

Horse Meat in Beef Products- Species Substitution 2013

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Summary

On 15 January 2013 the Food Safety Authority of Ireland, FSAI, published a press release on a small survey identifying horse and pig DNA in burger products, initiating a meat substitution scandal that involved most of Europe and maintained high and lengthy media and political salience. Herein we summarise the extent of the substitution, placing it in a historical, food authenticity, food safety and analytical context and drawing conclusions on the future measures recommended primarily to government. We conclude that history teaches us that this will happen again but not in quite the same way. We suggest it is now unlikely that widespread horse meat substitution will reoccur for decades but other frauds will arise and the way to guard against this is continued systematic vigilance. The challenge is to secure a cost effective, efficient scientific infrastructure to support that vigilance in a planned and sustainable manner.

Key Words

Horse meat, substitution, species, DNA, protein, ELISA

Note: we use the term "horse meat" throughout as "horsemeat" is not referenced in the major dictionaries; some legislative references to "horseflesh" are retained in the relevant context

*"Then by lowering the bushel, raising the shekel, by swindling and tampering with the scales we can buy up the poor for money, and the needy for a pair of sandals, **and get a price even for the sweepings of the wheat**"¹*

Introduction

On 15 January 2013 the Food Safety Authority of Ireland, FSAI, published a press release on a small survey identifying horse and pig DNA in burger products, initiating a meat substitution scandal that involved most of Europe and maintained high and lengthy media and political salience. Herein we summarise the extent of the substitution, placing it in a historical, food authenticity, food safety and analytical context and drawing conclusions on the future measures recommended primarily to government.

The 1991 edition of “Pearson’s Composition and Analysis of Foods” contains the statement that “*Horse meat has long been a common substitute for beef*”². But although the literature on species detection methods is extensive, well documented episodes of species substitution of horse for beef are sparse, hence the authors’ intention to record the 2013 episode.

The UK Parliamentary record appears to be the sole continuous public record in which periodic concerns about horse meat are extant. In 1886 a question was raised on foot of reports of diseased horses shipped from London to Rotterdam for slaughter for human consumption and “...*re-shipped to this country in the form of sausages and tinned meats...*”³. Following this the Sale of Horseflesh &c Regulation Act 1889⁴ required the sale of horseflesh for human consumption to be disclosed by way of a sign “...*in legible characters of not less than 4 inches in length and in a conspicuous position and so as to be visible throughout the whole time*”. Horseflesh was defined to include the flesh of asses and mules. The act created the offence of supplying horseflesh when another meat was asked for or including horse in a compound article of food which is not ordinarily made of horseflesh.

Anecdotal evidence of the admixture of horse meat with beef re-appeared in debates around the adoption of the Food and Drugs Act 1938⁵, into which the above provisions had been transferred (S. 38) and again in 1941⁶ and in 1943 in connection with the Horseflesh (Control and Maximum Prices) Order, 1941⁷. Alarm (mainly to do with the continued availability of horses for farm and other work) about the growth in the numbers of horses slaughtered was raised in 1948, the official response included reference to the difficulties of enforcement in this respect⁸. In the 1950’s the Rosebery⁹ and Northumberland¹⁰ Committees investigated and recommended on animal welfare in relation to the transport and slaughter of horses. The provisions of the 1889 Sale of Horseflesh Act were essentially retained, without the letter size requirement for the signage, in the Food and Drugs Act 1955¹¹ (S. 24), and the Food Act 1984 (S. 29)¹².

In the meantime, the “*great meat substitution scandal*” unfolded in Australia, stemming from the detection in 1981 of horse meat in Australian beef shipped to a plant in San Diego in the US¹³. This Australian episode rivalled in extent the current 2013 scandal and was documented in a Royal Commission Report. Considerable quantities of pet food were illegally diverted into the human food chain. The pet food included the flesh of donkeys, (feral) goats, kangaroos, buffaloes and horses, killed in the field without regard to hygiene. Mutton was substituted for lamb, and beef, sold as Halal food, that had not been slaughtered according to Islamic practice. Tighter regulation and the depressed economies of the

Australian export meat industry had significantly reduced the worst forms of malpractice by 1985^{14,15}. Saliency in the UK of the episode together with concerns about meat racketeering led to a private members bill to amend and strengthen the Food and Drugs Act 1955. The record of debate on the issue in Hansard makes much mention of species substitution and records campaigns in the media and by consumer and professional organisations to strengthen food law against meat racketeering¹⁶. Despite this the 1889 Sale of Horseflesh provisions, retained thus far, were not re-enacted in the Food Safety Act 1990.

In 1991 the Tribunal of Inquiry into the Beef Processing Industry (the “Beef Tribunal”) was set up in the Republic of Ireland, RoI, under Mr Justice Liam Hamilton and reported in 1994¹⁷. The “Beef Tribunal” investigated allegations made in a television programme, “World in Action” broadcast on 13 May 1991¹⁸ concerning alleged falsification of subsidy payment documents, use of false beef animal classification stamps, substitution of inferior product for beef going into intervention and other alleged fraud. No horse meat substitution was uncovered although falsification in respect of halal slaughter was alleged.

In the 1990’s, the then UK Ministry of Agriculture, Fisheries and Food, MAFF, instituted a research programme on food authenticity¹⁹. The research programme in 1999, undertook a substantial survey of meat speciation, but not for horse meat. Samples (n = 570) of sausages, burgers, pies, pâtés and recipe dishes were analysed for beef, pork, sheep, chicken and turkey with an undeclared species found in 83 samples (14.6 %). The methods available, then as now, were not quantitative and it was impossible to say whether the non-declared species were present as a result of deliberate substitution or accidental cross-contamination²⁰.

The food authenticity programme transferred to the newly formed Food Standards Agency in 2000, under the Food Standards Act 1999²¹. At the time Parliament was silent on horse meat, concerns being dominated by BSE, *salmonella* in eggs, a Scottish *E coli* outbreak, the conflict between agrifood support and consumer protection, and transparency in food policy making²².

The work of the FSA Food Authenticity Programme, Q01¹, is discussed below and described in detail elsewhere^{23,24}. In 2003 a relatively large survey for horse meat in salami was carried out in the UK under Q01. Following anecdotal evidence of donkey meat or horse meat in imported salamis an informal pilot study showed 3 out of 24 (12.5%) products contained low levels of horse meat. In the full survey of salami and similar products only a French chorizo was positive for horse at the limit of detection of the method which was 1% w/w. Given that only 1 out of 158 (0.6%) of the survey samples was positive it was unsurprising that the conclusions of the survey included: “*There was no evidence of a problem with undeclared horse meat or donkey meat in salami-type products*”²⁵.

Other substitution issues occurred at around the same time as the 2003 FSA salami survey and were dealt with effectively. For example, in late 2003 following the trial and conviction

¹ For information, details of the FSA Food Authenticity Research Programme Projects is given in Appendix 1 of this paper

in Nottingham of a number of defendants on criminal charges around the recycling of unfit poultry meat waste into the food chain²⁶ the FSA set up the Illegal Meat Task Force and the Waste Food Task Force. New rules on staining high risk unfit meat were introduced and procedures on veterinary inspection were tightened up.

The FSA Food Authenticity Programme (Q01) was very active in meat speciation research and surveys between 1998 and 2010, after which the program transferred to Defra where research and knowledge transfer continued albeit at a somewhat reduced pace, (see below, Discussion). In this period Illegal Unreported and Unregulated (IUU) fishing was reported to be a widespread phenomenon at levels of 30–40% of total catch, and sometimes more, legitimised by fraudulent labelling. This was recognised as a serious threat to fisheries sustainability in the UK, EU and worldwide with consequent attention devoted to stamping it out, underpinned by DNA testing²⁷. Fish speciation received considerable attention from Public Analysts, with an estimated 1990 samples DNA tested between 2007 and 2010²⁸.

Although no UK national surveys for horse meat were undertaken after 2003, individual Public Analysts occasionally included unfunded qualitative testing for it. For example between 2010 and 2012 56 samples, predominantly minced beef but also some burger and sausage products were tested in Wales for horse meat without any positive results²⁹.

