

Parasitic Burden and Pathologic Effects of Anisakis Sp.

(Nematoda : Anisakinae, Anisakidae) and

Contracaecum Sp. Larvae (Nematoda : Anisakinae,

Anisakidae) on Mugilids from Senegalese Estuaries

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Abstract

Consumption of mugilids is widespread in Senegal, especially in the estuaries, where there production is of economic importance. The prevalence, mean intensity and pathologic effects of Anisakis sp. and Contracaecum sp. larvae which infected mugilids from Senegalese estuaries were investigated. In 2009, individuals of mugilids belonging to 6 and 5 species were trapped from the estuaries of Saloum, Senegal and Casamance rivers respectively. The prevalence and mean intensity of Anisakis sp. and Contracaecum sp. are higher in Senegal and Saloum estuaries respectively. The increase in the load of *Contracaecum* sp., related to the season in L. falcipinnis, the season, the size, the weight and/or the sex in M. cephalus from the estuaries of Saloum and Senegal and M. curema from Saloum estuary were due to an accumulation of parasites over time. Liver, kidney, body cavity and mesentery and ovaries are the respective sites of infection of Anisakis sp. and Contracaecum sp. larvae. The immune response observed is the formation of a fibrous capsule surrounding the parasites with melanin granules all around the capsule. The implementation of the capsule and the presence of melanin granules suggest the involvement of lymphocytes, monocytes, neutrophils and macrophages. The lesions caused by Anisakis sp. and Contracaecum sp. larval settlement in target organs may cause the death of the host and/or a reduction in fertility.

Keywords: Anisakidae, Fibrous capsule, Prevalence, Mean intensity, Mugilids, Immune response, Casamance, Saloum, Senegal.

1. Introduction

Species of the family Mugilidae commonly known as mugilids are permanent and often abundant coastal, estuarine or lagoon fish. Locally popular, their economic importance is great and they are often the subject of specialized fisheries (Albaret and Legendre, 1985; Albieri et al, 2010; Wang et al, 2010; Durand et al., 2012). Nematode larvae in individual Anisakidae larvae have infected several species of marine, freshwater and brackish water fish including *M. cephalus*. The presence of larvae Anisakidae in fish may reduce their quality and be dangerous for consumers. Immunological studies of cellular responses induced by these parasites in birds (Nemeth et al 2012), mammals (Cattan et al., 1976; Migaki et al., 1982), human (Chord-Auger et al., 1995; Cu élar et al., 2010; Daschner et al., 2011; Gonzalez et al., 2010, 2011), and fish (Munday et al., 2003; Longshaw et al., 2004. Murphy et al., 2010; Abowei and Ezekiel, 2011; Buchmann, 2012) have been conducted by many authors around the world. In Africa, histopathological studies in fish from several families have been made but investigations on the effect of Anisakidae on mugilids are a first in Africa, especially in Senegal.

This paper's main purpose is to determine the parasitic load and study the effect of season, size, weight and sex of six species of mugilids (*Liza dumerili, Liza falcipinnis, Liza grandisquamis, Mugil bananensis, Mugil cephalus* and *Mugil curema*) from the Senegalese estuaries on parasitism by larvae of *Anisakis* sp. and *Contracaecum* sp. and the histopathological effects



induced by the establishment of these larvae in the host tissues.

2. Materials and methods

2.1 Sampling procedure

Casamance, Saloum and Senegal estuaries constitute the localities chosen within the framework of this study. The estuaries of the Casamance and Saloum rivers are "reverse" estuaries while the estuary of the Senegal river is normal. These ecosystems are characterized by a particular vegetation type, the mangrove swamp. Mangrove of the Casamance and saloum estuaries is composed of six species belonging to three families: Rhizophoraceae, Verbenaceae and Combretaceae. The mangrove of the estuary of the Senegal river is of minor importance in comparison with Casamance and Saloum mangroves. Low to sparse density and height rarely exceeding 5 m, it consists of two species: *Rhizophora racemosa* and *Avicenia africana*.

In these estuaries, samplings were done systematically per quarter. Three mugilid samplings were carried out in the dry season and one in the rainy season of 2009. The techniques of fishing used were the beach seines and the gill nets.

