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THE APPLICATION OF IMMUNOFLUORESCENCE TO THE SCREENING OF MEAT SUPPLIES AGAINST PATHOGENIC BACTERIA

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Methods for the detection of food poisoning bacteria are generally slow and laborious. This is particularly true of salmonella testing, and with conventional methods, meat would probably be processed and distributed by the time the test answer is known. We have therefore attempted to shorten the tests for salmonellae and for Clostridium botulinum by using the technique of immunofluorescence.

A salmonella test should be successful even with very low numbers of salmonella in a sample. We have found it impossible to dispense with the liquid enrichment procedure, which is required to raise the salmonella numbers to a detectable level. However, 1 or 2 days could be saved if the salmonella cells in the enrichment broths could be demonstrated by immunofluorescence examination, rather than by further culture on solid diagnostic media.

In early tests with pure cultures we found that salmonellae could be stained by immunofluorescence techniques after culture in selenite F broth at 43°C. It has also been established that selenite F enrichment at this temperature is very suitable for isolating salmonellae from meat (Gerogala & Boothroyd, 1965 a), and it was thought that a rapid salmonella test could be based on immunofluorescence examination of smears made from such selective enrichments.

The immunofluorescence examinations for salmonellae involved demonstration of cell O antigens only, as flagellar H antigens are poorly developed in selenite broth at 43°C. The O antigens are located in the cell wall, and immunofluorescent staining makes the cell outline clearly visible (Thomason, Cherry & Moody, 1957). At the start of our investigation we used the indirect staining technique, as it required only one fluorescent antiserum (e.g. goat antirabbit antiserum), which could then be used in conjunction with ordinary agglutinating salmonella antisera (Georgala & Boothroyd, 1964). Subsequently, fluorescent polyvalent antisera were prepared (Georgala & Boothroyd, 1965 b) and the indirect technique was dropped in favour of the direct staining technique. Nairn (1964) has edited an excellent text describing in detail these and other immunofluorescence techniques, which will also be illustrated here with photographic slides.

This paper describes results obtained with our immunofluorescence salmonella test, and also includes some details of our recent work with Cl. botulinum fluorescent antisera.

METHODS

Samples used in salmonella tests: The samples tested can be grouped as follows:-

(1) 128 samples of imported horsemeat and veal carrying heavy contamination.

(2) 706 routine samples, mostly of home produced beef, where only light contamination was encountered.

(3) 150 flesh plus skin samples taken from 23 suspect frozen chicken and ducks carrying heavy contamination.

The immunofluorescence salmonella test: 25 or 50gm. samples were cut into chunks and dropped into 100ml. single strength selenite F broth (Leifson, 1936). The enrichments were incubated for 18-24 hours in a 43°C waterbath, then a 10ml. quantity of each enrichment was centrifuged using a 16 place swing-out head centrifuge. Fixed smears were prepared from the sediments, as described in detail elsewhere (Georgala & Boothroyd, 1964), and stained by the indirect or direct staining technique.

For indirect staining the smears were covered with suitable agglutinating antisera for 20 min., washed, and then covered for 20 min. with rhodamine or fluorescein conjugated goat anti-rabbit antiserum. The smears were again washed, and then examined with a Reichert fluorescence microscope. In the direct technique the smears were simply covered for 20 min. with fluorescein conjugated polyvalent salmonella antisera (Georgala & Boothroyd, 1965 b), and then washed, mounted, and examined with the fluorescence microscope.

Assessment of stained smears: Smears were judged positive when they showed one or more salmonella-like cells per field at a standard magnification (a x 63 dry objective and x 8 oculars were used in these tests). Early experience had shown that a few fluorescent cells spread over a whole smear did not indicate the presence of salmonellae in the enrichment.

Cultural detection of salmonellae: In general the presence of salmonellae in the 43°C selenite enrichments of samples was confirmed by techniques similar to those described by Georgala & Boothroyd (1965 a). Brilliant green agar (Difco) was the main plating medium, although desoxycholate citrate agar (Oxoid) was sometimes used in addition. Suspect salmonellae were identified by a selection of the following procedures:-

Agglutination with polyvalent O and H salmonella antisera,
positive lysine decarboxylase test (Møller, 1955),
negative urease test (Christensen, 1946),
negative β galactosidase test (Le Minor & Ben Hamida, 1962),
negative KCN test (Møller, 1954),
correct reactions in SIM medium (Difco).

Purified salmonella isolates were then typed by slide agglutination with single factor O and H antisera.

Clostridium botulinum fluorescent antisera: Antisera against Clostridium botulinum Types A, B and E were prepared as described elsewhere (Boothroyd & Georgala, 1964). Cross staining was encountered, so the antisera were absorbed to eliminate unwanted antibodies. The absorbed antisera were tested for specificity and were then used in the detection of Cl. botulinum in cultures made from inoculated meats.

RESULTS

Salmonella tests: The results from the three groups of samples are summarized in Tables 1, 2 and 3. With the imported horsemeat and veal samples (Table 1) the immunofluorescence technique detected 87% of the salmonella containing samples, but also recorded 12% false positive results (i.e. fluorescence positive, cultural negative) in the total 128 samples examined.

