"ISOLATION OF SALMONELLA FROM THE MESENTERIC LYMPH NODES AND CECAL CONTENTS OF CULL SLAUGHTER SOWS - IMPLICATIONS FOR PUBLIC HEALTH"

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ABSTRACT

This experiment was conducted over a four month period by sampling 200 slaughtered cull sows for Salmonella.

The sampling was to determine the presence of Salmonella in cull sows at a Minnesota slaughter establishment. The weight range of the sows was 300 to 400 lbs.

Two samples of mesenteric lymph nodes and cecal contents were collected from each sow. Conventional methods, using enrichment and plating into selective media followed by biochemical and serological analyses were used to isolate and identify Salmonella serotypes.

The public health implications of these findings are emphasized.

Keywords: Public Health, Salmonella, sows.

INTRODUCTION

Pig production as an alternative source of protein should be encouraged by all means. However, it is important to bring to the attention of all, particularly to those in the developing countries, what massive losses can be caused in animal (pig) husbandry by Salmonella infection, which is also a zoonotic disease.

Salmonella is an important zoonotic disease of global distribution. It is important from an economic as well as public health point of view [1,2,3,4,5], because it is responsible for a considerable amount of animal disease and economic loss. It is also of immense importance as cause of human foodborne illness [6,7].

Of the numerous Salmonella serotypes that have been isolated, only a small number of these are predominantly host-adopted [8]. These serotypes include Salmonella typhi, Salmonella paratyphi A, B and C (man). Salmonella abortus ovis (sheep), Salmonella abortus equi horses), Salmonella gallinarum, Salmonella pullorum, (poultry), Salmonella dublin (cattle) and Salmonella choleraesuis (swine).

Although the importance of pork as a vehicle of transmission to man is not often considered by many investigators, results of studies of outbreaks of human salmonellosis in the United States and Canada. (A working Paper: Agriculture Canada Economic study of Salmonella Food Poisoning and control measures in Canada, November, 1984) ranked pork as third most important animal source of human infection from 1968 to 1977, while other studies ranked pork second [2,3]. Since each food product has its risk, our choice of sows (swine) as one example of food source that could be controlled to decrease the number of human infection traced to it, is based upon numerous pieces of information.

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This study was carried out:-

(i) to determine the prevalence of Salmonella in slaughter sows, previous studies having primarily involved slaughtered market hogs (220lbs weight, 6-8 months old) and not older swine [9].

(ii) to confirm the role that swine may play in the transmission of Salmonella to human.

(iii) to illustrate by other epidemiological studies some of the unusual modes of spread to man.

MATERIALS AND METHODS

Sampling procedure and preparation

Fifty mesenteric lymph nodes and fifty cecal contents were collected from slaughtered sows on each of 4 sampling days. The mesenteric lymph nodes and cecal contents from each sow were placed in numbered, disposable plastic bags. Samples were placed in a cooler with ice and then transported to the laboratory. The cecal contents were obtained through a cecal incision using packaged sterile tongue blades removed at the moment of collecting the cecal contents. The scissors were rinsed with cold water and dipped in hot water (>180°F) for 15 seconds after each sampling.

Upon arrival at the laboratory fat and capsular tissues were removed from the mesenteric lymph nodes individually. Each mesenteric lymph node (approximately 10g) was immersed in boiling water for 15 seconds and sliced into small pieces.

Individual mesenteric lymph nodes and cecal contents (10g) were inoculated into 10ml tetrathionate brilliant green bile broth (Muller Kauffman), as well as in 10ml of selenite brilliant green and incubated at 42°C for 18-24 hrs. The hot water in which the mesenteric lymph nodes were immersed was cultured after cooling. Subcultures were made onto brilliant green agar plates by streaking and incubating for 18-24 hours at 37°C. After incubation on selective mdeia the plates were examined for suspicious Salmonella colonies which were inoculated into the primary identification media: triple sugar iron agar, and lysine iron agar by stabbing the butt and streaking the slant. These were incubated at 37°C for 18 hours with caps loosened. Representative Salmonella colonies from the primary identification media were each inoculated into a set of biochemical media in the following order. TS1, Lysine, Indole, Glucose, Motility, Malonate and Urea. The tubes were examined as a set after incubation for 18 hours at 37°C. Following preliminary identification of "O" and "H" antigens by using polyvalent sera [10,11] all cultures were submitted to the National Veterinary Services Laboratory, Ames, Iowa for verification.

