are also determined.

The previous results of the FMEA are used in an outline design so that documentation as well as clarity and an easy to understand systematic are guaranteed. The form is established during the project with the help of the data base controlled software solution “FMEA system 2.5”.

Completed FMEA outline forms can be used for the documentation. Therefore it is always possible to retrieve the results of previous examinations and to pass on experience to the different enterprises in the chain. This outline form helps to establish the next steps in the FMEA.

In the sixth step a risk assessment (Figure 1) is realized. Firstly, current controls are recorded on the form. These may be preventive actions (e.g. all in-all out per section) to prevent causes of failure, or testing actions for the detection of the causes of failure (e.g. measurements of temperature).

6. RISK ASSESSMENT

The measure of the risk assessment is the risk priority number (RPN). The RPN consists of three assessment numbers, which are multiplied together:

- Occurrence (O) is the assessment number for the occurrence probability. This assessment number is fixed for every failure cause, considering all effective preventative actions.
- The assessment number Severity (S) is an assessment of how serious the effect of the potential failure mode is on the internal or external customer or upon the next process respectively.
- Detection (D) is the assessment number for the detection probability. This assessment number is fixed for every cause of failure under consideration of all effective testing actions.

Normally an assessment number on a scale of 1 (no risk) to 10 (high risk) is chosen. To make the assessment easier, verbal valuation criteria are assigned to the assessment numbers (Table 1).

Table 1: Assessment steps and criteria for the occurrence probability (O) of a cause of a failure

Valuation criteria for the occurrence (O)	
Assessment	The occurrence probability of the potential cause is…
10 9 8	High: The failure frequently appears.
7 6 5 4	Mean: The failure appears occasionally, but not often.
3 2 1	Low: The occurrence of the failure is low or almost non-existent

7. OCCURRENCE (O)

With respect to diseases as failure types it is usual to determine not only the probability of disease but also the morbidity rate from the disease. The morbidity number is the percent of animals of an herd that show well defined characteristics of a disease within a specific time limit.

8. SEVERITY (S)

The criteria for the determination of the importance of a disease should fit the following points:

- They should be able to be used for every disease (therefore general)
- The effect upon the product and the process quality should be determined
- The economic effects should be determined
- The effect upon the customer should be of importance.

The following criteria meet these requirements:

- Morbidity (M)
- Effect upon the Production (Pw)
- Effects upon the use of the product (V)
- The course of the disease (time-scale) (Ez)
- And the effects upon trade. (H)

Using these risk numbers the total value of the criteria "Severity" (S) is calculated using the above numbers as follows:

$$S = \frac{Mt+Pw+V+Ez+H}{5}$$

9. DETECTION (D)

Not every mistake will be noticed by the customer. This aspect, which is true in every branch, is also true for disease as a failure type: the more direct and encompassing the proofing system in the production stage is the less reduced value wares are passed on to the customer and therefore there is a concomitant reduction in the ability to detect such wares should they occur. Thereby however, the use of laboratory and fast tests allow the determination of sub-clinical disease within animals, which could be considered as a hidden failure.
The probability of detecting disease comes from the clinical diagnostic predictive value of a positive test result. This value shows with what probability a positive test result can be used as an indicator of clinical disease. The final result of the risk analysis is therefore arithmetically determined as the Risk priority Number (RPN), determined from the product of O x S x D.

10. OPTIMIZATION / NEED FOR ACTION

The risk priority number accepts a value between 1 and 1.000. It represents a ranking for the optimisation to be carried out in the seventh step by the corresponding proposals for a possible solution (Figure 1). Optimisation is required for high RPN's or high single assessments. Optimisations are carried out according to the following principle:

- Amending the strategy (Figure 1) to exclude the cause of failure or reduce the severity. In this case, the system must be restructured (step 2).
- Increasing the strategy's reliability, to minimize the occurrence of the failure cause.
- More effective detection of the failure cause.

If there is no need for action, the team has to define who has to perform which recommended action and set a time limit for its completion. The responsible person and the date up to which the actions should be implemented are recorded in the FMEA form.
The most promising suggested actions are discussed, selected and then performed. After the performance of the recommended actions a risk assessment is again carried out. Risk priority numbers, serving as the basis for the decision upon the release of the health management system are calculated once again. In the context of a final result assessment, a comparison of the two risk priority numbers (previous state and improved state) is carried out. In this manner the relationship between achievable improvement and effort required can be assessed. The determined RPNs then represent the remaining risk of occurrence of certain failures. On this basis the team decides once more whether the actions were successful or whether additional actions are necessary.

11. INTEGRATION OF FMEA IN A DATA MODEL

In order to achieve an objective determination of the risk for product quality and/or the production process from a specific failure the data for the estimation of the probability of occurrence as well as the significance and the developmental process of the failure must be available from the production process. These data accumulate at different times and at different points in the production process and must be brought together in order to properly conduct a FMEA analysis.
Should the data-base not be informative enough then the FMEA team requires – in addition to their own expert knowledge – external information sources such as the professional literature, professional discussions and external experts. Figure 2 lists the available data and information sources within the area of meat production, whereby the continually available data is listed in the upper half-ring and the discontinuous data sources are listed in the lower half-ring. Before FMEA can be integrated into an existing producer-marketing cooperative a number of preliminary steps must be taken. They are necessary if FMEA is to be an institutionalised instrument within the preventative quality control.

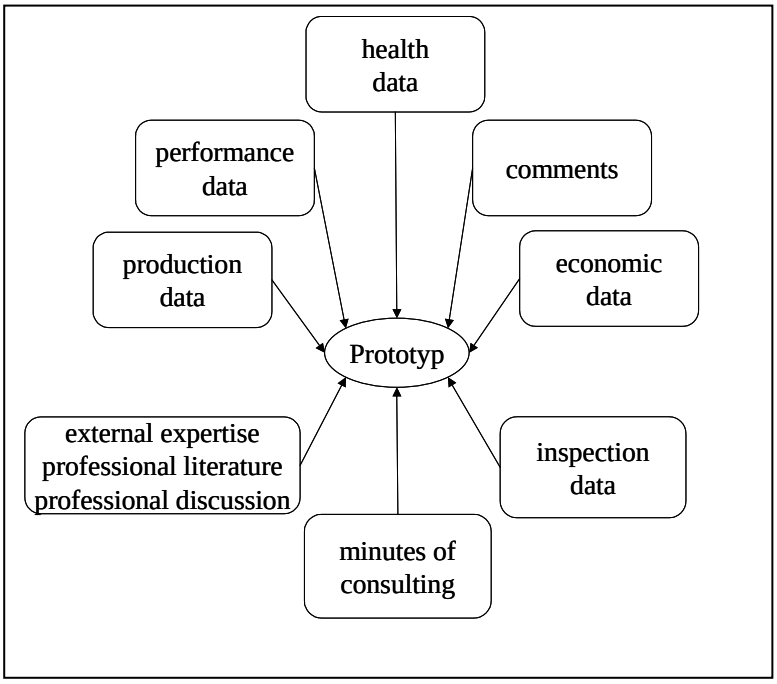

Figure 2: Data and information sources within the area of meat production

FMEA is an instrument for quality management and it is therefore to be expected that it access the data for quality safety. However, at the moment in the agricultural sector, the necessary computer based systems to support these activities are not developed. Therefore it is necessary to introduce FMEA parallel to the introduction of chain oriented communication and information systems. This is occurring at the moment in the Interreg project in the region Rhine-Wallis and Gronau.
A special databank and software system – SCIO – is being introduced to support the FMEA concept. This software-databank system has shown its usefulness in the automobile industry.

12. CONCLUSION

The Failure-Mode and Effects-Analysis (FMEA) is a preventative quality assurance method that has been implemented in many areas of the non-agricultural sector for years. It has now, for the first time, been introduced into the farming sector as part of a pilot project.
This analysis should enable the early recognition of potential failures and their influences on quality characteristics. Actions can then be taken to eliminate these non-conformities and prevent disorders.
At the present time, an extension and combination of the available data-bases in the agricultural sector are needed to supply the essential data basis for FMEA based risk evaluation.
In addition to preliminary analyses concerning organisation and content, it is necessary to implement activities in the data-processing area, such as: the setting-up and extension of an information and communication system that covers all stages of the production chain; the definition of interfaces; the creation of standardized communication

protocols to allow data exchange between agricultural management software and other programs; and the extension of the existing management software by taking new parameters and additional analytical possibilities into account.

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DETECTION OF RESIDUES OF ANTIBIOTICS IN FOODSTUFFS WITH MICROBIOLOGICAL TESTS USING *BACILLUS*

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1. INTRODUCTION

Since 1990 Maximum Residue Limits (MRLs) are set for residues of veterinary drugs in foodstuffs of animal origin by the European Council (EEC-Directive 2377/90 and amendments). Control on the presence of antimicrobial substances is usually performed by the use of microbiological tests. The poster presents data of a performance study of some recently developed commercial screening tests (Delvotest® MCS, Premi®Test and Premi®Test-Egg) using spores of *B. stearothermophilus* var. *calidolactis* as test organism.

2. MATERIAL AND METHODS

Blank meat fluid was obtained by heating (15 minutes at 64°C) blank muscle tissue (beef, pork and chicken) or applying the principle of freezing and thawing. Eggs from a tome of laying hens, not treated with antimicrobials the last 2 months, were mixed in order to obtain blank egg mix. Milk free from antimicrobials ("blank milk") and with a low bacterial load was collected from 4 cows in a good health status (low somatic cell count in the milk) and mixed. Blanks were further used to prepare the test samples (spiking).

For each veterinary drug substance tested, a stock solution (weight calculated on basis of active substance) and a whole batch of dilution series were prepared. The dilution steps were chosen depending on the expected detection level. All tests were undertaken according to the manufacturers instructions. All results were read visually. Each substance/concentration combination was replicated at least 10 times. The lowest concentration giving at least 90 % positive results was considered as the minimum detection level.

