Disease Interaction Between *Gyrodactylus* spp. And Motile *Aeromonas* In Wild Fish Populations In Navarra Rivers

Ruiz-Zarzuela, I[1]; de Blas, I [1]; Gironés, O[1]; Ortega, C[1]; Muzquiz JL[1]
[1]Laboratory of Fish Pathology, University of Zaragoza, Zaragoza, Spain.

Abstract

An observational study was performed during 4 years in 18 rivers of Navarra (Spanish northern region). More than 2 000 fish were captured in different seasons and sampling points, mainly salmonids (60.7% brown trout and 5.5% rainbow trout) and cyprinids (16.9% Eurasian minnow), and data were collected about lesions and presence of pathogens. So, bacteriological and parasitological diagnosis were carried out using classical diagnostic techniques. Also, main water characteristics were measured (physical-chemical and microbiological parameters). With all these data a epidemiological analysis was performed and statistical associations were found between presence of external and internal lesions, isolation of motile Aeromonas (specially *A. hydrophila*) and presence of *Gyrodactylus* spp. (a fish ectoparasite). Significative Odds Ratios were calculated in both situations. This communication discusses about the role of both pathogens and their interactions with relationship of presence of lesions in wild fish. We propose different models of epidemiological chain to explain obtained results considering influence of environmental factors as pH, water temperature and presence of fish farms.

Introduction

Disease in wild populations is a rare phenomena (except when occurs an outbreak due to introduction in the population of a virulent pathogen), but in general is difficult to find fish with lesions, probably due to predation pressure. So, in general sampling in wild populations is designed as a non probabilistic method, but at the end it is completely randomly.
Furthermore in most of cases, disease is associated with environmental conditions, and only in few cases the role of secondary pathogens is relevant. In this work we study the influence of different factors in the health status of fish.

Matherials and methods

Observational study was performed during 4 years in 18 rivers of Navarra (NE Spanish) in order to check health status of wild fish populations. Sampling was carried out four times every year with at least 15 fish each time by electrofishing. So 2 146 fish were captured in different seasons and sampling points (Table 1), mainly salmonids (60.7% brown trout and 5.5% rainbow trout) and cyprinids (16.9% Eurasian minnow). Most of fish were adults, but all age stages were represented in the sample. Potentially relevant epidemiological data were collected (watershed, presence of fish farm, family (salmonids or cyprinids), age, season, water temperature, pH, total dissolved solids, redox, conductivity, hardness, alkalinity, dissolved oxygen, DQO, carbonic dioxide, nitrites, nitrates, ammonia, phosphate, chloride, cupper, water microorganisms (aerobic bacteria growth at 22ºC and 37ºC, anaerobic bacteria, total coliforms, faecal coliforms and faecal streptococci)). Every fish was processed in order to determine health status: necropsy, microscopical observation of skin and gills, bacterial culture in solid mediums (trypticase soy agar, marine agar and blood agar) and cell culture for virus isolation.

Table 1 Distribution of sampled fish depending on specie and age

	Fingerling	Juvenile	Adult	Total
Salmonids	11,0%	43,7%	45,3%	1.469
Cyprinids	5,8%	21,9%	72,4%	677
Total	**9,2%**	**36,6%**	**54,2%**	**2.146**

All data were included in a database and they were processed with Win Episcope 2.0, WinEpi Tasas 2.0 and SPSS 12.0 for Windows. Diverse statistical techniques were applied Chi-square test, Odds Ratio, stratified Odds Ratio and logistic regression (using conditional forward method).

Results and discussion

Parasitism is a frequent phenomena in wild fish populations. Most was parasites found during the study period were opportunistic, and their prevalence and intensity of parasitation is associated with environmental conditions, and also with host susceptibility (Álvarez-Pellitero, 1988; Eiras, 1994; Scholz, 1999). Most important parasites were *Gyrodactylus* sp. in winter (26.9%), *Trichodina* sp. in spring (13.3%) e *Ichthyophthirius multifiliis* (5.2%) in autumn. For the rest of parasites the prevalences were lower than 4%, with maximum values in spring (except for *Argulus* spp. in autumn). In the case of bacteria most frequently isolated species belong to motile *Aeromonas* (12.98%), *Vibrio* spp. (10.5%) and *Pseudomonas* spp. (5.6%). No viruses were found in any of processed fish. In Table 2 is possible to observe stratified results for lesions, motile *Aeromonas* and *Gyrodactylus* spp.

Table 2 Observed prevalence of diseased and infected fish, stratified by specie and age (N=2146)

Strata	External lesions	Internal lesions	Motile *Aeromonas*	*Gyrodactylus* spp.
Salmonids	3.1%	5.3%	7.96%	21.51%
Cyprinids	2.2%	1.3%	23.19%	13.29%
Fingerling	0.0%	0.5%	11.50%	11.00%
Juvenile	2.5%	2.8%	12.75%	19.57%
Adult	3.5%	5.6%	13.38%	19.69%
Total	**2.8%**	**4.1%**	**12.98%**	**18.85%**

After a wide and intensive epidemiological analysis we only found that these pathogen are associated with lesions in wild fish (Table 3). Results indicate that infection by motile *Aeromonas* and parasitation by *Gyrodactylus* spp. produce an increment of the probability of external lesions and internal lesions. Specially important is the association with external lesions. These pathogens are normally considered as opportunistic agents, and it is frequent to find them in water and aquatic host, as part of gut microflora or located in skin and gills. For these reasons, generally as a secondary pathogenic role in fish (Aoki, 1999; Cipriano, 2001). Probably these epidemiological associations are due to the interaction of several biotic and abiotic factors that favour development of these pathogens (Koskivaara et al,1991; Cipriano, 2001).

