Susceptibility of selected freshwater coarse and salmonid fish species to *Lactococcus garvieae*

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Introduction

Lactococcosis, caused by the bacterium *Lactococcus garvieae*, has been reported in farmed Rainbow trout in many European countries including Italy, Spain, France, the United Kingdom and Portugal. This disease poses a significant threat to trout farming with mortality levels reaching 90% observed at water temperatures above 16-18°C (Pereira *et al.*, 2004). Despite its severity, there is little information on *L. garvieae* virulence in other freshwater fish (salmonids and non-salmonids) which may be farmed alongside Rainbow trout or reside in the vicinity of trout farms affected by lactococcosis outbreaks. These species could potentially be exposed to the pathogen or to infected or carrier fish in natural circumstances. Such a case occurred in 2000 when the first UK outbreak affected a large trout farm operating on a freshwater reservoir and nature reserve with natural populations of coarse fish.

An infected grayling showing exophthalmia

In a series of experiments we investigated the susceptibility of three salmonid species (Atlantic salmon, brown trout and grayling) and seven cyprinid fish species (carp, tench, rudd, barbel, chub, dace and roach) to *L. garvieae*. We first used intraperitoneal (ip) injection of a fairly high concentration of the bacterium as a challenge method to assess the virulence of the organism. Secondly, we used a cohabitation challenge to better mimic a natural infection route.

Material and methods

Bacterial isolate

A *L. garvieae* isolate from the 2000 UK outbreak was used in all trials. Its identity was confirmed using standard biochemical and molecular techniques and has since been stored at –80°C. When required the bacterium was cultured in TSB and re-suspended in PBSa to the desired concentration.

Challenge

Injected fish were anaesthetised using MS222 and ip injected with 0.1 ml of *L. garvieae* suspension.

- Carp, tench, rudd, barbel, chub, dace, roach and rainbow trout (RT) weighing between 11 and 35g were each injected with 1.09×10^6 CFU.
- Atlantic salmon, grayling, brown trout and RT weighing between 4 and 22g were each injected with approximately 10^2, 10^4 or 10^7 CFU.

Cohabitant fish were kept in the same tanks as rainbow trout ip injected with *L. garvieae* (direct cohabitation) or received outlet water from tanks containing *L. garvieae* injected fish (indirect cohabitation).

- Rudd, chub, roach and RT weighing between 7 and 22g were directly cohabited with RT injected with 8.5×10^3 CFU.
- Atlantic salmon, grayling, RT and brown trout between 4 and 22g were directly cohabited with RT ip injected with approximately 10^4 CFU.
- Brown trout, grayling and RT between 4 and 22g were indirectly cohabited with RT injected with 1×10^4 CFU.

Post challenge

All challenged fish were sampled (by inoculation of kidney material onto TSA) to check for presence of *L. garvieae*. Specific mortality and carrier status for each species was calculated. Any mortalities from which *L. garvieae* was not recovered were discounted from calculations.

Acknowledgements

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References

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Results

Cyprinid fish

The susceptibility of cyprinid fish to *L. garvieae*, and carrier status amongst surviving fish are summarised in Table 1.

Moribund and dead fish showed only a few signs of infection, including changes in swimming behaviour (lethargy, loss of orientation and surface swimming) and at times tail and fin erosion. Slight exophthalmia was observed in tench, roach and dace and internal haemorrhages observed in tench. None of the fish exhibited gross signs of lactococcosis at the end of the trial.

Salmonids

The results of exposure of salmonid fish to *L. garvieae* by ip injection are presented in Table 2. Comparative susceptibility of these fish to different doses of bacteria is shown in Figure 1. The results of exposure of salmonid fish to *L. garvieae* by cohabitation are given in Table 3.

All dead and moribund salmonids showed classic lactococcosis signs including exophthalmia, melanosis, internal haemorrhages, ascitis and enlarged spleen. All surviving fish, however, looked healthy.

Table 1: Mortality and carrier states in selected cyprinid fish exposed to *Lactococcus garvieae* by intra-peritoneal injection or cohabitation with infected fish

Challenge method	Fish species	Number of fish at D0[1]	Specific mortality (%)	Number of survivors at DF[2]	Carrier rate (% of surviving population)
Intra-peritoneal injection (1.09×10^6 CFU/fish)	Tench	29	6.9	27	22.2
	Carp	30	3.3	29	31.0
	Rudd	30	0	30	3.3
	Bream	31	3.2	30	40.0
	Chub	30	13.3	26	11.5
	Dace	31	19.4	25	20.0
	Roach	29	10.3	26	19.2
	Rainbow trout	10	100	0	-
Cohabitation with infected rainbow trout (8.5×10^3 CFU/fish)	Chub	33	3.0	31	3.2
	Rudd	30	3.3	28	0
	Roach	22	4.5	20	5.0
	Rainbow trout	30	76.7	7	57.1