RESULTS

One hundred and sixty-seven sows (83.5%) were positive for Salmonella with isolation from mesenteric lymph nodes and cecal contents (Table 1). Nine Salmonella serotypes were identified. Salmonella were isolated from 131 mesenteric lymph nodes (66%) and 60 of the cecal contents (30%) examined (Table 1). More Salmonella isolations were achieved from mesenteric lymph nodes than from cecal content (Table 2). The four most frequently identified Salmonella serotypes (S. agona, S. anatum S. derby, and S. infantis accounted for 71% (141/200) of the Salmonella-positive samples [12].

No organism was isolated from the hot water in which the individual mesenteric lymph nodes (approximately 10g) were immersed before culture in 10ml enrichment medium.

Salmonella choleraesuis was not isolated in our study. The Salmonella serotypes isolated in our study are those normally isolated from animal feeds [13, 14].

DISCUSSION

Numerous studies have revealed that a higher isolation rate of Salmonella from mesenteric lymph nodes of healthy domestic animals is achieved than from cecal contents and rectal swabs [15, 16, 17]. It has been reported in other studies that many Salmonella, producing subclinical infections in apparently healthy pigs can usually be isolated from the mesenteric lymph nodes [18, 19, 20]. Further studies have demonstrated that examination of mesenteric lymph nodes and tissues frequently reveals Salmonella of different serotypes than may be found in the faeces [21]. More Salmonella are found in cecal swabs and even more in cecal content [22, 23, 24].

The results of our study with an isolated rate of Salmonella species from the mesenteric lymph nodes (66%) and cecal contents (30%) of 167 (83.5%) out of 200 sows, indicate a high prevalence rate in sows especially cull sows suggest that the healthy cull sows may be a potential major source of transmission of Salmonella to young pigs, the creep, feed, the environment and human attendants, because previous studies confirmed that prevalence of Salmonella in new born pigs up to the age they are weaned is generally very low [25,26]. This confirmation further demonstrates that the potential for cull sows as a major source of transmission of Salmonella to young pigs,

TABLE 1

SALMONELLA ISOLATION FROM CAECAL CONTENTS (CC) AND MESENTERIC LYMPH NODES (MLN) OF SLAUGHTERED SOWS

Sample Batch No.	No. of Sows Sampled	No. of Sows Positive (%)	CC	MLN	No. of Rough Salmonella
I	50	39 (78.0)	12	28	
II	50	40 (80.0)	14	32	-
III	50	43 (86.0)	18	35	1
IV	50	45 (90.0)	16	36	-
TOTAL	200	167 (83.5)	60	131	1

MLN = Mesenteric lymph node CC = Cecal content

TABLE 2

SALMONELLA SEROTYPES ISOLATED FROM SLAUGHTERED SOWS

SERO TYPE	SERO GROUP	No. OF SOWS POSITIVE	MLN	CAECAL CONTENT	TOTAL MLN + CC
1. agona	B1	57	50	10	60
2. derby	B2	22	14	11	25
3. infantis	C1	3	1	2	3
4. litchfield	C2	12	-	12	12
5. newport	C3	1	1	-	1
6. anatum	E1	43	38	11	49
7. drypool	E2	9	7	4	11
8. java	E3	19	19	10	29
9. rough "O" iv 1,2		1	1	-	1
TOTAL		167	131	60	191

MLN = Mesenteric lymph node CC = Cecal content

the farm environment [27,28,29], the human attendants and to other pigs out of the farm during transportation for slaughter exist [30,31,32] because sows already infected on the farms but not excreting Salmonella may excrete Salmonella during and after transportation because of associated stress during loading, transportation and unloading [33].

We believe that the high isolation rate of Salmonella from our healthy cull sows 4-5 years is due to the fact that sows are kept longer on the farms for breeding. This we think the risk of exposure of acquiring of salmonellosis and the transmission to other pig is greater than other age groups of pigs on the farms.

