Epizootic investigation into the presence of bacterial kidney disease (BKD) in rainbow trout farms in Scotland 2005

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SUMMARY

This report summarises the findings of an epizootic investigation carried out by the Fisheries Research Services (FRS) Fish Health Inspectorate (FHI). Routine testing led to suspicion of the presence of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (BKD) at a rainbow trout (*Oncorhynchus mykiss*) farm in Scotland in March 2005.

A programme of sampling was carried out at sites connected to the original site by live fish movements. This led to suspicion of the presence of *R. salmoninarum* at one further site, and confirmation of the presence of *R. salmoninarum* at the original site and two further sites.

The sites where the presence of *R. salmoninarum* was confirmed entered into Control and Eradication programmes overseen by the FRS FHI. One site has successfully completed the programme. The cleaning and disinfection of fish holding facilities has been completed at other sites, and these have restocked with fish from sites without movement restrictions for notifiable disease. Sampling programmes will begin at these sites in late Spring 2007.
1 INTRODUCTION

1.1 Bacterial Kidney Disease (BKD)

Bacterial kidney disease (BKD), caused by the gram positive bacterium *Renibacterium salmoninarum*, is a serious condition of both wild and farmed salmonids. BKD was first recognised in wild Atlantic salmon (*Salmo salar* (L. 1758)) caught in the Aberdeenshire Dee (known as Dee disease) and the Spey (Mackie *et al*., 1933) in the early 1930s. The causative agent was not cultured until 1956 and was initially classified as a *Corynebacterium* (Ordal and Earp, 1956). The bacterium was renamed as *R. salmoninarum* in 1980 (Sanders and Fryer, 1980). Reports of the disease originate from Canada, Chile, Japan, USA and Europe. BKD was first recorded in cultured rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) in 1976 (Bruno, 1986). Natural outbreaks of BKD have been restricted to salmonids, and despite initial recording of the disease in fresh water, the disease is recognised to be more problematic for seawater farming operations.

Possible routes of infection for the pathogen include vertical (parent to progeny) and horizontal (infection by contact) transmission, from wild fish populations and from other wild macro-fauna that act as reservoirs of *R. salmoninarum*. The pathogen is reported to survive in faeces and pond sediment for up to 21 days (Austin & Rayment, 1985). Acute outbreaks of BKD are more likely to occur in infected stocks at temperatures of 13°C to 18°C (Bullock & Herman, 1988).

BKD manifests itself in a range of ways from asymptomatic to clinical signs (Plate 1) and low level continuous mortality. Clinical signs vary between infected populations as varying susceptibility of the hosts, and differential pathogenicity of bacterial strains exists. Clinical signs of BKD include darkened body colour, mottled appearance, abdominal distension, loss of balance, exophthalmia and small petechial haemorrhages at the base of the pectoral fins. Internally, gross signs include splenomegaly, pale liver, enlargement of the gall bladder and the kidney, pale pyloric caeca and a clear or cloudy ascitic fluid. An opaque, white pseudomembrane may form around the swim bladder, liver, spleen, heart and intestinal tract. The kidney may be swollen, appear grey in colour and exhibit white nodular patches. A yellow or bloody viscous fluid may occur in the intestine (Bruno & Poppe, 1996).

Plate 1: Fish exhibiting pathological lesions consistent with BKD, including soft, granular, swollen hind kidney and an opaque white pseudomembrane over the spleen
1.2 The Legislative Framework

BKD is a List III disease under Council Directive 91/67/EEC and a notifiable disease under the Diseases of Fish Acts 1937 and 1983. Historically, Fisheries Research Services (FRS) Fish Health Inspectorate (FHI) has monitored levels of BKD in Scotland on behalf of the Scottish Executive (SE). From 1993 to 1995, 100% of freshwater fish farms holding salmonids were subject to a sample of 30 fish per year. From 1995 until May 2004, fish were not routinely tested but sites were subject to a minimum of one inspection per year, samples were taken if clinical signs of disease were observed. Since May 2004, and as a requirement of the maintenance of Additional Guarantees in respect of BKD on imports of live fish and eggs, granted by Commission Decision 2004/453/EC, the FHI has implemented a programme of routine testing for BKD on behalf of the SE.