3. RESULTS

The Premi®Test (DSM-Gist b.v.) is a standard diffusion test for the detection of residues of antimicrobials in **meat**. Results are available within 2h50–3h20 (20 min prediffusion + 2h30-3h incubation (64°C)). The minimum detection levels of the Premi®Test for the different drug substances spiked in meat juice are given in table 1.

The Premi®Test-Egg (DSM-Gist b.v.) is a standard diffusion test for the detection of residues of antimicrobials in **eggs**. Results are available within 2h40–3h10 (10 min preheatment (80°C) + 2h30-3h incubation (64°C)). The minimum detection levels of the Premi®Test-Egg for the different drug substances spiked in egg mix are given in table 2.

The (DSM-Gist b.v.) is a standard diffusion test for the detection of residues of antimicrobials in **milk**, especially designed to be used in routine in milk control stations. It is a new version of the Delvotest® SP in multiplates (96 wells) without the need of a nutrient tablet. The Delvotest® MCS is the official test for raw milk testing (payment purpose) in Belgium since 01/01/2000. The visual reading of the colour of the test medium in the microtiterplates was performed after an incubation of 2h 40 at 64°C. The test sensitivity of the Delvotest® MCS is shown in figure 1 (penicillins and cephalosporins) and in figure 2 (other substances).

Table 1. Minimum detection levels (µg/l) of the Premi®Test in spiked meat juice (beef, pork and chicken) and MRLs in chicken meat (µg/kg). -*: no MRL fixed (EEC-Directive 2377/90 and amendments)

group	substance	Detection level (µg/l)			MRL (µg/kg)
		beef	pork	chicken	
β-lactams	Ampicillin	5	6	5	50
	amoxicillin	6	6	5	50
	oxacillin	30	30	25	300
	cloxacillin	60	60	40	300
	cephapirin	10	10	8	-*
tetracyclines	tetracycline HCl	300	250	300	100
	oxytetracycline	400	300	350	100
	chlortetracycline	250	150	250	100
	doxycycline	250	150	250	100
macrolides	spiramycin	3000	4000	4000	200
	tylosin	80	75	120	100
	erythromycin	300	200	300	400
lincosamides	lincomycin	400	400	400	100
aminoglycosides	streptomycin	5000	5000	5000	500
	dihydrostreptomycin	5000	6000	5000	500
	neomycin	500	1000	1000	500
	aminosidine	1500	2500	2500	500
sulphonamides	sulfachloropyridazine	40	40	30	100
	sulfadimidine	140	120	100	100
	sulfadimethoxine	60	60	40	100

Table 2. Minimum detection levels (D.L.) (µg/l) of the Premi®Test-Egg in spiked egg mix and MRLs in eggs (µg/kg).

substance	D.L. (µg/l) egg	MRL (µg/kg)	substance	D.L. (µg/l) egg	MRL (µg/kg)
ampicillin	5	-*	tylosin	75	-**
amoxicillin	4	-*	erythromycin	100	200
oxacillin	25	-*	lincomycin	150	50
cloxacillin	70	-*	streptomycin	2500	-*
cephapirin	8	-*	dihydrostreptomycin	1500	-*
tetracycline HCl	400	200	neomycin	600	500
oxytetracycline	800	200	aminosidine	1500	-*
chlortetracycline	1000	200	sulfachloropyridazine	40	-*
doxycycline	300	-**	sulfadimidine	70	-*
spiramycin	750	-*	sulfadimethoxine	30	-*

-*: no MRL fixed, -**: not for use in hens producing eggs (EEC-Directive 2377/90 and amendments)

The robustness of the Delvotest® MCS was also tested . The results of the Delvotest® MCS for 210 truck bulk milk samples and 90 farm milk samples were compared with those obtained with the Delvotest® SP, the improved agar diffusion test, the Charm Aim-96 and the *Bacillus cereus*-test. No false positive or false negative results were noticed for the Delvotest® MCS.

36 Milk samples with a somatic cell count >1. 10^6/ml were also tested and gave no false positive results after heating of the milk (10 min / 80°C). An abnormal high pH of the milk (> 7.2, mastitic milk) could provoke false positive results.

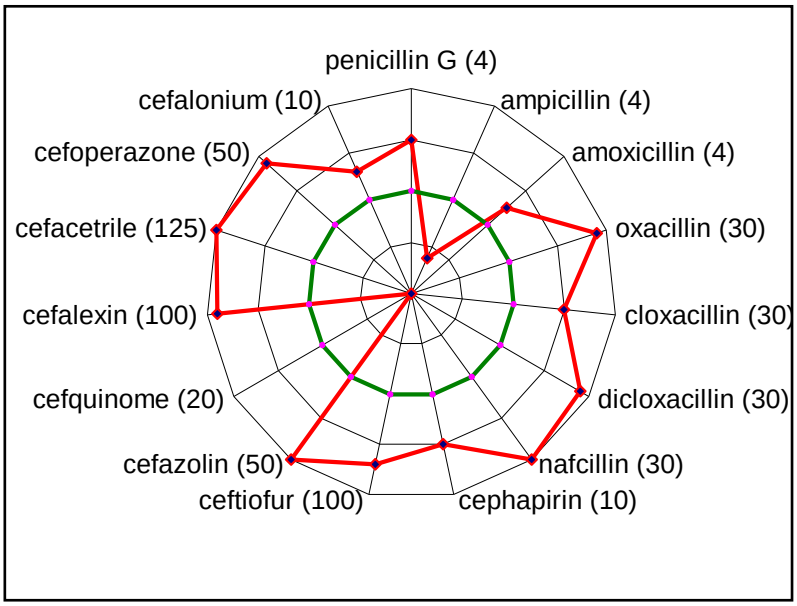

Figure 1: Test sensitivity of the Delvotest® MCS for penicillins and cephalosporins in relation to their respective MRL. Inner circle = 2 MRL, circle 2 = MRL, circle 3 = 0.5 MRL, outer circle = 0.25 MRL. For each substance the MRL in milk (µg/kg) is mentioned in between brackets.

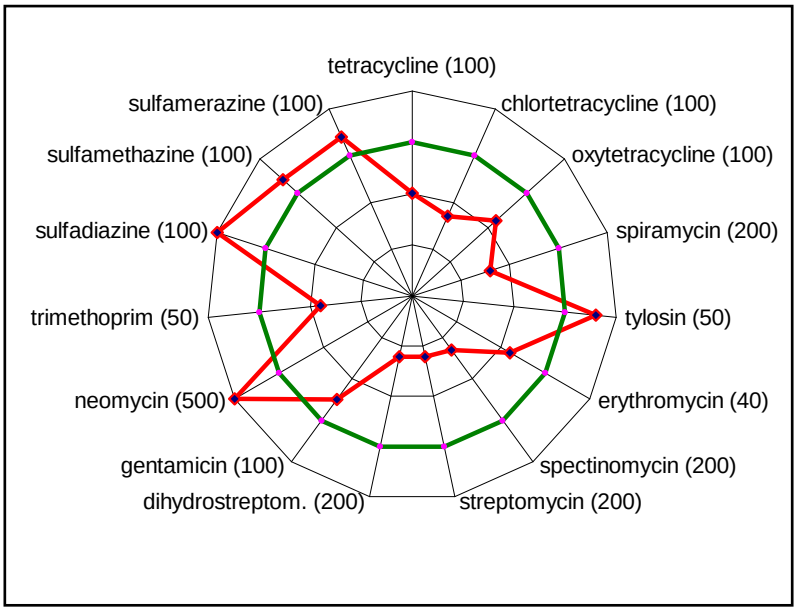

Figure 2: Test sensitivity of the Delvotest® MCS for other substances in relation to their respective MRL. Inner circle = 10 MRL, circle 2 = 2 MRL, circle 3 = MRL, outer circle = 0.5 MRL. For each substance the MRL in milk (µg/kg) is mentioned in between brackets.

4. DISCUSSION

In general all three tests are very sensitive for β-lactams (penicillins and cephalosporines) and sulphonamides. For most of the other compounds the sensitivity is not sufficient to monitor at MRL level.

The Delvotest® MCS detects most of the β-lactams at concentrations below their respective MRL. Therefore it was proposed to compensate this too high sensitivity when using the test in the frame of the official quality control of raw milk in Belgium. After a positive screening result the milk sample is retested in triple (heat treated milk, milk treated with penicillinase, milk 1:1 diluted) to confirm the positive result and to check if the inhibition

is caused by natural inhibitors or by β-lactams. In the last case, the result for the 1:1 with blank diluted milk is deciding if penalisation is applied or not.

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DE GLOBALE TRACEERBAARHEID IN DE BEDRIJFSKOLOM OOR VOEDINGSMIDDELEN.

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1. INLEIDING

Traceerbaarheid betekent o.a. dat men van een bepaald product kan nagaan waar het vandaan komt, welke productieprocessen het ondergaan heeft en waar zich de andere producten bevinden die dezelfde origine hebben of er bepaalde elementen mee gemeenschappelijk hebben. Men kan een onderscheid maken tussen interne traceerbaarheid en globale traceerbaarheid.

Interne traceerbaarheid is de opvolging van de producten binnen een onderneming. Deze is noodzakelijk om de eigen productieprocessen op te volgen en te optimaliseren en om een verband te kunnen leggen tussen de aangevoerde grondstoffen en de afgeleverde eindproducten.

Globale traceerbaarheid bekomt men door de aaneenschakeling van deze verbanden die gelegd zijn in de interne traceerbaarheid. Deze is nodig om:

- Producten kunnen te traceren te traceren doorheen de productiekolom om in geval van crisis, risicoproducten kunnen op te sporen en onschadelijk te maken.
- Betrouwbare garanties kunnen te geven over de kenmerken die aan een product toegekend worden.
- In geval van vaststelling van gebreken bij een product de verantwoordelijken hiervoor kunnen op te sporen (productaansprakelijkheid).