This is true because epidemiological investigations have demonstrated that infections arrive on a "clean farm" in six main ways [29,32] by purchasing an infected animal, contaminated feed having an infected human attendant, using contaminated water or by allowing infected rats and birds [33] to contaminate the animal feed or environment. Having arrived, infection becomes established in an animal, and the Salmonella organisms are excreted. All animals coming into contact with these faeces are bound to risk contracting the disease. The risk will be increased if faeces are allowed to contaminate the feeding or watering places because greater number of Salmonella will be ingested if sows are infected, definitely they may become a major source of transmission to their offspring, either in utero or very soon after birth [25,26]. Since the sows by husbandry practices are kept longer on the farms for breeding while other categories, such as feeder pigs, boars, and market hogs are disposed off as soon as they reach the market weight, which means infected sows will definitely remain chronic excreters of Salmonella and a major source of transmission to other pigs and human attendant directly or indirectly through faecal contamination of the environment.

Furthermore a study conducted in Holland suggested that Salmonella could be traced from slaughtered animal to human consumer, to the environment, back to animal and finally back to human consumer through cross-contamination, explains how man becomes involved in the Salmonella cycle [27,34,35].

We were not able to trace any outbreak of salmonellosis to cull sows because we did not know the marketing channels of these cull sows to the slaughter house. We believe from our study that in order to confirm the significant role played by cull sows in the transmission of Salmonella directly or indirectly to both humans and animals could be through retrospective studies, by tracing the positive cull sows to their farms of origin, by sampling the remaining pigs on the various farms of origin and later sampling farms buying offspring from these positive cull sows by culturing faecal specimens for Salmonella.

Data collected from various countries indicate wide variations in contamination of swine carcasses and retail pork products with Salmonella [36,37,38]. Some of the variations are probably due to differences in post-slaughter decontamination techniques or to methodological differences [27,39,40]. Salmonella from lymph nodes and other tissue may represent past infection rather than recent infection of contamination during slaughter [29]. On the other hand Salmonella are more likely related to farm exposure than to infection during transport and holding [29]. Isolation of Salmonella from the mesenteric lymph nodes of 54% of clinically normal pigs led to the opinion that the incision of the mesenteric lymph nodes may be a source of Salmonella for pork and edible offal in the abattoir [32,36]. Results of studies reported in 1983 of pork sausage of 35 different commercial brands for the presence of Salmonella showed that no variation in prevalence had occurred since 1946 [41].

The public health importance of porcine salmonellosis need to be emphasized because the 1978 Center for Disease Control (CDS) Salmonella surveillance annual summary [6] reported seven outbreaks of foodborne salmonellosis from eating pork, producing illness in 850 individuals. In 1979 there was only one occurrence of foodborne salmonellosis directly attributed to pork but approximately 1,200 human clinical cases resulted [42] and in one outbreak, all eight members of a family who ate a ham contaminated with Salmonella infantis [43] (one of the serotypes isolated in our study) became ill within 24 hours, although some ate raw ham while others ate it cooked. Twenty-three thousand Salmonella/g were isolated from raw ham that had been refrigerated two days after the outbreaks. The 100% attacked rate and the short incubation period are consistent with high dose and we also believe that 100% attacked rate might be due to cross-contamination with cutleries and cooking utensils in the family kitchen during cooking [44].

Some of the unusual modes of spread (of salmonellosis) to man include, (I) the transfer of S. cubana through contaminated crimson red dye from Peruvian highlands to medical centres in the United States, Western Europe and Japan which were using the crimson red dye for measuring intestinal motility, an outbreak termed "the red stool" [5], (II) multistate 85 cases of enteritis caused by Salmonella muenchen in Michigan, Georgia and Alabama cases associated

with marijuana [45].

Furthermore apart from some of these unusual spread of salmonellosis explained earlier, study conducted by Leavy and other researchers [46,47,48] found a large number of antibiotic-resistant gram-negative, bile salt-resistant bacterial contaminating vegetables and fruits sold at restaurants in the Boston area, suggesting that, such foods may be the source of the spread of resistant bacteria in many areas of the world. They explained that the origin of the resistant bacteria including (Salmonella) found in this study could be the use of fertilizers of excrement of animals being fed these drugs.