Where reasonable grounds exist for suspecting that waters are infected, the FHI, on behalf of the SE, has the power to restrict movements of live fish on and off sites under the Diseases of Fish Act 1937 (c.33), as amended by the Diseases of Fish Act 1983 (c.30). These movement restrictions can be in the form of a Thirty Day Notice (TDN) or a Designated Area Order (DAO).

Kidney samples from 30 fish (95% confidence of sampling a minimum of one infected fish if the level of infection is 10%), taken from 50% of fish farms in Scotland holding susceptible species annually, are tested by Enzyme-Linked Immunosorbent Assay (ELISA) for the presence of an antigen of \textit{R. salmoninarum}. This sampling has been carried out in conjunction with an existing sampling regime for other notifiable pathogens. To date, 429 sites have been tested as a requirement of the programme for maintenance of Additional Guarantees.

The criteria for suspicion and confirmation of BKD, for zoning and official surveillance following suspicion or confirmation of BKD, and for the control measures to be taken in the event of suspicion of BKD, are laid down in Commission Decision 2004/453/EC. Screening for, and diagnosis of, BKD is based on ELISA and the criteria for confirmation of BKD are laid out in Table 1. Culture of \textit{R. salmoninarum} is the only method used for confirmation in the absence of a positive by any other method. The Office International des Epizooties (OIE) guidelines for confirmation of BKD also include Polymerase Chain Reaction (PCR). The PCR technique used by FRS at the time of the epizootic investigation was used in conjunction with other recognised techniques (see Table 1).

<table>
<thead>
<tr>
<th>Hist B+</th>
<th>Hist B-</th>
<th>ELISA</th>
<th>PCR</th>
<th>IFAT</th>
<th>Culture</th>
<th>S or C?</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>S</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>[469]C</td>
<td>S</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>[469]C</td>
<td>C</td>
</tr>
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<td>[469]C</td>
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<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>[469]C</td>
<td>C</td>
</tr>
</tbody>
</table>

Hist B+ = histopath consistent with BKD, Gram positive bacteria seen
Hist B- = histopath consistent with BKD, no bacteria seen
IFAT = indirect fluorescent-antibody test
S = suspicion
C = confirmation
2 OUTBREAK OF BKD IN SCOTLAND

2.1 Chronology and Diagnostic Findings

On 9 March 2005, routine testing to meet the requirements for maintenance of Additional Guarantees in respect of BKD at Site 2005/01 gave grounds for suspicion of BKD. At the time of the inspection there were approximately 721,000 rainbow trout of 2004 (70g) and 2003 (550g) year class in 18 ponds on the site. Mortality on the site at the time was elevated in two ponds, site staff suspected enteric red mouth (ERM). One of five pools of kidney tissue tested positive for BKD by ELISA (Table 2 row 1). In this case no samples were taken for bacteriological culture. No clinical signs indicative of BKD were observed during testing and the water temperature was 4.1°C. A Thirty Day Notice (TDN) was placed on Site 2005/01 restricting movement of live fish on and off the site.

Site 2005/01 was revisited on 30 March 2005 and 150 fish were sampled and tested by ELISA and bacteriological culture. The water temperature during the inspection was 6.4°C. The sample size is 150 fish, as laid down in Commision Decision 2001/183/EC, to ensure sampling of a carrier (95% confidence) if the disease is present in at least 2% of the population (Cannon and Roe, 1982). Clinical signs indicative of BKD observed included splenomegaly and a grey coloured kidney. Again, the ELISA result gave suspicion of the presence of *R. salmoninarum* (Table 2 row 2). BKD was not confirmed as there were no positive results by bacteriological culture.