Table 3 Risk associated to infection (with 95% confidence interval)

Risk factor (infection)	External lesions	Internal lesions
Motile *Aeromonas*	5.853 (3.672, 9330)	1.786 (1.082, 2948)
Gyrodactylus sp.	7.132 (4.347, 11.701)	1.731 (1.103, 2.716)

In order to test interaction of environmental factors we have developed a logistic regression model to detect risk factors associated with presence of external and internal lesions (Tables 4 and 5). Model for external lesions has -2 Log likelihood of 296.699 and Nagelkerke R Square equal to 0.402. This model includes environmental variables as season (lesions are more probable in spring than in summer), pH and phosphate (curiously high values act as protection), microbiological quality of water (anaerobic bacteria), and infection by *Gyrodactylus* spp. and motile *Aeromonas.* It is relevant the role of parasitation by *Gyrodactylus* sp. in relation with the presence of external lesions, and it is the most important factor in the model. So perhaps would be necessary to reconsider its role as secondary pathogen, and the same for motile *Aeromonas*. And in the other hand, very few environmental variables were found that form part of the pathogenic chain in unspecific lesions. In the other model for internal lesions has -2 Log likelihood of 472.315 and Nagelkerke R Square equal to 0.225. In this case intrinsic variable as specie and age were the most important factors in the model, but also environmental variable play an important role in the model.

Salmonids and adults were the more exposed groups to suffer internal lesions but effect of survival bias and predation of weak fish should be considered. As we expected *Gyrodactylus* spp. doesn't take part of the model, and initial result of simple epidemiological analysis showed in Table 3 is discarded. It is important to consider that *Gyrodactylus* spp. are external parasites and it was difficult to explain its role in the causal mechanisms responsible of internal lesions.

Table 4 Logistic regression model for external lesions (N=1677)

Variable	Exposed group	Non exposed group	B	S.E.	Sig.	OR
Season	Spring	Summer	2.297	0.667	0.001	9.947
pH	6-7	< 6	1.483	0.815	0.069	4.405
Phosphate	0.05-0.09 mg/l	0.02-0.05 mg/l	-2.907	0.572	0.000	0.055
	> 0.09 mg/l	0.02-0.05 mg/l	-1.713	0.434	0.000	0.180
Anaerobic bacteria	> 10 fcu/20ml	< 10 fcu/20ml	1.144	0.377	0.002	3.139
Gyrodactylus spp	Present	Absent	2.417	0.368	0.000	11.208
Motile *Aeromonas*	Present	Absent	1.515	0.360	0.000	4.550
Constant			-23.14	2804.14	0.993	0.000

Table 5 Logistic regression model for internal lesions (N=1677)

Variable	Exposed group	Non exposed group	B	S.E.	Sig.	OR
Family	Salmonids	Cyprinids	2.306	0.453	0.000	10.035
Age	Adult	Fingerling	2.830	1.026	0.006	16.951
Extrem alkalinity	< 25 or > 250 mg/l	25-250 mg/l	1.437	0.323	0.000	4.210
Phosphate	0.05-0.09 mg/l	0.02-0.05 mg/l	-1.117	0.414	0.007	0.327
Aerobic bacteria 37ºC	> 1000 fcu/ml	< 1000 fcu/ml	1.176	0.286	0.000	3.242
Motile *Aeromonas*	Present	Absent	1.030	0.341	0.003	2.802
Constant			-8.072	1.141	0.000	0.000

As conclusion, we have detect several variables that could take part of the causal mechanism of unspecific lesions in wild populations, but role of the pathogens has been confirmed as a important part of this model. Further studies should be carried out to eliminate limitations of cross-sectional design.

References

Álvarez-Pellitero, P. (1988). Enfermedades producidas por parásitos en peces. En: *Patología en Acuicultura.* Espinosa de los Monteros J. and Labarta U. U (eds). Mundi-Prensa. Madrid. 215-326.

Aoki, T. (1999). Motile aeromonads (*Aeromonas hydrophila*). En: *Fish Diseases and Disorders. Volume 3*. Viral, Bacterial and Fungal Infections. Woo P.T.K. and Bruno D.W. (eds). CABI-Publishing. Oxon. 427-453.

Cipriano, R.C. (2001). *Aeromonas hydrophila* and motile aeromonad septicemias of fish. *Fish Disease Leaflet,* 68.

Koskivaara, M., Valtonen, E.T. and Prost, M. (1991). Seasonal occurrence of gyrodactylid monogeneans on the roach (*Rutilus rutilus*) and variations between four lakes of differing water quality in Finland. *Aqua Fennica*, 21, 47-55.

Scholz, T. (1999). Parasites in cultured and feral fish. *Veterinary Parasitology*, 84, 317-335.

Acknowledgments

This work has been granted by the Project 01/06 from Consejería de Ordenación del Territorio y Medio Ambiente of the Goverment of nNavarra (Spain).