

FMDV Survival in meat Required input from a risk assessment point of view

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Background

EUFMD RG has requested the DFVF risk assessment group to share compiled information about the FMDV survival in meat products. The information below is taken from an ongoing study “Risks associated with imported meat from swine vaccinated against foot and mouth disease virus. A qualitative assessment”. Briefly, the scope of this risk assessment was a description of risk scenarios in the context of the new vaccination policy for FMD given by Council Directive 2003/85/EC of September 29th 2003. Only vaccination in swine was considered in the assessment. The assessment was of qualitative nature as the legal and illegal marked response in the trade of meat from vaccinated swine in European is impossible to be predicted.

The risk assessment framework

The release assessment is part the formal risk assessment process according to OIE standards. It describes the scenarios for introduction of infectious virus into a country by one or many specified introductory pathways and assesses the probability of any of these pathways to result in a release. The release of virus from imported meat from vaccinated animals depends on the vaccine efficacy, the presence of virus in the tissues at the time of slaughter and the survival of virus during the processing and transport.

Survival of FMDV in non-cured meat

The literature on virus survival in animal products has quite miscellaneous focus points, and nothing that directly relates to the definition of meat as used in the EU-legislation. Definitions of products of animal origin is described in Council Directive 64/433/EEC of 26 June 1964, in which the exact definitions of meat (articles 1 and 2) was latest changed by directive 83/90/EEC of 7 February 1983. In the revised text, five points of article 2 (a, b, d, e and f) are relevant for the definition of fresh meat:

- (a) “Meat”, means all parts of domestic bovine animals (including buffalo), swine, sheep, goats and solipeds which are suitable for human consumption;
- (b) “Fresh meat” means meat, including meat vacuum-wrapped or wrapped in a controlled atmosphere, which has not undergone any treatment other than cold treatment to ensure preservation;
- (d) “Carcase” means the whole body of a slaughtered animal after bleeding, evisceration and removal of the limbs at the carpus and tarsus, removal of the head, tail and the udder, and in addition, in the case of bovine animals, sheep, goats and solipeds, after flaying;
- (e) “Offal” means fresh meat other than that of the carcase as defined in (d), even if it remains naturally connected to the carcase;
- (f) “Viscera” means offal from the thoracic, abdominal and pelvic cavities, including the trachea and oesophagus;

This implies that fresh meat of pigs can consist of any protein tissue (normal understanding of meat), fat, skin, bone, lymphoid tissues and residual blood in larger vessels.

The literature has some information with regard to post-mortem survival of FMDV in animal tissues. However the data was generated in a time, when implementation of the information in risk assessment was not an issue, and consequential that structure of the published data may not be particularly helpful. Moreover, the published data fall into two major categories. One category of data presented as

survival times of FMDV in single tissues and one category of data presented as FMDV survival in meat cuts (i.e. multiple tissues). An overview of the literature on the subject is given in Tab. 1.

Survival of FMDV in cured meat products

The virus survival of FMDV has been investigated in various cured products. Under an assumption that none of the curing processes enhances virus survival of FMDV, the numbers presented in Tab. 2 should be accepted as minimum values for FMD virus survival in these tissues irrespective of whether the meat was cured or not.

The effect of pH in meat of cattle and pigs

The pH value in infected animal products has long been recognised as an important factor for survival of FMDV. The pH drop is assigned to lactic acid arising from anaerobic post-mortem metabolism, and thereby dependent on the glycogen reserves in the tissues at the time of slaughter. This largely restricts the pH drop to the pure muscle tissue with only minor distribution to surrounding tissue due to compartmentalisation by connective tissue. It has been found that pH in large lymph nodes was unaffected by the pH drop in the adjacent muscle. The post-mortem drop in muscle pH is relatively fast. It has been hypothesised that any survival of virus in muscle tissue should be referred to the minute amount of blood in the capillaries - which is subsequently subject to acidification from the surrounding muscle tissue. The inherent hypothesised difference between blood in capillaries on one side and other tissues and blood in larger vessels on the other side in their sensitivity to acidification should be explained by the physiological capacities of capillaries with regard to exchange of gas and metabolites. The effect of pH in meat is supported by the fact that FMDV survival in minced meat is reported to be zero, and the instant disappearance of virus is thought to be caused by redistribution of the lactic acid across any compartments existing prior to mincing. Finally the pH effect in pure meat is reflected in the set-up of models used for studying of heat and other treatments.

An anecdotal substantiation of the pH effect was provided in the study of (Henderson and Brooksby, 1948), who had to thaw their samples of frozen meat in a buffered solution in order to recover virus. This study indicated that the pH drop is reinitiated after thawing if the meat is frozen immediately after slaughter.

