Reflections on adulteration, meat speciation, testing and associated issues

The recent 'Horse Burger' incident involving British supermarket group Tesco and the feeding of meat products that were designated halal but showed traces of pig DNA to prisoners in British jails, beg some interesting questions.

At the outset, we need to appreciate that DNA is not meat, and that 'traces of horse or pig DNA' are basically amounts that are too small to quantify accurately. Since the test has not been a conclusive negative, these results are referred to as 'traces'.

Firstly, we need to address the issue of super sensitive tests such as PCR, which can virtually pick up a few molecules of foreign DNA. This might be in the meat but, in manufactured products, it could be in one of several additives. These tests will give us positive results on products which, had they been tested a decade or so ago with the then commercially available tests, would have given negative results.

Secondly, we need to focus on the fact that toxic substances, such as lead, arsenic and melamine, all have to comply with value limits which test results must not exceed, whereas for horse and pig DNA there must be a total absence – remember pig and horse DNA is found in large quantities in pork and horse meat respectively and many people eat these meats out of choice!

Surely, this is unreasonable and the current situation has arisen because those who should have known better about the sensitivity of modern testing methods have failed to inform or have over reacted to the emotional comments (hysteria?) of consumers and customers.

We hear that in the USA a similar debate is occurring over fish and the substituting of high value species by low value ones.

We need to differentiate safety issues from labelling or commercial issues. For the former we must keep the public safe, for the latter has the time come to define levels of acceptability that are sensible and practical and will minimise the recall and destruction of perfectly safe products?

Remember, in this world of ours millions are still starving!

In the beginning . . .

On 16th January 2013 the British Food Standards agency stated that there were two distinct types of case:

- In all but one of the cases, the levels of horse and pig DNA were extremely low.
- In the one exceptional case, the level of horse meat accounted for 29% of the meat content.

The causes of these two problems are therefore likely to be different and the focus of the investigations into the causes will be different. The FSA has now set out a four-point plan for its investigation, which it will be implementing in conjunction with other Government departments, local authorities and the food industry:

- To continue the urgent review of the traceability of the food products identified in FSAI's survey. The retailers and the UK processor named in the survey were asked to provide comprehensive information on the findings by Friday 18th January.
- To explore further, in conjunction with the Food Safety Authority of Ireland, the methodology used for the survey to understand more clearly the factors that may have led to the low level cases of cross-contamination.
- To consider, with relevant local authorities and the Food Safety Authority of Ireland, whether any legal action is appropriate following the investigation.
- To work with the Department for Environment, Food and Rural Affairs (Defra), the devolved rural affairs departments and local authorities on a UK-wide study of food authenticity in processed meat products.

Indian viewpoint

Meat speciation is an area which requires specialised focus in food quality management systems. It is a vital field to ensure food safety for consumers and it upholds the laws related to meat and meat products.

The adulteration of inferior quality meat into superior quality meat is a common practice all over the world. Using meat speciation techniques we can easily solve the problems of misdemeanours related to the Prevention of Cow Slaughter Act and the Prevention of Food Adulteration Act in India, and similar laws worldwide.

Various methods are available from physical, chemical, anatomical, histological and biological to sophisticated molecular techniques.

All these methods have pros and cons so the knowledge of all these techniques is of immense value for those having some involvement in this specialised field.

If the meat is in carcase form, it can be easily identified with the physical, chemical, anatomical and histological methods, but reproducibility and quantitative identification is not possible.

In biological methods the simple method of antigen-antibody reaction can be used for visual identification. In electrophoresis, the migration of the protein moiety according to their molecular weight under the influence of electric field principle is applied.

The band patterns produced in this technique is then visualised for result interpretation.

In molecular techniques DNA and RNA amplification is done to produce the fingerprints as the characteristics of an identical genetic material for a particular meat species.

Finally, the development of PCR techniques makes it easy to identify the meat species even from the cooked and spoiled meat in which protein is easily destroyed. Real time PCR is the revolution in this field in which we can identify and monitor the product during its amplification.

However, Indian scientists feel no single technique is sufficient for differentiation of all types of meat species and meat products.

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European service

Neogen Europe's meat speciation analysis can be carried out using qualitative ELISA (Enzyme Linked Immunosorbent Assay) and qualitative PCR analysis techniques to identify a range of animal species in raw or cooked products across a wide variety of food and feed matrices. They have also developed PCR analysis methods for the qualitative detection of a variety of fish species.

Meat Speciation Qualitative by ELISA Analysis can be used for beef, pork, poultry and sheep meat, whereas PCR Analysis covers chicken, cow, goat, horse, pork, rabbit, sheep and turkey.

Their Fish Speciation Qualitative PCR Analysis covers Atlantic salmon, British pollock, cod, coley, haddock, hake, rainbow trout and whiting.

ILS testing

At ILS in the UK, meat speciation is undertaken using ELISA tests kits for various types of meat. These kits can be used to test for both raw and cooked meats. The kits utilise anti-species albumin antibodies coated onto microwells in a direct sandwich type ELISA. Albumin from a sample extract binds to the antibody coated wells.

Any unbound material is removed by aspiration and washing.

A peroxidase enzyme labelled anti-species antibody then binds to the captured albumin from the samples, to complete the 'sandwich'. Excess conjugate is removed and the peroxidase activity is determined by the addition of TMB substrate which develops a blue colour.

ILS can test raw or cooked meats for pig, cow, poultry and sheep.

They can also sub-contract fish speciation to another accredited laboratory. This testing method uses DNA and there are eight species of fish that can be differentiated and, here again, cooked or raw products can be analysed.

The eight species of fish are – cod, hake, coley, haddock, pollock, whiting, trout and salmon.