In deze uiteenzetting zullen we uitgaande van een voorbeeld uit de zeugenhouderij beide begrippen verduidelijken en een schematisch beeld geven van de globale traceerbaarheid die in België in opbouw is.

2. ZEUGENHOUDERIJ: EEN SCHAKEL IN EEN REEKS PRODUCTIEPROCESSEN.

2.1. Het zeugenbedrijf als onderneming.

Alvorens een varkenshouder een bedrijf kan oprichten moeten er een ganse reeks voorwaarden vervuld zijn. Hij moet beschikken over een bouwvergunning, een uitbatingsvergunning, een milieuvergunning, een aangifte bij de mestbank en wellicht nog veel meer. Alvorens hij dan hierin dieren mag houden, moet hij nog beschikken over een sanitair attest waarop o.a. het aantal zeugen- en vleesvarkenplaatsen vermeld staat.
Met andere woorden: een varkensbedrijf is een onderneming die onderworpen is aan een ganse reeds voorafgaande voorwaarden en vergunningen.

In de gebouwen is een specifieke infrastructuur aanwezig die toelaat dieren te huisvesten en te verzorgen: stallen voor drachtige zeugen, kraamhokken, biggenbatterijen, vleesvarkensstallen, automatische voederinstallaties, computergestuurde verluchtingssystemen enz.

In deze infrastructuur worden dieren gebracht. Het is de combinatie van de technische infrastructuur en de levende dieren die samen de productie-infrastructuur vormen.

2.2. Het productieproces

Door voeder te verstrekken aan de zeugen, samen met goed drinkwater in goed verluchte stallen, zullen deze op zeker ogenblik bronst vertonen. De varkenshouder zal de zeugen laten dekken of insemineren met sperma van een beer die hij aangekocht heeft of met sperma dat kant en klaar aangekocht is.

Het is voor de economische resultaten van het zeugenbedrijf zeer belangrijk dat de gegevens over bronst en dekking goed geregistreerd worden, zodat op gepaste tijdstippen bijzondere controles kunnen gebeuren evenals noodzakelijke preventieve behandelingen.

Kort voor het einde van de dracht worden de zeugen overgebracht naar een andere productieafdeling in de onderneming, namelijk de kraamstal. Bij het werpen zal de varkenshouder zorgvuldig notities maken van de werpdatum, van het aantal levend en dood geboren biggen, van informatie over het verloop van de geboorte en van eventuele behandelingen. Deze gegevens zijn nodig om o.a. via periodieke overzichten een beoordeling te maken van de individuele productieresultaten van iedere zeug, maar ook bij de dagelijkse gang van zaken, al was het maar om te weten wanneer de biggen moeten gespeend worden.
In het geval dat de biggen gespeend worden op 3 weken en aanstonds afgevoerd worden, zijn de pas gespeende biggen het eindproduct van het productieproces.

Met de aangevoerde basisproducten (voeder, water, lucht, sperma, medicamenten, …) wordt met de aanwezige productiemiddelen (gebouwen, technische voorzieningen, aanwezige zeugen, …) en de kennis en het werk van de varkenshouder een bepaalde hoeveelheid eindproduct (biggen) gevormd, samen met een aantal nevenproducten die hetzij een positieve economische waarde hebben (oude zeugen) of in tegendeel bijkomende kosten veroorzaken door de verdere verwerking (mest).

2.3. Kenmerken van de geproduceerde producten

Afhankelijk van de aangevoerde basisproducten en het gevolgde productieproces kunnen aan het eindproduct bepaalde kenmerken toegekend worden die belangrijk zijn voor de handelswaarde en voor het volgende of latere productieprocessen waaraan het product zal onderworpen worden.

2.3.1. Kenmerken in relatie tot de grondstoffen

De genetische inhoud van het gebruikte sperma zal in combinatie met dit van de aanwezige zeugen de genetische waarde van de biggen bepalen. Dit is meestal rechtstreeks bepalend voor hun verkoopprijs.

De aard en de samenstelling van de aangekochte voeders kunnen een invloed hebben op de bijkomende kenmerken die aan de biggen toegekend worden (vrij van bepaalde contaminanten, gehalte aan genetisch gemanipuleerde organismen (GGO's), aanwezigheid van groeibevorderaars, …). Dergelijke dingen zijn soms zeer belangrijk in geval een bepaald lastenboek moet nageleefd worden voor het bekomen van een label.

2.3.2. Kenmerken in relatie tot het productieproces.

Ook de manier waarop een varkenshouder te werk gaat, kan aan het eindproduct bepaalde bijkomende kenmerken bezorgen. Indien er niet zorgvuldig omgegaan wordt met het gebruik van medicamenten, riskeert men een slecht residu statuut, en om een goed Aujeszky statuut te behouden moet er gepast gevaccineerd worden en een aantal sanitaire voorwaarden gerespecteerd. Ook de manier van houden van zeugen (groepshuisvesting of individueel) kan van belang zijn in bepaalde gevallen.

Het betreft hier allemaal elementen die niet eenvoudig vast te stellen zijn op het eindproduct zelf (de biggen). Om toch voldoende garanties te kunnen geven, worden de hiervoor noodzakelijke gegevens niet alleen door de veehouder geregistreerd, maar ook centraal op een zodanige manier dat hierop controles mogelijk zijn en de garanties betrouwbaar gemaakt worden.

2.4. Te centraliseren gegevens voor betrouwbare garanties

Nemen we als voorbeeld het Aujeszky statuut. Om te kunnen beschikken over een A3-statuut moeten alle zeugen regelmatig gevaccineerd worden en moeten de resultaten van de serologische testen op een representatief aantal dieren, negatief zijn. Dit kan men slechts op een betrouwbare manier garanderen indien men weet hoeveel zeugen er in het bedrijf gehouden worden, hoeveel vaccinaties er uitgevoerd werden, hoeveel bloedstalen genomen zijn en wat de uitslag van de laboratoriumtesten was.

Daarenboven moeten de biggen die van dit bedrijf afgevoerd worden op een gecontroleerde wijze geïdentificeerd zijn, zodat men zeker weet dat ze inderdaad van dit bedrijf afkomstig zijn en er geen verwisseling gebeurd is.
Volgende zaken dienen dan ook te gebeuren:

- identificatie van alle biggen op speenleeftijd met een oormerk; de aflevering en het verbruik van deze oormerken wordt administratief gecontroleerd.

- 3 maal per jaar een bezoek door de bedrijfsdierenarts waarbij informatie verzameld wordt over het aantal aanwezige dieren en over het gebruik van de oormerken ter plaatse
- registratie van de uitgevoerde vaccinaties met vermelding van de diercategorie, het aantal gevaccineerde dieren en het gebruikte vaccin.
- opvolging van aan- en afvoer van dieren en zichtbaar maken van de statuten op de begeleidende vervoersdocumenten

Door een combinatie van deze gegevens kan aan elk lot biggen dat het zeugenbedrijf verlaat een betrouwbare garantie gegeven worden aangaande een aantal kenmerken die van invloed zijn op hun handelswaarde of mee bepalend zullen zijn voor de waarde van het eindproces van het volgende of verder afgelegen productieprocessen waar zij onderdeel van uitmaken.

Een lot zou men in dit kader kunnen definiëren als een bepaalde hoeveelheid geïdentificeerd product (aantal biggen met bepaald oormerk) van dezelfde herkomst (identificatie van het zeugenbedrijf), met dezelfde kenmerken (bepaalde statuten), die op een bepaald ogenblik een logische eenheid vormen (gelijktijdig afgevoerd in dezelfde vrachtwagen).

3. VOLGENDE STAPPEN IN DE VOEDSELKETEN

3.1. Biggenbedrijf

Door een vervoerder worden meerdere loten biggen van verschillende zeugenbedrijven verzameld en afgeladen in een biggenbedrijf waar ook andere vervoerders loten biggen aanvoeren. Samen met de voeders, het drinkwater, de verluchting en eventuele medicatie vormen zij de basisgrondstoffen voor het productieproces dat hier doorgevoerd wordt: met de aanwezige productiesystemen (stallingen, voederinstallatie, …) en de verzorging van de varkenshouder wordt een nieuw eindproduct gevormd, namelijk “mestbiggen” die in een vleesvarkensbedrijf verder gehouden worden tot slachtrijpe vleesvarkens.

Hierbij komen opnieuw de volgende onderdelen voor:
- Interne tracering: door zijn eigen notities zal de biggenhouder de interne gegevens bijhouden en gebruiken om zijn productieproces te optimaliseren.
- De kenmerken van het product: de kenmerken waarover de aangevoerde biggen beschikten (statuten, manier van houden, …) kunnen door de vermenging met andere biggen en door de manier van werken in het biggenbedrijf, gewijzigd zijn, dezelfde blijven of aangevuld worden met nieuwe kenmerken.
- Verband tussen aangevoerde grondstoffen en afgevoerde producten: bij afvoer worden de biggen opnieuw geïdentificeerd en worden nieuwe loten gevormd. Door de uitgevoerde registraties kan er een verband gelegd worden tussen de aangevoerde en de afgevoerde biggen.
- Centralisatie van een aantal gegevens: hierdoor kan er garantie gegeven worden aangaande de kenmerken die aan het biggenbedrijf of de biggen zelf toegekend worden en kan de tracering doorlopen over de processen heen.

Het leggen van het verband tussen aangevoerde grondstoffen en de afgewerkte producten is niet altijd even eenvoudig en gaat soms gepaard met verlies van informatie. De tracering bij varkens, waar men soms bijkomende identificaties aanbrengt en waar de dieren niet individueel opgevolgd worden maar per lot, is minder gedetailleerd dan b.v. bij runderen waar één enkele identificatie van kort na de geboorte behouden blijft tot na het slachten. Hierdoor zal men soms bij het opzoeken van de herkomst van varkens, niet terechtkomen bij een enkel bedrijf, maar bij een beperkt aantal mogelijke herkomstbedrijven.

