QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES
DIVISION OF ANIMAL INDUSTRY BULLETIN No. 147

CONTAMINATION OF BONELESS BEEF WITH ORGANISMS POTENTIALLY PATHOGENIC TO MAN

By LARISSA FOMIN, B.Sc., and G. C. SIMMONS, B.Sc.

SUMMARY

Ninety-two beef samples from a North Queensland abattoir were examined for the presence of a number of potential human pathogens using culture and guinea pig inoculation. Salmonellas isolated from 16 samples included *S. bredeney, S. chester, S. anatum, S. adelaide* and *S. give*. Coagulase positive staphylococci were isolated from 80 samples. Phage patterns of some of the typable strains suggested human contamination. No *E. coli* strains belonging to types 026/B6, 055/B5, 0111/B4, 0119/B14, 0125/B15, 0126/B16, 0127/B8 and 0126/B12 were isolated. *Brucella abortus, Mycobacterium bovis, Pseudomonas pseudomallei,* Leptospira, Q-fever and psittacosis organisms were not detected.

I. INTRODUCTION

In recent years increased attention has been given to the possibility of meat being contaminated with micro-organisms potentially pathogenic to human beings.

While meat inspection procedures can minimize the possibility of meat originating from animals with overt infections, the possibility exists that pathogenic micro-organisms may be carried into the meatworks by apparently healthy animals, and subsequently transferred to the meat during the preparation of the carcasses. In addition, pathogens of human origin may contribute to the contamination of the meat.

Using the facilities available, a wide range of pathogens was sought from boneless beef prepared in a North Queensland abattoir. Except for entero-pathogenic *Escherichia coli,* all the micro-organisms were known to be present in the regions from which the cattle were obtained and cattle carrying the micro-organisms could enter the meatworks.

Although salmonellas were sought primarily, the methods used included those that may indicate the presence of other human pathogens such as *E. coli* (selected serotypes), *Staphylococcus aureus, Pseudomonas pseudomallei, Mycobacterium bovis, Brucella abortus, Leptospira pomona, L. tarassovi* and *L. hardjo* and the causal organisms of Q-fever and psittacosis.

II. MATERIALS AND METHODS

(a) Meat

The meat came from cattle subject to routine ante-mortem and post-mortem meat inspection. Cattle killed on the 23 days of sampling included 1,803 cows, 303 bulls and 8,441 bullocks. On one day bullocks only were killed, but on the other 22 days cows and bulls were also included. The cattle were killed and the carcasses placed in a chiller room maintained at a temperature of approximately 0°C. Approximately one hour would elapse from the time that the skin was removed to the time that the carcass was placed in the chiller. During this period the surfaces of the carcass would be exposed to contamination from the atmosphere in the meatworks and from handling procedures. Multiplication of the contaminants could occur. The carcasses were boned out the following day in a room at 10°C and would again be exposed to contamination during the boning and packing procedures.

The 92 samples examined were all ox chuck. Four samples of meat were sent to the laboratory on one day each week for 23 weeks. Each sample weighing 100 g consisted of six pieces of meat taken at random from a carton just prior to closing the carton for freezing. Cartons were selected at random from the day's production. The samples were placed in a double layer of plastic bags and transported on ice to the laboratory within 24 hr of sampling. The samples were minced using an electrically driven mincer (Sunbeam Mixmaster MX24C1 with mincing unit MA1C1, Sunbeam Corporation Ltd., Australia).

An emulsion of the meat was prepared by weighing 10 g of minced meat into a 6 oz oval emulsion bottle, adding 100 ml nutrient broth and blending for 3 min by means of a Silverson Blender.