2.2 Parasite Prevalence and Mean Intensity

The prevalence (P) or parasitism rate is the percentage ratio of the number of infected hosts (N) by a given parasite species on the number of fish examined (H).

P(%) = N / H * 100

The parasite mean intensity (I) refers to the total number of individuals of a parasite species (n) by the number of infected fish (N).

I = n / N

2.3. Effect of weight, size and sex of Mugil cephalus and season on parasitism

We consider intensity or parasite load as a dependent variable or response variable and the weight or size, the season and the sex as variables. Among the explanatory variables, there is a continuous variable which is the weight or the size and two categorical variables or factors, the season and the sex, which are at two levels. A linear regression relationship allows us to study the effect of size, weight and/or sex of each host and the season on the parasite load. The meaning of the data is established at 0.05. R software was used for statistical analysis.

2.4 Histopathological Study

Collected first, livers, kidneys and mesentery of host fish underwent three ethanol baths to 95% on the first day, then 3 butanol baths on the second day and then three paraffin baths on the third day before being lumped in "Leuckart bars" placed on a glass plate. Cuts of 5 to 7 microns thick are made two to three days after making the block, with a paraffin microtome Minot kind. They were then plated and bonded to albumin on cleaned slides of ethanol 95%. Drying was done in a paraffin oven at 60 °C. The slides thus prepared were stained with Groat haematoxylin and dyes of Masson Trichome, which helped differentiate tissues.

3. Results

3.1 Prevalence and Mean Intensity

Ecological parameters prevalence and mean intensity of *Anisakis* sp. and/or *Contracaecum* sp. larvae parasites of *L. dumerili*, *L. grandisquamis*, *M. bananensis*, *M. cephalus* and *M. curema* are summarized in the tables 1 to 6 below.



The prevalence of *Anisakis* sp. recorded in the estuary of the Senegal river is higher than that found in estuaries of the Saloum and Casamance rivers whatever the host species. It also varies according to the season. Indeed, this ecological parameter is higher in rainy season than in dry season except for *M. bananensis* originating in the Saloum estuary.

In hosts of the genus *Mugil*, the mean intensity of *Anisakis* sp. obtained in Senegal estuary is higher than that recorded in the estuaries of the Casamance and Saloum rivers, with a significant difference in *M. bananensis* and *M. cephalus*. In *L. grandisquamis*, parasite intensity is higher in the Saloum estuary than in the estuaries of the Casamance and Senegal rivers. In host species where the parasite was met throughout the year, the mean intensity is higher in rainy season than in dry season, but a significant difference was only observed in *M. cephalus* from the estuary Saloum.

The prevalence and mean intensity of *Contracaecum* sp. identified in the Saloum estuary are generally higher than those recorded in the estuaries of the Casamance and/or Senegal rivers regardless of host species. These ecological parameters vary seasonally. Indeed, in species where the parasite was met throughout the year, the prevalence and mean intensity is higher in dry season than in rainy season except for *M. curema* native to the Saloum estuary for the prevalence and *L. dumerili* from the estuary of the Casamance river for the mean intensity. The difference between the values of the mean intensity according to the season is significant in *M. cephalus* and *L. falcipinnis* from the Saloum estuary.

Contracaecum sp.								
P (%) I								
Localities	RS	RS	DS					
Casamance	10 (H=10, N=1)	50 (H=6, N=3)	5	3.33±3.21				
Saloum	NE	100 (H=60, N=60)	NE	50.06±41.25				
Senegal	11.76 (H=17, N=2)	54.35 (H=46, N=25)	9.5±10.61	10.25±7.08				

Table 1. Prevalence and mean intensity of Contracaecum sp. larvae parasite of L. dumerili

RS: rainy season

DS: dry season

NE=host not examined

Table 2. Prevalence and mean intensity of Contracaecum sp. larvae parasite of L. falcipinnis

Contracaecum sp.								
	P (%) I							
Localities	RS	DS	RS	DS				
Casamance	NF	NF	NF	NF				
Saloum	35.85 (H=53, N=19)	81.08 (H=37, N=30)	10.47±17.96	56.52±69.1				
Senegal	1.2 (H=25, N=3)	25.81 (H=63, N=16)	2±1.73	4.79±6.68				

