Immunological identification of meat species

PATTERSON, R.L.S., JONES, S.J. and KANG'ETHE, E.K.

ARC Meat Research Institute, Langford, Bristol, UK

Introduction

A question which arises sometimes is whether or not meat is actually from the stated species of animal. Although improvements in technology and processing have led to more economical transportation and utilisation of deboned carcass meat, this has also facilitated the use of cheaper undeclared meats, because unequivocal identification of species becomes very difficult once meat has been taken off the carcass and the anatomical features have been destroyed. Meat of similar pigmentation, for example beef and horse meat, beef and mutton, or poultry and pig meat are virtually impossible to distinguish by eye once they have been frozen *en masse* in large blocks, or flaked and incorporated into comminuted meat products. However, apart from deliberate misrepresentation, many meat products in Europe as well as British-style sausages, burgers and pies, may contain the flesh of more than one species, and the analyst requires rapid methods of species identification.

Methods for determination of species origin have been available for some time based upon immunological antigen/antibody reactions using various forms of the precipitin test. Precipitating antisera for the different meat species are available commercially and can be used qualitatively in Ochterlony-type double immunodiffusion tests[1] or semi-quantitatively in "rocket" immunoelectrophoresis.[2] One disadvantage of these methods is that they require concentrated antibody preparations which becomes very expensive in large scale testing. Gel electrophoresis[3] and isoelectric focussing[4,5] are alternative methods which have had considerable success in identification of the species origin of fresh meats and fish, but which are unsuitable for quantitative analysis of mixtures containing the flesh of more than one species.

Enzyme-linked immunosorbent assay (ELISA)[6,7], has emerged in the last few years as a rapid, convenient and relatively cheap method of assaying antigens and antibodies quantitatively in many diagnostic tests in clinical medicine. One way that ELISA differs from the precipitin reaction is that visualisation of the antigen-antibody interaction is through colour formation by enzyme conversion of a substrate rather than by formation of a precipitate through an agglutination reaction. Recently, we adapted a particular form of ELISA for use in meat species identification.[8] The same technique has also been applied to the detection and estimation of soya protein in food products[9], and it is clear that many more applications will be developed in future wherever food components are capable of acting as antigens.

Experimental

Many antisera are now commercially available, but although produced in a host animal (rabbit, sheep, goat) in response to injection of a *single* antigenic substance, for example, a serum albumin, they may comprise a number of different types of antibodies depending upon the number of antigenic determinants (sites) present on the injected antigen. Such antisera are termed heterologous, polyclonal antisera. Purchased antisera were therefore purified by immunoadsorbent chromatography[10]. This procedure consisted of coupling the corresponding antigens, for example, beef serum albumin (BSA), horse SA and sheep SA to cyanogen bromide-activated Sepharose 4B and filling the resulting suspensions into individual chromatography columns. Each column was then equilibrated with 0.15M phosphate buffered saline (PBS) at pH 7.2. Isolation of the "monospecific" fractions from the prepared antisera was achieved by circulating the first antiserum, say anti-HSA, through the columns packed with the other two immunoadsorbents (BSA and SSA) continuously for 48 hours. Once cross-reacting antibodies to BSA and SSA had been removed, the now diluted anti-HSA serum was circulated through the HSA-immunoadsorbent column. Unbound protein remaining in the column was washed out with PBS and the adsorbed antibodies, now monospecific to HSA, recovered by elution from the adsorbent with glycine-HCl buffer (0.1M, pH2.5) containing 10% dioxane. After concentration and dialysis against PBS, the solution of mono-specific antibodies was stored frozen at -20°C. The trapped cross-reacting antibodies retained by the BSA and SSA immunoadsorbents were removed from the other two columns in the same way and the columns regenerated according to the method of the manufacturer. Specific antibodies to BSA and SSA were obtained likewise, using the same immunoadsorbents, first by removal of cross-reacting fractions and then the immobilisation and collection of the specific fractions.

Since the species antisera had all been raised in rabbits, they were therefore rabbit immunoglobulins. The enzyme used to visualise and quantify the assay was horse radish peroxidase which had been conjugated to a second antibody raised against rabbit immunoglobulin (ie an anti-antibody), and which now acted as a common means of detection of all the species-specific antibodies. In the presence of substrate (o-phenylenediamine + hydrogen peroxide) increased development of the yellow chromophore, measured as absorbance at 492 nm, indicated increased antigen content in the original meat extract.