Hoe gedetailleerder de opvolging van een product kan gebeuren, hoe gedetailleerder de tracering kan gebeuren en hoe kleiner het aantal ondernemingen zal zijn waartegen maatregelen moeten genomen worden in geval van crisis. Dit geldt eveneens binnen een onderneming: hoe gedetailleerder de registraties zijn en hoe kleiner de loten zijn die tijdens de productieprocessen effectief van elkaar gescheiden zijn, hoe minder product zal moeten in beslag genomen worden in geval van infecties of contaminaties.

3.2. Transport

Tussen het zeugenbedrijf en het biggenbedrijf is er transport van dieren. Ook bij afvoer van het biggenbedrijf naar het vleesvarkensbedrijf is dit het geval. Bij het transport wordt dus telkens een bepaalde hoeveelheid van een bepaald geïdentificeerd product op de ene plaats opgeladen waarbij de verantwoordelijkheid over dit product overgedragen wordt van de producent naar de vervoerder. Het vervoer wordt beëindigd bij het afladen van de

goederen, waarbij deze overgedragen worden van de vervoerder naar de verantwoordelijke van het volgende productieproces.

Belangrijk in het traceringmechanisme is dat wanneer er een overdracht is van producten (dieren of andere) van een verantwoordelijke naar een andere, dit geregistreerd wordt en ter beschikking gesteld wordt van de controlerende overheid. Hierbij volstaat het niet dat dit enkel door een van beide partijen gemeld wordt. Ook de andere partij moet hiervan een melding doen of ten minste hetgeen gemeld werd kunnen controleren en verifiëren.

4. TRACEERBAARHEID VAN PRODUCTEN VAN DIERLIJKE OORSPRONG

De ganse voedselketen is een aaneenschakeling van productieprocessen waarbij telkens de afgewerkte producten en/of nevenproducten van het ene proces dienen als uitgangsproduct voor het volgende. Het transport maakt de verbinding tussen beide.
Telkens worden een aantal gegevens centraal geregistreerd zodat tracering snel mogelijk is en gericht kan ingegrepen worden in geval van crisis. Daarnaast wordt deze informatie gebruikt om betrouwbare garanties te geven aan de volgende of verder afgelegen afdelingen van de voedselketen.
Dit is niet alleen het geval binnen de sector van de levende dieren, maar ook in de sector die erbij aansluit, namelijk bij de verwerking van de producten van de dieren en evenzeer bij de sector die grondstoffen aanvoert naar de levende dieren (veevoeders, medicamenten, …).

Gebaseerd op de ervaring van de traceerbaarheid van de levende dieren (Sanitel) die noodzakelijk was voor de bestrijding van besmettelijke ziekten, beginnen ook de andere sectoren zich steeds meer te organiseren en afspraken te maken om gegevens uit te wisselen en de traceerbaarheid uit te breiden over de ganse keten.
Een dergelijk uitgebreid systeem onderling samenhangend maken is niet eenvoudig en vraagt een progressieve opbouw. Alhoewel België in vergelijking met andere Europese landen reeds goed gevorderd is, is er nog heel wat weg af te leggen. Uitgaande van de onderdelen die in de laatste jaren reeds uitgebouwd werden en functioneel zijn, zal in een schematisch overzicht de verdere opbouw verduidelijkt worden.

4.1. Niveau 1: locale registratie van de basisgegevens (Sanitel lokaal)

In de provinciale verbonden voor dierenziektenbestrijding is een operationele eenheid met hardware, software en personeel aanwezig die instaat voor de omkadering van de gebruikers van Sanitel en de registratie van de informatie in de database.
De verschillende contactpunten van waar zij informatie bekomen en aan wie zij gegevens kunnen terug geven zijn o.a.:

- de veehouders
- de diergeneeskundige inspecteurs van het ministerie van landbouw
- de bedrijfsdierenartsen
- de diergeneeskundige laboratoria
- de vervoerders van dieren
- …

4.2. Niveau 2: koppeling naar andere diensten binnen de sector (Sanitel netwerk)

De plaatselijke gegevensbank van een provincie is via het grote Sanitel netwerk gekoppeld aan de plaatselijke gegevensbanken van de andere provincies. Informatie over dieren die verhandeld worden tussen provincies kunnen zo uitgewisseld worden.
Niet alleen met de databases van de andere provincies is er informatie-uitwisseling, maar er zijn ook verbindingen met andere netwerken:

- Centrale dienst van het ministerie van landbouw
- De slachthuizen en dierenverzamelplaatsen (markten)
- Private ondernemingen zoals het destructiebedrijf of de veeteeltverenigingen
- De nieuwe communicatiekanalen via het voice responce system en de webtoepassingen
- Het netwerk van de laboratoria voor diergeneeskundige onderzoeken
- …

Een verbinding tussen deze systemen is slechts mogelijk dank zij duidelijke afspraken aangaande de basisidentificaties van de operatoren. Het geeft het grote voordeel dat steeds meer informatie terug ter beschikking kan gegeven worden en er steeds meer kruiscontroles zijn op de nauwkeurigheid van de gegevens.

4.3. Niveau 3: koppeling naar andere sectoren.

4.3.1. Stroomafwaarts

Indien er een goede opvolging is van de levende dieren, kan men de nodige garanties geven aan de volgende segmenten van de keten die instaan voor de verwerking van de producten van de dieren en zo verder tot bij de consument. Het systeem dat de gegevens over de levende dieren bevat moet dus verder door kunnen getrokken worden naar o.a. de slachthuizen en van daar verder naar de vleesverwerkende bedrijven, de groothandel en de detailhandel.

Essentieel hierbij is dat er een duidelijke ondubbelzinnige verbinding kan gemaakt worden tussen de twee kolommen. De identificatie die gegeven wordt aan de dieren die de kolom van de levende dieren verlaten, moet overeenstemmen met de identificatie die het begin is van de kolom van de vleesverwerkende sector, te beginnen in de slachthuizen. In België is hiervoor het programma BELTRACE in opstartfase. Dit verzekert een directe en gecontroleerde link tussen Sanitel en de kolom van de vleesverwerkende sector die in ontwikkeling is.

4.3.2. Stroomopwaarts

Door de grote impact van de dioxinecrisis werd op zeer pijnlijk wijze ervaren dat een samenwerking tussen de kolom van de levende dieren en de vleesverwerkende sector onvoldoende is om volledige kwaliteitsgaranties te geven aan de consument. Er wordt immers nog geen rekening gehouden met de producten die aangevoerd worden op de beslagen en in dit geval vooral dan met de voeders.

Er worden door de Belgische autoriteiten heel wat controles uitgevoerd bij de veevoederproducenten, maar een registratie van welke voeders waar geleverd worden, was er tot voor kort nog niet. Hierdoor was het niet mogelijk snel te weten op welke bedrijven gecontamineerd voeder terechtgekomen was, welke dieren hiervan gegeten hadden en of er reeds dergelijke dieren geslacht waren en in welk stadium van de verdere verwerking zij zich bevonden. De veevoederfirma's konden lijsten voorleggen met namen en adressen van personen aan wie zij voederleveringen gefactureerd hadden, maar deze stemden vaak niet overeen met de plaatsen waar de dieren gehouden werden (beslagnummers).
Vandaar dat ook deze sector nu bezig is met het voorbereiden van een geïntegreerd systeem dat niet alleen toelaat de tracering binnen de kolom sneller en efficiënter te doen, maar ook een ondubbelzinnige link legt naar de kolom van de levende dieren.

4.4. Gebruik van deze gegevens

4.4.1. Ter controle en als beheersorgaan in geval van crisis

Uit de massa van informatie die in deze drie kolommen aanwezig is, wordt telkens de essentie ter beschikking gesteld aan de centrale overheid: het federale agentschap voor de voedselveiligheid. Zodra er zich problemen aanmelden kan dit agentschap zeer snel een zicht krijgen op de omvang van het probleem en de gepaste maatregelen nemen.

Door het feit dat het geheel transparant aan elkaar gekoppeld is, kan men steeds stroomopwaarts naar de oorzaak van het probleem gaan zoeken, zonder afhankelijk te zijn van de medewerking van de betrokkene. Anderzijds kan men stroomafwaarts de probleemsituaties snel lokaliseren en enkel daar ingrijpen waar het nodig is.

4.4.2. Gebruik door de sectoren

Deze massa informatie is niet enkel te gebruiken als een beheersorgaan in geval van crisis door de overheid, maar bevat ook een schat aan gegevens die ook in het voordeel van de sectoren zelf kunnen gebruikt worden. Mits het akkoord van de betrokkenen kunnen deze gegevens ook gebruikt worden door bepaalde labelprogramma's, voor het uitbetalen van premies of compensaties, enz.
De toepassingen die mogelijk zijn en ook gevraagd worden zijn zeer talrijk. Bepaalde labels die aan het eindproduct gekoppeld worden, geven garantie over de origine van de dieren, andere over de huisvestingsvorm, weer andere over verstrekte voeders (vrij van GGO), enz.
De mensen van de commerciële afdelingen zijn zeer vindingrijk in het bedenken van nieuwe criteria en anderzijds kunnen acties van bepaalde drukkingsgroepen een bestaand systeem helemaal omkeren. Een goed programma moet dan ook een voldoende brede basis hebben (zeker ook technisch) om op nieuwe vragen in te spelen, zonder hetgeen reeds bestaat telkens te moeten veranderen. Een voorbeeld is de weerstand tegen het gebruik van GGO's in de voeding en wie weet in de toekomst misschien tegen bepaalde vaccins!