RS: rainy season; DS: dry season; NF=parasite not found

Table 3. Prevalence and mean	intensity of Anisakis sp.	and Contracaecum sp.	larvae parasite of
L. grandisquamis			

	Ani	Contracaecı	um sp.			
	Р((%)		Ι	P (%)	Ι
Localities	RS	RS DS		DS	DS	DS
Casamance	6.67 (H=30,	=30, 3.37 (H=148,		1±0	2.01 (H=148,	18±9.54



	N=2)	N=5)			N=3)	
Saloum	NE	6.67 (H=30, N=2)	NE	1.5±0.71	63.64 (H=33, N=21)	4.52±3.82
Senegal	23.07 (H=13, N=3)	NF	1±0	NF	NF	NF

RS: rainy season; DS: dry season; NE=host not examined; NF=parasite not found

Table 4. Prevalence and mean intensity of *Anisakis* sp. and *Contracaecum* sp. larvae parasite of *M. bananensis*

Anisakis sp.					Contracaecum sp.			
	Р(P (%)		Ι		%)	Ι	
Localities	RS	DS	RS	DS	RS	DS	RS	DS
Casamance	NE	100 (H=1, N=1)	NE	1±0	NE	100 (H=1, N=1)	NE	26±0
Saloum	60 (H=20, N=12)	81.81 (H=11, N=9)	1.25±0.45	1.33±0.5	52.63 (H=19, N=10)	100 (H=9, N=9)	10.6±14.06	11.89±9.44
Senegal	NE	90.47 (H=21, N=19)	NE	2.84±1.74	NE	53.85 (H=13, N=3)	NE	20.8±20.7

RS: rainy season

DS: dry season

NE=host not examined

Table 5. Prevalence and mean intensity of *Anisakis* sp. and *Contracaecum* sp. larvae parasite of *M. cephalus* (Dione et al. 2014)

Anisakis sp.						Contracaecum sp.			
	P (%)		Ι		P (%)		Ι		
Localities	RS	DS	RS	DS	RS	DS	RS	DS	
Casamance	NE	NE	NE	NE	NE	NE	NE	NE	
Saloum	51.02 (H=49, N=25)	32.5 (H=80, N=26)	1.6±0.71	1.58±0.64	22.44 (H=49, N=11)	51.25 (H=80, N=41)	3.09±1.81	11.71±14.87	
Senegal	90 (H=60, N=54)	73.83 (H=107, N=79)	3.09±1.92	2.38±1.5	6.66 (H=60, N=4)	25.23 (H=107, N=27)	3.5±3	5.93±7.23	

RS: rainy season

DS: dry season

NE=host not examined

Table 6. Prevalence and mean intensity of *Anisakis* sp. and *Contracaecum* sp. larvae parasite of *M. curema*

	<i>Anisakis</i> sp		Contracaecum sp.					
	P (%) I			P (%) I				
Localities	RS	DS	RS	DS	RS	DS	RS	DS
Casamance	NF	NF	NF	NF	NF	NF	NF	NF
Saloum	70.59 (H=17, N=12)	37 (H=100, N=37)	1.4±0.97	1.16±0.37	78.57 (H=14, N=11)	65.31 (H=98, N=74)	13.45±10.03	21.75±52.91
Senegal	NE	84.62 (H=13, N=11)	NE	1.64±1.03	NE	11.11 (H=9, N=1)	NE	1 ±0

RS: rainy season

DS: dry season

NE=host not examined

NF=parasite not found

3.2 Effect of Size, Weight and Sex of the Host and Season on the Parasitism

For *L. dumerili*, *L. grandisquamis* and *M. bananensis* from the estuaries of the Casamance, Saloum and Senegal rivers, season, weight, height and sex can not explain the variations in the load or parasite intensity of *Contracaecum* sp. or *Anisakis* sp larvae.

In *L. falcipinnis* from the Saloum estuary, the variations in the intensity of *Contracaecum* sp. can not be elucidated by the season, the other above variables are not related to this ecological setting. Individuals operating in the estuary of the Senegal host larvae *Contracaecum* sp. with a load which changes can not be explained either by season, size, weight or sex.