Table 1. Immunofluorescence and cultural tests on 128 imported horsemeat and veal samples (indirect staining)

Fluorescence technique	Cultural technique	No. of samples
positive	positive	53
negative	positive	8
positive	negative	16
negative	negative	51

Table 2. Immunofluorescence and cultural tests on 706 routine meat* samples (direct staining)

Fluorescence technique	Cultural technique	No. of samples	
positive	positive	14	
negative	positive	1	
positive	negative	49	
negative	negative	642	*mostly home produced beef

Table 3. Immunofluorescence and cultural tests on 150 samples taken from 23 suspect chicken and duck (direct staining)

Fluorescence technique	Cultural technique	No. of samples
positive	positive	62
negative	positive	2
positive	negative	0
negative	negative	86

The immunofluorescence technique detected 14 of the 15 positive samples among the samples of home produced meat (Table 2), but also included 6.9% false positive results in the total of 706 samples examined.

No false positive results were recorded with the samples from suspect poultry (Table 3), and the immunofluorescence technique detected 62 of the 64 positive samples.

The salmonella types isolated in these investigations were - Salm. anatum, S. indiana, S. meleagridis, S. minnesota, S. newport, S. oranienburg, S. orion, S. paratyphi B, and S. typhimurium.

Cl. botulinum fluorescent antisera: These fluorescent antisera showed cross-reactions between Cl. botulinum types A and B. Similar cross-reactions had also been reported by Walker and Batty (1963). A single strain of Cl. sporogenes was also stained by the fluorescent botulinum antisera. Absorption of the sera removed these unwanted reactions and the antisera are now far more specific.

The types A and B fluorescent antiserum have been used successfully for detecting botulinum growth in anaerobic cultures of experimentally inoculated meat products, while our type E antiserum has been of assistance in detecting Cl. botulinum Type E in Baltic fish (Dr. Johannsen - personal communication).

DISCUSSION

The performance of the 18-24 hour immunofluorescence salmonella detection technique suggests that it might have applications in the routine screening of meats and poultry for salmonellae. The absorbed fluorescent botulinum antisera described here have also proved useful, particularly for research into the survival and growth of Cl. botulinum in inoculated meat products.

The serological cross-reactions encountered with the immuno-fluorescence salmonella test were fewer than expected. Furthermore, these false positive results were reduced considerably when the direct staining technique was used instead of the indirect technique. In theory the direct technique should be no more specific, but in the tests recorded in Table 2 and 3 it gave "cleaner" preparations, with less staining of back-ground debris. Direct staining is easier and quicker than indirect staining, but suffers from a serious disadvantage in requiring high quality fluorescent polyvalent salmonella antisera. The salmonella antisera prepared for these tests could only detect those commoner salmonellae belonging to the larger Kauffman White groups. These are the salmonellae most frequently encountered in food poisoning incidents and in various foods in many countries (Hobbs, 1962; van Oye, 1964). If organisms from other salmonella groups were expected, further polyvalent antisera could be prepared against the group antigens involved.

The rapid salmonella test described here could best be used as a presumptive test. Enrichments producing positive fluorescent smears after 18 hours could be streaked on to conventional diagnostic agars on the same day. A confirmed answer would then be available the following day. In this way a large proportion of the salmonella negative samples (e.g. 642 of the 691 negative samples shown in Table 2) could be cleared for production within 18-24 hours with a high degree of certainty, and normally need not be tested further by conventional means. The total time and labour for salmonella testing would be reduced, allowing a higher sampling rate. In addition, delays in clearing meat for production would be kept to a minimum.

Like other salmonella detection systems, the immunofluorescence technique is a compromise, and can miss a small number of contaminated samples, particularly those lightly contaminated samples requiring prolonged enrichment. However, with an adequate sampling rate the technique should be suitable for detecting the appearance of serious contamination in material generally free of salmonellae. It could therefore be valuable in laboratories engaged in routine checking of raw materials for use in food production.

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SUMMARY

A rapid 18-24 hour technique has been developed for detecting salmonella contamination in carcass meats, boneless meats, poultry, and other meat products. The technique is based on 43°C selenite enrichment of samples, followed by immunofluorescent detection of salmonella cells in the enrichments. A high proportion of salmonella containing samples are detected by the rapid technique but some false positive results are also encountered. The rapid test therefore lacks precision, but it could be used as a "presumptive" salmonella test to prevent contaminated materials from reaching the processing lines of food factories.

Immunofluorescence has also been applied to the detection and identification of Clostridium botulinum Types A, B and E. Specific fluorescent cell antisera have been prepared, and these have been of assistance in detecting Cl. botulinum in foods.

ZUSAMMENFASSUNG

Die Verfasser haben ein Schnellverfahren (18-24 Stunden) zum Nachweis von Salmonellen im Fleisch geschlachteter Tiere und in knochenfreiem Fleisch, Geflügelfleisch und anderen Fleischprodukten entwickelt. Das Verfahren beruht auf der Anreicherung der Proben in Selenitbrühe und darauffolgendem Nachweis von Salmonella-Zellen in den angereicherten Proben mittels der Immunofluoreszenz-Technik. Das Schnellverfahren erfasst einen hohen Prozentsatz aller Salmonellen-haltigen Proben, liefert aber auch mitunter positive Fehlresultate bei Proben, die keine Salmonellen enthalten. Das Verfahren ist also wenig präzis, könnte aber als "präsumtiver" Test in Lebensmittel-betrieben dazu dienen, salmonellenhaltiges Material vor der Verarbeitung auszuschliessen.

Die Immunofluoreszenz-Technik ist auch zum Nachweis und zur Identifizierung von Clostridium botulinum Typ A, B und E angewandt worden. Die Verfasser haben spezifische Antiseren gegen fluoreszierenden Zellen dargestellt, die zum Nachweis von Cl. botulinum in Lebensmitteln herangezogen wurden.