The 2013 Situation

On 15 January 2013 the Food Safety Authority of Ireland, FSAI, published a press release³⁰ identifying horse and pig DNA in burger products mainly described as “beef”. The FSAI summarised their findings: *“A total of 27 beef burger products were analysed with 10 of the 27 products (37%) testing positive for horse DNA and 23 (85%) testing positive for pig DNA. In addition, 31 beef meal products (cottage pie, beef curry pie, lasagne etc.) were analysed of which 21 were positive for pig DNA and all were negative for horse DNA. Reminiscent of the main FSA survey in 2003, all 19 salami products analysed tested negative for horse DNA. Traces of horse DNA were also detected in batches of raw ingredients, including some imported from the Netherlands and Spain.”* In all but one of the positive samples, low levels of horse and pig DNA were found. However, in one instance a level of 29.1% equine DNA was reported. Although it was initially unclear if this was relative to the beef DNA content FSAI added that the level of horse DNA indicated that horse meat accounted for approximately 29% relative to the beef content. On 16 January 2013 the affected products were removed from the shelves.

In early February it was made known that meat pies and pasties supplied to prisons in England and Wales were labelled and served as halal but contained traces of pork DNA and on 4 February the UK Food Standards Agency (FSA) and the UK food industry agreed a testing regime with the results to be made public. Further findings of horse meat in various products followed and on 12 February FSA and police raided two meat handling/production premises in West Yorkshire and West Wales, involved in the alleged supply of horse meat. On 22 February FSA released the first results of industry testing with 3599 (over 99%) negative at or below the level of 1% (DNA or meat), results for 35 samples were positive

representing the 13 positive products already withdrawn, (see below for a discussion of the pragmatic 1% threshold)

Further industry results were published at intervals and on 13 June the first of the agreed quarterly reports was issued by FSA on industry testing showing a further 3 beef products had tested positive for horse meat since the results reported on 1 March. In total up to 13 June 2013 24,480 industry tests for horse meat had been carried out with a total of 47 positive results, (0.19%)³¹.

Coincidentally in 2013 Cawthorn *et al* reported the analysis in South Africa of processed meat products (minced meats, burger patties, deli meats, sausages and dried meats) by ELISA and DNA-based approaches for soya, gluten and 14 animal species. The results revealed that 95 out of 139 (68%) samples contained species which were not declared with the incidence being highest in sausages, burger patties and deli meats. Soya and gluten were identified as undeclared plant proteins in a large number of samples (>28%), while pork (37%) and chicken (23%) were the most commonly detected animal species. Unconventional species such as donkey, goat and water buffalo were also discovered in a number of products. Overall, this study appeared to confirm that the mislabelling of processed meats was commonplace in South Africa³².

Two related strands (UK and EU) of official sampling and analysis took place in response to the horse meat issue. In the UK, in February 2013, FSA initiated a study to investigate the presence of undeclared equine and porcine DNA in meat products containing beef available at retail, wholesale and catering businesses. There were three phases to this work, sampling for which was undertaken by enforcement officers from 28 Local Authority (LA) Trading Standards or Environmental Health Departments throughout the UK, including in Scotland, Wales and Northern Ireland. The aim was to select food representative of that on the market, with additional emphasis on brands at the lower end of the market (particularly for burger type products). Market research and household expenditure data were used to inform the choice of outlets and brands³³.

Formal three-part samples (three individual packs for a batch) were taken according to the Food Law Code of Practice³⁴ from a variety of commercial retail, wholesale and catering outlets throughout the UK and submitted to the local authority-appointed Public Analysts. For understandable reasons the three samples were not to be mixed before division into three, but used as if mixed and sub-sampled. An analytical protocol was issued to each participating Public Analyst laboratory by FSA detailing sample homogenisation, analysis and reporting of results. However the responsibility for ensuring that the sampling and analysis were forensically sound enabling enforcement action to be taken by the LA where non-compliant samples were identified remained with the LA's and in particular their Public Analysts.

In Phase 1, samples of raw comminuted (minced) beef products (n = 224) including burgers, minced beef, beef sausage or meat balls were sampled between 4 and 18 February 2013. In Phase 2, samples of beef-based ready meals (n = 140) including frozen, chilled or canned lasagne, chilli con carne, cottage pie, ravioli, cannelloni and spaghetti bolognese were taken between 13 and 25 February 2013. Samples taken in Phases 1 and 2 were tested for horse and

for the presence of undeclared pork. Phase 3 of the UK official survey dovetailed with a European Commission survey (official control programme) for horse DNA (see below) and consisted of 150 samples. These included products marketed or labelled as containing beef as a major ingredient such as minced meat, meat products and meat preparations (such as kebabs with seasoning). Products such as gelatine, beef dripping, stock cubes, steak, stewing steak and ready meals which contain beef that was not minced were taken between 25 February and 8 March 2013.

In all a total of 514 samples were taken for testing by 51 local authorities. More than 98 % of the products were negative for horse DNA or, where tested, pig DNA at the reporting limit of 1%. Two beef products, (2 out of 514 = 0.4%) which had been noted previously, were found to contain horse DNA at levels greater than the 1% reporting limit. None tested positive for the veterinary drug phenylbutazone. Three other products, which had been announced previously, were found to contain pig DNA above 1%; in addition one product, labelled as halal, was found to have trace levels of pig DNA (4 out of 514 = 0.8%)³⁵.

The European Commission was drawn into the horse meat scandal owing to the pan-European nature of the episode³⁶. A Commission Recommendation of 19 February 2013 required from Member States a coordinated control plan with a view to establishing the prevalence of such fraudulent practices. The measures required were official controls for horse meat in foods destined for the final consumer or for mass caterers, which were marketed and/or labelled as containing beef; and official controls on horse meat destined for human consumption to detect phenylbutazone residues, (product scope – fresh chilled or frozen meat of horses, asses, mules or hinnies). A total of at least 2250 samples was recommended with indicative monthly recommended sample numbers of 150 each from France, Germany, Italy, United Kingdom, Spain, Poland, 100 each from Romania, Netherlands, Belgium, Greece, Portugal, Czech Republic, Hungary, Sweden, Austria, Bulgaria, 50 each from Lithuania, Slovakia, Denmark, Ireland, Finland, Latvia, and 10 each from Slovenia, Estonia, Cyprus, Luxembourg, and Malta. The coordinated control plan was directed to be carried out for a period of one month starting from the date of adoption of the Recommendation or at the latest by 1 March 2013 with results reported to the Commission³⁷. The Commission authorised funding at 75% of eligible costs amounting to €300 per sample for DNA and phenylbutazone testing, some €1.36M in total³⁸.

The results of the EU-wide testing revealed that 7,259 samples were analysed by the competent authorities in the 27 EU countries, of which 4,144 were tested for the presence of horse meat DNA and 3,115 were tested for the presence of phenylbutazone. Of those tests, 193 were positive for horse DNA (4.66%) and 16 showed positive traces of phenylbutazone (0.51%)^{35,39}.

In addition to the above many LA's undertook their own local surveys and also checked the authenticity of school and care home supplies. The results of these tests are not currently centrally available.

The FSA has published a timeline containing significant events in the unfolding history of the episode⁴⁰, a report detailing the FSA response⁴¹, a short focussed independent review of the

Food Standards Agency's response⁴² and an action plan to implement recommendations from the independent review⁴³. Further FSA actions are available in FSA Board papers throughout 2013. A ministerial statement and report by the authorities in the Republic of Ireland, RoI, were issued in March 2013⁴⁴. Oral evidence in a series of meetings was taken by the House of Commons Select Committee on Environment, Food and Rural Affairs under the heading "Food contamination"⁴⁵. In June 2013 Defra commissioned Professor Chris Elliott to lead an independent review of Britain's food system in the light of the horse meat fraud⁴⁶. Also in June 2013 the report of an expert group commissioned by the Scottish Government to carry out a review of the lessons to be learned from the horse meat incident was published⁴⁷.

As late as July 2013 a frozen meat pie product was found by its distributors to contain horse DNA and was withdrawn from sale on 19 July. The product, with a best before date of 22 January 2014, was manufactured in Latvia and supplied to small retail shops in the UK, many of which specialise in products from Eastern Europe⁴⁸ and it is to be presumed this product was a hangover from the main episode.

Methods of Analysis

A systematic review of modern methods for the detection and estimation of horse meat in meat is beyond the scope of this paper. However, a brief summary is given.

Early attempts to detect horse meat relied on the higher glycogen content of horse meat compared to beef and on serological methods. There followed methods for meat speciation based on fatty acid and triglyceride analysis, immuno-, gel, or isoelectric focusing electrophoresis of soluble proteins, and much development of enzyme linked immunoassay (ELISA)⁴⁹.