Intensity= $\begin{bmatrix} 0 \\ 44.26 \end{bmatrix}$ Season + 10.47 ; R²=13.8%; p=0.008

In *M. cephalus*, the variations in the parasite load of *Anisakis* sp. can not be explained either by size or by weight or by sex or season. In contrast to *Contracaecum* sp., the parasite load is related to the size, weight and season in the Saloum estuary while in the estuary of Senegal, it is correlated to the size, weight and gender. The following models are used to show the linear regression relationship existing between the parasite load and the different explanatory variables.

In individuals infected from the Saloum Delta, the variations in the parasite intensity can be explained by the size or weight and season. Thus, individuals with large size or weight harbor much more *Contracecum* sp. larvae than those of small size or weight. In addition, the parasite load was higher in the dry season than the rainy season.

Intensity=1.06*(Size) +
$$\begin{bmatrix} 0 \\ 8.11 \end{bmatrix}$$
 season - 22.95
; R²=27.66%; p=0.002
Intensity= 0.07*(Weight) + $\begin{bmatrix} 0 \\ 7.59 \end{bmatrix}$ season - 5.46
; R²=26.78%; p=0.002

In the estuary of the Senegal river, the variations in the parasite load of *Contracaecum* sp. larvae can be explained by size, season and sex or weight and sex. Indeed, the number of larvae found in a host increases with the size or weight. It also depends on the season that is to say, is higher in dry season than in rainy season and it is even higher in males than individuals of indeterminate sex.

Intensity=1.74*(Size) +
$$\begin{bmatrix} 0 \\ 4.66 \end{bmatrix}_{Season}$$
 + $\begin{bmatrix} 0 \\ -8.84 \end{bmatrix}_{Sex}$ - 31.8
; R²=71.11%; p=0.000
Intensity=0.14*(Weight) + $\begin{bmatrix} 0 \\ -11.12 \end{bmatrix}_{Sex}$ - 3.2
; R²=85.66%; p=0.000

Anisakis sp. larvae parasites of *M. curema* from the estuaries of the Saloum and Senegal rivers have a parasitic intensity whose changes can be associated with the season, the size, the weight or the sex of the host. As the larvae *Contracaecum* sp. parasitizing hosts individuals from the Saloum estuary, they have a parasite load which is related to the above explanatory variables. Indeed, the linear relationship between the environmental parameters and the explanatory variables shows that host individuals which have a size or heavy weight are the most infested. This relationship also shows that the parasitic intensity is higher in dry season than in rainy season and the female individuals harbor more *Contracaecum* sp. larvae than males.

Intensity=3.97*(Size) +
$$\begin{bmatrix} 0 \\ 1.22 \end{bmatrix}_{season}$$
 + $\begin{bmatrix} 0 \\ -7.93 \end{bmatrix}_{sex}^{-80.35}$; R²=12, 67%; p=0,021
Intensity=0.29*(Weight) + $\begin{bmatrix} 0 \\ 1.26 \end{bmatrix}_{season}$ + $\begin{bmatrix} 0 \\ -7.95 \end{bmatrix}_{sex}^{-24.82}$; R²=16, 64%; p=0,005

3.2 Histopathological Study

The kidney is noisy in its posterior part (Figure 7a) by *Anisakis* sp. resulting in the formation of capsules in which the worm is wound several times (Figure 7b) to give sectional multiple sections of the worm (Figure 7c). In the liver, the larvae of *Anisakis* sp. have encysted (Figure 7e) and histological sections also show worms at different levels (Figure 7f). The turn of parasites, liver and kidney tissue was disorganized revealing gaps and black granules (Figure 7d). Two or 3 larvae of *Anisakis* sp. can be found in a capsule (Figure 8a, b, c).

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Figure 7. Encapsulation of *Anisakis* sp. larvae in the kidney (a, b, c, d) and liver (e, f) of *M*. *cephalus*. An. l.=larvae of *Anisakis* sp.

Figure 8. Encapsulation of *Anisakis* sp. larvae in the kidney (photo a) of *M. bananensis* with 2 (photo b) or 3 (photo c) larvae in each capsule.

In the mesentery of different host fish larvae of *Contracaecum* sp. have encysted in adipose tissue and melanin granules can be distinguished in some places (Figure 9a). Between the larval cuticle and host tissue, spaces or meats exist (Figure 9b).

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Figure 9. Encapsulation of *Contracaecum* sp. larvae in the mesentery of *M. curema*. Cont. l.= larvae of *Contracaecum* sp.