5. GLOBALE TRACEERBAARHEID

Als deze kolommen die de informatie bevatten over de producten van dierlijke oorsprong internationaal op elkaar afgestemd zijn en doorverbonden worden met de kolommen die informatie bevatten over de producten van plantaardige oorsprong, over de verwerking van de nevenproducten (afvalverwerking), over de verpakkingsmaterialen enz., komt men tot de globale traceerbaarheid van de voedingsmiddelen. Het totale

schema wordt hierdoor steeds complexer maar het blijft gebaseerd op de aaneenschakeling van dezelfde basisprocessen.

De snelheid waarmee een dergelijk globaal systeem opgebouwd wordt is afhankelijk van uitwendige druk op de verschillende sectoren en van het voordeel dat de sectoren zelf er kunnen uit halen. Het voorkomen van crisistoestanden brengt dergelijke ontwikkelingen meestal in een stroomversnelling.

Men kan er dan ook van uit gaan dat de gegevens die men nodig heeft om te traceren en de manier waarop deze worden verzameld, zullen blijven evolueren in functie van de technische mogelijkheden, de vraag binnen de sectoren en de eisen van de consument.

GARANTIESYSTEMEN IN DE DIERLIJKE PRODUCTIE: DE VOEDINGSSECTOR GECONFRONTEERD MET EEN NIEUW VOEDINGSBELEID

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1. VERANDERINGEN VERTALEN IN BELEID:

Beleid, ook voedingsbeleid, wordt mee bepaald door maatschappelijke evoluties en prioriteiten.

Bij de oprichting van de EU, zo'n 50 jaar geleden, was het voedingsbeleid een belangrijk instrument voor de realisatie van de doelstellingen van de EU. Tot voor kort werd het voedingsbeleid vooral afgestemd op het Gemeenschappelijk Landbouwbeleid en de realisatie van de eenheidsmarkt. Productiviteit, zelfvoorziening en technologische vooruitgang waren belangrijke prioriteiten. Op EU niveau werd getracht om de wetgeving van de verschillende lidstaten op mekaar af te stemmen en gelijk te schakelen, om op die manier het vrij verkeer van diensten en goederen te kunnen realiseren. De complexe reglementering op het sanitaire en fytosanitaire domein kwam op die manier tot stand. De primaire doelstelling was de realisatie van de eenheidsmarkt door het harmoniseren van normen en regelgeving. Tegelijk werd door deze harmonisering ook een hoog niveau van voedselveiligheid bereikt. Dit beleid was erg succesvol op economisch vlak: de graad van zelfvoorziening werd zo groot dat de Europese voedselindustrie een belangrijke speler werd op de wereldmarkt en de eenheidsmarkt werd gerealiseerd.

Belangrijke wijzigingen in de maatschappelijke prioriteiten en in de agrovoedingsketen zelf liggen aan de basis van een grondige hertekening van het beleid.

Binnen de agrovoedingsketen werden de krachtverhoudingen grondig herschikt. De agrovoedingsketen evolueerde van een vraagmarkt naar een aanbodmarkt. Vroeger was het afzetten van het product een vanzelfsprekendheid, vandaag moeten producenten grote inspanningen leveren om te voldoen aan de eisen van hun veeleisende klanten en om tegelijk competitief te blijven. De distributie is binnen de keten zeer dominant geworden en legt haar voorwaarden op aan de voorafgaande schakels, om te kunnen een antwoord geven op de angsten en vragen van de (soms grillige) consument.

De houding van de consument t.o.v. voedsel en de manier waarop het geproduceerd wordt is zeer kritisch geworden. Voedsel was waarschijnlijk nooit meer gecontroleerd en veiliger dan vandaag. Er wordt voortdurend vooruitgang geboekt op het vlak van meet- en monitoringmethodes, zodat de kennis over voedsel en de problemen die ermee samenhangen steeds groter wordt. En toch overheerst bij de consument het wantrouwen, dat soms omslaat in paniek. Dit heeft ongetwijfeld te maken met de toenemende complexiteit van de productieprocessen. Vroeger kwam voeding dikwijls uit de onmiddellijke omgeving, de consument (hij noemde toen nog niet zo) was min of meer vertrouwd met de productiemethodes en ging voorzichtig met voeding om. Vandaag is voedingsproductie een mondiaal gebeuren. Grondstoffen voor diervoeding, voedsel en ingrediënten komen dikwijls uit andere continenten en het product wordt op zijn beurt over de ganse wereld verspreid. Door nieuwe technologieën wordt de productieketen langer en ingewikkelder. De consument (een stedeling) gebruikt steeds meer gebruiksklaar voedsel en wordt minder geconfronteerd met het primaire product. Dikwijls zijn voedingswaren gemengd en bereid voor ze bij de consument terechtkomen.

Op maatschappelijk vlak wordt het gebruik van nieuwe technologieën en de stijgende productiviteit kritisch bekeken. Economische aspecten, die gedurende lange tijd de drijfveer waren om ons hoog niveau van welvaart te bereiken, worden afgewogen aan en moeten het steeds meer afleggen tegen andere afwegingen

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(volksgezondheid, leefmilieu, ethische aspecten). De prioriteiten werden herschikt, mede vanuit het besef dat er grenzen zijn aan groei en vooruitgang. De nieuwe prioriteiten worden vertaald in het begrip duurzaamheid.

Voor het voedselbeleid zijn voedselveiligheid en volksgezondheid de absolute prioriteit geworden, waaraan al het overige ondergeschikt gemaakt wordt.

2. HET WITBOEK OVER VOEDSELVEILIGHEID:

In het witboek over voedselveiligheid[2] schetst de EU Commissie een volledig nieuw kader voor de organisatie van en het toezicht op voedselveiligheid. De Commissie wil met deze nieuwe wetgeving de gezondheid van Europese consumenten promoten door het instellen van regels van voedselveiligheid die tot de strengste en beste ter wereld behoren. Het witboek omvat een actieplan van 84 punten die ten laatste eind 2002 moeten gerealiseerd zijn. De doelstellingen kunnen als volgt worden samengevat:

- Het oprichten van een Europese voedselautoriteit (EVA).
- De realisatie van een coherente Europese wetgeving m.b.t. voedselveiligheid.
- Een betere en meer coherente organisatie van controles op de voedselketen.
- Permanente dialoog met en informatie van de consumenten.
- Een internationale dimensie: het Europese voedselbeleid hard maken op wereldvlak.

De laatste doelstelling is zeker niet de minst belangrijke, alhoewel er in het witboek het minst over gezegd wordt. De regels voor voedselveiligheid zijn in Europa dikwijls strenger dan in de rest van de wereld. Denk maar aan de discussie over het gebruik van natuurlijke hormonen, of aan het moeilijke debat over de genetisch gewijzigde organismen (GGO's). Door de handelspartners van de EU in de rest van de wereld worden deze verregaande bepalingen dikwijls gezien als een vorm van protectionisme. Toch is het van groot belang dat de strenge Europese regels op wereldvlak aanvaard worden om te kunnen garanderen dat ingevoerde producten even veilig zijn als eigen Europese producten.

De Europese voedselautoriteit (EVA) moet een onafhankelijke instantie worden waarin de gevaren verbonden aan voedsel op een wetenschappelijke manier worden onderzocht. De EVA zal, op basis van een wetenschappelijke risicobeoordeling, adviezen geven aan de overheid. De opdrachten worden in het witboek gedefinieerd als volgt:

- Onafhankelijke wetenschappelijke risicobeoordeling.
- Ontwikkelen en operationeel houden van bewakingsystemen op voedselveiligheid.
- Communicatie over voedselveiligheid en nutritionele aspecten.
- Beheer van rapid alert systemen.

Het risicobeheer (maatregelen om risico's te vermijden of te beheersen) behoort tot de bevoegdheid van de Commissie, die de wetenschappelijke adviezen vertaalt in normen en reglementering voor voedselveiligheid.
In het witboek worden tevens een aantal principes naar voor geschoven waarop de nieuwe reglementering zal gebaseerd worden:

- Een geïntegreerde benadering van de ganse keten van grondstof tot voedsel.
- Het responsabiliseren van operatoren en producenten.
- Traceerbaarheid van voeder, voedsel en ingrediënten.
- Systematisch gebruik van risicoanalyse bij de beoordeling van problemen.
- Toepassing van het voorzorgsbeginsel[3], wanneer nodig.

3. HET FEDERAAL AGENTSCHAP VOOR DE VEILIGHEID VAN DE VOEDSELKETEN (FAVV):

Tot op heden was de bevoegdheid voor de voedselveiligheid in België verdeeld over verschillende ministeries en diensten. Deze versnippering van bevoegdheden stond een efficiënt toezicht op de voedselproductieketen dikwijls in de weg. Met de oprichting van het FAVV worden alle controlediensten op de voedselketen

[2] White paper on food safety, COM(1999) 719 final

[3] COM (2000) 438 final (europa.eu.int/comm/food)

geïntegreerd in één globale structuur, onder de bevoegdheid van de Minister van Volksgezondheid. Dit is een logische vertaling van de herschikte prioriteiten. In het verleden was voedselveiligheid soms een aanhangsel van andere beleidsdomeinen (bvb. landbouw), in de toekomst wordt voedselveiligheid een kerntaak van het FAVV, waaraan andere bevoegdheidsdomeinen ondergeschikt gemaakt worden. Het FAVV werd opgericht met de wet van 4 februari 2000[4].

Het doel van het FAVV wordt als volgt omschreven: " de veiligheid van de voedselketen en de kwaliteit van voedsel teneinde de gezondheid van de consumenten te beschermen". In de wet worden de bevoegdheden van het FAVV vastgelegd:

- Controle, onderzoek en keuring van voedselproducten en grondstoffen.
- Controle en keuring van productieprocessen.
- Het verlenen van erkenningen en vergunningen.
- Uitwerken en integreren van identificatie- en traceringsystemen van voedsel en grondstoffen.
- Verzameling, beheer en verspreiding van alle informatie aangaande haar opdracht.
- Opzetten van beleid inzake preventie, sensibilisatie en informatie.
- Het toezicht op het naleven van de wetgeving.