ELISA methods for species identification have proliferated owing to their ease and relatively lower cost but suffer lack of sensitivity below around 1% w/w. Moreover the amount of capture antibody present is rapidly saturated by high amounts of antigen, restricting their linear range and necessitating successive serial dilution of the sample to avoid underestimating elevated concentrations of adulterant⁵⁰. Quantification is possible, although ELISA kits are generally sold as semi-quantitative at best, but precise results can be vitiated at low levels by lack of knowledge of the tissue type and suggestions of ingress of meat "drip".

In the mid-2000's FSA funded the development of real-time PCR assays for the specific detection of duck, pheasant, deer, horse, donkey and wild boar in commercial products⁵¹. The approach was found to be applicable to the detection of duck, pheasant, deer, horse and donkey and in 2005 Chisholm *et al.* reported development of real-time PCR assays specific for horse and donkey. Primers, designed to hybridize to the mitochondrial cytochrome b gene, were 3' mismatched to closely related and other commercial species. Amplification of non-target species DNA was prevented by truncation of primers at the 5' position, thereby conferring complete specificity. The assays were highly sensitive and detected the presence of 1pg of donkey template DNA or 25pg of horse template DNA when assessed using

dilutions of DNA in water. Model food samples, spiked with horse or donkey muscle at 4%, 2% and 1% w/w and cooked/processed commercial products (as opposed to raw meat) containing horse, were successfully tested for the presence of horse or donkey, demonstrating the applicability of the assays to food products.⁵² A set of standard operating procedures was provided for use by public analysts and other laboratories. Additionally a chip-based technology, the Food Expert ID system developed by bioMerieux, was evaluated for the identification of species in food products. The range of species included on the chip allowed screening for tuna and white fish mixtures, meat mixtures from the DNA quality project (Q01033 & Q01034) and exotic meats from the Exotic Meat project (Q01083) to a detection level of 5% and below.

The current methods of analysis remain essentially qualitative (see below, Discussion) and can be summarised as follows:

- DNA based:
 - PCR (Polymerase Chain Reaction) – this is used for screening for presence or absence of the species sought with a defined limit of detection. The method is qualitative
 - Real time PCR – this generally offers greater sensitivity and specificity than PCR alone and if applied to nuclear DNA is capable of being quantitative
 - DNA sequencing – this is often used to provide unequivocal identification of a species DNA sequence through reference to validated sequence databases, and can be performed on products resulting from PCR analysis to confirm findings

- Protein based:
 - Enzyme linked immunosorbent assay (ELISA) – these testing kits apply antibodies raised to proteins of meat species to identify the species present; the method is qualitative but can in some circumstances be semi-quantitative and has been used for many years to check for undeclared species such as beef and pork in lamb

Whatever method is used it must be documented and validated – i.e. the laboratory using it must have evidence that the procedure works, to defined quality parameters, in their hands. In routine practice, analysts must have been trained in the method and blank material and authentic standards and mixtures at defined concentrations should be included in each analytical run. The results must be interpreted by a scientist qualified and experienced to do so. Accreditation to ISO/IEC 17025 and participation in a Proficiency testing (PT) scheme are regarded as good indicators of sound science in the testing laboratory.

The majority of results of horse meat reported to date appear to have been as % DNA in extracted DNA rather than as % horse meat in the sample.

Referee Analysis

Official sampling requires that a formal three part sample is taken. If a dispute should arise between the official and the counter-analysis, the retained portion of the sample may, in statutorily defined circumstances, be submitted to the Government Chemist for a definitive investigation⁵³. Five samples of beef products were referred to the Government Chemist in March 2013 as a result of the FSA-instigated LA sampling (see above). For three of the samples the Government Chemist was asked to determine if horse meat was present and for two samples was asked to determine if pork was present. As an independent referee, the Government Chemist is not able to comment on individual cases. However, in general the Government Chemist's findings confirmed those of the Public Analysts in that either horse or pork was found in the relevant samples. The individual findings, in the form of an official certificate, were sent to each Local Authority that referred a sample, requesting the Local Authority to pass it on to the food businesses concerned. The Government Chemist has also sent all the findings to the Food Standards Agency.

Owing to several inquiries asking for information it was considered that it might be useful to set out brief details of the approaches taken in these referee cases on species substitution. It should be noted that the approach taken in the referee cases was essentially qualitative since the state of the art of the science at the time needed further development.

The usual records were made of the integrity, seals and marking of the samples. Opening of the samples and sample preparation took place in a laboratory area not previously used for horse meat work and the benches were cleaned down with a general laboratory cleaning reagent (e.g. microsolv) followed by ethanol and were covered with disposable paper tissue before work commenced. Newly laundered lab coats, safety glasses and disposable gloves were worn by staff and access to the work area was restricted. Digital photographs of the official labels, seals and samples were taken and kept in secure electronic storage.

A new kitchen blender was used for each sample and was cleaned as above prior to use. Kitchen blenders were disposed of after each use to ensure minimal chances of cross contamination.

The referee sample burger products were homogenised and distributed into polypropylene screw capped containers in approximately 25g aliquots and frozen. Random 25g aliquots were removed and allowed to come to room temperature for analysis thus preventing any further freeze/thaw cycles. The aliquots were mixed and multiple replicates taken for DNA extraction and protein extraction.

Packaging and retail packaging were retained and retail labelling details were captured. Referee sample sausages were incised and the sausage meat removed from the sausage casings. The sausage meat was homogenised as above. The usual access, segregation and cleaning restrictions and protocols for DNA work were observed. The validity of key steps in the analytical procedures was attested by being witnessed by a second designated scientist, all

work was fully recorded in case notes made at the time and all transcriptions of data were checked.

For investigation for horse both DNA and ELISA approaches were taken.

DNA was extracted from homogenised 1g aliquots by incubation and lysis of cellular components in an SDS buffer with Proteinase K, binding of isolated DNA to positively charged silica beads, followed by multiple washing steps and an ethanol precipitation to clean, elute and concentrate the extracted DNA. Equine (*Equus* genus) nuclear DNA was tested for by real time polymerase chain reaction by the method of Köppel *et al*⁵⁴. The assay applied has been validated for specificity against a range of 14 common meat species and 35 herbs, spices, nuts and cereals with cross reactivity to horse (*Equus caballus*) only observed for mule (*mulus*) and donkey (*Equus asinus*).

Mitochondrial DNA was analysed by a commercially available Real Time PCR kit designed to detect horse (*Equus caballus*), (Primerdesign “Genesig - Real-time PCR detection of Horse contamination: *Equus caballus*” kit, PrimerDesign Ltd, Southampton, UK).

Two real-time PCR instruments were deployed, the Applied Biosystems™ 7900HT Fast Real-Time PCR System (ABI 7900) and the BIO-RAD CFX™ Real-Time PCR System (BioRad).

Protein analysis was carried out as follows. Protein was extracted from 5g aliquots, heat treated at 95 - 100°C and examined by ELISA for heat resistant horse-species specific muscle related glycoproteins, (ELISA-TEK® Cooked Horse Speciation Kit, ELISA Technologies Inc., Gainesville, Florida, USA).

Similarly, for investigation for pork both DNA and ELISA approaches were taken.

DNA was extracted from 1g aliquots and was examined in triplicate for pork (*Sus* genus) nuclear DNA by real time PCR by the method of Köppel *et al*. The assay applied has been validated for specificity against a range of 14 common meat species and 35 herbs, spices, nuts and cereals with no cross reactivity observed for pork.

Mitochondrial DNA was analysed by a commercially available Real Time PCR kit designed to detect pig (*Sus scrofa*), (Primerdesign “Genesig - Real-time PCR detection of Pork contamination: *Sus scrofa*” kit, PrimerDesign Ltd, Southampton, UK). The real time PCR instrument deployed was the ABI 7900.

Interpretation of all DNA results as detected or not detected was relative to the validated LOD of the respective assays. This was based on best measurement practice and kit manufacturer’s instructions, where applicable.

Protein analysis was carried out by extracting protein from 5g aliquots, heat treated at 95 - 100°C and examined by ELISA for heat resistant pork species-specific proteins, (Biokits® Cooked Pork Speciation Kit, Neogen Corporation, Ayr, KA6 5HW, Scotland).

Interpretation of results obtained by all of the above approaches was aided by concurrent analysis of gravimetric reference mixtures, prepared under controlled conditions e.g. at 1% w/w, 0.5% w/w and 0.1% w/w of raw horse in raw beef or raw pork in raw beef. From these it was possible to *infer* what the response from PCR amplification of DNA might reflect in terms of the likely equivalent w/w material on the assumption that tissue types are matched exactly between test sample and gravimetric mixture. This approach does not have general applicability and a quantitative DNA/DNA approach is the subject of current validation work. Reference materials of authenticated meat species and gravimetric mixtures of same are available from LGC Standards.

