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Isolation And Identification Of InfectiousPancreatic Necrosis Virus From Rainbow Trout (salmo Gairdneri Richardson) In South Africa

Bragg, Robert Richard

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6. Conclusion

The procedures for the passage and propagation of four fish cell lines were established. The cell lines most suited for the isolation of viruses from trout in South Africa are the BF2 and RTG2 cells. This was based on average incubation periods for the cells to reach 100% confluency, as well as sustained susceptibility to the viruses and incubation periods needed for the viruses to produce CPE. At first, the FHM cell line had all the attributes which would make it one of the lines of choice. It was, however, discovered that this cell line inexplicably lost its susceptibility to the viruses. This loss of susceptibility could not be attributed to any changes in culture conditions, equipment, materials or passage number of the cultures. Although it is known that FHM is refractory to some serotypes of IPN virus, it was found that none of the serotypes of IPN virus, IHN virus or VHS virus would grow on this cell line once it had lost its susceptibility. No explanation could be supplied by other laboratories working with the FHM cells when approached. It was also noted that the BF2 cells began to detach and degenerate after about 14 days of incubation. This did not pose a problem as the viruses normally showed CPE after about three to four days post inoculation. This was, however problematic when attempting to isolate virus from fish. It was recommended (McDainal, 1975; Amos, 1985) that samples be inoculated for 14 days, followed by blind passage and another 14 days incubation. The BF2 cells were found to start detaching before the end of the incubation period. The incorporation of negative control wells in the 24 well plates was imperative to prevent recording of false positives which were in effect degenerating BF2 cells. This problem was not encountered with the other cell lines. Hill (1976a) recommended that cells be incubated for 7 days followed by a blind passage and incubation for an additional 7 days. During 1986, it was decided to reduce the incubation period to 7 days as used by Hill (1976a) and no further problems were encountered using the BF2 cells for the isolation of viruses from fish.

Samples of fish cell lines were also successfully frozen in liquid nitrogen for periods varying from 2 weeks to 2 years and reconstituted. Samples of viruses were also successfully stored and reconstituted. The different serotypes of IPN virus could be stored by addition of 50% glycerol, freezing at -20C or in liquid nitrogen, or by freeze drying. Samples of the other viruses were successfully stored by freezing or freeze drying. They did not survive in 50% glycerol.

The procedures for the serological identification of viruses from trout were established. It was found from sensitivity and specificity studies that the method of choice was the serum neutralization test. Both the indirect fluorescent antibody technique and the indirect immunoperoxidase assay were difficult to interpret due to non-specific staining. It was found that

the VR299, Ab and Sp serotypes of IPN virus possess sufficient common antigens to make specific identification using IFAT very difficult. Hill (1976) and Amos (1985) recommended the use of both serum neutralization and IFAT for serological identification and for this reason, IFAT was carried out on viruses isolated in South Africa.

A recently developed technique (VIBCA), although having the advantage of being very rapid, was not very sensitive and required high virus titres to be successful.

To alleviate the problem of the subjectivity of IFAT and immunoperoxidase assay, due to non-specific staining, a new technique was developed. This technique was based on the fact that Protein A, produced in the cell membrane of S. aureus Cowan strain A, binds IgG in such a way that the antigen binding sites are unaffected. Thus, a technique, based on the principles of IFAT was established. However, in this case, the indicator, being a bacterium which is easily visualized, could be counted, thus providing tangible quantification of the non-specific reactions. This technique was termed 'Immunostapnylococcus-Protein-A test'. As with fluorescent antibody techniques, there is a direct and an indirect method of doing ISPA. In the case of direct ISPA, virus specific antibodies were bound to the bacteria by simply incubating the bacteria with the antibodies.

The final and most important aspect of this work was to attempt to isolate viruses from rainbow trout in South Africa. During 1985, a virus was isolated from a farm in the Eastern Transvaal from fish imported from Denmark. The virus was serologically identified as the VR299 serotype which is unknown in Europe. Upon further investigation it was found that ova were imported onto this site from the USA and virus could be isolated from these fish. Neutralizing antibodies were detected in the serum collected from both the Danish and American imports. IPN VR299 virus was isolated again in 1985, this time from a consignment of ova imported from the USA. All importations from the USA were stopped at this stage.