Table 2: Summary of test results leading to suspicion or confirmation of the presence of *R. salmoninarum*

<table>
<thead>
<tr>
<th>Site</th>
<th>Date sampled</th>
<th>ELISA (Positive pools/no. of pools tested)</th>
<th>Culture (Positive fish/no. of fish tested)</th>
<th>PCR (Positive pools/no. of pools tested)</th>
<th>Histopathology (Positive fish/no. of fish tested)</th>
<th>Status Confirmed or suspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2005/01</td>
<td>09/03/05</td>
<td>1/5</td>
<td>None taken</td>
<td>None taken</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>2005/01</td>
<td>30/03/05</td>
<td>1/150</td>
<td>0/150</td>
<td>1/1</td>
<td>S</td>
</tr>
<tr>
<td>3</td>
<td>2005/03</td>
<td>08/04/05</td>
<td>4/23</td>
<td>2/150</td>
<td>4/4</td>
<td>C</td>
</tr>
<tr>
<td>4</td>
<td>2005/04</td>
<td>19/04/05</td>
<td>3/150</td>
<td>1/150</td>
<td>3/6</td>
<td>C</td>
</tr>
<tr>
<td>5</td>
<td>2005/05</td>
<td>20/04/05</td>
<td>1/91</td>
<td>0/150</td>
<td>0/2</td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>2005/01</td>
<td>18/05/05</td>
<td>1/4</td>
<td>3/150</td>
<td>–</td>
<td>Suspect BKD (1/6)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Kidney pathology not attributed to BKD (1/6)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2005/05</td>
<td>24/05/05</td>
<td>0/5</td>
<td>0/150</td>
<td>–</td>
<td>Suspect BKD (2/7)*</td>
</tr>
<tr>
<td>8</td>
<td>2005/03</td>
<td>26/05/05</td>
<td>None taken</td>
<td>1/150</td>
<td>–</td>
<td>Suspect BKD Histology (1/9)*</td>
</tr>
<tr>
<td>9</td>
<td>2005/04</td>
<td>31/05/05</td>
<td>None taken</td>
<td>0/150</td>
<td>–</td>
<td>None taken</td>
</tr>
</tbody>
</table>

PCR = Polymerase Chain Reaction, only carried out on ELISA-positive samples.

* Severe kidney and moderate liver pathologies indicative of bacterial kidney disease (Figure 3).

€ Mild to moderate kidney pathology indicative of a systemic bacterial infection, suspect bacterial kidney disease

# Moderate kidney pathology and the presence of small gram positive bacilli indicative of bacterial kidney disease
ELISA positives prompted an epizootic investigation to ascertain the source, and possible pathways of transmission, of the bacterium. Fish at Site 2005/01 are not moved to any other fish farm. Therefore, the epizootic investigation concentrated on sites that had supplied fish to Site 2005/01.

Site 2005/01 had received stocks from Site 2005/02 and Site 2005/03 and a site in England (Site A). One hundred and fifty fish from Site 2005/02 and 150 fish from Site 2005/03, on 7 and 8 April 2005, respectively, were sampled by ELISA and bacteriological culture. No clinical signs indicative of BKD were observed during testing at Site 2005/02 and the water temperature was 5°C. Further tests for differential diagnosis were carried out on one fish. All 150 fish from Site 2005/02 tested negative for BKD by ELISA. Bacteriological culture was completed for the fish subject to differential diagnosis and was negative. However, as ELISA results for BKD from this site were negative, all other bacteriological culture plates were discarded.

No clinical signs indicative of BKD were observed during testing at Site 2005/03 and the water temperature was 6.8°C. However, positive ELISA and bacteriological culture results were obtained from samples taken from Site 2005/03 (Table 2 row 3). Thus, the presence of *R. salmoninarum* on site was confirmed at Site 2005/03. Site 2005/03 had approximately 3.65 million rainbow trout on site and mortality was up to 7,800 fish per tank per week at the time of sampling. However, the mortalities were not attributed to BKD by site staff.