Some larvae of *Contracaecum* sp. were found encysted on ovarian filaments of *L. grandisquamis* (Figure 10) or in the ovaries of *L. dumerili* (Figure 11), *L. grandisquamis* and *M. bananensis*. The capsules containing the parasite are peripheral and located in the ovary wall and rely on meats. The stacks of eggs near the cyst suffered disruption to the contact zone. The capsule wall is very thick and we noted some areas of degradation products of oocytes (Figure 9. c, d). This degradation of oocytes can be very deep and result in the formation of a crust (Figure 12).

Figure 10. Fixing *Contracaecum* sp. larvae on ovarian filaments of *L. grandisquamis*

Figure 11. Encapsulation of *Contracaecum* sp. larvae in the ovary of *L. dumerili* (photos a and b before and after ovaries opening respectively, c and d). Cont. l. = larvae of *Contracaecum* sp.

Figure 12. Necrosis of oocytes forming a crust

4. Discussion

In the Saloum and Senegal estuaries, the variations in the parasite load of *Anisakis* sp. can not be elucidated by the sex, size, weight and season. No model has therefore been developed for these larvae. That could be explained by the parasitic intensity and relatively low prevalence, corresponding to a wide dispersion of larvae in the host population. Considering parasitism by *Contracaecum* sp. larvae, we found in *M. cephalus* from the estuaries of the Saloum and Senegal rivers and in *M. curema* from the Saloum estuary a regression relationship between the parasite load and the size or weight, the season and the sex. Indeed, in the estuary of the Saloum river, the parasite load in *M. cephalus* is correlated to the size or weight and season, while in the estuary of the Senegal river, it is based on either the size, sex and season, weight and sex. In *M. curema*, the variations in the intensity of *Contracaecum* sp. can be explained by the season, size, weight and sex of the host. In *L. falcipinnis* native of the Saloum estuary it is only the season that has provided explanations to load variations.

The low R^2 obtained with the predefined templates in the Saloum estuary explain only a small part of the variation in parasite intensity. So there would be other variables that best explain these variations. The R^2 obtained with linear models at the estuary of Senegal suggest the sex of the host, in addition to the weight or size and the season, is correlated with parasite load. Indeed, individuals of indeterminate sex of *M. cephalus* lodge fewer *Contracaecum* sp. larvae than

males. Females of *M. curema* are more frequently infected than males. These results allow us to say that the effect of gender on parasitism is stronger between immature and mature than between mature individuals only. The physico-chemical environmental conditions such as temperature or salinity and the presence of birds, definitive hosts of these nematodes also explain the variations noted in the load of *Contracaecum* sp.

The increase of the parasite burden with the size or weight of *M. cephalus* in both estuaries and *M. curema* from the Saloum estuary means that larvae of *Contracaecum* sp. have been accumulated, after repeated infestations in fish hosts over time. We confirm this accumulation of parasites over time already mentioned by several authors (Valles -Rios et al., 2000; Bergmann and Motta, 2004; Al- Zubaidy, 2009) particularly in the Senegal estuary where male fish lodge more larvae of *Contracaecum* sp. than individuals of indeterminate sex. However, some authors (Cremonte and Sardella, 1997, Gonzalez and Acuna, 2000; Sylva and Eiras, 2003; Abattouy et al., 2011; Tantanasi et al., 2012; Tejada, 2013) found no relationship between parasitism by *Anisakis* spp. and sex of the host fish, but noted an increased risk of infection with the weight, size or season or three variables. Given the importance of fish weight for the consumer, it is necessary to give the following preventive measures: it is safer to consume *M. cephalus* and *M. curema* from these estuaries of the Saloum and Senegal rivers with average weight to reduce the risk of contamination because it is the big fish that contain more nematode larvae.

In this study we did not find Anisakidae larvae in the flesh of fish, but in the kidney, the liver, the body cavity, the mesentery and the ovaries. Therefore these larvae were not able to reach the fish. Migration depends on factors related to fish muscles such as wealth even more fat than their preference for high fat tissue was observed (Lymbery et al., 2002; Stromnes and Anderson, 2003; Abattouy et al., 2011). Many authors (Smith and Wootten, 1975; Wootten and Waddell, 1977; Smith 1984, Wharton et al., 1999; Kino et al., 1993; Abollo et al., 2001; Martins et al., 2005; Mo and al., 2013) supported the idea that Anisakidae larvae perform a post-mortem migration in the fish. In contrast, other authors (Mladineo, 2001; Tejeda, 2010), speak of migration at catching fish.

