J. Hyg., Camb. (1974), **72**, 155
Printed in Great Britain

Experimental *Salmonella* infection in calves. 1. The effect of stress factors on the carrier state

By H. GRØNSTØL*
Meat Research Institute, Langford, Bristol, BS18 7DY
A. D. OSBORNE
Department of Veterinary Medicine, University of Bristol, Langford, Bristol
and S. PETHIYAGODA
Department of Bacteriology, University of Bristol, Bristol

(*Received* 1 *June* 1973)

SUMMARY

Four calves were infected with $2{\cdot}4 \times 10^8$ *S. dublin* and penned separately. Fluctuations in environmental temperature did not affect the symptoms of the disease or the duration of excretion. Faecal samples were superior to rectal swabs for detecting excretors.

After transport stress, *Salmonella* was isolated from several organs at slaughter in an excreting calf, and uninoculated control calves became cross-infected. During slaughter, carcasses became contaminated on their surfaces. From one contaminated carcass *Salmonella* was recovered after chilled storage at 0° C. for 1 week and also after freezing at −20° C. for 1 month.

INTRODUCTION

Salmonellosis is one of the most important types of food poisoning. In 1968 it accounted for 73% of all reported incidents in England and Wales (Vernon, 1970). Being a zoonosis, the main vehicle seems to be meat and meat products. The organisms have been found in all species of animals used for meat production, but veal has been incriminated in the most serious outbreaks of salmonellosis in this country (Anderson, 1968). Calves suffering from a clinical *Salmonella* infection may give rise to outbreaks of food poisoning if they are slaughtered and the meat used for consumption. However, healthy, latently infected animals give more cause for concern, because they are extremely difficult to detect. Nottingham & Urselmann (1961) found *Salmonella* after slaughter in 13% of 1100 apparently healthy calves, while Edel, Guinée & Kampelmacher (1969) found 22·7% of 1000 calves examined at slaughter positive for *Salmonella*. On the farm, Rankin, Taylor & Burrows (1969) found the incidence of *Salmonella* excretion to be approxi-

* Present address: Statens Veterinaere Forsoksgard for Smafe, Hoyland, Sandnes, Norway.

mately 1 %, and there is evidence that there is a build-up of infection in calves during transport and time in holding pens. Anderson, Galbraith & Taylor (1961) found that 0·5 % of the calves in the market were infected with *Salmonella* and calves kept at dealers' premises for only a few hours before slaughter had an infection rate of 0·6 %. However, calves kept in lairage for 2–5 days before slaughter showed an infection rate of 35·6 % at slaughter.

Stress factors appear to increase the susceptibility of calves to salmonella infections (Buxton, 1960; Gibson, 1965). Therefore, a study has been made of some of the factors which in practice may contribute to this increase, such as temperature fluctuations and transport stress. Additionally, the effect of slaughter operations on cross-contamination of carcasses has been investigated.

Most published work is based upon infections with *S. typhimurium*. Because calfhood infections with *S. dublin* closely resemble those with *S. typhimurium* (Gibson, 1961), and *S. dublin* is the most important serotype in cattle (Sojka & Field, 1970), this serotype was selected for the experimental infection of young calves.

MATERIALS AND METHODS

Animals

Four Friesian calves (B47, B49, 440 and 719), 2 weeks old, were kept in pens. Before the experiment started, faecal samples were examined daily for 1 week to exclude the possibility of a natural infection. Towards the end of the experiment, which lasted for 6 weeks, 2 new Friesian calves, 2 weeks old, were introduced to study the effect of cross-contamination.

Housing

The calves were kept in separate pens throughout the experiment. Two of the calves (B47 and 719) were maintained at a temperature of 14–16° C. The other two were stressed by reducing the daytime temperature to between $-2°$ and $+5°$ C. by opening the doors and turning off the heating. During the night, the temperature was maintained at 14–16° C.

S. dublin

The four calves were inoculated orally with $2{\cdot}4 \times 10^8$ viable cells of *S. dublin*. The strain (06/8 B_2), kindly supplied by Dr A. H. Linton, University of Bristol, had been isolated from an outbreak of salmonellosis in calves and was resistant to neomycin, tetracycline and ampicillin.

