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Transport Capacity of *Salmonella typhimurium* by *Ascaridia galli* Body Parts

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Abstract: ***The relationship between Salmonella tyohimurium and Ascaridia galli is a particular important issue in poultry, where both parasites are likely common. Several lines of evidence suggest that the nematode is responsible for the mechanical transmission of Salmonella. In an in vitro system, the parasite-parasite relation between Salmonella typhimurium and Ascaridia galli was studied. A total of312 adult's worms (149 males and 163 females) were usedand 105 worms were ligatured by a thread tied around the body behind the mouth and anus. We evaluated the transport capacity of Salmonella typhimurium by different body parts of A. galliby placing the nematode and the bacteria in an in vitro medium and then incubated in a Salmonellas elective media. We observed association on the surface of body wall, digestive tract and on the uterus/eggs of both ligatured and unligatured male and female Ascaridia galli worms. These worms may effectively appear as vectors of Salmonella transmission. Control of Ascaridia galli may also contribute to the control of Salmonella infections.***

Keywords: Salmonella, Ascaridia galli, poultry, transmission

1. Introduction

Many decades ago, the association of gram-negative enteric bacteria and helminth parasites has been reported (Spaldonova *et al.*, 1969; Otten & Dickerson, 1972).For approximately 60 years, there has been occasional reports on the association of *Salmonella* with helminths in different hosts.

The co-occurrence of *Salmonella* sp. and the nematode *A.galli* in chickensis especially noteworthy (Chadfield *et al.*, 2001; Eigaard *et al.*, 2006). Chadfield *et al (*2001) showed that *Salmonella* were able to attach on *A.galli* eggs. Few years later, Gamit *et al.* (2017) proposed that *A.galli* migration might lead to the mechanical transmission of pathogens such as the enteric bacterial *Salmonella.* Moreover, there are several possible means of transmission of *Salmonella* by *A.galli*. *A. galli* may carry *Salmonella* on its body (Gamit *et al*., 2017) or on *its* eggs (Chadfield *et al.*, 2001). The aim of the present study was to evaluate the transport capacity of *Salmonella* by *A.*galli and the impact on the control of *Salmonella*.

2. Material Methods

The study was carry out in the Research Unit of Biology and Applied Ecology, in the Research Unit of Physiology and Animal Health in the University of Dschang, Cameroon.

Experimental animals: Local chickens used in the study were bought from the Dschang market. "Arbor acres" chickens were obtained from commercial poultry and the sample of bacteria was supplied by Professor LoVerde Philip of the University of Texas Health, USA.

Isolation of parasites

Local chickens with patent *A. galli* infection were sacrificed, adult worms were removed from the intestine and rinsed twice in sodium phosphate-buffered saline (PBS, pH: 7.2). *A.galli* eggs were removed from the worm's uteri, and then incubated for 21 days in 0.1 N sulfuric acid to obtain embryonnated eggs (Permin *et al.*, 1997b).After eggs embryonnation, "Arbor acres" chickens obtained from a commercial hatchery and previously raised up to 45 days were infected with *A.galli* eggs (Permin *et al.*1997b). The chickens were sacrificed 30 days post-infection, and the adult worms were removed from the intestines.

1) Bacteria-parasite association

The adult worms were removed from the chicken's intestine, rinsed twice in minimal essential medium in a base of Earl Salts, enriched with L-glutamine (MEM) to remove any contaminants from the worm's surface (Melhem and LoVerde, 1984). Individual worms in groups of ≤ 20 were incubated for one hour at 37°C in a Tissue culture medium (TCM consisting of MEM and 10% heat-inactivated fetal calf serum) with a suspension of approximately 10^6 bacteria per ml (supplied by M. LoVerde, Texas Health University). After incubation, TCM was decanted and replaced with 5ml of sterile 0.2M sodium phosphate-buffered saline (PBS; pH 7.2). To remove any loosely adhering bacteria, the nematodes were washed 20 times; each wash consisted of 5 ml of sterile PBS, followed by vortexing for 5 seconds (LoVerde *et al.*, 1980).