Extracts of meat or meat mixtures were prepared by homogenization in saline of the finely minced material, followed by centrifugation at 10,000 r.p.m. for 30 min. at 4°C. The supernatant was then filtered through Whatman No.3. paper to remove floating particles of fat, and stored frozen in 3 ml aliquots. The extract, now containing the serum albumins residing in the musculature after slaughter, was then dispensed in diluted form (1-50) into wells in a micro-ELISA plate, and the plate incubated for 3 hours at 4°C to allow absorbtion of the antigens on to the polystyrene surface. After washing, the species-specific antisera were added and further incubation (2 hours) completed. Residual antisera were thoroughly washed out and the second antibody/enzyme conjugate added and incubated overnight at 4°C. Substrate was added and allowed to react for 30 minutes, after which colour development was stopped by addition of 12.5% sulphuric acid. The intensity of the yellow colour developed was measured by a micro-ELISA plate reader.

Results and Discussion

Immunoadsorbent chromatography was successful in reducing the cross-reactivity of most of the sera: for example, the crude antiserum to BSA cross-reacted strongly with pure SSA and extracts of sheep, goat and deer meat (venison) prior to chromatography, but not afterwards. However, it should be noted that the antisera, although now with greatly improved specificity, were not necessarily fully monospecific: whilst the anti-horse and the anti-beef serum did not show cross-reactions with the opposing antigens (serum albumins) or with sheep, goat or pig antigens, the "purified" anti-sheep still cross-reacted strongly with goat, and weakly with beef and deer antigens. Further purification by improved affinity chromatography, or development of a monoclonal antibody, might be successful in reducing the degree of cross-reactivity in the serum. Unfortunately, monoclonal antibodies are not yet available commercially for identification of the common meat species.

Horsemeat was clearly differentiated from beef, pork and lamb at the optimum dilution of the meat extracts (Figure 1a), and was also detected easily in mixtures with beef at levels between 3 and 80% (Figure 1b). Although small differences in absorbance values were found for beef containing less than 3% horsemeat compared with those for pure beef, they were not statistically significant. Greater efficiency of extraction of the serum albumins from the muscle mass would probably improve sensitivity at low concentrations: also introduction of an "inhibition" stage in the ELISA procedure, which results in an inverse relationship between antigen concentration and intensity of colour developed would improve sensitivity at low levels. Figure 1c shows clear differentiation of beef and veal from pork, horsemeat and lamb with the anti-BSA serum, whilst the cross-reactivity of the anti-SSA serum with goat serum albumin is seen in Figure 1d, due to the very close phylogenic relationship of the two species: a degree of cross-reactivity of this antiserum with beef serum albumin is also evident.

These results demonstrate that a conventional serum may not be fully monospecific even after purification by immunoadsorbent chromatography and that some care should be exercised when interpreting results from sera purified in this way. It should be noted that the use of antisera raised against serum albumins or most other native proteins will be effective only in the differentiation of raw, unheated meats: a completely new and different set of antisera specific to heat-stable proteins will be required for heat processed and cooked meats.

In addition to improving the specificity of the antisera, work is in hand to provide analysts in the meat and meat processing industry with a simplified version of the ELISA procedure. Initially, the individual reagents ie, species reference materials, antisera, coating buffers, and colour-forming chemicals will be available through The Sigma (London) Chemical Company, who intend producing kits at a later stage containing all the necessary equipment and reagents to enable a user with the minimum of laboratory facilities to test for a range of meats. The kits will most likely be based upon the use of cheap, disposable polystyrene test-tubes (approximately 50 x 10 mm diam) or similar spectrophotometer cells, instead of micro-ELISA plates. It is hoped that such a system will provide a first stage screening procedure, enabling a rapid check to be made of the identity

of meat species in incoming bulk supplies. Apart from use in detecting the incorporation of illegal meats in meat supplies intended for human consumption, a rapid procedure is required for the detection of undeclared, legal meats used as substitutes for more expensive meats, common examples in the UK being the inclusion of mutton in beef and turkey in pigmeat.

Summary

An enzyme-linked immunosorbent assay (ELISA) procedure is described which allows identification of different meats by use of antisera raised against the species serum albumins. Purification of the crude antisera using affinity chromatography is described; however, interfering cross-reactions were still detected between phylogenically similar species such as sheep and goat.