Bacteriological examinations

Ante-mortem samples. Daily, 1 g. of faeces taken from a sample removed from the rectum of each calf, using a polythene sleeve, was suspended in water to give a 10 % solution, and tenfold dilutions were made. Counts of *S. dublin* were made on bismuth sulphite agar (WB) and brilliant green agar (BG) by spreading duplicate 0·02 ml. drops from 20 SW stainless steel cannulae (Astell Ltd, Brownhill Road, Catford, London S.W. 6) on the surface of the plating media and counting

characteristic colonies after incubation for 48 hr. at 37° C. At the same time, enrichment cultures of 1 g. of faeces were made in selenite broth (SB) (Stokes & Osborne's modification, 1955) at 37° C. Plating from the enrichment medium was made on WB and BG after 24 and 48 hr., and the plates were incubated at 37° C. for 48 hr. After 48 hr. typical colonies were identified serologically and examined for antibiotic resistance. During the first period of the experiment rectal swabs were also taken but were discontinued because they were found to give far less consistent results than faecal samples.

Post-mortem samples. After captive bolt stunning, calves were killed by exsanguination and skinned and dressed following normal slaughterhouse practice. Samples of muscles, organs and viscera were taken with aseptic precautions. All but intestinal samples were placed in water at 100° C. for 6 sec. (Kampelmacher, Guinée & Janssen, 1964). The samples were then cut in small pieces with sterile scissors and placed in SB, approximately 10 g./100 ml. enrichment broth.

Before spraying, the surfaces of the carcasses were sampled using cotton-wool swabs, *ca.* 15 × 10 cm., moistened in peptone water. One swab was used on the outside and one on the inside of each carcass. Sterile plastic gloves were used during the handling of the swabs which, after use, were kept inside the gloves until they were put into enrichment broth (Kitchell, Ingram & Hudson, 1973).

Serology

Agglutination tests were made using antigens prepared from two strains of *S. dublin*, the vaccine strain (Smith, 1965) and a field strain.

Preparation of 'O' antigen. 0·1 ml. quantities of an overnight culture at 37° C. were spread on several nutrient agar plates, incubated at 37° C. and the culture harvested using approximately 5 ml. of saline per plate. After adding 10 times the amount of absolute alcohol, the pooled suspension was heated in a water bath at 53 ± 1° C. for 2 hr., centrifuged at 1000 rev./min. for ½ hr. and the deposit re-suspended in saline with a trace of merthiolate as a preservative. The antigen suspension was standardized using a nephelometer and a standard 'O' suspension (Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS).

Preparation of 'H' antigen. 1 ml. of an overnight nutrient broth culture was added to 100 ml. of nutrient broth in a 250 ml. flask and incubated at 37° C. in an orbital incubator. After overnight incubation, formalin was added to give a final concentration of 2 %. After standing overnight, the suspension was centrifuged (1000 rev./min. for ½ hr.) and the deposit was re-suspended in 1 % formalin in saline. The antigen was standardized using a nephelometer and a standard 'H' suspension (Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS).

RESULTS

Before the experiment started, sera from the four calves were negative for 'H' and 'O' agglutinins, and none of the animals were found to harbour *Salmonella*. Within 24 hr. of inoculation, all the calves showed symptoms of salmonellosis, with temperatures rising to 104–106° F. on the fourth day. High numbers of

Table 1. *Excretion of* S. dublin *in faeces of calves after oral infection with a single dose of* $2{\cdot}4 \times 10^8$ *cells*

(Counts are numbers per g. of faeces.)

No. of days after inoculation	Calves kept under fluctuating temperature conditions		Calves kept at constant temperature	
	B49	440	719	B47
2	$1{\cdot}6 \times 10^6$	$1{\cdot}7 \times 10^6$	$1{\cdot}6 \times 10^6$	$7{\cdot}2 \times 10^4$
3	$1{\cdot}0 \times 10^4$	$6{\cdot}0 \times 10^3$	$3{\cdot}2 \times 10^4$	$1{\cdot}0 \times 10^4$
4	$8{\cdot}0 \times 10^5$	$1{\cdot}8 \times 10^4$	$9{\cdot}6 \times 10^4$	$3{\cdot}8 \times 10^5$
5	$4{\cdot}0 \times 10^4$	$8{\cdot}0 \times 10^3$	$4{\cdot}0 \times 10^4$	$6{\cdot}0 \times 10^6$
6	$1{\cdot}0 \times 10^6$	$< 10^3$	$8{\cdot}0 \times 10^3$	$7{\cdot}6 \times 10^5$
7	$< 10^3$	$< 10^3$	$< 10^3$	$< 10^3$
10	$< 10^3$	$< 10^3$	$< 10^3$	$< 10^3$
14	$< 10^3$	—	$< 10^3$	$< 10^3$
21	$< 10^3$	—	$< 10^3$	—
28	$< 10^3$	—	—	—
35	$< 10^3$	—	—	—
42	$< 10^3$	—	—	—
49	—	—	—	—

salmonellas were excreted for 5–6 days (Table 1) and low numbers ($< 10^3$/g. faeces), which could be detected only after enrichment, for 10–42 days at which time the animals were slaughtered. The symptoms were no more severe in the stressed calves than in the unstressed.