Post hoc DNA sequencing confirmed the species identity in each referee case⁵⁵.

Discussion

Many aspects could be discussed in relation to the 2013 horse meat episode. We confine ourselves to those areas of which we have most knowledge namely measurement science, food safety and authenticity and food law. The economics and structure of the food supply network are extremely important to this issue but must be left to others. Discussion and recommendations of the various reports on the matter are not necessarily rehearsed herein and should be separately consulted.

Phenylbutazone

One aspect of the horse meat episode that concerned many observers was the possible presence of residues of the veterinary medicine phenylbutazone arising from the substitution of beef with horse meat and it is appropriate to consider this in more detail here.

Phenylbutazone is an anti-inflammatory medicine legitimately used in horses, e.g. for welfare reasons in older companion-animal horses. For a medicine to be administered to food producing animals there must be a safety assessment, and some drugs are banned completely, e.g. the nitrofurans antibiotics. Others are allowed, after a stringent assessment of a dossier of information including that provided by the manufacturer. If a medicine is allowed to be used in food producing animals, generally a period of time (the “withdrawal period”) must elapse prior to slaughter, to allow residues to deplete to a safe level. The elimination rate of phenylbutazone follows exponential decay and traces can remain in horse meat in previously-treated horses for a long time. In addition the assessment of a safe residue level of phenylbutazone in the meat of food producing animals was never completed because key information is not available. Thus if phenylbutazone is administered the animal must never enter the food chain.

Phenylbutazone was marketed as a medicine for human use in the United States for the treatment of rheumatoid arthritis and gout in 1952. Accounts of serious and sometimes fatal adverse effects such as aplastic anemia and agranulocytosis appeared in the literature within three years of its use and it was largely withdrawn. Phenylbutazone is now only used in

human medicine for some people who suffer from ankylosing spondylitis, a type of arthritis and it is recommended that it should be used only by a specialist in severe cases where other treatments have been found unsuitable. It is important to note however that the levels used to treat humans are thousands of times higher than would be expected to be found in horse meat of treated animals. Although it should not be forgotten that some of the effects of phenylbutazone are reported to be idiosyncratic, that is to say not dependant on dose, and in theory might occur at any dose⁵⁶. Table 1 illustrates typical ranges of concentrations of phenylbutazone in humans (when it was used), horses and residues typically found (if present) in horse.

Table 1 – Typical Ranges of Phenylbutazone Residue Levels

Substrate	Phenylbutazone Concentration
Human therapeutic levels ⁵⁷ <i>Note – no longer used in humans except in rare cases</i>	40 – 150 mg L ⁻¹ plasma
Horse therapeutic levels ⁵⁸	0.9 – 24 mg L ⁻¹ plasma
Horse tissue ⁵⁹	0.24 – 6.5 µg kg ⁻¹ plasma, kidney

Thus it can be seen that the levels that have been found in some horse samples are very low – some 6000 or more times lower than from the doses used in human medicine. Horse kidneys are tested to check for phenylbutazone residues, as this is the edible tissue where residues would be most concentrated. Residue concentrations in the corresponding meat would be even lower.

The EU regulates the traceability of food animals (“Food Chain Information”, FCI), enacted into UK Legislation. This includes control of the use of veterinary drugs. If a medicine is administered, then this must be recorded in the FCI. Horses treated with phenylbutazone must have their FCI forms (“horse passport”) marked accordingly, and they are permanently excluded from the food chain. All EU Member States conduct prescribed analytical testing of samples from farms and abattoirs to ensure that these rules are being followed. In the UK, this is run by the Veterinary Medicines Directorate, VMD, and overseen by the independent Veterinary Residues Committee. It includes testing of horses that are slaughtered in the UK for food export to Europe. In recent years a number of these samples have been found to contain phenylbutazone at low (parts per billion) concentrations. Subsequent investigations have concluded that FCI regulations were not followed, and that there may be specific issues with the traceability of horses which – whilst not considered food animals by their owners – are subsequently sold on for food. The VRC raised concerns about this issue in their 2011 annual report, and the VMD have circulated advice to vets, dealers and abattoirs. In the surveys carried out to date, phenylbutazone has been found in around 0.5 % of the samples tested and although phenylbutazone should not be present, the risk is estimated to be very small.

Analytical Considerations

Difficulties in quantifying, by ELISA and DNA approaches, any one meat species in admixture with other species and ingredients were evident throughout the Food Authenticity Programme and were, for DNA, summarised in 2010 by Primrose *et al.*²⁴. To begin with, it cannot be assumed that the amount of DNA present is a true reflection of the amount of meat present because the DNA may have been degraded during processing which may also, along with other ingredients affect the amount of DNA that can be extracted. Clearly these considerations impact upon processed foods e.g. ready meals that are multi-ingredient and cooked for sale much more than e.g. “all beef” beefburgers sold raw where ground skeletal muscle (of whatever species) is the main ingredient. Moreover in real-time PCR, the copy number of the marker gene (for the adulterant) is measured and compared to the copy number of a “normalising” gene. This ratio can then be compared, with caution, to results from standard mixtures to infer the amount (e.g. percentage) of the adulterant. PCR suffers from inhibition and amplification efficiency issues due to matrix effects which can influence these calculations and although work in several authenticity projects found ways to minimise their influence and, again the variation is less problematic for foods with a limited number of ingredients, the results for composite ingredient foods were found to be too variable to be useful⁶⁰.

The main problem for DNA methods however, lies in the tension between detection and quantification. Some of the genes that are targeted in authenticity tests are located on mitochondrial DNA. Mitochondria are organelles that produce energy through oxidative phosphorylation, are involved in many cellular processes⁶¹ and are present as multiple copies in each cell. Copy numbers in the range 1000-8000 per mammalian cell, including multiple copies within each mitochondrion are typical^{62,63}. Although this makes mitochondrial detection much more sensitive compared with that of nuclear genomic DNA, particularly in highly processed samples, it makes *exact* quantification almost impossible because many factors can influence organelle copy number and as a result the copy number varies within the tissues of a single animal and from one animal to another. Ballin *et al* reviewing species determination in food and feed by ELISA and DNA approaches confirm the above, note that genome size is also an influence (e.g. 3-fold size difference between the chicken and cow genome) as are tissue-variable fat and water contents⁶⁴. These authors recommend that quantitative species determination should be by real time PCR of genomic DNA expressed as genome/genome equivalents rather than on a weight/weight (w/w) basis.

Thus the majority of DNA approaches for meat speciation are qualitative in nature; that is, the species-specific DNA fragment will either be reported as detected or not detected, with an associated limit of detection (LOD). There is currently no officially recognised, standardised or approved approach for quantifying the levels of meat species adulteration, and although this state of affairs is by no means uncommon in food standards work, opinion is also divided between expressing results in terms of w/w tissue measurements or DNA/DNA copy numbers. Whilst the former may help to promote understandable results in line with public interest and conforms with the European Union’s view of defining threshold levels for prohibited/adulterant species, the latter is scientifically more achievable and traceable. As

shown above there is no direct conversion between DNA/DNA and w/w tissue measurements and such a comparison is significantly affected by many factors including species, genome size, tissue type, matrix background, other ingredients, processing, level of degradation, and PCR efficiency.

Despite the above limitations, DNA approaches for meat speciation appear to be preferred because of potential advantages over protein detection methods, including specificity, sensitivity, the presence of DNA in virtually all tissue types, choice of targets and potential for development of a quantitative estimate without the risk of saturation (of antibody). By including standard w/w mixtures of specified species in the analytical procedure it is possible to infer an approximate w/w composition in the target sample however this inference should currently only be used for illustrative purposes as, in addition to the caveats rehearsed above, it relates solely to the tissue used to prepare the w/w reference mixtures and their use cannot necessarily be extrapolated to the analysis of other mixtures.