De bestaande controlediensten zullen binnen het FAVV herschikt worden op basis van een volledig nieuwe organisatiestructuur, die gebaseerd is op een multidisciplinaire aanpak. Het is de bedoeling om de diensten zo te reorganiseren dat zij zo efficiënt en zo goed mogelijk kunnen toezien op de complexe agrovoedingsketen, van voeder tot voedsel. De uitbouw van goede systemen voor uitwisseling van informatie en het opzetten van efficiënte structuren voor het beheer van voedselcrisissen zullen erg belangrijk zijn voor een goede werking van het FAVV.

In de wet werd voorzien dat het FAVV zich voor een aantal taken kan laten bijstaan door derden en dat een aantal opdrachten aan derden kunnen uitbesteed worden. Hier wordt dus ruimte gecreëerd voor de organisatie van autocontrolesystemen in de voedingssector. Het spreekt vanzelf dat dergelijke autocontrolesystemen niet vrijblijvend zijn, maar aan strenge regels zullen moeten voldoen en dat het FAVV zal blijven toezien op de toepassing ervan.

Bij het FAVV worden twee adviescomités ingesteld: een wetenschappelijk comité dat wordt samengesteld uit nationale en internationale deskundigen, en een raadgevend comité samengesteld uit vertegenwoordigers van de overheid, de consumenten en de producenten. Het wetenschappelijk comité kan het best worden vergeleken met de EVA op Europees niveau. Het is in dit comité dat de wetenschappelijke evaluatie zal gebeuren van de risico's op het vlak van voedselveiligheid. De adviezen van dit comité zullen bepalend zijn voor het voedselveiligheidsbeleid in België.

Tenslotte wordt bij het FAVV een permanent meldpunt gecreëerd waar de consument terecht kan voor objectieve informatie en individuele klachtenbehandeling met betrekking tot de kwaliteit en de veiligheid van voedsel. Op deze manier wordt het FAVV een rechtstreeks aanspreekpunt voor de consument, wanneer deze met vragen zit over voedsel.

Met de oprichting van het FAVV worden de beleids- en controlediensten die bevoegd zijn voor voedselveiligheid grondig herschikt. Het FAVV staat nog in de kinderschoenen en de volledige uitbouw ervan zal nog enkele jaren in beslag nemen. Het opzetten van deze nieuwe structuur is een unieke kans om een grote stap vooruit te zetten in het Belgische voedselveiligheidsbeleid.

4. AUTOCONTROLE: BEDRIJVEN ZIJN VERANTWOORDELIJK VOOR DE VEILIGHEID VAN VOEDINGSPRODUCTEN.

Voedselveiligheid is een gedeelde verantwoordelijkheid tussen de overheid en de ondernemingen. Een goede verdeling, goede afspraken over wie wat doet zijn noodzakelijk om te komen tot een coherente en complementaire ketenbenadering. De producenten zijn zich bewust van de nieuwe prioriteiten en passen zich aan. GMP (good management practices), HACCP (gevarenanalyse op kritische controlepunten), autocontrole

[4] Wet van 4 februari 2000 houdende oprichting van het Federaal Agentschap voor de Veiligheid van de Voedselketen, B.S. 18/02/2000.

vinden steeds meer ingang in bedrijfseigen kwaliteitsbewakingssystemen en worden een essentiële voorwaarde om toegang te krijgen tot de markt.

Het bepalen van de voorwaarden voor autocontrole is daar een onderdeel van. In het witboek wordt duidelijk gesteld dat producenten zelf verantwoordelijk zijn voor de kwaliteit en de veiligheid van de producten die zij voortbrengen. De overheid moet instaan voor normering en voldoende inspecties. De overheid kan autocontrole aanmoedigen door zijn inspecties vooral te richten op bedrijven die te weinig of geen inspanningen leveren op dit vlak. Uiteindelijk kan het noodzakelijk zijn om bepaalde vormen van autocontrole te verplichten.

De discussie over hoe autocontrole een rol kan spelen in de relatie tussen de bevoegde overheid (het FAVV) en bedrijven of sectoren uit de voedingsindustrie moet nog worden aangevat. De wet tot oprichting van het FAVV creëert een wettelijke basis voor het invoeren van autocontrole. Het opstarten van deze discussie is heel dringend, aangezien het ontbreken van duidelijke afspraken met de overheid momenteel de evolutie in de bedrijven afremt.

Een basis voor discussie kan gevonden worden in een werkdocument van de Codex Alimentarius (FAO, WHO) over bedrijfseigen kwaliteitsbewakingssystemen: « Proposed draft guidelines for the utilization and promotion of quality assurance systems to meet requirements in relations to food »[5].

De wetgeving vereist van de bedrijven uit de voedingsindustrie dat zij een systeem uitbouwen om de veiligheid van hun producten te waarborgen. De bedrijven moeten via het beheersen van kritische punten in hun productieproces zowel ziekteverwekkende kiemen als verontreinigingen in het voedsel voorkomen. Zij moeten hiervoor de HACCP principes toepassen. Dit acroniem staat voor “Hasard Analysis in Critical Control Points”. In heel wat voedingsbedrijven zijn kwaliteitssystemen aanwezig die verder gaan dan wat de wet voorschrijft. In deze systemen worden niet alleen garanties gegeven op het vlak van de voedselveiligheid maar ook inzake algemene kwaliteitsaspecten.

Een aantal bedrijven hebben er voor gekozen om hun kwaliteitssystemen of hun productiemethode door een onafhankelijke derde (een certificatie- of inspectieorganisme) te laten controleren. Deze instellingen zullen nagaan in welke mate de bedrijven voldoen aan bepaalde normen of lastenboeken. Deze controles kunnen uiteindelijk uitmonden in een certificaat. Het certificaat kan betrekking hebben op het systeem dat door de bedrijven gebruikt wordt om de kwaliteit te waarborgen (ook systeemcertificatie) ofwel op de geproduceerde producten of op een combinatie van beide.

Instellingen die hiertoe door BELCERT (Ministerie van economische zaken) geaccrediteerd zijn kunnen certificaten afleveren op basis van een welomschreven norm of lastenboek waarvan aangetoond is dat zij een toegevoegde waarde hebben of met andere woorden eisen bevatten die verder gaan dan de wettelijke voorschriften. Zij moeten tevens aantonen dat zij hun eigen kwaliteitssysteem hebben en dat zij onafhankelijk zijn. Er zijn verschillende types van accreditatie:

- een accreditatie voor het certificeren van kwaliteitssystemen (volgens de Europese norm NEN 45012)
- een accreditatie voor het certificeren van producten (volgens de norm NEN 45011)
- een accreditatie voor het uitvoeren van inspecties (volgens de norm NEN 45004).

De laboratoria die analyses uitvoeren kunnen daartoe geaccrediteerd worden door BELTEST (volgens de norm NEN 45004).

In de voedingsindustrie zijn vooral ISO 9000, HACCP, en BRC ingeburgerd.

Verschillende branchevereningingen uit de Belgische voedingsnijverheid stelden “gidsen voor goede hygiënepraktijken” (GGHP) op.

Bij de uitbouw van deze kwaliteitssystemen wordt veel aandacht besteed aan een geïntegreerde benadering van grondstof tot afgewerkt product (ketenbenadering). Daartoe werden in België een aantal initiatieven genomen:

- In het “overlegplatform voor de verwerking van plantaardige grondstoffen” (OVPG) wordt een IKKB systeem voor de plantaardige productie uitgewerkt.

[5] www.codexalimentarius.net

- In OVOCOM werd een kwaliteitssysteem voor dierenvoeder uitgewerkt onder de benaming GMP dierenvoeders.
- Het Verbond van Belgische Tuinbouwveilingen (VBT) ontwikkelde een aantal kwaliteitssystemen die nauw aansluiten bij EUREP-GAP, een referentie voor de goede landbouwpraktijk die werd uitgewerkt door een aantal Europese retailers.
- De Belgische zuivelsector ontwikkelde de “integrale kwaliteitszorg melk” (IKM).
- De promotie organismen VLAM en ORPAH ontwikkelden een aantal kwaliteitssystemen voor verschillende sectoren (vlees, groenten en fruit, aardappelen, …).
- Voor de biologische productie ontwikkelde het overlegplatform BIOFORUM het logo en lastenboek BIOGARANTIE.

Op de website www.qualityfood.be heeft de Belgische federatie van de voedingsindustrie (FEVIA) onder de naam “kwaliteit van A tot Z” een volledig overzicht ontwikkeld van de kwaliteitsborgingssystemen in de Belgische voedingsindustrie.

CONSUMER RISK PERCEPTION AND RISK COMMUNICATION: THE CASE OF MEAT DEMAND IN BELGIUM

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ABSTRACT

Meat consumption levels have decreased throughout Europe during the last five years. Beef consumption is definitively most affected. This is not surprising given the occurrence of several beef safety crises, which affected consumer perception and confidence. This paper focuses on the concepts of risk perception and risk communication with illustrations from the case of meat demand in Belgium. Though clear-cut answers as to how to communicate lack, it is clearly shown that – in absence of adequate risk communication strategies – strong reactions at the consumer level may be expected in the event of future crises.

