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THE SURVIVAL OF SALMONELLA ENTERITIDIS IN ANIMAL FEED AND DEEP LITTER

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The survival of Salmonella enteritidis in fish meal, maize, mixtures for laying hens and in deep litter was studied for four months. S. enteritidis survived best in deep litter and worst in fish meal and feed. The recovered bacterial strains from the fish meat and poultry meat were compared. Our experiment showed that the meal strain had survived in all material for three months. The growth of S. enteritidis animal strain was inhibited in maize and feed after the two month storage.

Key words: Salmonella, survival, feed, deep litter

INTRODUCTION

The control of Salmonella infection is important in livestock production, in order prevent salmonelosis in humans. Te detection of Salmonella is very significant for its spreading and elimination. Its presence in animals, feeds and environment may pose a potential health hazard to the human population.

S.enteritidis has frequently been recognized as a causative agent in animals and humans. Particulary S. enteritidis is an important means of spreading in poultry industry. In many cases infected animals do not develop clinical signs. Bacterial entry and colonization the alimentary tract, survive and multiply in tissues, producing toxins and regulating of the expression of virulence determinants in the environment. The vertical transmission, environmental contamination and hatchery cross contamination are a major source of S. enteritidis infection.

There are many other sources or potential sources of salmonellosis and one in particular is the hatchery environment. Litter, debris and down particles can serve as a reservoir of bacteria (Magwood 1967). The scientific literature indicates that, generally, the number of salmonella found in feed and feed ingredients ranges from 1 to 40 organisms per 100 g (Stott et. al., 1975 and Patterson 1971). The contamination rate of 67% for animal protein products was reported by Shrimpton (1989).

Feed is an important source of salmonelosis in poultry reported Williams (1981), Frank Jones and Raleigh (1990).

Futhermore, recent data have shown that salmonella isolated in broilers probably came from the breeder- multiplier house environment (Cox et al., 1991). Feed has historically been incriminated as a source for introduction of salmonella to poultry. Reports by the Ministry of Agriculture in Great Britain, in recent years, have shown that 5% of all animal protein is contaminated with Salmonella serotypes Meclroy 1996. The same investigations have assessed that feed is a major source of Salmonella infection in poultry flocks.

The environment may also act as a source of infection, but it should be kept in mind that although if salmonella can survive for long periods in the environment, no significant multiplication usually occurs (Richard et al., 1996).

The transmission of S. enteritidis to people by contaminated eggs endangers public health and presents economic problem in the United States, the United Kingdom and Western Europe, Bogel (1994).

The incidence of human salmonelosis has markedly incresed over the last 10 years Salmonella contaminated food, pariculary, chicken meat and eggs.

Feeds, meal fish as well as the environment are usually sources of Salmonella infection for animals, and represent suitable media for bacteria growth. The purpose of this study was to examine the survival rate of S. enteritidis in feeds and deep litter in the course four months.

MATERIAL AND METHODS

Bacterial strains. Two strains of S. enteritidis were studied: one strain isolated from fish meal (SFM) and another strain from pathology material of chicken (SPC). Bacterial strains had been prepared by series of laboratory passages.

Bacterial media. Three media were used: differential Wilson Blauer agar, selective MacConcey agar and nutrition broth media (Torlak, Belgrade) to prepare the inocula. 0,1% Pepton water was used as a diluent for an enumeration of the surviving bacteria.

Inoculated material. Commercial mixed feed for layng hens, (Yugoslavia) contained no added antimicrobial agents. Fish meal was of important. Deep litter was sampled from a field after wheat harvest. Maize was home grown.

Thirty grams of each material were put in each flask. They were sterilized by heating at 121^0C for 15 min before inoculation.

Method of inoculation. After incubation nutrient broth media with test bacteria, were diluted by 0.1% sterile pepton water to obtein a suspension containing approximately $5x10^8$ cfu/ml. The material in the flasks was contaminated with 1 ml suspension of bacteria. So prepared material was mixed and then incubated at the room temperature for four months.

S. enteritidis was recovered every 30 days, during four months. Duplicate samples of contaminated material (1g) were test tubes transfered to tubes, containing 9 ml of 0,1% pepton water, and were made decimal dilution of 10^1 o 10^6 and 1 ml of each and subsequently dilution spread on the surface of petri dishes of MacConcey and Wilsonblair was done incubated at 37^0C 24^h to 48^h. After that the counting of colonies. Typical colonies were examined biochemicall and serologically.

RESULTS

The inoculated material allowing survival of both test strains of S. enteritidis after four months are shown in Table 1 and 2. At room teperature storaged after 120 days viable animal strain SCP could be recovered only from straw. After the same period the environmental strain SFM could be recovered from mixed feed for laying hens, as well as from deep litter.