Site 2005/02 had received fish from Site 2005/03 on 24 March 2005. It was subsequently revisited and 300 fish sampled by ELISA and bacteriological culture. Again, all ELISA results were negative and the bacteriological culture plates discarded.

The Centre for Environment, Fisheries and Aquaculture Science (Cefas) Fish Health Inspectors sampled 150 fish from the supply site in England (Site A, Fig. 1) and ELISA results for BKD were negative.

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**Figure 1:** Schematic diagram of live fish movements between sites confirmed as having BKD and sites under suspicion of the presence of BKD
2.2 **Site 2005/03 as a Large Scottish Rainbow Trout Producer**

Site 2005/03 had received fish from Site A in England, Site 2005/08 and Site 2005/09, and had also received egg imports directly from certified BKD free sources. Site A in England had previously been tested due to contact with Site 2005/01. Site 2005/03 is one of the largest suppliers of fingerling rainbow trout in the UK. As such, it has supplied a total of seven rainbow trout farms in the UK with a total of approximately nine million five gram fry during the 12 month period prior to the launch of the epizootic investigation. It is estimated that Site 2005/03 is responsible for approximately 50% of Scottish rainbow trout fry production.

Movements of live fish from Site 2005/03 are primarily to on-growing sites. Sites which received fish from Site 2005/03 in the twelve month period prior to confirmation of the presence of BKD were inspected and 150 fish were sampled for BKD by ELISA and bacteriological culture at each site. Water temperatures at these sites ranged from 6°C to 10.5°C. All contact sites in England were also tested. Dates of the last live fish movement to each site taken from stock movement records are given in Table 3. A schematic diagram of movements of live fish on and off Site 2005/03 is given in Figure 1.

**Table 3: Summary of most recent live fish movements from Site 2005/03 to on-growing sites involved in this investigation**

<table>
<thead>
<tr>
<th>Recipient site</th>
<th>Date of last live fish movement</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005/07</td>
<td>04/04/05</td>
</tr>
<tr>
<td>2005/02</td>
<td>24/03/05</td>
</tr>
<tr>
<td>2005/05</td>
<td>05/03/05</td>
</tr>
<tr>
<td>2005/01</td>
<td>09/11/04</td>
</tr>
<tr>
<td>Site in England (Site B)</td>
<td>22/09/04</td>
</tr>
<tr>
<td>2005/06</td>
<td>04/09/04</td>
</tr>
<tr>
<td>2005/04</td>
<td>03/09/04</td>
</tr>
</tbody>
</table>

2.3 **Follow-up Testing**

At this point of the epizootic investigation Sites 2005/02, 2005/06, 2005/07, 2005/08 and 2005/09 had all tested negative for BKD by ELISA on 150 fish. Movement restrictions were allowed to lapse and the sites were considered BKD free. The presence of BKD in the stocks at Sites 2005/03 and 2005/04 was confirmed by bacteriological culture. Sites with positive results for BKD from the FRS investigation only are summarised in Table 2.

Acute outbreaks of BKD are more likely to occur in infected stocks at temperatures of 13°C to 18°C (Bullock & Herman, 1988). Sites 2005/01, 2005/03, 2005/04 and 2005/05 had previously tested positive by ELISA and were revisited when water temperatures were more conducive to clinical expression of BKD and 150 fish were tested (Table 2 rows 6-9). Site 2005/05 gave a suspect BKD result by histopathology (no bacteria observed) but tested negative by bacterial culture, suspicion of BKD was subsequently ruled out and movement restrictions were revoked on 20 August 2005. Site 2005/01 tested positive for BKD by bacteriological culture, the presence of BKD was therefore confirmed at this site.
Following the confirmation of the presence of *R. salmoninarum* at Site 2005/03, movements of live fish off the site were permitted to other sites confirmed as having BKD in Strathclyde region. The risk of spread of BKD was not considered to be significantly increased as the recipient site was already known to have BKD and was subject to movement restrictions.