(b) Detection of Pathogens

Cultural and animal inoculation methods were used as follows:

Salmonella.—25 g of minced meat were weighed into an 8 oz screw-cap jar containing 100 ml mannitol selenite broth (Oxoid) and 6 ml 10% (v/v) tergitol. A further 25 g were weighed into another jar containing 100 ml tetrathionate broth (Difco) containing 1 ml of 0·5% (w/v) brilliant green and 6 ml 10% (v/v) tergitol. The broths were incubated at 37°C for 18-24 hr. After incubation the broths were shaken and a loopful (5 mm/diam.) was plated onto Difco brilliant green agar (BGA) containing 0·08 mg sulphadiazine per ml and another loopful onto S.S. agar (Difco). These were then incubated at 37°C for 18-24 hr. A maximum of three suspicious colonies from each plate was inoculated into Kohns I and II media (Oxoid). Strains which gave salmonella reactions in the Kohns media were tested for agglutination with salmonella antisera (poly-O, poly-H, groups B, C_1, C_2, D, E and *S. adelaide*). Strains failing to agglutinate with these antisera were tested with the Moeller's lysine decarboxylase test (Difco) and the ONPG test (Cowan and Steel 1965). Strains giving negative lysine decarboxylase tests and/or positive ONPG reactions were discarded. Salmonella strains were sent for identification to the Salmonella Reference Laboratory, Adelaide.

In addition to the method described above, a tube method (Harper and Shortridge 1969) was used for isolating Salmonella: 0·1 ml each of the tetrathionate and selenite broth cultures was pipetted into the inner tube. This was incubated at 37°C and observed for up to 7 days for the advancement of growth. Growth was removed from the outer tube using a finely pulled Pasteur pipette and plated onto BGA medium. After 24 hr incubation a maximum of three suspicious colonies from each plate was picked off into Kohns I and II and tested as above.

Staphylococcus aureus.—1 ml of the meat emulsion was pipetted into a 100 ml cooked meat medium (Records and Vawter 1945), containing 7·5% (w/v) sodium chloride. After 24 hr incubation at 37°C a loopful was plated onto Baird-Parker Medium (Oxoid) containing 50 μg sulphamezathine per ml and salt cream agar (SCA) prepared as described by Willis (1960) with 6·5% (w/v) sodium chloride added but with Filde's extract omitted. After 48 hr incubation a maximum of three suspicious colonies was picked off each plate and inoculated onto nutrient agar slopes. Coagulase positive *Staph. aureus* strains were detected by means of the tube coagulase test (Cowan and Steel 1965) using rabbit plasma. These strains were then phage-typed by Dr. A. Frost, Department of Preventive Medicine, Veterinary School, University of Queensland, St. Lucia. In addition to the International Phage Types belonging to groups I, II, III and IV, the following phages were used: 13, 54, 81, 101, 102, 110, 187, 367, 373, 425, 600 and 1363.

Escherichia coli.—A loopful of the meat emulsion was plated on MacConkey agar (Oxoid) and incubated for 24 hr at 37°C. A maximum of three lactose fermenting colonies was inoculated onto urease slopes. Urease negative cultures were tested against Burroughs Wellcome polyvalent *E. coli* antiserum which included types 026/B6, 055/B5, 0111/B4, 0119/B14, 0125/B15, 0126/B16, 0127/B8 and 0126/B12.

Brucella abortus.—The medium used was based on that described by Nelson *et al.* (1966). To each 100 ml of the basal medium (4·3% (w/v) Albimi Brucella agar and 0·9% (w/v) glucose)) were added 180 units polymyxin B, 750 units bacitracin, 3 mg cycloheximide and 5 ml sterile bovine serum.

The medium was inoculated with a loopful of meat emulsion. After 5 days' incubation at 37°C in 10% carbon dioxide, a maximum of three suspicious colonies was picked off into serum nutrient broth (Oxoid), incubated in 10% carbon dioxide for 48 hr and tested for growth on blood agar aerobically and in the presence of 10% CO_2. Strains which grew in the presence of 10% CO_2, but not aerobically, were tested using a slide test for agglutination against *Br. abortus* antiserum and were examined for acid fastness by a modified Ziehl-Neelsen method which involved staining for 10 min with dilute carbol fuchsin, decolorizing with 1% (v/v) acetic acid, washing and counter-staining with 1% methylene blue for 30 sec.

