EXHIBIT 26

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, May 1976, p. 661-663

Vol. 31, No. 5
Printed in U.S.A.

Salmonella in Horses: a Source of Contamination of Horsemeat in a Packing Plant Under Federal Inspection

GARRY D. ANDERSON AND DON R. LEE*

Department of Biology, East Texas State University, Commerce, Texas 75428

Received for publication 22 December 1975

Cecal samples from 270 slaughter horses revealed that 41 samples (15.1%) contained *Salmonella*. Of 233 horsemeat samples tested, *Salmonella* was isolated from 62 samples, or 26.6%. Only 2 of 158 human stool specimens from the plant workers revealed *Salmonella*. Predominant serotypes isolated from the horsemeat were *Salmonella enteritidis* Good and Anatum, whereas the serotypes Agona and Derby predominated the horse cecal isolates. Preliminary data indicate that the high percentage of meat contamination is surface contamination due to poor slaughtering technique.

Very little information is found in the literature concerning the incidence of *Salmonella* in horses. Prost and Riemann (12) discussed the incidence of *Salmonella* in slaughter horses and indicated that the occurrence is low. However, Meara (7) recently reviewed this topic and showed with meat imported into the United Kingdom that 42% of horsemeat samples and 50% of horse offal samples were contaminated with *Salmonella*. Horsemeat from South America imported into Holland showed 15% *Salmonella* contamination to carcass meat and 57% *Salmonella* contamination in boneless meat. Schothorst and Kampelmacher (14) have shown that in meat imported from South American countries into European countries, *Salmonellae* were isolated from 278 of 800 samples (34.7%) of frozen carcass or boneless horsemeat.

Salmonella contamination in fresh meat from horses is an international problem. People in this country do not consume a relatively large amount of this product, but there are approximately a dozen horsemeat-packing firms operating in this country. The plants are producing fresh meat, both whole carcasses and boneless, to be exported fresh and frozen into foreign countries for human consumption. The purpose of this communication is to discuss our findings during a 1-year study in one of these plants.

MATERIALS AND METHODS

Between October 1974 and August 1975, 270 horse cecal samples, 158 human stool samples, and 233 horsemeat samples were obtained as random samples from a horsemeat-packing plant near the university campus. Buffered glycerol saline transport medium (BBL) was used to transport the stool specimens. No transport medium was added to the meat samples, but the isolation procedure for meat was begun the day the samples were obtained.

The methodology of *Salmonella* isolation is well documented (1, 9), and the techniques described in the United States Department of Agriculture's *Microbiology Laboratory Guidebook* (1) were used for meat analysis. The Texas State Department of Health procedure, which was originally described by Edwards and Ewing (5a), was used for human and horse stool analysis.

Meat sampling. Meat surfaces were sampled with sterile or alcohol-sanitized knives. Typically, whole carcasses were sampled on the forequarter or foreleg. Boneless meat was most commonly taken from open boxes in the boning room. Meat samples were placed in sterile jars, capped, and transported to the laboratory. The testing procedure was begun on the same day as sampling. A 25-g sample of meat was obtained by using a sterile technique. This 25-g quantity was placed into sterile blender jars with 250 ml of sterile lactose broth and 1.5 ml of Tergitol 7 (Sigma Chemical Co.). Blending proceeded for 2 min. The pre-enrichment incubation was performed overnight at 35 C in the blender jars. Enrichment, isolation, and biochemical identification strictly followed the *Microbiology Laboratory Guidebook* (1).

RESULTS AND DISCUSSION

The value of serotype distribution in the genus *Salmonella* is a widely recognized and used epidemiological tool. During an 11-month period, extending from October 1974 through August 1975, experiments were conducted in a horsemeat-packing plant to study the distribution of *Salmonella* serotypes in the plant. This survey concerned only those isolations from horsemeat, the horse cecum, and stools of plant personnel. A total of 14 serotypes were isolated (Table 1). Two serotypes, Agona and Anatum, were encountered in all three places of isolation. The horse cecum proved to be a prominent

place for isolating *Salmonella*, with eight serotypes isolated from it. Table 2 depicts these eight serotypes with frequency of isolation and percentage of total horse cecal isolates. The serotypes Agona and Derby were the most commonly encountered serotypes, being found 17 and 10 times, respectively, in 270 horse cecal samples. Two serotypes, Montevideo and Siegburg, were isolated exclusively from the cecum; however, each of these serotypes was isolated only once.