**Plate 2:** Histological slide of a granuloma in the liver of a fish sampled at Site 2005/01. The granuloma is seen as the lighter shaded area to the left of the arrows, and a loss of structure can be observed.

### 2.4 Further Testing

In the event of suspicion of BKD, Commission Decision 2004/453/EC requires EU Member States, having Additional Guarantees on imports in respect of BKD, to place all of the farms situated in the same water catchment area or coastal area as the suspect farm under official surveillance. No fish, eggs or gametes must leave these farms without the authorisation of the official service. Thus, farms in the same water catchment area as suspect infected sites were inspected and tested. No positive results were obtained from testing farms in any of the relevant catchment areas. There is a site upstream of the Site 2005/01; however, this site was not tested as it is separated from Site 2005/01 by an area of weed bed. This area of weed bed was inspected by FRS FHI and was deemed to block the upstream migration of fish. The risk of transmission of the pathogen across this ‘barrier’ was therefore perceived to be insignificant by FRS FHI.

Testing took place in CATCHMENT 1, downstream of Site 2005/03, on the 7 September 2005, as part of a routine wild fish monitoring programme. Thirty brown trout (*Salmo trutta* (L. 1758)) were tested for BKD by culture. The results were negative. A further five brown trout, five rainbow trout and two sticklebacks (*Gasterosteus aculeatus* (L. 1758)) were sampled for BKD. The tissue from the
brown trout was pooled, the tissue from the rainbow trout was tested individually and the tissue from the sticklebacks was pooled. The pool containing kidney material from the two sticklebacks tested positive for the presence of *R. salmoninarum* by Real Time Polymerase Chain Reaction (QPCR). Culture samples were not taken from the fish in the QPCR positive pool due to lack of material. All other fish were tested by bacteriological culture and the results were negative. The significance of the single positive QPCR test from sticklebacks is unknown. There are currently no plans for further sampling of CATCHMENT 1.

Upstream of Site 2005/03 is a reservoir used as a fishery with native fish species and stocked rainbow trout. Testing took place at the reservoir, which lies approximately 600 m upstream of the water intake to Site 2005/03, on the 17 and 18 November 2005. Brown trout (77) and rainbow trout (18) of various sizes were tested by ELISA. Due to the size range of fish tested some were tested individually and some in pools of up to five fish. The total number of pools tested was 33 and all were negative by ELISA for BKD.

### 2.5 Put-and-take Fisheries

A letter was sent to all known put-and-take fisheries that had received fish from any of the suspect or confirmed sites in the previous year. The letter included a description of the clinical signs of BKD and requested that the FHI be contacted if stocks of fish were suspected of being infected with BKD. To date, the FHI has received no responses to indicate the occurrence of BKD in the stock at any of the recipient put-and-take fisheries.

### 2.6a Eradication at Site 2005/03

Site 2005/03 was subject to a progressive site clearance programme such that all stock on site at the time of confirmation of BKD was removed. The site was progressively cleaned and disinfected and restocked with fish from sites with no movement restrictions in place for notifiable disease. The site was subsequently subject to four 150-fish tests by ELISA to ascertain if the eradication programme was successful. The results from the testing programme were negative, and the movement restrictions were revoked.