In addition, each of two guinea pigs was inoculated with 1 ml of the meat emulsion, guinea pig A intramuscularly and guinea pig B intraperitoneally. Guinea pigs A and B were killed and bled after 4 and 6 weeks respectively. The spleens and inguinal lymph nodes were cultured on blood agar and brucella agar, incubated 3–5 days in 10% CO_2 and examined for Brucella. A tube agglutination test was used to detect *Br. abortus* antibodies in the serum.

Mycobacterium bovis.—To 5 ml meat emulsion, 5 ml 4% (w/v) sodium hydroxide were added and shaken for 5 min. This was centrifuged at 3000 rev/min for 30 min and the deposit neutralized with 10% (v/v) phosphoric acid using phenol red as indicator. After recentrifugation, the deposit was swabbed onto two Lowenstein-Jensen slopes containing 35 μg nalidixic acid and 50 units penicillin/ml of medium. The slopes were examined for growth and for presence of acid fast cells by the Ziehl-Neelsen stain after 2, 4, 6 and 8 weeks' incubation at 37°C.

The guinea pigs used for detection of Brucella were also examined for lesions of tuberculosis.

Other pathogens.—The blood agar plates inoculated with guinea pig spleens and lymph nodes as described under Brucella were also examined for *Pseudomonas pseudomallei.* The guinea pig serum was tested by a tube agglutination test for *Leptospira pomona, L. tarassovi* and *L. hardjo* antibodies and by a complement fixation test for PLG and Q-fever antibodies.

Guinea pigs that died before the end of the test period were autopsied and the spleen, heart, blood and bone marrow were cultured on blood agar (incubated 2 days in 10% CO_2) and MacConkey agar for aerobic bacteria.

III. RESULTS

Salmonellas were isolated from 16 of the 92 meat samples by the tube method and two of these samples were also positive by the broth-agar method. On one occasion *S. anatum* was isolated from all four meat samples submitted on the same day, with one sample yielding *S. give* also. On three occasions three out of four meat samples submitted on the same day yielded the same Salmonella serotype, namely *S. bredeney* twice and *S. chester* once. On three occasions, only one out of four samples was positive, with *S. chester* occurring twice and *S. adelaide* once.

Coagulase positive staphylococci were isolated from 80 (88%) of the meat samples examined, but 71·8% of these strains were untypable by the range of phages used. Typable strains were isolated from 45·6% of the samples. Of the typable strains, 40% belonged to phage types commonly considered to be potentially human pathogens, i.e. strains which were lysed by phages in groups I and/or III or by phage 81, which does not belong to any of the International phage groups. Of these, 12% belonged to group I, 38% to group III, 40·5% to type 81 and 9·5% of the strains were lysed by phages from more than one group. Potential human strains were isolated from 23% of the meat samples.

No brucellas were isolated from any of the meat samples. No acid fast organisms of the typical mycobacterium morphology were isolated.

Of the 220 lactose fermenting colonies picked off the MA plates, 194 were tested serologically. None agglutinated with the polyvalent *E. coli* antiserum.

Eighteen of the inoculated guinea pigs died prior to completion of the test period. *Pasteurella multocida* was isolated from two of these. *E. coli,* Acinetobacter, Klebsiella, Proteus and coliforms were isolated from the organs of the other dead guinea pigs.

Only two of the guinea pigs killed at the end of the test period showed enlarged lymph nodes or abscessed at the site of inoculation. Haemolytic *E. coli* was isolated from a local lesion in one of the animals. An enlarged inguinal lymph node in the other animal was cultured for mycobacteria but was negative. None of the serological tests done on guinea pig sera were positive.