Horsemeat showed the most contamination, with a total of 12 serotypes isolated from it. Table 3 shows the frequency of isolation of these 12 serotypes and the percentage of total meat isolations. The serotypes Good and Anatum proved to be the most commonly encountered serotypes in the meat, being found 19 and 18 times, respectively, out of 233 horsemeat samples. Six of the serotypes in horsemeat were found exclusively as meat contaminants; however, five of these were found only once.

The serotype St. Paul proved to be an interesting case. It was isolated eight times for a frequency of 12.9% in horsemeat only; it never was detected in the horse cecum. By categorizing all the serological types taken from meat into areas of isolation in the abattoir (Table 4), it can be seen that the serotype St. Paul was isolated from samples of boned meat taken from the boning room. This perhaps could suggest other sources of contamination besides horse feces.

In preliminary experiments involving 18 intact carcasses, contamination appeared only on surface samples and never in deep muscle samples. Six carcasses showed surface contamination, but no subsurface sample revealed *Salmonella*. Questions have been posed in the literature (12) concerning the possibility of a carrier animal's tissues being contaminated with *Salmonella*. Schothorst and Kampelmacher (14)

TABLE 1. *Salmonella serotypes isolated from horsemeat, horse ceca, and plant employees*

Serotype	Place of isolation		
	Horse-meat	Horse cecum	Human stool
Agona	x	x	x
Anatum	x	x	x
Derby	x	x	
Good	x	x	
Paratyphi B	x	x	
Newport	x	x	
Montevideo		x	
Siegburg		x	
St. Paul	x		
Drypool	x		
Oranienberg	x		
Alabama	x		
Heidelberg	x		
San Diego	x		

TABLE 2. *Salmonella serotypes isolated from the horse cecum*

Serotype	Isolation frequency	% of total horse cecal isolates
Agona	17	41.5
Derby	10	24.4
Anatum	5	12.2
Good	3	7.3
Paratyphi B	2	4.9
Newport	2	4.9
Montevideo	1	2.4
Siegburg	1	2.4

TABLE 3. *Salmonella serotypes isolated from horsemeat*

Serotype	Frequency	% of total meat isolation
Good	19	30.6
Anatum	18	29
St. Paul	8	12.9
Drypool	4	6.5
Newport	3	4.8
Oranienberg	3	4.8
Agona	2	3.2
Alabama	1	1.6
Derby	1	1.6
Paratyphi B	1	1.6
Heidelberg	1	1.6
San Diego	1	1.6

TABLE 4. *Salmonella serotypes encountered in horsemeat in different areas of the abattoir*

Boning room (boned meat)		Kill floor		Hanging room (carcass meat)	
Serotype	Frequency	Serotype	Frequency	Serotype	Frequency
Anatum	7	Good	7	Anatum	8
Good	5	Anatum	3	Good	7
St. Paul	5	St. Paul	2	Drypool	2
Newport	3	Drypool	1	San Diego	1
Oranienberg	2	Oranienberg	1	Heidelberg	1
Drypool	1	Agona	1	Paratyphi B	1
Derby	1			St. Paul	1
				Alabama	1
				Agona	1

have stated that external contamination is much more common than internal contamination.

A large number of serotypes were found both in the horse cecum and in horsemeat (Table 1). This supports the idea that a major source of meat contamination is horse feces. However, no explanation can be given for the large variation in the isolation frequency of certain serotypes from meat and the horse cecum. Both samplings were random, and no carcasses were sampled for both cecal and meat isolations.

The distribution of *Salmonella* in the environment has been the subject of research effort in recent times. Cherry et al. (2) have suggested the use of *Salmonellae* as an index of surface water pollution. Environmental contamination should be a common concern of all meat-processing firms. Conversely, a number of researchers (3, 6, 13) have shown the significance of packing plant runoff as a source of environmental contamination.

In a study involving an ecological survey of a freshwater lake, Cook et al. (4) reported the isolation of *Salmonella enteritidis* serotype Agona, a newly emerging serotype, from a freshwater lake that received poultry processing wastes. In 1973 the serotype Agona was the seventh most common human *Salmonella* isolate at the Center for Disease Control; consequently, the authors described it as an emerging serotype. They linked the worldwide emergence of this serotype to the need for and wide use of fish meal in animal feeds. Crumrine et al. (5) have even shown that during storage, grain insects are involved in *Salmonella montevideo* transmission in wheat.