USA's gold standard

RenekaBio Meat Speciation ELISA kits are able to detect and identify species content in cooked, raw, and thermally processed meat, meat products and animal feed. Their test kits have been the gold standard in the US (used by USDA and SENASA) and are utilised by the food processing industry, organisations, and government agencies worldwide.

Options from EuroProxima

Preventing the adulteration of feed and food products with non-desirable or low quality animal products is important for health, regulatory and economic reasons. The identification of species is performed to assure consumers that the products they purchase are safe, wholesome and properly labelled.

To cover the whole range of meat products EuroProxima offer three types of non-competitive sandwich ELISA kits that detect different proteins.

Raw Meat Species Kits are for the speciation of uncooked products and these detect species specific serum albumin in meat, meat products and milk. They detect 1% impurity with alternative species.

Cooked Meat Species Kits are for speciation of cooked products. These kits detect a heat resistant species specific muscle protein in cooked, canned or processed products up to a temperature of 133°C at 3 bar. They detect 1% impurity with alternative species.

MELISA-TEK Meat Species Kits are the ultimate kits for the detection of porcine or bovine species in rendered meat and bone meals. The Troponin-I protein that is detected in this test is species specific and

heat resistant for material heated over 133°C and 3 bar.

They detect as little as 0.05% skeletal muscle in processed materials. Genetic ID has comprehensive tests for detection and identification of animal by-products in animal feed and for determination of the species of meat products.

These tests help clients comply with current EU, Japanese, US, and other country regulations prohibiting animal products in feed.

These tests are also highly effective and useful for species identification of meat products and to detect adulteration of meat products with tissue from other species.

PCR technology

Genetic ID have designed a wide range of primer sets and tests for PCR analyses of DNA isolated from animal feed samples. These tests include the following:

- Ruminant-specific test.

Selectively detects members of the ruminant family by targeting a genetic sequence that is found only in this family, which includes cows, sheep, goats, deer and elk.

- Bovine-specific (beef) test.

Targets a sequence unique to cattle and very closely related bovine species.

- Ovine-specific (mutton) test.

Targets a sequence unique to sheep and very closely related ovine species.

- Porcine-specific (pork) test.

Targets a sequence unique to pigs and very closely related porcine species.

- Duck/goose tests.

Targets a sequence unique to the common duck and goose, and that differentiates them from other avian species such as chicken and turkey.

- Chicken/turkey tests.

Targets a sequence unique to the common chicken and turkey, and that differentiates them from other avian species such as goose and duck.

Assays are performed by Real Time PCR using TaqMan probe based technology. Results are reported qualitatively 'species detected' or 'species not detected'.

The limit of detection of the tests varies depending on the composition of the sample, the nature of the animal-derived material (tissue meal versus bone meal, versus blood meal, versus fresh tissue), and the manner in which the animal-derived material has been processed. For instance, animal feed ingredients are often heated to high temperatures.

UK 1998 MAFF Survey

As part of its surveillance programme the UK's Working Party on Food Authenticity carried out a survey to investigate the accuracy of meat speciation label declarations on meat products sold through retail outlets in the UK.

Samples of meat products were collected in January 1998. These included sausages, burgers, pies, pâtés and recipe dishes, which were obtained from large, small and discount supermarkets, butchers shops and other small independent retailers.

The total number of samples included in the survey was 570. Collection of the samples was undertaken by 17 local authorities.

The sampling plan was designed to collect samples in each area approximating to the proportion of market shares of broad categories of meat products, and to the percentages normally sold by each type of retailer as far as the available market data at the time allowed. All the products were pre-wrapped and product information was obtained from the product labels and recorded on a specially designed sample information sheet by the enforcement officer.

All products were subjected to a screening test using the Cortecs Cooked Meat Species Identification Kit which is based on an ELISA test. Where samples exhibited a positive response to a meat species not declared on the label a further confirmatory DNA test was applied. Since the ELISA test does not differentiate between poultry species, those samples where a positive poultry response was recorded were also subjected to DNA analysis to identify the species of poultry that was present.

In the survey, 83 (14.6%) samples were identified as having labels which did not declare a species of meat detected within them. However the methods available did not offer enough quantitative precision for it to be possible to say whether the non-declared species were present as a result of deliberate substitution or accidental cross-contamination.

Top 10 trouble-shooting tips

In the latest issue of our sister publication International Food Hygiene (Vol 24 No. 1, page 23), Leatherhead Food Research provided an article entitled 'Horse meat found in beef burgers – what went wrong?' To help manufacturers identify where production problems in general may occur, they published a list of 'Top 10 Trouble-shooting Tips' which are summarised below. Certain of these tips can be useful in helping to identify whether or not a product contains horse meat and when and where things might have gone wrong.

1. Keep it simple with basic tests at first.
2. Microscopy is important but start with the eye.
3. Employ a range of analytical tests, both physical and chemical.
4. Compare the product with the correct standard or control.
5. Trend analysis is important even if in specification.
6. Find out what's changed – ingredients, processing, packaging.
7. Check if the product has been abused during storage.
8. Evaluate the likely impact of consumer misuse or malicious action.
9. Determine the source of the contaminant or cause of the problem.
10. Do not be afraid of a quick fix.

Australia

In the 1981 Australian meat substitution scandal a variety of circumstances and factors enabled the substitution of frozen beef meat packs with beef/kangaroo and possibly other species. The meat exporting companies concerned were able to engineer this scam through criminal activity, such as forging export certificates and stamps, and because the compliance testing and assay procedures at the time were not stringent enough to detect the substitution at source.

This situation changed, and all export meat abattoirs had their bonded product randomly sampled on site, using an eight species ELISA meat speciation system.

The introduction of the system by the Meat Inspection Branch has essentially eliminated meat substitution and paved the way for improved export surveillance of the quality of certified export meat products from Australia.