A range of gravimetrically prepared raw horse meat in raw beef meat (w/w) materials was produced at LGC in early 2013, in line with internationally recommended standards for reference material production⁶⁵ and authenticated for species identity using real-time PCR, ELISA and DNA sequencing. By distribution of these gravimetric mixtures to Public Analysts involved in the three phases of official sampling in the UK and making them commercially available a benchmark was created that facilitates comparison of results. In addition Defra commissioned LGC to assess the LOD of methods associated with the three phases of official sampling and analysis (see above). The three methods were a PCR-capillary electrophoresis, PCR-CE, approach based on FSA-funded work⁶⁶ (LOD reported as approx 1% w/w), a PrimerDesign kit using real-time PCR (validated LOD of approx <100 mitochondrial copies) and a Neogen BioKits PCR kit (validated LOD approx 0.1% w/w). Public Analysts were consulted over any deviations from the official protocol in the application of the methods they chose, and such deviations were replicated at LGC to ensure the representativeness of results. The results obtained show that all three methods have the capability of reaching a LOD of less than 0.1% w/w raw horse meat in a raw beef (meat) background if Quality Procedures and Good Laboratory Practice for molecular biology methods are adhered to. However greater guidance is needed to standardise and harmonise expression of results from meat analysis in order to afford comparability at the European level⁶⁷.

The PCR-CE approach described above and applied by many in the recent official survey appears to have been based on the FSA Food Authenticity Programme Q01107 project that ran from 2006 to 2007. It is interesting to speculate what might have been the outcome if, Q01107 had been followed up at the time. Equally, Q1104 on a proteomic approach to the determination of meat species, although limited in what it achieved, showed promise and a proteomic approach complimenting DNA techniques might usefully be further explored.

Food Law

In the UK, it is a criminal offence under Sections 14 and 15 of the Food Safety Act 1990 to sell food that is not of the nature, substance or quality demanded by the consumer, or to falsely or misleadingly describe or present food. If all or most of the meat in a product labelled “beefburger” is horse meat the product is not of the nature or substance demanded. If low levels of horse meat are present the product is not of the quality demanded. There are also specific offences under the Food Labelling Regulations 1996, the Meat Products Regulations 2003 (which stipulate compositional criteria for burgers) or the Consumer Protection from Unfair Trading Regulations 2008. Consumers do not expect horse meat in beefburgers and for those who wish to avoid pig meat the description and labelling of the food must be accurate and honest to allow them to reject products not meeting their specific requirements.

One of the overarching measures in EU food law is Regulation (EC) No 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.⁶⁸ The relevant provisions are:

Article 8 – Protection of consumers' interests

Food law shall aim at the protection of the interests of consumers and shall provide a basis for consumers to make informed choices in relation to the foods they consume.

It shall aim at the prevention of:

- (a) fraudulent or deceptive practices
- (b) the adulteration of food and
- (c) any other practices which may mislead the consumer

Article 16

Without prejudice to more specific provisions of food law, the labelling, advertising and presentation of food or feed, including their shape, appearance or packaging, the packaging materials used, the manner in which they are arranged and the setting in which they are displayed, and the information which is made available about them through whatever medium, shall not mislead consumers

Article 17 – Responsibilities

Food and feed business operators at all stages of production, processing and distribution within the businesses under their control shall ensure that foods or feeds satisfy the requirements of food law which are relevant to their activities and shall verify that such requirements are met

There is a public expectation of regulatory oversight by government and EU Regulation 882/2004⁶⁹ harmonises official controls on feed and food. The regulatory landscape in the UK is a complex one. The Food Standards Agency has overall responsibility for enforcement policy as the central competent authority for the UK. Day to day enforcement is the responsibility of local authorities/local government. Hence the FSA as central competent

authority depends for official action on local authorities which in turn depend on the scientific support offered by their statutorily-appointed Public Analysts.

Mislabelling of food is illegal, potentially harmful, penalises the honest trader, and undermines consumer choice and value for money, and when driven by financial gain is food fraud. The presence of bulk quantities of horse meat when only beef was stated on the label or specified is almost certainly the result of fraud, and sale of such food contravenes S.14 of the Food Safety Act 1990 and Art.8 of Regulation 178/2002. Although the 1889 Sale of Horseflesh Act is no longer extant its proscription of including horse in a compound article of food which is not ordinarily made of horse meat might be appropriate for inclusion in a contemporary report.

In fact there are strict requirements for the origin traceability of beef that as yet do not apply to other species (but see below) and arise from concerns about health risks in the transmission of BSE from cattle to humans and other animals. Council Regulation (EC) 1760/2000 replaced earlier regulations and established a system for beef labelling and Commission Directive (EC) 1825/2000 gives the detailed rules on the labelling of beef. An amendment (Commission Regulation (EC) 275/2007) gives more flexibility on the labelling of trimmings and mince from batches prepared from a mixed origin of beef cuts. The rules apply to all sales of raw beef, whether chilled or frozen, beef mince including uncooked beef burgers (without any added ingredients), and require information on where the animal was born, reared, slaughtered and cut up. This information is required to be printed on pre-packed beef labels, but can be displayed on a notice for beef sold loose in butchers for example. Each beef product is given a reference number which serves as a batch code and permits the product to be linked back to the source animal, group of animals or batches of beef used in the trimmings for example for minced beef⁷⁰.

Cleaning procedures between production runs of legitimately traded meat species may not be sufficient to remove all of the previous run/species. If bulk horse meat was wittingly or unwittingly present in a run there could be traces in the next batch and this may be an explanation for some of the 2013 findings. However, since horsemeat is not culturally an acceptable ingredient in meat products in the UK or RoI and hence not supposed to be used in meat processing plants there should be no question of such carry over at the “trace” level into processed meat products.

For those for whom specific meat species must be avoided in relation to their faith and for vegetarians and vegans, on the basis that specific or animal species in general should not be present, the question has usually been resolved using the limit of detection of the technique deployed to detect (the) animal species. Analytical techniques can rarely facilitate a true “zero tolerance” approach as the scope of the technique is determined by its LOD. Outwith considerations of faith, morals or ethics “action levels” above which exception could be taken by consumers in general to one species in another is something for debate in the light of clarity around LOD’s and the economics of processed meat production. Work funded by Defra at LGC on cross contamination of beef by pork after processing should yield interesting results⁷¹.

On a related issue, for people with food allergies there is a very real risk from undeclared and fraudulent switching of food ingredients in the supply chain. People with allergies depend on accurate and honest labelling to protect them and there have been fatalities arising from the unwitting consumption of allergens^{72,73}.

Other than food law there are event-specific matters of contract law and criminal conspiracy that are outside the remit of this paper.

Food Authenticity Policy, Research and Knowledge Transfer in the UK

Phillips and French⁷⁴ noted that in 1874 a Parliamentary Select Committee considered that the public was being “*cheated rather than poisoned*” by food adulteration but that official prioritisation of food safety over food authenticity was cemented by the transfer of food regulation policy to the newly created Ministry of Health in 1919, “*the new Ministry [rejected] the need for any legislation that was not justifiable on health grounds*”.

Nevertheless, MAFF instituted in the 1990’s a food authenticity research programme. To the best of our knowledge the first MAFF project to employ DNA methods to the authenticity analysis of food reported in 1995 and addressed the detection and quantification of common wheat (*T. aestivum*) in durum wheat (*T. durum*) pastas and semolinas.⁷⁵ From this flowed a series of studies on DNA techniques to counter the adulteration of durum wheat^{76,77,78,79}.

When the FSA replaced MAFF in 2000 the dominant concerns were food safety. It was at that time feared that the invariably-fatal human variant Creutzfeldt–Jakob disease, vCJD, considered by most scientists to be caused by ingestion of meat from bovines with BSE, might kill thousands. A Scottish *E coli* outbreak in 1996 had led directly to the deaths of 17 people⁸⁰. Nonetheless, the FSA continued MAFF research on food authenticity as the Food Authenticity Programme, Q01. This has been one of the most scientifically successful series of research in Europe if not the world, into food authenticity and its converse, food fraud. Many novel analytical authenticity approaches including high-resolution NMR, carbon isotope ratio analysis and DNA techniques were developed to tackle previously intractable frauds (^{19, 23, 24}and see Appendix 2).

Q01 was very active in meat speciation research between 1998 and 2012 funding at least 13 DNA-based research projects, 3 on ELISA and 2 in the field of proteomics (Appendix 2). In particular, two projects dealt with horse meat. Q01083, “Development of methods for the identification of duck, pheasant, venison, horse, donkey and wild boar in meat products” aimed to provide rapid species-specific DNA-based assays for the above species in meat products and ran from 2003 to 2004 at the then Central Science Laboratory, CSL (now Food and Environment Research Agency, FERA)⁸¹. Q01107 “The adaptation and validation of real-time PCR methods, for exotic species identification, for analysis on a capillary electrophoresis chip system” aimed to transfer existing DNA methods for verifying commercial and exotic meat species including horse, donkey, pheasant, duck and venison as well as pork, beef, lamb, chicken and turkey to a more efficient and versatile lab-on-a-chip

capillary electrophoresis system. This work ran from 2006 to 2007 again at CSL (now FERA)⁸².