Horsemeat could be detected in mixtures with beef at levels of between 3 and 80% using an antiserum to horse serum albumin. Beef and/or veal could be differentiated from lamb and pork with an anti-bovine antiserum, and pork and horsemeat from lamb or mutton with an anti-sheep antiserum. The sheep antiserum cross-reacted strongly with goatmeat and also weakly with beef.

A simplified version of the test procedure for use by the meat industry is discussed.

References

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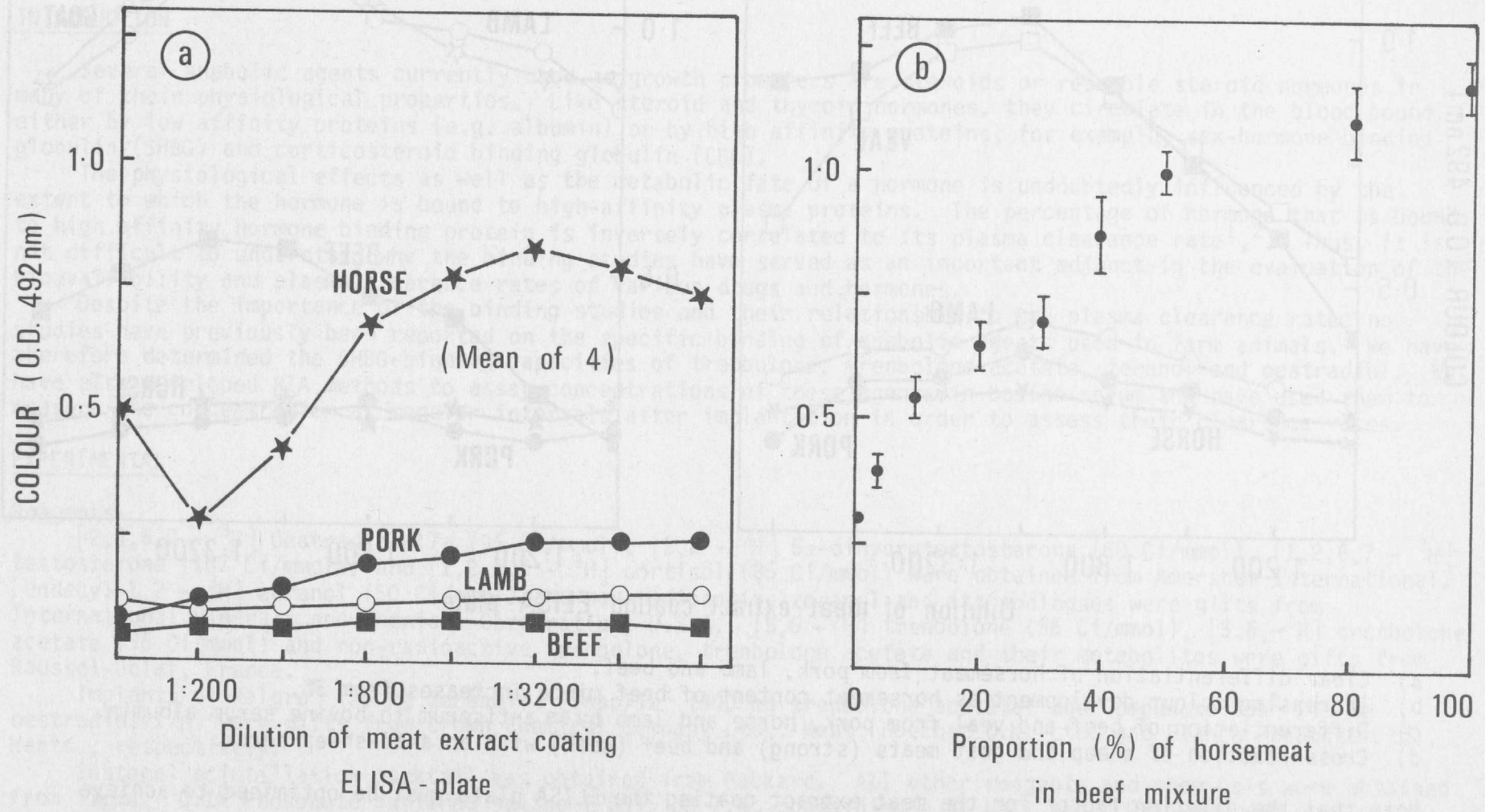