If the migration of Anisakidae larvae was done after the death of fish, immediate evisceration could prevent. Large firms conduct early fish gutting and nevertheless anisakidose cases continue to be identified in some countries like Japan and the USA. Migration of nematodes for catching fish is plausible. As soon as the fish is trapped, it is in a state of stress, which can lead to birth and signal flow in the body. These stress signals can be picked up by the parasites in particular Anisakidae larvae that will migrate into the fish where they will be safer. That is a good strategy for these larvae to stay alive in the meantime, by chance, to move to another host, such as human. The site of infection in the host fish is an important determinant of the risk of human anisakiasis (Quiazon et al., 2011). The greater presence of larvae of *Anisakis* sp. in the kidney is a potential risk of contamination because it is usually consumed. Another prophylactic measure would be to remove the kidney during evisceration.

The installation of *Anisakis* sp. larvae in renal and hepatic serous and/or general cavity of *L. grandisquamis*, *M. bananensis*, *M. cephalus* and *M. curema* and that of Contracaecum sp. larvae in the mesentery and/or ovaries of *L. dumerili*, *L. falcipinnis*, *L. grandisquamis*, *M. bananensis*, *M. cephalus* and *M. curema* are causing an inflammatory reaction leading to the formation of a fibrous capsule around the parasite. The shell is made of a hard and stratified epithelium and is therefore slightly permeable to nutrients, Berland (1998). Its implementation involves several cells including neutrophils, fibroblasts, lymphocytes and macrophages (Elarifi, 1982; Dezfuli et al., 2007; Murphy et al., 2010). The complete encapsulation of the larvae of

Anisakis sp. in the liver, kidney and of *Contracaecum* sp. in the mesentery and/or ovaries of host prevents migration and therefore the infested sites lesion. However, the translucent areas and debris from tissue surrounding the capsule show degradation be it small, of infected organs.

Then, how lysis of host tissues is made while the larvae are not in direct contact with them? According to Larsen (1980), the cephalic tooth of Anisakidae larvae functions as a needle piercing the capsule to permit the entry of host cells. He also states that the cephalic gland secret histolytic enzymes that lead the process of maintaining an opening in the capsule.

The lesions of the kidney and liver, essential functional organs could cause a more or less long-term death of the host. The pathogenic action of the larvae of *Anisakis* sp. was shown by Petter (1969). The experience has been to ingest larvae of *Anisakis* sp. into a mugilid (*M. cephalus*), the death of the fish was observed 6 days after ingestion. The larvae of *Anisakis* sp. are pathogenic for these fish hence the importance of paying attention to these parasites. Given their large size, they cause mechanical compression of tissues causing malfunctions of infected organs.

The consequence of many lesions of the mesentery was slimming host fish seeing that it is an important lipid reserves. These results could be compared with those of Petter et al., (1974) although parasitic nematodes in question and the hosts are different, but also with those of Lymbery et al., (2002) in which larvae of *Contracaecum* sp. found in *M. cephalus* and *Aldrichetta forsteri* are causing major morphological changes in the liver. The pathogenic action of larvae of *Contracaecum* sp. was also studied by Acha and Szyfres, (1987) who reported a decrease in weight of the host and the lipid content of the liver or even the death of young fish when larvae invade the cardiac region. The presence of necrotic plaque in the ovaries can deeply affect the fertility of the host. Indeed, necrosis oocytes lead to decreased fertility of the host and the result in longer term would be a reduction of the fish stock.

5. Conclusion

Prevalence and mean intensity of *Anisakis* sp. and *Contracaecum* sp. larvae are higer in the estuaries of the Senegal and Saloum rivers respectively. Variations of the load of *Anisakis* sp. can not be explained by the locality, the season, the size, the weight or the sex of hosts. In contrast, variations of *Contracaecum* sp. intensity are related to the season, the size, the weight and/or the sex of some hosts. The settlement of the anisakid larvae may induce the death of the host or a reduction in fertility.

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