From 2000 to 2008 fifteen food authenticity surveys were carried out: on tuna labelling (2000), added water in chicken (2001 and 2003, with added protein) and shellfish (2002), meat content, added water and hydrolysed protein chicken breast in catering (2001), maize oil authenticity (2002), declaration of irradiation (2002), varietal labelling of potatoes (2003), undeclared horse/donkey meat in salami-type products (2003), Basmati rice authenticity (2004), fat content of minced meat (2004), soya in meat products (2006) and generally (2007) and production method, geographic origin and speciation of fish (2007 and 2008)⁸³.

Owing to the flexibility, relatively lower costs and probative value of DNA techniques in food authenticity Q01 also carried out knowledge transfer (KT) of DNA methods to Public Analysts. The effectiveness of KT of 5 DNA methods (fish species, meat and exotic meat species, bushmeat species, Basmati rice, and orange juice adulteration with mandarin juice) has been assessed as high²⁸. KT of other analytical approaches followed (e.g. methods for detection of substitution of cod with Vietnamese Catfish; substitution of kangaroo meat with beef while KT on DNA sequencing has been particularly successful⁸⁴. For other meat-related FSA (Defra) authenticity projects see Appendix 2 below.

Q01 activity was discussed at the meetings of the Food Standards Sampling Co-ordination Working Group, however scrutiny of the published minutes (2003-2008) reveals that attention was focused on fish species, pork in chicken, and bushmeat; horse meat substitution was not discussed⁸⁵. In view of the essentially negative findings of the 2003 survey it was probably difficult to make a case for activity on substitution by horse meat in the face of finite resources and many competing demands. Thus, although food authenticity and food fraud remained live issues for government, substitution of horse meat for beef, after over 100 years of legislative cognizance and public scrutiny, seems to have disappeared from official view around 2007.

Horizon Scanning

Active horizon scanning has developed in sophistication and extent in recent years. The European Food Safety Agency (EFSA) developed an approach to identify emerging risks in the food and feed chain, with a report⁸⁶ containing a comprehensive overview of sources (including weblinks) and prioritisation criteria - a two-step process based on the UK Food Standards Agency use of the National Intelligence Model and the Dataquest approach⁸⁷. It is supported by the EFSA Emerging Risks Exchange Network⁸⁸ and the EFSA Stakeholder Consultative Group on Emerging Risks⁸⁹. The FSA's Emerging Risk programme aims to provide a co-ordinated approach to the collation and analysis of intelligence relating to food safety. It applies specialist "intelligent" software (Memex Patriarch®) along with an existing incident classification system and the National Intelligence Model credibility matrix to identify emerging food safety issues. An early success for the National Food Fraud Database was the seizure of hundreds of bottles of counterfeit vodka containing potentially harmful levels of methanol. This was the result of further detailed intelligence received by the FSA

after it published an initial alert to local authorities across the UK about counterfeit vodka⁹⁰. However, presumably because it had disappeared from official thinking and was not a food safety issue, none of the above captured the risk of horse meat substitution, which as is noted below, was known to some sections of the trade in mid-2012.

The Food Industry

Clearly the food industry, already reasonably well regulated, must self-regulate further with more determination, skill and application. Food and animal feed must be safe, authentic and properly labelled, responsibility for which falls to those who make and sell it⁶⁷. All players in the food supply chain thus need to have a very accurate and up-to-date awareness of exactly what is going into their products, a point emphasised by the Institute of Food Science & Technology (IFST)⁹¹. Scientifically-sound testing has a significant part to play in this along with financial and commodity intelligence gathering. It is instructive to quote here *in extenso* from the official RoI investigation report⁴² [at paras 3.4.2 and 3.4.3] that:

“In the RoI products had been sourced from some 19 different Polish suppliers over a sustained period and these stocks were stored in QK Cold Stores, Naas. QK Meats subsequently admitted that, based on its own risk assessment, it had tested 15 consignments from 9 of its 19 different Polish suppliers. Seven of these tests had shown to be, on a qualitative basis, positive for equine DNA. The first such positive test result was on 27th June 2012 and the company then contacted the Polish supplier whose representative visited the plant and arranged to take back the consignment. Further positive tests results on other consignments of Polish labelled product were obtained by the company in October, November, December 2012 and January 2013.”

Causes

A variety of possible causes for the horse meat substitution uncovered in 2013 have been advanced in the media mainly around the length of the supply chain and the need for very cheap flesh meat ingredient. Examples include the reclassification of “desinewed meat”, DSM, so that it could not be described as “meat” in the labelling of meat products is suggested to have led to firms seeking alternative supplies of a cheap meat ingredient⁹². The banning of DSM from beef may have forced some UK and Irish meat product producers to look elsewhere for supplies of cheap raw material – making the UK vulnerable to the wider European problem of beef adulterated with horse meat, which may not have been a UK issue up until then. The recent European Union ban on the export of live horses from Romania, in an attempt to prevent the spread of equine infectious anaemia, is alleged to have led Romanian farmers to begin exporting slaughtered horse meat in 2011, a commodity more readily diverted into substitution than live horse export⁹³. An alleged ban on donkey-carts on Romanian roads was also advanced as a possible root cause⁹⁴. Police and food law enforcement investigations continue and it is more profitable to await the outcomes of these investigations than speculate further here.

Conclusions

Species (and other) substitution of high value foods such as meats and fish is well known and appears perennial with anecdotal evidence from 1886 onwards. Modern UK surveys have demonstrated solid evidence with common meat species substitution standing at 14.6% in 1999 while in 2003 horse meat substitution was found at 12.5%* (pilot survey) and 0.6% (main survey). In 2012, based on FSAI findings substitution prevalence of 37%* for horse and 85%* for pig were reported in January 2013. In the UK industry testing from February to June 2013 indicated substitution with horse meat at a prevalence of 0.19%. The official survey in the UK indicated a similar prevalence of 0.4% substitution by horse meat and 0.8% by pig meat. The EU wide survey in the same period found the prevalence of horse meat substitution to be 4.7%. A South African study reported in 2013 substitution prevalence for common species of 23% - 37%. (* Denotes small sample numbers).

While quantification remains elusive in 2013 some products appear to have contained substantial amounts, in some cases up to 100%, horse meat. Thus the possibility of undeclared and unwanted meat species in meat products and fraudulent switching of food ingredients in the supply chain generally is a well-known risk. What is unknown is when such an event is actually going to occur. Horse meat seems to have been consigned as a purely historical adulterant in official UK authenticity policy thinking around 2007 with apparent abandonment of a FSA Food Authenticity Programme Q01107 project that ran from 2006 to 2007. This PCR-CE approach does not appear to have been effectively followed up until the horsemeat episode itself and it is interesting to speculate what might have been the outcome if this work had been followed up at the time with further validation and perhaps a survey. The last centrally coordinated authenticity survey was on the speciation of fish in 2008. A conclusion to be drawn from this is that apparent difficulties in analytical approaches for recognised adulterants should be followed up with vigour to yield robust validated method(s) that are widely disseminated. In this context it should be noted that an analytical method for “desinewed meat” in admixture with muscle tissue that can be termed “meat” for food labelling purposes remains to be demonstrated and validated.

Public Analysts did not altogether abandon testing for horse meat however such testing as was carried out was minimal as it was not specifically requested by local authorities and hence was unfunded (i.e. self-funded by individual laboratories). It was included on the initiative of individual Public Analysts and returned negative results. However the fact that it happened at all is instructive in that it was carried out at marginal cost by immunological screening tests rather than DNA methods and would only have been capable of detecting gross adulteration by horse meat. The principal that a large number of screening tests economically carried out yields more protection than few and costly analyses is well illustrated by the application of screening for coliforms as indicator organisms for the microbiological safety of water. This concept might with profit be applied to food authenticity testing where Public Analysts retain a spectrum of rapid sorting techniques (such as pollen identification for honey authenticity) and can then go on to apply definitive follow-up methods when a problem is uncovered.