Note 1: D0 = day of the intra-peritoneal injection challenge
Note 2: DF = day of the termination of the trial (respectively D20 for ip injection and D27 for cohabitation trials)

Table 2: Mortality and carrier states in salmonid fish exposed to *Lactococcus garvieae* by intra-peritoneal injection

Challenge method	Fish species	Number of fish at D0[1]	Specific mortality (%)	Number of survivors at DF[2]	Carrier rate (% of surviving population)
Ip injection					
2.0×10^2 CFU/fish	Atlantic salmon	15	13.3	13	84.6
	Grayling	20	65	7	85.7
	Rainbow trout	20	100	-	-
2.0×10^4 CFU/fish	Atlantic salmon	15	6.7	14	85.7
	Grayling	20	90	2	100
	Rainbow trout	20	100	-	-
2.0×10^7 CFU/fish	Atlantic salmon	15	40	9	100
	Grayling	20	100	-	-
	Rainbow trout	20	100	-	-
Ip injection					
6.1×10^1 CFU/fish	Brown trout	5	20	1	100
6.1×10^3 CFU/fish	Brown trout	6	0	6	16.6
	Rainbow trout	5	80	1	0
6.1×10^6 CFU/fish	Brown trout	5	20	4	100

Note 1: D0 = day of the intra-peritoneal injection challenge
Note 2: DF = day of the termination of the trial (D24)

Table 3: Mortality and carrier states in salmonid fish exposed to *Lactococcus garvieae* using a direct or indirect cohabitation challenge

Challenge method	Fish species	Number of fish at D0[1]	Specific mortality (%)	Number of survivors at DF[2]	Carrier rate (% of surviving population)
Direct cohabitation with Rainbow trout infected by ip injection (1.6×10^4 CFU/fish)	Atlantic salmon	57	0	54	18.5
	Grayling	60	40	22	54.5
	Rainbow trout	30	93.3	1	100
Direct cohabitation with Rainbow trout infected by ip injection (1.0×10^6 CFU/fish)	Brown trout	60	0	60	76.6
	Grayling	57	40.3	34	35.3
Indirect cohabitation with Rainbow trout infected by ip injection (1.0×10^6 CFU/fish)	Brown trout	60	1.7	57	31.6
	Grayling	59	37.3	33	18.2
	Rainbow trout	30	86.7	4	25

Note 1: D0 = day of the intra-peritoneal injection challenge
Note 2: DF = day of the termination of the trial (respectively D21 for trial direct cohab with salmon and grayling; and D24 for direct cohab with brown trout and grayling and all indirect cohabs)

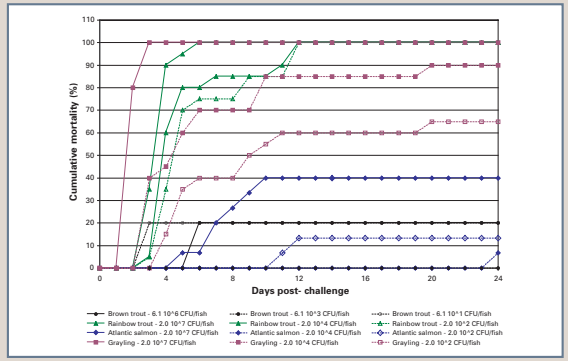

Figure 1: Comparative susceptibility of salmonid fish exposed to various doses of Lactococcus garvieae by intra-peritoneal injection

Conclusions

- With a LD50 at D20 below 2×10^2 CFU/fish, the first UK *L. garvieae* isolate is at least as virulent for rainbow trout as its Portuguese and Italian counterparts for which LD50 of 10^1-10^2 CFU per fish were reported (Ghittino *et al.*, 1998; Pereira *et al.*, 2004).
- The *L. garvieae* isolate is highly virulent for grayling. Other salmonids such as Atlantic salmon and brown trout are susceptible to the pathogen and can develop the disease
- Coarse fish such as carp, tench, barbel, chub, dace and roach can all succumb to *L. garvieae* infections. Rudd populations appeared relatively resistant with only 1 fish dying of lactococcosis in the whole of our trials.
- Covert *L. garvieae* infections can occur in fish that have survived a lactococcosis outbreak. Similar findings had been observed by Muzquiz *et al.* (1999). Our direct and indirect cohabitation challenges demonstrated that healthy carrier fish could be detected in all salmonid and cyprinid species tested apart from rudd.
- The proportion of carrier fish within a population previously exposed to *L. garvieae* was significant, particularly amongst salmonid fish (>18%). Carrier fish can act as a reservoir of the bacterium which may be shed back into the environment when conditions are favourable and transmit the infection to other fish (Austin and Austin, 1999). Carrier fish are known to play a major role in the epizootiology of other bacterial diseases such as furunculosis (Hiney *et al.*, 1997). Our findings, along with data on the relative susceptibility of various fish species, are therefore important for the management of surviving stocks and predicting the impact of lactococcosis on wild and farmed fish species.