Table 1. Survior numbers of Salmonella enteritidis environment strain in feed and deep litter when used on WB and MC agar

Inoculum material	Storage time in days							
	30		60		90		120	
	WB*	MC**	WB	MC	WB	MC	WB	MC
Fish meal	$1.15x10^5$	$1.30x10^5$	$4.0x10^5$	$25.0x10^5$	$1.15x10^5$	$1.0x10^4$	0	0
Maize	$1.8x10^6$	$3.6x10^6$	$3.5x10^5$	$23.0x10^5$	$10.0x10^3$	$10.0x10^3$	0	0
Mixed feed	$51.0x10^6$	$70.0x10^6$	$13.0x10^5$	$36.0x10^5$	$1.15x10^5$	$10.0x10^5$	$3.2x10^5$	$1.9x10^5$
Deep litter	$100.0x10^6$	50.0x106	$10.0x10^5$	$8.0x10^5$	$57.0x10^6$	$52.0x10^6$	$18.0x10^6$	$6.0x10^6$

* WB Wilson Blair agar
** MC Mac Conkey agar

Table 2. Survior numbers of Salmonella enteritidis animal strain in feed and deep litter when used on WB and MC agar

Inoculum material	Storage time in days							
	30		60		90		120	
	WB*	MC**	WB	MC	WB	MC	WB	MC
Fish meal	$1.0x10^5$	$1.0x10^5$	$5.0x10^5$	$9.0x10^5$	0	0	0	0
Maize	$30.0x10^3$	$30.0x10^3$	$6.0x10^4$	$1.0x10^4$	0	0	0	0
Mixed feed	$45.0x10^5$	$10.0x10^5$	$8.0x10^5$	$18.0x10^5$	$3.5x10^5$	$20.0x10^3$	0	0
Deep litter	$10.0x10^6$	6.0x106	$4.8x10^5$	$3.2x10^5$	$15.0x10^5$	$13.0x10^5$	$8.0x10^6$ $6.0x10^6$	

* WB Wilson Blair agar
** MC Mac Conkey agar

The results for the survival of S. enteritidis environmental strain in different material are presented in Table 1. In cases of fish meal, maize and mixed feed, there were reductions in viable cells counts during the thre month storage. In the case of the litter there were an increase in viable count of Salmonella three month after the storage. There were the same increases in viable counts in the experiment with S. enteritidis strain SCP (Table 2). Strains SPC i SMF were the most resilient in straw.

The bacterial strain SMF survived well in fish meal, mixed feed and deep litter. While the animal strain SCP survived not so well in the mixed feed thas in the litter.

S. enteritidis strain compared with the animal strain SCP survived worse than than the envronmental strain. The strain SCP was inhibited in maize and mixed feed two months after storage.

The recovered bacterial strains in two selective media are compared. The results obtained from the qualitative growth of both strains, showed an optimal growth on WB, while environment strain grew exclusively on MC agar.

In both experiments survivor strains have grown on surface, as R and S form colonies. In primary biochemicaly characteristic not found deviation. Serologicaly test of aglutination was tipicaly.

DISCUSSION

The aim of our experiment was two fold. First to assess the survival of S. enteritidis, isolated from different sources in or on materials present in the environment poultry, second to evaluate the two media for the recovery of these bacteria from it containated materials.

More investigators have found lower Salmonella isolation frequencies from feces, but Doughtery is isolation frequencies were higher in new litter (Doughtrey 1976). Other authors have shown that Salmonella is isolated but they concern less frequently from the flock reared on an old litter (Lahellec et al., 1985).

Our results show that protein ingedients and litter could be, an important source of salmonelosis in poultry. In the examined environment the strain survived better during the two months. A survey conducted in Great Britan in 1990. showed that 7% of vegetable raw materials, was contaminated by S. enteritidis and S.typhimurium. These bacteria have been found in soya, sunflower and wheat. George Mcllory (1996), Himathongkham et al. (1996) in their experiments have shown that thermal death rate.

Another potential material used for poultry bedding and both of Salmonella in poultry feed depends time, temperature and moisture contents bacterial strains survived comparatively well in this dry material. Once containated bedding will readily act as a potential source of Salmonella for poultry.

This is a matter of minor importance, however, it is particular by necessary to investigate the ecology of this species in the poultry environment. The reduction of contamination Salmonella in livestockis a major epidemiological in problem in poultry feed control.

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PREŽIVLJAVANJE SALMONELLE ENTERITIDIS U STOČNOJ HRANI I DUBOKOJ PROSTIRCI

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SADRŽAJ

U radu je ispitivano preživljavanje S. enteritidis u stočnoj hrani i dubokoj prostirci, u toku četiri meseca. Upoređivan je rast i razmnožavanje dva soja S. enteritidis, soj izolovan iz uginule kokoške SPC i soj izolovan iz ribljeg brašna SFM. Ispitivane bakterije najbolje su se održale u dubokoj prostirci, a lošije u stočnoj hrani. U eksperimentu je dokazano preživljavanje soja S. enteritidis iz ribljeg brašna u periodu od tri meseca. Salmonella životinjskog porekla nije rasla, u kukuruzu i smeši, posle dva meseca skladištenja.