Even quite sophisticated horizon scanning, albeit targeted at food safety, appears to have failed to provide intelligence that flagged up horse meat as a potential adulterant in beef products until FSAI uncovered it. However, it is evident from the RoI investigation that some elements of the food industry were aware, in mid-2012, of the presence of horsemeat in the supply chain. Reluctance to share such intelligence persists. The FSA Chief Executive reported to the Board on 4 June 2013⁹⁵ that a Food Business Operator, FBO, had discovered low levels of horse meat contamination in a beef shipment product. The FBO destroyed the material and took steps to secure their supply chain but was not prepared to share the specific intelligence involved with the FSA. Clearly there will be continuing difficulties for regulators to capture industry intelligence, in the absence of any national or European laws with regard to the mandatory disclosure of such information, making it all the more important for surveillance to be carried out at regular intervals. It is crucial that all stakeholders are aware that food authenticity is being policed on a continual basis providing a disincentive to conceal malpractice. Thus we conclude that future official surveillance (including sampling and analysis) should systematically include over time the known potential frauds (Table 2) supplemented by outputs from the various Emerging Risk programmes which henceforth should include food authenticity issues.

It is further suggested that the efforts of over 400 local authorities in the UK must be centrally coordinated in such programmes of activity, ultimately well-publicised to provide transparent deterrence. The food industry also has a significant role to play and FSA appears to have agreed with industry a transparent industry-funded testing scheme with quarterly reports on the FSA website. It is suggested that this too should be extended in scope in line with the known risks (Table 2). One food fraud that was effectively dealt with by industry, aided by FSA-sponsored analytical method development, was the substitution of orange juice by mandarin juice. This episode might with profit be re-examined to learn why it succeeded so well.

The root causes of the 2013 horse meat episode remain definitively to be elucidated but fraud must feature highly in the likely outcomes of pending investigations. Fraud is outside the normal safeguards which operate to ensure food is traceable and safety checks have been undertaken, endangering consumers as well as subverting choice and trust, potentially robbing whole industry sectors of hard-won reputations. In 2013 the horse meat found in processed beef products all appears to have come from approved premises and was produced appropriately for use in food. This was not clear at the outset of the episode and might not always be the case. While UK government ran a highly-creditable food authenticity research programme the prioritisation of food safety over food authenticity has been a dominant theme in food policy. It is suggested that owing to the damage that can be occasioned and the potential for adverse safety events the prioritisation of food safety over food mis-description (fraud) should be abandoned in favour of a holistic approach prioritised on the basis of the individual merits in each case.

The Royal Commission into the Australian 1980's horse and other meats scandal uncovered many deficiencies in industry regulation at the time, including lack of attention to the accuracy of information regarding commodities, a lax control of export approval stamps, and the poor sharing of information between officials. There was also evidence of corruption on

the part of some meat inspectors, the Australian meat industry appeared to be generally tolerant of malpractice which, to the Australian Federal Police, remained a low priority. The Royal Commission led to considerable tightening of the Australian meat inspection system, increased testing of products, increased penalties for substitution, the replacement of the Bureau of Animal Health with a new Export Inspection Service within the Department of Primary Industry and new legislation. While there is currently no suggestion that any of the above factors played a part in the UK or RoI in the 2013 scandal the various authorities might, as a precaution, have regard to the Australian findings in the current Europe-wide investigations.

In the UK and EU a reporting threshold of 1% was adopted as a pragmatic approach based on the lowest physical admixture (1%) tested in the published literature and the experience of regulators, enforcement and industry of an appropriate level at which to distinguish trace contamination from deliberate adulteration⁹⁶. It was never made clear whether this is 1% on a w/w basis or otherwise. It is also interesting to note that 0.9% w/w is the threshold above which the presence of EU-authorized GMO's must be disclosed in the labelling of foods and feeds⁹⁷. The 1% threshold worked well in moving the response forward and allowing FSA, Defra and industry to deal with a rapidly evolving situation. It placed the responsibility for a front line *forensically-robust* analytical response with the Public Analysts, the scientists best placed by training, qualifications and experience to deal with it. Analytically, laboratory capability in the UK, particularly in the Official Control System (Public Analysts) was strengthened by the research on DNA-based methods undertaken by the FSA Food Authenticity Programme 2000-2010 and the knowledge transfer carried out then and subsequently by Defra²⁸. However the strengthening of Public Analysts' ability to respond was not as much as it might have been if given better support nationally. In addition international guidance is needed to standardise and harmonise expression of results from DNA meat speciation analysis in order to afford comparability at the European level. Strengthening of expertise in FSA and Defra on food authenticity to levels comparable with those of the FSA Food Authenticity Programme should be considered. Moreover, science should not be confined to scientists. Policy officials would benefit from regular familiarisation seminars on science topics.

The European Commissioner for Health and Consumer Policy, Tonio Borg, has proposed a 5-point Action Plan to address the shortcomings identified from the horse meat issue in Europe's food supply chain. The Plan includes the following actions to be implemented by 2014:⁹⁸

- 1 Develop synergies between enforcement authorities, ensure rapid exchange of information on intentional violations of food chain rules, promote the involvement of Europol in investigations
- 2 Ensure that rules on horse passports are enforced correctly, that passports are delivered only by competent authorities and that national databases are created
- 3 Require that financial penalties for intentional violations of food chain rules be established at sufficiently dissuasive levels, and that control plans

- in the Member States include unannounced controls [inspection, sampling and analysis]
- 4 Adopt rules on mandatory origin labelling of meat (sheep, goat, pig, poultry, horse, rabbit, etc.) and deliver a report in autumn 2013 on the possible extension of mandatory origin labelling to all types of meat used as ingredient in foods
 - 5 Present and assess the results of the controls currently carried out in the EU countries

The consequences of the above plan will determine much of what will be put in place in the UK in the future and it is timely that Regulation 882/2004 on official controls is under review. The Commission's stated position is that where financial penalties are used in relation to intentional violations of food chain law, they are at a level which is sufficiently dissuasive and higher than the economic gain expected from the fraud. It will also be expected that Member States include in their control plans and perform regularly mandatory unannounced official controls (including inspections and testing) directed at combating food fraud and the Commission wish to be able to impose (not just recommend) coordinated testing programmes in specific cases, in particular in case of fraud.

Will all this happen again? History teaches us that the answer is yes, but not in quite the same way. We suggest it is now unlikely that widespread horse meat substitution will re-occur for decades but other frauds will arise and the way to guard against this is continued systematic vigilance. The challenge is to secure a cost-effective, efficient scientific infrastructure to support that vigilance in a planned and sustainable manner.

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Appendix 1

FSA Food Authenticity Research Programme Projects

Reference	Title	Details	Web Address	Duration and Contractor
Q01002	Detection of meat species in fresh and processed food - production and use of monoclonal antibodies reactive with insoluble muscle protein desmin	The research aimed to develop monoclonal antibodies for species-specific meat proteins in order to identify the species of meat present in meat products.	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01002/	Study Duration: September 1998 to September 2000 Contractor: Nottingham Trent University
Q01003	Differentiation of species of meat in particular cooked products by DNA methods	The research project aimed to optimise existing PCR based methods for the quantitation of meat species in cooked meat products.	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01003/	Study Duration: April 1998 to September 1999 Contractor: Laboratory of the Government Chemist (LGC)
Q01023	The immunological determination of meat content in cooked meat products	The research aimed to develop an ELISA method to determine the amount of lean meat present in cooked meat products.	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01023/	Study Duration: February 2000 to September 2002 Contractor: Laboratory of the Government Chemist (LGC)
Q01033/43	Real time analysis of PCR-based DNA methods for the limit of detection, sensitivity and specificity	The research aimed to assess the effect of food processing and the food matrix on the usefulness of DNA methods for identifying meat and fish species and to develop real-time PCR methods that will allow species-specific identification in several matrices with determined detection limits. It focused on identification of	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01033_43/	Study Duration: August 2000 to May 2003 Contractor: Central Science Laboratory (CSL) (now FERA, Food and Environment Research Agency) and Eurofins Scientific

Reference	Title	Details	Web Address	Duration and Contractor
		meat and fish species.		
Q01036	Combined competitive PCR/multicolour fluorescence for accurate quantification of markers in DNA from foods	The research aimed to investigate the possibility of developing a competitive PCR assay for the quantification of meat species in meat products.	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01036/	Study Duration: April 2000 to September 2002 Contractor: University of Nottingham
Q01049	The identification of meat species in vegetarian foods by QRT-PCR	The research aimed to apply DNA sequences that are common to all animals as a means of detection. Contaminating beef when present as a homogenised blend of meats from animals of differing species can be detected at about 0.05%, however, the exact level upon the actual food itself. Analysis of commercial products showed evidence of very occasional low-level contamination consistent with ineffective manufacturers cleaning procedures	http://www.foodbase.org.uk/results.php?f_report_id=93	Study Duration: 2002 to 2003 Contractor: RHM Technology, (now Premier Analytical Services, part of Premier Foods)
Q01052	Development and application of genomic markers for the quantification of meat in meat products	The research aimed to develop working TaqMan PCR methods to identify the species and determine the amount of meat in sausage products.	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01052/	Study Duration: April 2001 to June 2002 Contractor: Campden and Chorleywood Food Research Association (now Campden BRI)