It should be noted that the drop in pH provides a progressively more unfavourable environment for virus survival, and absence of drop below 6.0 should not be interpreted as certain virus survival. This mechanism renders a cut-off for survival of FMDV partially meaningless and is reflected by the lack of consensus in the literature. Cut-off values between 5.9 and 6.5 have been encountered.

Standard comparator muscles

Deboned and matured beef is considered safe with regard to transfer of FMDV. This is exemplified by the requirements for trade of beef in the directive 2003/85/EC, where trade of deboned fresh beef, matured at >2°C for >24 hours to a pH in the middle of the *Longissimus dorsi* muscle below 6.0 (annex VIII) is generally allowed from 30 days after the last vaccination in the vaccination campaign (article 58-8). This establishes *Longissimus dorsi* as a standard comparator muscle of beef with regard to pH, and there seems to be consensus that all other muscles should possess the same the same virucidal pH. The term 'standard comparator muscle' is an expression more than a fixed standard, since the choice of standard comparator muscles may vary between studies and other contexts such as control purposes.

No such standard seems to exist for pigs in the context of infectious diseases. If, however, the scope is turned towards the meat science literature, there is an abundance of literature reporting pH values in pig meat. For example, (Berg et al., 2003) found no differences in pH 24 hours post-mortem across 48 pigs in four treatment groups. They investigated the pH in three different muscles (*M semimembranosus*, *M. Longissimus (dorsi?)* and *M Gluteus medius*) of each pig and the means was in

the range of 5.3 to 5.4 with a variability described by a maximum standard deviation of 0.1 (S.E.x square root of N = $0.031 \times 12^{0.5}$).

A pH between 5.6 and 5.8 24 hours post-mortem in loin (*M. Longissimus dorsi*) was found in Italian heavy pigs (Corino et al., 2003). The post-mortem pH development in *M. Longissimus lumborum* and *M. Biceps femoris* has been found in the range of 5.4-5.6 (Lambooij et al., 2004). The ultimate pH (at least 24 hours post mortem) in a larger number of studies was reviewed and it was found that the majority of results indicated ultimate pH values below 6.0 in pig meat (Bendall and Swatland, 1988). However, exceptions have been reported. One cause of the exception was variation between muscles. In one study of 2893 pigs reported variation in mean of ultimate pH between the four standard comparator muscles in that study with mean ultimate pH of 5.75, 5.86, 6.14 and 6.15 (Gallwey and Tarrant, 1978). Besides the within animal (between muscle) variation of pH, also between animal and between race variation has been reported.

At least four different profiles exist for post-mortem metabolism in meat of Danish Landrace pigs. One did, despite an apparent pH drop, only reach ultimate pH between 5.7 and 6.3 in the standard comparator muscle of that study (Briskey and Wismer-Pedersen, 1961). Due to the age of the study, the fraction of pig carcasses with that profile must be considered uncertain in relation to the Danish situation of today - and in relation to the genetic constitution of pig production in other countries. In a recent study of 165 Danish Landrace crossbreeds and 190 Duroc crossbreeds, it was found that pH of *M. longissimus dorsi* was in the range 5.32 to 5.88 with a mode (>50% of observations) in the interval 5.54-5.67 for both races after 24 hours (Lindahl 2004, personal communication). The between-race variation can e.g. be reviewed (Bendall and Swatland, 1988). It appears that same breed may have varying mean ultimate pH in the same standard comparator muscle. Thus, it may be that the between-race variation has a subcomponent of between-lineage variation. It has previously been hypothesised that stress related glycogen depletion prior to slaughter may cause less post-mortem drop in pH - and possibly account for some of the between-animal variation seen. However, the effect of stress prior to slaughter had no effect (van der Wal et al., 1999).

Discussion

The knowledge about FMD virus survival in beef is extensive whereas data for pork and other swine meat products are sparse and mostly related to specific products. The experiments have typically been conducted on small sample sizes. The experimental design (number of animals per group, virus type, viraemic concentration in tissues, processing) is necessarily very context-specific. The biostatistical treatment of the published results, according to our impression, has not been to the standards of food hygiene or pharmacological research. In effect, the use of published figures in risk assessment models (qualitative or quantitative) introduces uncertainty because the results may not apply to fresh meat in general or to the speciality product under consideration. Furthermore, the statistical uncertainty (measurement error and biological variability) is not yet well represented in our risk models.

The question needs to be addressed whether vaccination is an important biological factor in these models beyond its impact on virus concentration in blood and tissues.