2) Determination of *A. galli* body part that transport *Salmonella*

To determine the transport part of the worm, 312 adult's worms (149 males and 163 females) were used, 105 worms were ligatured by a thread tied around the body behind the

mouth and anus before any experiment. Three parts were experimented on ligatured and unligatured worms: the body surface, the digestive tract and the gravid uterus were examined for *Salmonella* using *Salmonella* selective media.

- **Unligatured worms**

To evaluate the transport capacity of the worm's body surface or wall, 50 worms (32 males and 18 females), one per plate were then streaked on *Salmonella-Shigella* agar, incubated at 37°C, and examined for the presence of *Salmonella* colonies on the streaks or around the worm after 24 and 48h.

To determine whether *Salmonella* were present in the digestive tract and/or the uterus/eggs of *A. galli*, 61 adult worms (26 males and 35 females) of *A. galli* were used and treated as above. The worms were dissected to remove the digestive tract and the uterus/eggs. The digestive tract and the uterus gravid were plated and scored as above.

- **Ligatured worms**

For the ligature experiment, 105 adult's worms (46 males and 59 females) were used. However, before the worms were place in the *invitro* culture medium, the only openings of the digestive system were closed by using a sterile thread. The worms were ligatured by tying a thread just behind the mouth and before the anus. The ligatured worms were incubated with bacteria for one hour, each worm was removed, and 50 of the ligatured worms (26 males and 24 females) were plated and scored as above (surface of the tegument). A cut was made just behind the rest of 55 worms, and the posterior part of the worm was washed 20 times in sterile PBS. The worms were dissected to remove, plate and score the digestive tract and the uterus gravid as described above.

As a control procedure, the phosphate buffer was monitored for *Salmonella* contamination. Part of 0.1ml of the 20thwash described above was placed on selective medium, incubated at 37° C, and examined for *Salmonella* colonies after 24 and 48 h. Results were only considered in those experiments in which the sample from the 20thwash did not show any colonies, indicating that all loosely adherent bacteria had been removed. Another control for the natural occurrence of *Salmonella-Ascaridia galli*in our *Ascaridia galli*-infected chickens was carried out with96 worms (45 males and 51 females). They were aseptically removed from sacrificed chicken, and placed in PBS, washed twice in 5 ml of buffer as previously described; forty six(46)of the worms(27 males and 19 females) were then plated on *Salmonella-Shigella* agar, incubated at 37°C, and examined for *Salmonella* colonies after 24 and 48hrson the surface of the body. The other 50 worms respectively 18 males and 17 females were monitored for *Salmonella* contamination of digestive tract and 15 females for the uterus gravid and score as described above.

3. Results

For the experimentation carried out with 312 adult worms, *Salmonella typhimurium* was shown to occur in each part of *Ascaridia galli* tested (table 1) comprising82% of males of and 75% of females part of worms examined. The rate of infection of the body wall, the digestive tract and the gravid uterus were 72.22%and 100%; 65.38% and 88.90% respectively.

Table 1: *In vitro* association of unligatured *Ascaridia galli* with *Salmonella typhimurium* incubated for one hour

		Number of *Ascaridia galli* tested (%)	Positive for *Salmonella*
Part of *Ascaridia galli*		Male (n= 58)	Female (n= 53)
Surface of tegument	Infected	32(100.00)	13(72.22)
	Uninfected	00	05
Digestive tract	Infected	17(65.38)	11(64.00)
	Uninfected	09	06
Uteri/eggs	Infected	-	16(88.90)
	Uninfected	-	02

NB: Each data set represents the sum of at least three experiments. The control wash was free of salmonellae after the 20th wash in all cases.

Ligatured worms of *A. galli* gave definitive evidence of the transmission of *Salmonella* by *A. Galli* body surface (Table 2). In these experiments, *Salmonella* was cultured from 66.53% of the male worms' body part such as 73.07% on the surface tegument and 60% on the digestive tract respectively; and from 56.39% of the female worms' body part with 79.17% on the surface tegument, 70% on the digestive tract and 20% on the gravid uterus. Moreover, *S. typhimurium* was cultured from 100% and 70.00% respectively on the male and female worm partial piece (table 2).