1. INTRODUCTION

The evolution of meat consumption in Belgium since 1955 reveals that distinct long-term changes have taken place. Animal protein and fat intake have risen along with increasing wealth in the West-European society. Over time, a gradual shift away from red to white meat types was observed. Top meat consumption levels were reached during the first half of the nineties, with considerable consumption decreases being noticed since, especially when considering per capita at-home meat intake. Over a period of six years (1995-2000), Belgian at-home fresh beef and veal consumption fell more than 28%; pork and poultry consumption decreased with about 7% and 3%, respectively. Out of home meat consumption may have increased but figures are not readily available. Nevertheless, available data from supply balance sheets and household panels systematically point towards former significant consumption declines, which exemplify a general “malaise” against fresh meat. While a negative trend in recent meat consumption is not surprising given the already elevated levels of animal protein intake, it is clear that timely lows in consumption resulted from meat safety crises, the consequent loss of confidence at the consumer level, worsening perception of meat and lack of adequate risk communication responses by industry and government. This paper first presents some insights in the concepts of (risk) perception and communication. Next, illustrations from the case of meat demand in Belgium are provided. Findings are based on several consumer surveys and secondary data, which have been collected, analysed and reported since 1996.

2. (RISK) PERCEPTION AND COMMUNICATION

Scientists frequently wonder why consumers see things differently than the reality behind. Scientific objectivity relates to facts and it is often referred to as the only one truth. Examples are product specifications in terms of technological quality and microbial food safety (e.g. pH of pig meat, number of micro-organisms present e.g. in yoghurt, probability of getting ill by eating a certain quantity of a good). In the consumer psychology and behaviour disciplines, it is widely recognised that there exists a distinct filter or gap between the external (objective) and the internal (subjective) world (1). This filter, also called a perception filter, accounts for the difference between scientific objectivity and human subjectivity. The paramount importance of perception lies in the fact that perception – not necessarily scientific facts – determines preference and choice, which is ultimately what is of interest to the industry. As will be illustrated in the case of meat in Belgium, the perception filter or the gap between reality and consumer perception is largely shaped by communication through mass media reports or advertising.

With the emergence of food safety challenges during the last decade, research interests focused specifically on the topic of risk perception. Definition holds that risk perceptions are socially constructed, and that individual behaviours are driven by perceptions or beliefs about risks, and not by the technical estimates provided by experts (2). The latter part of this definition corroborates the concept of the previously mentioned perception filter. The important addition lies in the social dimension of risk perception. The idea behind is that certain aspects of hazard events interact with psychological, social, institutional and cultural processes in ways that might attenuate or intensify perceptions of risk (3, 4). This process is called "social amplification of risk" and it tries to explain why society responds to a particular hazard in a way, which may appear disproportionate to the technical risk estimates. Such processes, with risks apparently being blown out of proportion, have for instance occurred during the BSE, dioxin and Coca-Cola crises in Belgium.

An explanation for the fact that certain risks trigger more alarm than others lies in the presence of so-called "fright factors" (5). Risks are generally more worrying if perceived to be involuntary, inequitably distributed, inescapable, novel, man-made and poorly understood by science. Furthermore, perceptions of risk are stronger if they cause hidden and irreversible damage, pose danger to future generations, threaten a form of death arousing particular dread and damage identifiable rather than anonymous victims. Many of these fright factors hold for food safety risks and they certainly did so in the meat safety cases of previous years. Additionally, the meat safety risks had numerous characteristics, which made them likely to become a major media story. These characteristics are called "media triggers" and include: question of blame (e.g. the feed industry or government), attempted cover-ups (e.g. dioxin crisis communication), links with personalities (e.g. ministers), massive exposure (e.g. everyone consumes eggs, at least in some form), strong visual impact (e.g. the mad cow) and link to crime (e.g. the "hormone mafia").

How to communicate about risk to consumers and the general public is certainly amongst the most frequently asked questions in public health policy and food industry debates. Risk communication becomes particularly difficult in cases where a lot of uncertainty is involved, and where clear advice is hard to provide (e.g. the BSE case). One of the most important issues relates to trust in information sources and transmitters. Trusted sources like medical doctors and consumer organisations are perceived to be knowledgeable and concerned with public welfare (2). Distrusted sources (e.g. government) are perceived to distort information, to have been proven wrong in the past, and to provide biased information. Trust is associated with moderate accountability. Industry is seen as over-accountable, whereas popular press is perceived to have too little accountability to be trusted.

3. CONSUMER PERCEPTION OF MEAT (RISKS)

Key fresh meat attributes from the consumer viewpoint are obtained from qualitative research and literature review. Next, consumer perception of fresh meat, including pork, has been assessed on scales during two consumer surveys. Methodological details are fully reported in the respective publications that are referred to. Inspection of attribute rating profiles of April 1998 revealed that problems of the pork image mainly pertained to pork's perception as the most fat, the worst tasting, the least healthy and the overall lowest quality meat (6). Beef is perceived as the most expensive and least safe and trustworthy meat. Poultry receives overall the best perception scores, except on animal welfare. It is to be noticed that all fresh meat types received a negative average perception score with respect to animal welfare. While meat safety concerns are still paramount today, also other findings pointed toward a growing importance of animal welfare consciousness when making meat consumption decisions in the near future (7).

The same exercise of measuring consumer perception was repeated two years later, in April 2000, after the occurrence of the Belgian dioxin crisis. Like the previous meat safety crises (hormone abuses, antibiotic residues, BSE), the dioxin scare received considerable attention from the mass media, which brought the issue to the public's attention on May 27, 1999. Media reports initially suggested a playing-down or cover-up by the Ministries of Agriculture and Public Health, of the risks to human health resulting from a dioxin contamination in animal feed. The cause of the dioxin problem dated back to the end of January 1999 and pertained to PCB and dioxin contaminated transformer oil that entered the food chain. It initially led to abnormal laying hen mortality and decreasing egg hatchability. Consequent analyses indicated dioxin levels that exceeded the legal standards; for example those applying to chicken fat, by 1,500 times. The 28th of May, all chicken and eggs were removed from the Belgian shelves. Additionally, some of the broiler feed appeared to have been recycled into pig feed, thus also involving pork meat into the debate and product recall. The initial blocking of meat products in Belgium was soon followed by import bans of Belgian meat and egg products by other EU countries, backed up by the EU veterinary committee's decisions. Any remaining restrictive measures were only lifted on April 18, 2000 by EU decision 2000/301, which denoted the formal end of the crisis.

Pork and especially poultry were affected by the dioxin crisis, which was finally reflected in their perceptual profiles. This led to significant shifts toward the "with hormones"-pole of the semantic differential scale (or stronger associations with containing hormone) for both meats' perception. Additionally, poultry perception on "quality", "trustworthiness" and "safety" significantly worsened after the dioxin crisis. No other shifts of the pork and poultry perception profiles were discovered, which is reasonable in absence of substantial changes in sensory, price, convenience or animal welfare issues over the considered time interval (8). Over the two-year interval, beef perception improved on attributes related to safety and healthiness. This is what could have been expected in absence of major media focus on beef issues during the data collection interval. Remarkably, consumption (behaviour) evolution did not follow attitude changes: beef consumption continued to decrease during the data collected interval, pork and poultry consumption almost managed to stabilise.

Considerable bias was discovered between meat facts or scientific indicator criteria and consumer perception of these facts. This phenomenon has specifically been addressed related to health, leanness and sensory characteristics of pork (9), but also related to meat quality labels (10). Pork perception was found to be worst as compared to beef and poultry on "leanness", "healthiness", and attributes that relate to eating or sensory quality, i.e. "taste" and "tenderness". On the contrary, it was scientifically shown that pork could be low in fat and cholesterol, or excelling in taste and tenderness, depending on the specific cut and handling throughout the meat chain. Similar conclusions were drawn related to the perception of quality labels. In 1998, a considerable part of the interviewed consumers claim to buy labelled meat but fail to recall any label unaided. Additionally, features and benefits are assigned to quality labelled meat that do not correspond to the actual performance of the label. Two years later, the knowledge and perception of labels appear to have improved. In 2000, higher shares of the respondents claim the need for labels. Furthermore, the percentages claimed buying and market share on the one hand, and unaided knowledge and recall on the other hand, are better matching.

Consumers were also asked about their levels of concern related to certain risks from consuming meat. Invariably, more than 40% of the respondents claimed to be highly concerned or concerned about any presented stimulus: hormones, BSE, antibiotics, Salmonella, dioxin, fat/cholesterol. Remarkably, high numbers of respondents reported concern about dioxin in beef and hormones or BSE in poultry or pork. This denotes a lack of knowledge at the consumer level and the existence of general concern about any potential risk from meat consumption.

4. IMPACT OF COMMUNICATION

Claimed attention to mass media publicity is found to have a highly negative influence on consumer behaviour and decision-making processes toward fresh meat. Consumers who attended mass media coverage of fresh meat issues, reported significantly higher meat consumption decreases with reference to the past as well as intentions towards the future. Additionally, these consumers perceived beef and pork significantly worse on attributes that relate to health, trustworthiness and product safety. It was finally found that consumers who pay a high level of attention to media reports express higher health consciousness, more misperception of health risks and higher levels of concern about potential health hazards that were frequently reported in mass media. While the impact of attention to mass media publicity was shown to be very significant, high levels of attention to personal communication from butchers or to advertising were found to have some but far more limited impact. Meat consumers who pay high levels of attention to information from butchers reported more positive perception scores, but this did not translate further into associations with health concern, claimed behaviour or behavioural intention (11).