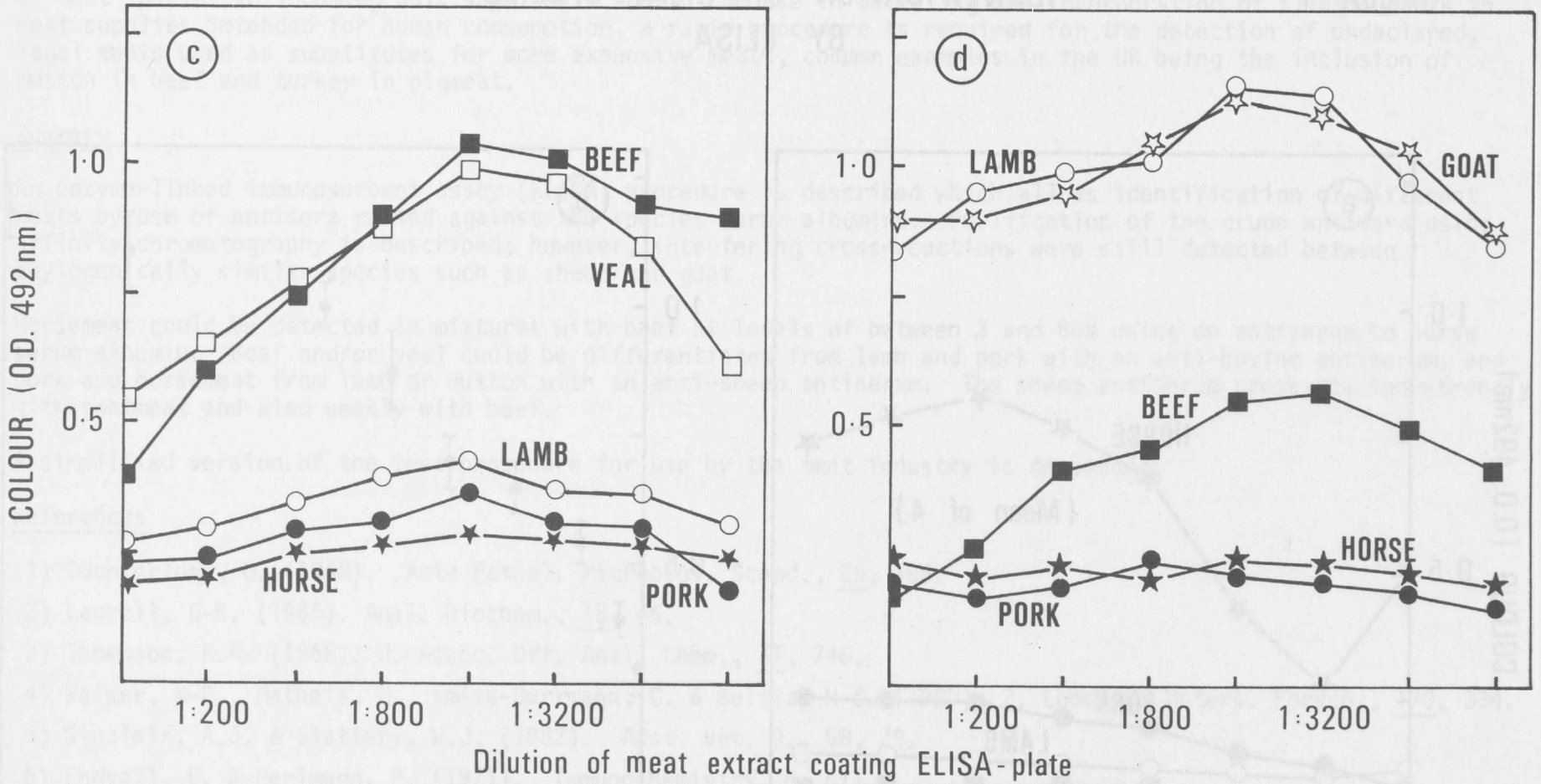
Figure 1 **IDENTIFICATION OF MEAT SPECIES BY ELISA**





a) Clear differentiation of horsemeat from pork, lamb and beef.
b) Increasing colour development as horsemeat content of beef mince increases from 3%.
c) Differentiation of beef and veal from pork, horse and lamb by an antiserum to bovine serum albumin.
d) Cross-reaction of sheep and goat meats (strong) and beef (weak) with an anti-sheep serum.

Note that the dilution factor for the meat extract coating the ELISA plate must be optimised to achieve maximum differentiation.