Since *S. typhimurium* was found in each ligatured and unligatured worms, it was assumed that *Salmonella* might enter *A. galli* through the mouth before reaching the digestive tract and the uterus gravid.

Table 2: *In vitro* association of ligatured *Ascaridia galli* and *Salmonella enteric* serovar *typhimurium* incubated for one hour

		Number of *Ascaridia galli* tested (%)	Positive for *Salmonella*
Part of *Ascaridia galli*		Male (n= 46)	Female (n= 59)
Surface of tegument	Infected	19(73.07)	19(79.17)
	Uninfected	07	05
Digestive tract	Infected	12(60)	14(70)
	Uninfected	08	06
Uteri/eggs	Infected	-	03(20)
	Uninfected	-	12

NB: Each data set represents the sum of at least three experiments.
The control wash was free of salmonellae after the 20th wash in all cases.

Naturally occurring *Salmonella-Ascaridia galli* association were found in less than 1.00% of the control worms, specifically the uterus/eggs of the female worms. In the case of *Salmonella* positive uterus/eggs worm, only two *Salmonella* colonies were observed on culture and they were not around the uterus/eggs but on the streak (table 3).

Table 3: *In vitro* natural occurrence of *Ascaridia galli* and *Salmonella typhimurium* used in this study

		Number of *Ascaridia galli* tested (%)	Positive for *Salmonella*
Part of *Ascaridia galli*		Male (n= 45)	Female (n= 51)
Surface of tegument	Infected	00	00
	Uninfected	27	19
Digestive tract	Infected	00	00
	Uninfected	18	17
Uteri/eggs	Infected	-	02
	Uninfected	-	15

NOTE. Dots = not tested.

4. Discussion

In nature, an association between *Salmonella* and *Ascaridia galli* is common. Chadfield *et al*. (2001) demonstrated that infected eggs of *A.galli* can carry *Salmonella* and LoVerde *et. al*. (1980) demonstrated that pili are important in the attachment of *S. typhimurium* to *Schistosomamansoni.* This suggest that pili may be the appendages used for the attachment of *Salmonella* to the body surface of *Ascaridia galli* in this study. It is also shown that salmonellae are able to colonize the digestive tract (Melhem & LoVerde, 1984) as well. The mode of attachment of *Salmonella* to the digestive tract of *Ascaridia galli* is not clear.

Authors have previously reported that migration of *A.galli* may lead to the mechanical transmission of enteric pathogens like *Salmonella* (Gamit *et al.,* 2017). Moreover, the interaction between *Salmonella* and schistosomiasis suggest that parasitism other than schistosomiasis (e.g., lymphatic filariasis, *Loaloa* and other soil transmitted helminth infections) may have similar unexplored interactions with *Salmonella* (Hsiao *et al.,* 2016).The relationship of *Salmonella* and *A.galli* has been reported in chickens, where adult female *Ascaridia galli* worms were found in a portion of commercial poultry egg (Gamit *et al.,* 2017). Furthermore, *Salmonella* also infect poultry eggs, and such eggs become a potential source of infection to persons who consume raw eggs (Okorie Kanu *et al.,* 2016).

The results of this work highlight some important points on the mechanism of *Salmonella* transmission by A. *galli* body parts. Additionally female of *A.galli* worms appeared to be more implicated than the male because they have another contamination pathway, in which eggs pass through feces. However, in nature the relationship has often been described as synergistic. We suggest that this property of *Ascaridia galli* together with the adhesive property of *Salmonella*, enables *Ascaridia galli* to provide a long-term focus for bacterial multiplication in the intestine, thus, in part accounting for the protracted course of chicken' salmonellosis dually infected with *Salmonella* and *Ascaridia galli.* Control of *Ascaridia galli* in chickens may represent an important key to reduce potential *Salmonella* infection. Therefore, further studies on this association and its importance in salmonellosis and ascaridiasis processes are needed.

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