No extensive data have been compiled on the *Salmonella* serotypes isolated from given animal species. Morse and Duncan (8), however, give an adaptation of a table from the work of W. H. Ewing describing the ecological divisions of *Salmonella*. Three groups are included: I, II, and III. Group I are serotypes more or less adapted to man, group II are animal-adapted serotypes, and group III are unadapted serotypes. It would be very valuable indeed to have data relating different serological types to different animal species. The serotype Typhimurium appears to be an important horse pathogen (8, 10, 11); however, other serotypes have been shown in horses and have produced disease (8, 11). Of the 23 serotypes reported to have been isolated from horsemeat by Schothorst and Kampelmacher (14), our investigation revealed 9 of the same serotypes, with a striking similarity in the most frequently isolated serotypes.

Concerning this research, it must be pointed out that slaughter animals, especially horses, are a special case. Slaughter horses have usually been trucked for extensive distances. Many times they are injured or unhealthy, housed poorly, fed and watered improperly, and sometimes held for long times, as much as a week, in dirty, confined pens at the slaughter plant. Carrying rate for *Salmonella* can become quite great under these circumstances. This becomes a fact with which the meat-processing firm must deal, and stress of slaughter animals, as pointed out by Meara (7), should be minimized.

ACKNOWLEDGMENTS

The technical assistance of Jo Wilson is greatly appreciated. Special thanks is extended to Gloria Pierce of the Texas State Department of Health for serotyping the isolates.

LITERATURE CITED

1. **Animal and Plant Health Inspection Service.** 1974. Microbiology laboratory guidebook. U.S. Department of Agriculture, Washington, D.C.
2. **Cherry, W. B., J. B. Hanks, B. M. Thomason, A. M. Murlin, J. W. Biddle, and J. M. Croom.** 1972. *Salmonellae* as an index of pollution of surface waters. Appl. Microbiol. 24:334-340.
3. **Claudon, D. G., D. I. Thompson, E. H. Christenson, G. W. Lawton, and E. C. Dick.** Prolonged *Salmonella* contamination of a recreational lake by runoff waters. Appl. Microbiol. 21:875-877.
4. **Cook, W. L., R. A. Champion, and D. G. Ahearn.** 1974. Isolation of *Salmonella enteritidis* serotype Agona from eutrophic regions of a freshwater lake. Appl. Microbiol. 28:723-725.
5. **Crumrine, M. H., V. D. Folty, and J. O. Harris.** 1971. Transmission of *Salmonella montevideo* in wheat by stored-product insects. Appl. Microbiol. 22:578-580.
5a. **Edwards, P. R., and W. H. Ewing.** 1972. Identification of Enterobacteriaceae, 3rd ed. Burgess Publishing Co., Minneapolis.
6. **Hrubant, G. R., R. V. Daugherty, and R. A. Rhodes.** 1972. Enterobacteria in feedlot waste and runoff. Appl. Microbiol. 24:378-383.
7. **Meara, P. J.** 1973. Review: salmonellosis in slaugher animals as a source of human food poisoning. J. S. Afr. Vet. Assoc. 44:215-233.
8. **Morse, E. V., and M. A. Duncan.** 1974. Salmonellosis—an environmental health problem. J. Am. Vet. Med. Assoc. 165:1015-1019.
9. **National Academy of Sciences.** 1971. Reference methods for the microbiological examination of foods. Food Protection Committee of National Research Council, Washington, D.C.
10. **Pierce, R. L., and C. E. Gates.** 1973. *Salmonella* serotypes encountered at the South Dakota Animal Diagnostic Laboratory. Appl. Microbiol. 25:317-318.
11. **Pocurull, D. W., S. A. Gaines, and H. D. Mercer.** 1971. Survey of infectious multiple drug resistance among *Salmonella* isolated from animals in the United States. Appl. Microbiol. 21:358-362.
12. **Prost, E., and H. Riemann.** 1967. Food-borne salmonellosis. Annu. Rev. Microbiol. 21:495-528.
13. **Rhodes, R. A., and G. R. Hrubant.** 1972. Microbiol population of feedlot waste and associated sites. Appl. Microbiol. 24:369-377.
14. **Schothorst, M. V., and E. H. Kampelmacher.** 1967. Salmonella in meat imported from South American countries. J. Hyg. 65:321-325.