The negative impact from television publicity was confirmed through econometric probit analysis and the estimation of an Almost Ideal Demand System (AIDS) for fresh meat including information effects. Probabilities to cut fresh meat consumption were boosted as consumers reported to have paid high attention to television coverage of meat issues. Attending negative press pushes probability levels of decreasing meat consumption in the next year to around 65%. Furthermore, negative TV coverage pushes meat reduction rates for young people to levels that would have occurred naturally with the ageing process (12). Similarly, parameters of the television coverage indices were largely significant and negative in the AIDS model, contrary to the estimates of the advertising expenditure variables, which were insignificant. For example, in the case of beef in Belgium during the second half of the nineties, a negative press over advertising impact ratio of five to one was found (13). It means that five units of positive news are needed to offset the impact of one similar negative message, or, that the meat industry has to communicate five times stronger (in terms of frequency, assuming that the effectiveness is similar) than mass media report negative messages. This ratio is particularly alarming when realising that

negative press is granted for free, while engaging in the advertising business is tremendously expensive for the whole industry. From the total number of TV reports during the period 1996-1999, the number of positive messages amounted to only 12% and 6% for beef and pork, respectively. The impotence of advertising is explained by the fact that a minimum threshold performance or expenditure level, which can be particularly elevated in an era dominated by bad press, was not reached (14).

5. CONCLUSIONS

The case studies on meat demand in Belgium provide a clear picture of the impact of negative press related to meat safety issues in absence of adequate response strategies. In the private sector, it is axiomatic that perception is everything, or, that there exists only reality, namely the perceived one. Perceptions can be changed either by modifying the underlying product or simply by managing perceptions e.g. through communication. Governments who are the main responsible for public health communication feel generally uneasy with this approach, and in their concern not to be seen as manipulating opinion, frequently adopt approaches and behaviours which destroy trust and confidence (15). This attitude of governments is alarming given the paramount importance of trust when engaging in risk communication. Last but not least, it is important to notice that consumer behaviour does not consistently follow attitude change or perception. This was illustrated with the case of the dioxin crisis in Belgium. It points to realise that quality is much more than just safety; it also pertains to sensory or convenience attributes. In conclusion: although the monitoring of safety is a key element in quality management, the other elements that contribute to quality perception by consumers should not be overlooked; both in the quality management and communication phases.

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ESTIMATION OF THE SENSITIVITY AND THE SPECIFICITY OF TWO DIAGNOSTIC TESTS FOR *TRYPANOSOMA EVANSI* IN ABSENCE OF A GOLD STANDARD

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1. INTRODUCTION

Estimation of the diagnostic sensitivity and specificity of a new test requires the existence of a gold standard.
In trypanosomosis, parasite detection is generally assumed to be the gold standard but lack of sensitivity of the latter leads to biased results of the test characteristics of the new test.
New approaches where no information is needed on the true disease status of the individual (the disease status is a latent variable, hence the term Latent Class Analysis LCA), are therefore prospected.
According to Hui and Walter[1] if two tests are applied to comparable individuals from two populations with different disease prevalences, then assuming conditional independence of the errors of the two tests, the diagnostic sensitivity and specificity of both tests and the true prevalences in both populations can be estimated.
This is demonstrated with field data obtained with the direct agglutination test CATT/*T.evansi* and the micro haematocrit centrifugation technique (MHCT) in camels from Niger.

2. MATERIALS AND METHODS

2.1 Data

Data were obtained from a field study in Niger in collaboration with the Institute of Tropical Medicine. Dromedary camels were sampled *ad random* in different geographic locations. The whole dataset (N=929) was divided into two, geographically distinct, major populations (population 1 (N1=452) and population 2 (N2=477)) for further processing.

2.2 Diagnostic tests

The MHCT, a parasite detection technique, was conducted in the field according to Woo[2]. When a trypanosome was observed the sample was considered MHCT positive.
The CATT/*T.evansi* detects antibodies against the predominant Variant Surface Glycoprotein RoTat 1.2 [3], 4] and was performed according to Verloo *et al*.[5]. Serum samples with end titres 1/8 or higher were considered CATT/*T.evansi* positive.

2.3 Data analysis

2.3.1 Classic

Prevalence in both populations and diagnostic sensitivity and specificity of the CATT/*T.evansi* assuming the MHCT as a gold standard is calculated for both populations together with their binomial 95% exact confidence intervals.

2.3.2 Hui Walter

When comparing both tests in two comparable populations, assuming equal performance of both diagnostic tests in both populations and conditional independence of both tests the likelihood of observing the different combinations of the test results in each population can be defined. Bayesian estimation of the parameters (prevalence in population 1 and 2, sensitivity and specificity of the MHCT and CATT/*T.evansi*) point estimates and 95% credibility intervals (the Bayesian analogue of confidence interval) are obtained by Gibbs sampling [6] which is an iterative Monte Carlo Markov-chain technique. Because the model is identifiable (degrees of freedom equals the number of parameters to be guessed) and large sample sizes were available, no prior information (NIP beta 1,1) was incorporated.
Code for the Gibbs sampler was written in S-Plus. Posterior densities were calculated after 10000 iterations and a 1000 burn-in period.

3. RESULTS

Results of both tests in both populations are visualized in two contingency tables (Table 1).

Table 1: Contingency tables with the test results of both populations

Contingency table population 1

CATT/*T.evansi*	MHCT +	MHCT -	
+	13	92	*105*
-	0	347	*347*
	13	*439*	*452*

Contingency table population 2

CATT/*T.evansi*	MHCT +	MHCT -	
+	90	217	*307*
-	4	166	*170*
	94	*383*	*477*

3.1 Classic

Assuming the MHCT is the gold standard (sensitivity = specificity = 100%) the diagnostic sensitivity and specificity of the CATT/*T.evansi* in both populations is 96,26% (90,70%-98,97%) and 62,41% (59,0%-65,72%). Prevalence in population 1 is 2.88% (1.54%-4.87%) and in population 2 is 19.71% (16.23%-23.56%)

3.2 Hui Walter

Gibbs sampler results obtained from the Hui and Walter Model are visualized in Figure 1. For the MHCT and the CATT/*T.evansi* the sensitivities are 32.18% (26.29% - 38.81%) and 96.0% (90.89% - 99.10%) and the specificities 99.78% (98.91% - 99.99%) and 84.18% (78.86% - 90.45%). The prevalence in population 1 is 9.36% (4.93% - 15.84%) and in the second population 60.56% (53.95% - 67.02%)
All results are summarized in Table 2.

Fig1: Gibbs sampler output of the Hui Walter LCA model

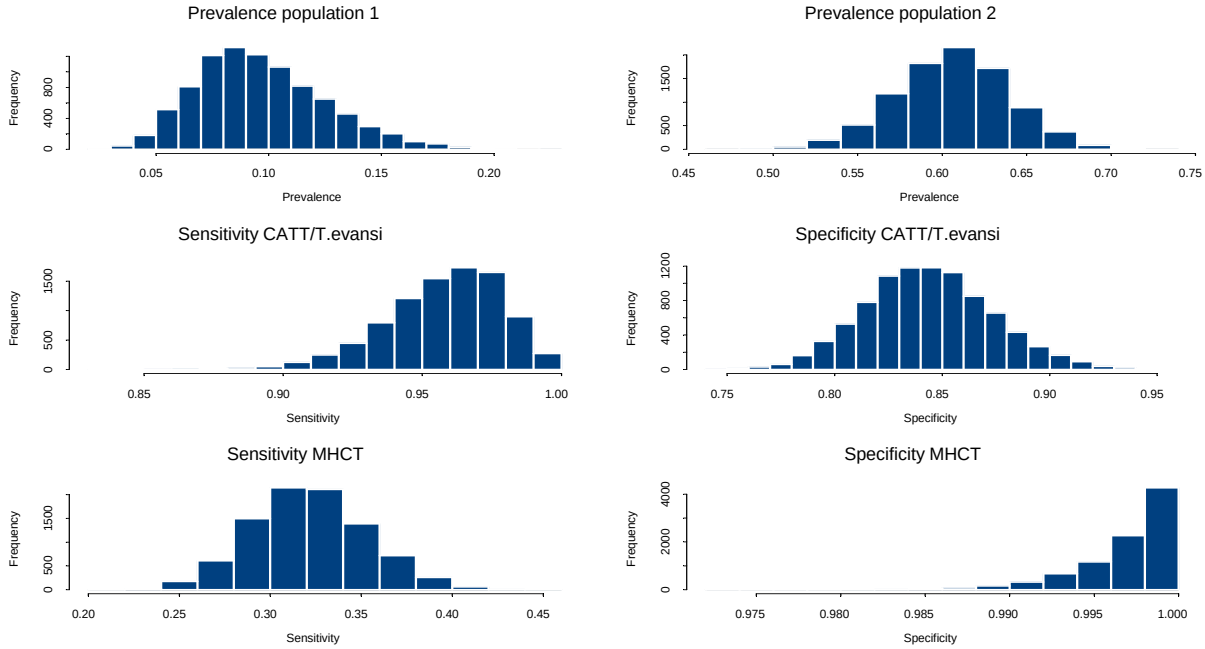

Table 2: Summarized results (in %)

		Prevalence		Sensitivity		Specificity	
		Population 1	Population 2	MHCT	CATT	MHCT	CATT
Classic	Point estimate	2.88	19.71	*100*	96.26	*100*	62.41
	95% CI	1.54 - 4.87	16.23 – 32.56		90.7 – 98.97		59.0 – 65.72
Hui Walter	Point estimate	9.36	60.56	32.18	96.0	99.78	84.18
	95% CI	4.93 – 15.84	53.95 – 67.02	26.29 – 38.81	90.89 – 99.10	98.91 – 99.99	78.86 – 90.45

4. DISCUSSION

Application of the Hui Walter model under the assumption of conditional independence give realistic estimates of the sensitivity and specificity of both CATT/*T.evansi* and MHCT including estimates of the true prevalence in both populations.
Results in the classic approach are heavily biased (underestimation of the specificity of the CATT/*T.evansi* and the prevalences in both populations) due to the low diagnostic sensitivity of the MHCT.
The assumption of conditional independence is in this case acceptable because the MHCT and CATT/*T.evansi* are biologically two different tests[7]. Equal test performance in both populations is also acceptable because we tested the same species (Camel) in similar habitats.
We conclude that, following thorough consideration of the assumptions, LCA provides a powerful tool in the field of test evaluation.

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