Table 2 lists the 'O' and 'H' agglutinin titres in the sera of the calves and shows that the calf excreting the organisms throughout the experiment (B49) also had the highest titres. 'H' titres seem a more reliable means of assessing infection than 'O' titres.

When faecal samples from three of the four inoculated calves were negative for *S. dublin* (42 days), the animals were transported for 7 hr. in a trailer together with two uninfected control calves, but separated from them by a double partition. The following day all six calves were slaughtered. Faecal samples were taken 2 hr. before slaughter, and *S. dublin* was demonstrated in the excretor (B49) and the two control calves. *S. dublin* was isolated from either the inner or outer surface or both of five of the six carcasses; the exception was an inoculated animal (719) (Table 3). The persistent excretor (B49) was positive for *S. dublin* from several sites, but the other inoculated calves were negative except for the surfaces of two and a liver sample from another. The organisms were demonstrated in abomasal contents and on the surface of the carcasses of the two control calves.

After slaughter one half of each carcass was hung in a chill room, where they were cooled to 0° C. at a relative humidity of 91% and stored for one week. During storage they lost 6·6–12% of their weight and the surfaces became very dry. Swabs taken after 1 week showed no *Salmonella* except on B49. This carcass and one other were frozen to −20° C. and tested again after 1 month. B49 was still positive, the organisms being isolated from swabs both from the outside and the inside of the carcass, while the other was negative.

Table 2. *'O' and 'H' agglutinin titres in sera of calves during the experiment*

No. of days after inoculation	B49				440				719				B47			
	MH	AH	MO	AO	MH	AH	MO	AO	MH	AH	MO	AO	MH	AH	MO	AO
0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	1/40	—	1/40	—	—	—	—	—	—	—	—	—	1/160	1/40	—	—
15	—	—	1/40	1/40	—	—	1/40	—	—	—	—	—	1/80	1/80	—	—
20	1/320	1/320	1/80	1/160	1/40	1/40	—	1/80	1/40	1/40	—	1/80	1/320	1/320	—	1/320
28	1/320	1/320	1/160	1/80	1/80	1/40	—	—	1/40	1/40	—	—	1/160	1/320	—	—
35	1/640	1/640	1/40	1/20	1/160	1/160	—	1/40	—	1/20	—	—	1/160	1/160	—	—

MH = 'H' – antigen-suspension from 'Mellovax' Vaccine strain.
MO = 'O' – antigen-suspension from 'Mellovax' Vaccine strain.
AH = 'H' – antigen-suspension from field strain.
AO = 'O' – antigen-suspension from field strain.

Table 3. *Isolations of* S. dublin *from post-mortem samples*

	Inoculated calves				Control calves	
Organ (samples)	B49	440	719	B47	1217	1218
Swab from outside of carcass	+	−	−	−	−	+
Swab from inside of carcass	+	+	−	+	+	+
Lung	+	−	−	−	−	−
Liver	+	+	−	−	−	−
Spleen	+	−	−	−	−	−
Gall bladder	+	−	−	−	−	−
Small intestine	−	−	−	−	−	−
Colon	+	−	−	−	−	−
Kidney	−	−	−	−	−	−
Mandibular lymph nodes	−	−	−	−	−	−
Mesenteric lymph nodes	−	−	−	−	−	−
Prescapular lymph nodes	−	−	−	−	−	−
Crural lymph nodes	−	−	−	−	−	−
Muscle from forelimb	−	−	−	−	−	−
Muscle from hindlimb	−	−	−	−	−	−
Abomasal contents	−	−	−	−	+	+

DISCUSSION

All four inoculated calves showed symptoms of salmonellosis for 1 week, with fever, diarrhoea and dullness, but no loss of appetite. Fluctuations in environmental temperature did not seem to affect them but this could be explained by the fact that otherwise conditions of management were excellent, and the calves were penned singly.

Three of the calves ceased to excrete *Salmonella* 2–4 weeks after infection, but one continued to excrete the organisms until slaughtered at 6 weeks without showing symptoms of disease. After transporting this animal, *S. dublin* was recovered from lungs, liver and spleen at slaughter. The presence of the organism in lungs and spleen suggests that the infection could have been re-activated by the stress of transport typical of that to which very young calves are commonly subjected when moved from one part of the country to another.