### 2.6b Eradication at Site 2005/01 and Site 2005/04

Site 2005/01 and Site 2005/04 remain subject to Designated Area Orders for BKD. Plans have been submitted for cleaning and disinfection programmes in order to facilitate the revocation of the DAOs from these farms. Control and eradication programmes are on-going at these sites. Cleaning and disinfection of fish holding facilities has been completed, and the sites have restocked with fish from sites without movement restrictions for notifiable disease. Sampling for the presence of *R. salmoninarum* as part of the control and eradication programme as laid down in Commission decision 2004/453/EC will begin in late spring 2007.
3 POSSIBLE PATHWAYS FOR THE INTRODUCTION AND SPREAD OF BKD

3.1 Horizontal Transmission
It is possible that sites were infected by movements of live fish from a single point source. Transmission of BKD is known to occur via the oral route and erosion of the skin. Possible sources have been listed as (i) diseased fish, (ii) contaminated water (although the pathogen is not known to survive well outside the host in water) and (iii) contaminated feed. The connection of sites with positive test results to live fish movements originating from Site 2005/03 is consistent with Site 2005/03 being the source of infection. It was not possible to identify the source of infection at Site 2005/03. There are also difficulties in identifying the source of infection at other positive sites, as positive results were obtained from fish from multiple sources. It is possible that Site 2005/03 served as the source of infection on these sites, though there was evidence for horizontal transmission within farms.

3.2 Vertical Transmission
Evelyn et al (1986) showed that the bacteria are present in the coelomic fluid and vertical transmission via eggs is documented (Fryer & Sanders, 1981, Bruno & Munro, 1986). Infection can occur prior to ovulation directly from the ovarian tissue (Evelyn 1993). However, all rainbow trout eggs entering Site 2005/03 were from stocks, originating in the USA, certified as free from BKD.

3.3 Wild Fish and Macro Faunal Carriers
All salmonid fish species can act as vectors of BKD with varying efficacy. However, rainbow trout are thought to be one of the least susceptible salmonid species (Mitchum & Sherman, 1981). There is no method of intake water treatment currently in use at Site 2005/03, so a water-borne source of *R. salmoninarum* can not be ruled out and, as stated above, the pathogen is reported to survive in faeces and pond sediment for up to 21 days (Austin & Rayment, 1985). It should noted however, that the reservoir upstream of the water intake to Site 2005/03 tested negative for BKD by ELISA.
4 PRESENT SITUATION

BKD-related mortalities were not reported from any of the sites or waterways involved during the epizootic investigation. However, clinical signs of BKD were observed by the FHI during the epizootic investigation. Fish tested at Site 2005/03 exhibited soft, grey, swollen kidneys and fish examined at Site 2005/01 exhibited grey, granular, swollen kidneys.

Movement restrictions (Thirty Day Notice) served on all sites tested as part of the investigation have since elapsed where negative ELISA results were obtained. Designated Area Orders (DAO) have been revoked where any suspicion of BKD was not substantiated.

Site 2005/03 completed an eradication and testing programme on 27 October 2005 and the movement restrictions were revoked. The site has subsequently been inspected in February 2006, a 150 fish BKD sample by ELISA in April 2006 and a 30 fish BKD sample by ELISA in February 2007. All subsequent testing has proved negative for BKD.

The results from the testing of CATCHMENT 1 and the reservoir upstream of Site 2005/03 have not led to the suspicion or confirmation of the presence of *R. salmoninarum* in the catchment.

Site 2005/01 and Site 2005/04 currently remain subject to movement restrictions (DAO) for BKD. Plans for cleaning and disinfection of the sites were submitted in 2005 in order to facilitate the revocation of the DAOs. Control and eradication programmes are on-going. Progressive fallowing, cleaning and disinfection of the fish holding facilities has been completed at both sites. These sites have been restocked with fish from fish farms without movement restrictions for BKD. In late spring 2007 Site 2005/01 and Site 2005/04 will be subject to the first 150 fish sample for the presence of *R. salmoninarum* as part of the control and eradication programme as laid down in Commission decision 2004/453/EC.

5 ACKNOWLEDGEMENTS

The cooperation of the fish farming businesses involved in the epizootic investigation is gratefully acknowledged.
6 BIBLIOGRAPHY


Commission Decision (2001/183/EC) laying down the sampling plans and diagnostic methods for the detection and confirmation of certain fish diseases and repealing Decision 92/532/EEC.