IV. DISCUSSION

Although the microbiological aspects of keeping quality and hygiene in relation to specific organisms such as salmonellas have been the subject of many articles, published work on the contamination of meat with human pathogens such as Brucella and Q-fever organisms is much less extensive. An attempt to isolate these organisms must take into account that they are more likely to occur on the surface of the meat than in the tissue. Hence the amount of meat sampled is of secondary importance to the area of surface swabbed or emulsified. The failure to detect the presence of micro-organisms other than salmonellas and staphylococci could have been due to the limitations of the method used and quantity sampled, as a relatively high degree of contamination may have been necessary for the pathogens to be detected. It has been pointed out (World Health Organization 1971) that cases of brucellosis in man produced by the ingestion of infected meat or meat products are very few. Nevertheless, the possibility of the contamination of meat warrants some attempt to determine whether brucellas can be detected on meat produced under circumstances where it is likely to be contaminated.

The beef was examined for human phage types of staphylococci and human enteropathogenic serotypes of *E. coli* in order to obtain an indication of human contamination of the meat. No human serotypes of *E. coli* were isolated. There is little evidence of previous work of this kind. Pohl and Thomas (1967) reported their failure to isolate *E. coli* of the kind causing gastro-enteritis in man from fresh meat, but they did not list the serotypes tested.

Although a considerable amount of work has been done on methods for isolating staphylococci (Baird-Parker 1962; Jay 1963; Crisley, Peeler and Angelotti 1965) there is little published material on surveys for the presence of *Staph. aureus* in fresh meat. Jay (1961, 1962*a*, 1962*b*) found a large percentage of non-frozen market meats to contain coagulase positive staphylococci. The phage patterns of most of the typable strains isolated by Jay (1961, 1962*b*) were known to be involved in human outbreaks of food poisoning, indicating human sources of contamination. The results of the present work indicate that *Staph. aureus* contamination of meat may be partly of human origin, though the presence of phage types not known to occur in man and also of untypable strains indicates that other types of contamination also occur. Further work on isolating phages which lyse the untypable strains may give more information on the possible sources of contamination.

Interest in the contamination of meat with Salmonella has led to a large volume of literature on this topic. Increasingly sensitive methods for isolating salmonellas have been sought. In the present study the extended tube method showed a distinct advantage over the standard plating method. On the limited data available no reasons for the isolation of certain serotypes on the same day can be given.

V. ACKNOWLEDGEMENTS

The funds for this project were provided by the Australian Meat Research Committee. We wish to acknowledge the co-operation of officers of the Queensland Department of Primary Industries and officers of the Commonwealth Department of Primary Industry.

REFERENCES

BAIRD-PARKER, A. C. (1962).—*J. appl. Bact.* 25:12.

COWAN, S. T., and STEEL, K. J. (1965).—"Manual for the Identification of Medical Bacteria". (Cambridge University Press: London.)

CRISLEY, F. D., PEELER, J. T., and ANGELOTTI, R. (1965).—*Appl. Microbiol.* 13:140.

HARPER, J., and SHORTRIDGE, K. F. (1969).—*J. Hyg., Camb.* 67:181.

JAY, J. M. (1961).—*J. Fd Sci.* 26:631.

JAY, J. M. (1962*a*).—*Appl. Microbiol.* 10:247.

JAY, J. M. (1962*b*).—*Appl. Microbiol.* 10:252.

JAY, J. M. (1963).—*J. appl. Bact.* 26:69.

NELSON, C. J., ANDERSON, R. K., KIMBERLING, C. V., and PIETZ, D. E. (1966).—*Am. J. vet. Res.* 27:1515.

POHL, P., and THOMAS, J. (1967).—*Annls Med. Vet.* 111:110.

RECORDS, E., and VAWTER, L. R. (1945).—Bacillary haemoglobinuria. *Tech. Bull. Nev. agric. Exp. Stn* No. 173.

WILLIS, A. T. (1960).—*J. Path. Bact.* 80:379.

World Health Organization (1971).—*Wld Hlth Org. tech. Rep. Ser.* No. 464.

(Received for publication December 13, 1971)

G. C. Simmons is an officer of Pathology Branch, Division of Animal Industry, Queensland Department of Primary Industries, stationed at Animal Research Institute, Yeerongpilly. Miss Fomin participated in this work while an officer of the Institute.