The other three experimentally infected calves showed no symptoms of re-infection after stress and bacteriological examination of their organs and viscera for *Salmonella* gave negative results except for a single liver sample (calf 440). As one calf in the group was excreting *Salmonella*, they would all have been subjected to cross-infection, but having already passed through a stage of infection, they were apparently resistant to re-infection. Faecal samples positive for *Salmonella* obtained the next day from the two uninoculated control calves indicated that cross-contamination had occurred, despite the control calves being separated from the inoculated calves by a double partition. The fact that at post-mortem examination organisms were only detected in abomasal contents indicated that invasion had not, however, taken place. It is difficult to explain how cross-infection occurred. Had it been by contaminated droplets one would have expected initial lung infection with generalization and there was no evidence

of this. One can only assume that some faecal splashing had occurred, giving opportunity for the control calves to lick infected material.

During slaughter all the calves except one (719) became contaminated on the surface of the carcasses although handled under hygienic conditions. This demonstrated that when one calf is infected at slaughter, several carcasses may become contaminated on their surfaces, and confirms the findings of Sandbu (1960) who examined calves slaughtered a few weeks after salmonellosis due to *S. typhimurium*. Although none were found to be excreting *S. typhimurium* at slaughter, the organism was isolated from several organs and the viscera and also from the surface of the carcasses. Once there, the survival and growth of *Salmonella* will be decided by the conditions under which the meat is handled and stored.

The survival and growth of salmonellas on carcass meat will also depend upon how many are present initially on the surface. Theile (1970) found that less than 50 *S. dublin*/cm.^{2} would not survive cooling at 4° C. for more than 5 days. Greater numbers would survive up to 23 days, but they would not grow at that temperature. Four of our calves had probably a light contamination only, and no salmonellas were recovered after 7 days at 0° C. However, from calf B49 which was heavily infected, salmonellas were readily recovered after 7 days at 0° C. and also after a further month at −20° C. The rate at which *Salmonella* as superficial contaminants died during prolonged chilled or frozen storage has yet to be determined.

We are indebted to Dr A. G. Kitchell and Dr T. A. Roberts for helpful criticism in the preparation of this paper.

REFERENCES

ANDERSON, E. S. (1968). Drug resistance in *Salmonella typhimurium* and its implications. *British Medical Journal* iii, 333.

ANDERSON, E. S., GALBRAITH, N. S. & TAYLOR, C. E. D. (1961). An outbreak of human infection due to *Salmonella typhimurium* phage-type 20a associated with infection in calves. *Lancet* i, 854.

BUXTON, J. C. (1960). Salmonellosis in calves. *Veterinary Record* **72**, 640.

EDEL, W., GUINÉE, P. A. M. & KAMPELMACHER, E. H. (1969). Studies about the occurrence of salmonellae in calves after slaughter. *Tijdschrift voor Diergeneeskunde* **94**, 1170.

GIBSON, E. A. (1961). Salmonellosis in calves. *Veterinary Record* **73**, 1284.

GIBSON, E. A. (1965). *Salmonella* infection in cattle. *Journal of Dairy Research* **32**, 97.

KAMPELMACHER, E. H., GUINÉE, P. A. M. & JANSSEN, L. M. (1964). Studies on the use of surface decontamination of *Salmonella*-contaminated mesenteric lymph nodes by boiling water. *Tijdschrift voor Diergeneeskunde* **89**, 504.

KITCHELL, A. G., INGRAM, G. C. & HUDSON, W. R. (1973). Microbiological sampling in abbattoirs. In *Society for applied Bacteriology Technical Series* **7**. *Sampling – Microbiological Monitoring of the Environment* (ed. R. G. Board and D. W. Lovelock). London: Academic Press.

NOTTINGHAM, P. M. & URSELMANN, A. J. (1961). *Salmonella* infection in calves and other animals. *New Zealand Journal of Agricultural Research* **4**, 449.

RANKIN, J. D., TAYLOR, R. J. & BURROWS, M. (1969). *Salmonella* infection in young calves. *Veterinary Record* **87**, 582.

SANDBU, P. (1960). Den hygieniske betydning av latente salmonellainfeksjoner hos slaktedyr. *Nordisk Veterinarmedicin* **12**, 29.

SMITH, H. W. (1965). The immunization of mice, calves and pigs against *Salmonella dublin* and *Salmonella cholera-suis* infections. *Journal of Hygiene* **63**, 117.

SOJKA, W. J. & FIELD, H. I. (1970). Salmonellosis in England and Wales 1958–1967. *Veterinary Bulletin* **40**, 515.

STOKES, J. L. & OSBORNE, W. W. (1955). A selenite brilliant green medium for the isolation of *Salmonella*. *Applied Microbiology* **3**, 217.

THEILE, H. (1970). Tanasitat von Salmonellen bein Kuhlhaus-temperaturen. Veterinary Dissertation, Free University of Berlin.

VERNON, E. (1970). Food poisoning and salmonella infection in England and Wales, 1968. *Public Health, London* **84**, 238.