Reference	Title	Details	Web Address	Duration and Contractor
Q01053	Quantitative and qualitative detection of DNA targets in meats of known provenance	This research aimed to develop a TaqMan PCR assay to differentiate at least 5 different meat species through one mitochondrial target and address some of the fundamental parameters that could affect the overall robustness and accuracy of quantitative Polymerase Chain Reaction.	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01053/	Study Duration: June 2001 to July 2003 Contractor: Veterinary Laboratories Agency Virology Department
Q01055	Quantitation of meat in fresh and processed foods - an evaluation of the use of antibodies to the insoluble muscle protein desmin	This research aimed to extend the use an indirect ELISA and dot blots for the specific detection of poultry meat species and the total meat content of meat products, complementary to the DNA-based assays but quantitative in nature without being excessively technically demanding.	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01055/	Study Duration: January 2003 to January 2005 Contractor: Nottingham Trent University
Q01064	Development and validation of methods for the determination of non-muscle tissues in meat products	This research aimed to build upon DNA PCR work to maximise assay sensitivity and robustness by the application of nested PCR and real time detection techniques, followed by validation using admixtures that have undergone a variety of processing conditions to provide robust tissue detection assays.	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01064/	Study Duration: June 2002 to August 2003 Contractor: LGC

Reference	Title	Details	Web Address	Duration and Contractor
Q01070	Optimisation of real-time polymerase chain reaction methods for accuracy and precision	This research aimed to investigate the underlying causes of real time quantitative PCR imprecision and inaccuracy and produce a set of guidelines for design and optimisation of assays	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01070/	Study Duration: June 2002 to May 2003 Contractor: Central Science Laboratory CSL (now FERA)
Q01083	Development of methods for the identification of duck, pheasant, venison, horse, donkey and wild boar in meat products	This research aimed to provide rapid species-specific DNA-based assays for duck, pheasant, venison, donkey, horse and wild boar in meat products	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01083/	Study Duration: July 2003 to December 2004 Contractor: Central Science Laboratory CSL (now FERA)
Q01084/87/88/89/90	Final optimisation and evaluation of DNA based methods for the authentication and quantification of meat species.	This collaborative project aimed to optimise and evaluate DNA methods developed from previously funded FSA projects based on real-time polymerase chain reaction (RT-PCR) for meat species identification and measurement	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01084a/	Study Duration: September 2003 to March 2005 Contractor: CSL(now FERA), RHM Technology (now Premier Analytical Services, part of Premier Foods), Veterinary Laboratory Agency, Eurofins, Campden and Chorleywood Food Research Association (now Campden BRI)

Reference	Title	Details	Web Address	Duration and Contractor
Q01104	A proteomic approach to the determination of meat species within a mixed meat product	A method was developed and optimised for the extraction, enrichment and processing of proteins from different meat mixes, which were either fresh or heavily cooked meat. Mass spectrometry were then used to identify species-specific peptide biomarkers. The use of stable isotope labelling technology was investigated for quantification of these peptides, using chicken in pork as a model system. Using stable isotope labelling, it was possible to detect chicken mixed with pork in amounts as low as 0.5% in cooked meats. The overall results have confirmed the feasibility of a proteomic approach for quantification and identification of meat ingredients, although further studies to fully validate this approach for all species are needed.	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01104/	Study Duration: July 2006 to July 2009 Contractor: School of Biological Sciences, Royal Holloway, University of London
Q01107	The adaptation and validation of real-time PCR methods, for exotic species identification, for analysis on a capillary electrophoresis chip system	This research aimed to transfer existing DNA methods for verifying commercial and exotic meat species including horse, donkey, pheasant, duck and venison as well as pork, beef, lamb, chicken and turkey to a more efficient and versatile lab-on-a-chip capillary electrophoresis system.	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01107/	Study Duration: July 2006 to April 2007 Contractor: Central Science Laboratory, CSL (now FERA)

Reference	Title	Details	Web Address	Duration and Contractor
Q01109	Adaption of DNA analysis techniques for the identification of illegally imported bushmeat for use on the Agilent 2100 bioanalyser	This research aimed to identify 11 species including chimpanzee, gorilla, bushbuck, African sheep, dwarf zebu, zebu, duiker (4 sub-species), bush pig, cane rat (2 sub-species), and porcupine using PCR. The method was validated to ensure that it is suitable for use by trialling it in a public analyst laboratory. A Standard Operating Procedure (SOP) was produced.	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01109/	Study Duration: June 2006 to October 2007 Contractor: Food DNA Services
Q01129	The development and validation of DNA marker methods for the verification of meat from wild boar	This research aimed to distinguish pure wild boar meat from pure breed or cross-breed pig meat and produced a standard operating procedure (SOP) for the verification of meat from wild boar, which is suitable for the analysis of cuts of meat.	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01129/	Study Duration: January 2009 to February 2010 Contractor: Food and Environment Research Agency (FERA)
Q01130	Verification of meat from traditional cattle and pig breeds using Single Nucleotide Polymorphism (SNP) DNA markers	This research aimed to develop a breed identification assay based on SNP DNA markers	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01130/	Study Duration: 1 October 2008 to 30 November 2011 Contractor: information not readily available

Reference	Title	Details	Web Address	Duration and Contractor
Q01132	Inter-laboratory validation of a method to determine the species of origin of gelatine found in chicken by mass spectrometry	This research aimed to optimise a proteomics method to determine the species origin of gelatine (bovine, porcine or avian) in water binding injection powders, and a method to extract and enrich added gelatine in chicken, so that the proteomics method can be used to speciate added gelatine in chicken samples.	http://webarchive.nationalarchives.gov.uk/20100907111047/http://www.food.gov.uk/science/research/choiceandstandardsresearch/authenticityresearch/q01list_meat/q01132/	Study Duration: March 2010 to February 2012 Contractor: Food and Environment Research Agency (FERA)

Appendix 2 – Potential Authenticity/Fraud Problems in the Food Chain (Largely after Primrose, Woolfe and Rollinson²⁴)

Type of Fraud	Example
Substitution of one ingredient by a similar but cheaper one	Using whiting or pollack in place of cod
	Substituting bonito for tuna or sea trout for salmon
	Labelling cheaper varieties of potatoes as “King Edward” variety
	Substituting ground peanuts for ground almonds
Extending or adulterating food with a cheaper or base material	Adding high nitrogen compounds such as melamine
	Adding non-approved GM varieties
	Adding water to increase weight of chicken breasts
	Mixing long-grain rice with Basmati rice
	Mixing cow’s milk with buffalo milk before production of buffalo mozzarella cheese
	Adding common wheat to durum wheat pasta labelled 100% durum wheat
	Extracting soluble coffee from beans mixed with skins and husks
	Adding cheaper vegetable oils to named higher value vegetable oils
	Adding water, sugar, acids and colouring to fruit juices
	Adding desinewed meat (note a method to detect desinewed meat in mixtures with other meat remains to be validated)
	Diverting waste meat into the human supply chain
	Diverting meat intended for pet food into the human supply chain
	Presence of undeclared ingredients
Meat species substitution: legitimately traded commercial species and donkey, horse, goat, kangaroo, buffalo,	
Mechanically separated meat (MSM) in processed meat products	
Extending or adulterating food to increase value	Adding mandarin or tangerine juice to orange juice to improve colour of juice
	Adding glycerol to wine to improve body
	Counterfeit spirits

Type of Fraud	Example
Non-declaration or false declaration of processes	Labelling poultry as fresh even though it has been previously frozen
	Failure to declare that food has been irradiated
	Failure to declare that juice has been prepared from concentrate
Over-declaration of a quantitative ingredient	Including hydrolysed protein as part of the meat content
False claims regarding geographical or production origin	Labelling South American beef as British beef
	Declaring farmed fish as “wild”
	Labelling conventionally produced food as organic
	Claiming that extra virgin olive oil is from a particular geographical region.