The public health and economic importance of Salmonella should be considered seriously because (i) the total medical cost for 200 persons, who were at risk during an outbreak of salmonellosis, was estimated at US$20,000.00 and the economic loss from absence from work at US$28,000.00 [2]; (ii) an estimate of the annual incidence of salmonellosis in the United States of America puts it at 2,000,000 cases, and on a national scale, this disease may be responsible for loss amounting to US$1.2 billion each year. It can be concluded that salmonellosis, as a foodborne illness, may have a very significant socio-economic impact, including agriculture, the food industry in addition to that on the patient and his family [2,3,4,7].

The actual incidence of human salmonellosis is difficult to assess, since many countries do not have a system of epidemiological surveillance, and in those countries with such a system, sporadic and mild cases are generally not reported. In countries that do have a reporting system, the number of outbreaks have increased considerably in recent years [5,6,7,45,46]. This increase may be attributed to better reporting, the availability of monitoring and epidemiological surveillance system [49,50,51,52,53].

From our point of view, the quality of reporting the incidence of salmonellosis from pork products depends upon the commitment of foodborne disease surveillance, by the state or local health departments. The departments interest in foodborne disease and its investigative and laboratory capabilities are central determinants to the quality of the investigation.

The likelihood of an outbreak coming to the attention of health authorities varies considerably depending on consumer and physician awareness, interest and motivation to report the incidence. For example, large outbreaks, restaurant associated outbreaks, and outbreaks involving serious illness, hospitalization or deaths are more likely to come to the attention of health authorities than cases of mild illness following a family cookout.

Many investigators who found high isolation rates of Salmonella from pork products concluded that since historically, people cook pork well to avoid trichinosis, this same practice may be effective in reducing human Salmonella outbreaks.

Vaccination against salmonellosis has not proved of great value in animals, except in pigs where a specific live attenuated vaccine against S. choleraesuis is employed. Killed vaccines (eg. against S. typhimurium) have been used, but while they give protection against the septicemic form of the disease, they have not proved effective against alimentary infection and subsequent excretion patterns (Linton et al., 1979) [23, 54].

CONCLUSIONS

Studies of Salmonella outbreak have demonstrated that in animals and human, contaminated food and feed, of animal origin, can be the source of infection. Researchers have suggested that the impact of these agents of human foodborne Salmonella infections should be decreased by controlling the ten to twelve most frequently associated with human infection and disease rather than to decrease the number of all Salmonella that can infect humans [53].

We believe that one can reduce human infection by controlling the impact of salmonellosis in domestic animals. Thus the prevention of salmonellosis in meat has to start on the farms, by sampling pigs on the farms and during slaughter and tracing it back to the farms. Benefits accrue in two specific areas, economic and better public health, and both areas must be addressed if control and prevention programs are to assure a safe and abundant supply of food of animal origin [55].

The institution of efficient feed sterlization methods is necessary to prevent the introduction of salmonellosis to the farms. Gamma radiation, as alternative process of treatment of animal feed and bone meal, has been tried successfully to sterilize animal feeds and therefore merits consideration [53].

The education of food handlers and consumers that food of animal origin can be hazardous, is also important in the prevention of salmonellosis.

Based on our findings, salmonellosis, as a foodborne illness, may have a very significant socio-economic impact on the national economy, including agriculture, the food industry, the patient and his family [53]. It is also evident from our study, including surveil-

lance data from those of the Centres for Disease Control [1,5,6,39,40,41,42,43,44,45,46] that procine Salmonella is very prevalent, or is on the increase. This may be due to the fact that either efforts are not being made to control it or efforts made in the past years were not effective in controlling Salmonella infection in pigs.[56,57,58,59,60,61,62].

We suggest that both epidemiologist and their colleagues in private practice must rely on each other to diagnose and report cases of salmonellosis in order to develop even better methods of diagnosis.

Finally, practising veterinarian and practising physicians should request the services of epidemiologists to identify actual or potential epidemics, to ferret out the causes, if possible and to suggest remedies when indicated. The epidemiologist may also provide consultation to both practising physician and veterinarians, in difficult or unusual cases. He may do this directly on request and by voluntarily providing his colleagues in private practice with information on salmonellosis occurence in their various communities.

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