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Table 1. Virus survival for FMDV in non-cured products and tissues of pigs and cattle

Tissue (origin), number of animals	FMDV survival in days last pos./first neg.	Source
Bone marrow (pig)	42 / ?	Tab. 8 in (Cottral, 1969)*
Muscle (pig)	/ 1	(Cottral et al., 1960)
Blood on surface of carcass (pig)	4 / ?	(Cottral et al., 1960)
Blood clot in heart (pig)	34 / ?	(Cottral et al., 1960)
Lymph nodes and blood (pig)	70 / ?	(Cottral, 1969)
Lung, kidney and spleen (pig)	42	Tab. 9 in (Cottral, 1969)*
Stomach and tongue (pig)	10	Tab. 9 in (Cottral, 1969)*
Brain (pig)	17	Tab. 9 in (Cottral, 1969)*
Muscle (pig), N=2	1 (70) 1 d: Refrigerated meat, 70 d: Frozen meat	(Savi et al., 1962)
Blood, brain, lung, bone marrow, lymph nodes, stomach, intestines, tongue, fat and parotid (pig), N=2	10 (210), 10 d: Refrig. tissues, 210 d: Frozen tissues	(Savi et al., 1962)
Spleen, kidney and liver, N=2	1 (210), 1 d: Refrig. tissues, 210 d: Frozen tissues	
Bone marrow (cattle)	80 / ?	
Bone marrow	194 / fin	(COX et al., 1961)
Bone marrow (cattle), N=8 ?	210 / ?	(Cottral, 1969)*
Lymph nodes (cattle)	120 / ?	(Cottral, 1969)*
Hemal nodes (cattle)	120 / ?	(Cottral, 1969)*
Tongue and cheek (cattle)	33 / ?	Tab. 8 in (Cottral, 1969)*
Intestines (cattle)	6 / ?	Tab. 8 in (Cottral, 1969)*
Muscle (cattle), N=2	1 / 3, (60 / fin), Positive reactions at 60 days were thought to be due to contamination.	(Cottral et al., 1960)
Bone marrow, N=2	73	(Cottral et al., 1960)
Blood and lymph nodes (cattle), N=2	60 / fin	(Cottral et al., 1960)
Synovial fluid (cattle)	19	(Gailiunas et al., 1960)
Blood, lung, bone marrow, lymph nodes, rumen, tongue, parotid and uterus (cattle), N=2	8 (210), 8 d: Refrig. tissues 210 d: Frozen tissues	(Savi et al., 1962)
Liver and spleen (cattle)	1 / 2	(Cottral et al., 1960)

* Contains references to original work.

Table 2. Virus survival in different tissues of cured products of pigs (*cursive = cited ~ unverified information*)

Reference	Curing	Product	FMDV survival in days	Comment
(Mebus et al., 1993)	Iberian	Ham-muscle	0 ^{a,b} (<14) ^c	P(0 pos at day 14 prev at day 0) = 0.91
	Iberian	Shoulder-muscle	0 / 14	
	Iberian	Loin-muscle	0 / 14	
	Serrano	Ham-muscle	0 / 14	
(McKercher et al., 1987)	Parma	Ham-muscle	÷ ^b (<72h) ^c	American experiment
	Parma	Ham-muscle	0 ^b (<30h) ^c	Italian experiment
(Savi et al., 1962)	-	Ham-muscle	25	
(Mebus et al., 1993)	Iberian	Ham-fat	0 ^{a,b} (<14) ^c	P(0 pos at day 14 prev at day 0) = 0.29
	Iberian	Shoulder-fat	0 ^{a,b} (<14) ^c	
	Serrano	Ham-fat	140 ^d	
(McKercher et al., 1987)	Parma	Ham-fat	0 ^b ~ 72h (<30) ^c	American experiment
	Parma	Ham-fat	96 ^b	Italian experiment
(Dhennin et al., 1980)		Ham-fat	176 / 183	
		Shoulder fat	155 / 169	
(Savi et al., 1962)	-	Ham-fat	46	
(Mebus et al., 1993)	Iberian	Ham-bone marrow	56	
	Iberian	Shoulder-bone marrow	84	
	Serrano	Ham-bone marrow	84	
(McKercher et al., 1987)	Parma	Ham-bone marrow	30 ^b	American experiment
	Parma	Ham-bone marrow	30 ^b	Italian experiment
(Savi et al., 1962)	-	Bone marrow of hams	89	
(Mebus et al., 1993)	Iberian	Ham-In. popliteus	112	
	Serrano	Ham-In. popliteus	168 ^d	
(Cottral et al., 1960)		Bone marrow of hams	183	
(Dhennin et al., 1980)		<i>Salted bacon</i>	183 / 190	cited in (Mebus et al., 1993)
(Savi et al., 1962)		Bacon	10	Tab. 10 (Cottral, 1969)
(Panina et al., 1989)		Different Italian salamis	0 ^b (<72h)	
^a Only 2 of 62 pigs had virus in meat on the day of slaughter. ^b The last virus positive sample ^c The first virus-free sample - only included for samples negative ^d Reported to be associated with hemorrhagic specimen				