During 1986, IPN VR299 virus was isolated from four sites during routine monitoring. All of these sites, except one had a history of ova imports from the same site in the USA. Up to this stage, no mortalities had been associated with IPN. A case of high mortality in fingerlings was investigated and IPN virus was isolated. Aeromonas salmonicida, a known pathogen of fish, was also isolated and the mortalities were attributed to the bacterial infection. These fingerlings had originated from ova imported from the USA in 1985.

A survey of fingerlings produced for sale was undertaken, and IPN VR299 virus was isolated from seven sites, one of which had been

tested before and found to be positive, thus confirming the original isolation. Neutralizing antibodies were detected in the serum from fish from two sites, the remaining five sites did not submit serum for testing.

The fact that IPN VR299 virus was isolated from sites in South Africa, and antibodies were found in the serum of fish from sites where the virus was isolated is conclusive evidence that IPN virus occurs in South Africa.

The virus which was isolated was the VR299 serotype, which is a North American serotype, and a similar virus was isolated from ova imported from a site in the USA. All, but one site had imported ova from this site in the USA. These facts indicate that the virus was introduced into South Africa via infected trout ova imported from the USA.

8. REFERENCES

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Appendix 1: List of ingredients in Eagles minimum essential medium, Eagles basal medium and non-essential amino acids.

Ingredient	Eagles Basal Medium mg/l	Eagles MEM mg/l	100x NEAA mg/l
L-Alanine			8.90
L-Arginine HCl	21.06	126.4	
L-Asparagine H_2O			15.00
L-Aspartic acid			13.30
L-Cystine disodium salt	14.21	28.42	
L-Glutamic acid			14.70
L-Glutamine	292.3	292.3	
Glycine			7.50
L-Histidine HCl.H_2O	10.50	41.90	
L-Isoleucine	26.23	52.50	
L-Leucine	26.23	52.50	
L-Lysine HCl	36.53	73.06	
L-Methionine	7.46	14.90	
L-Phenylalanine	16.51	33.02	
L-Proline			11.50
L-Serine			10.50
L-Threonine	23.82	47.64	
L-Tryptophan	4.08	10.20	
L-Tyrosine disodium salt	22.51	45.02	
L-Valine	23.43	46.90	
D-Ca pantothenate	1.00	1.00	
Choline chloride	1.00	1.00	
Folic acid	1.00	1.00	
i-Inositol	2.00	2.00	
Nicotinamide	1.00	1.00	
Pyridoxal HCl	1.00	1.00	
Riboflavin	0.10	0.10	
Thiamin HCl	1.00	1.00	
$CaCl_2.2H_2O$	264.9	264.9	
KCl	400.0	400.0	
$MgSO_4.7H_2O$	200.0	200.0	
NaCl	6800	6800	
$NaHCO_3$	1680	2000	
$NaH_2PO_4.2H_2O$	158.3	158.3	
D-Glucose	1000	1000	
Phenol red sodium salt	17.00	17.00	

Appendix 2. Complete list of all cases which were investigated from January 1983 to the end of August 1987.

Date	Case No.	Site	Sample	No.	Cells	Results
4/1/83	F1/83	Searun R.T.[1]	S[6]	6	RTG2	Negative
9/1/83	F3/83	NPB Himeville	S	5	RTG2	Negative
11/1/83	F4/83	Machado R.T.[2]	F[7]	3	RTG2	Negative
20/6/83	F14/83	J.H. Steyn	F	5	RTG2	Negative
15/7/83	F19/83	Machado R.T.	F	6	RTG2	Negative
25/7/83	F24/83	Misty Hills	F	8	RTG2	Negative
6/3/84	F6/84	Poortjie	S	5	FHM	Negative
5/4/84	F11/84	Waterval	F	20	FHM	Negative
22/5/84	F19/84	Pleasantways	S	4	FHM	Negative
6/9/84	F34/84	Waterval	S	4	RTG2	Negative
21/9/84	F36/84	Poortjie	F	10	RTG2/FHM	Negative
7/12/84	F44/84	G.J. Wille	F	6	RTG2/FHM	Negative
29/1/85	F2/85	Maloney's eye	F	2	RTG2	Negative
5/2/85	F5/85	TPA Lydenburg	F	9	RTG2	Negative
6/2/85	F6/85	Kritzinger	F	5	RTG2	Negative
20/2/85	F8/85	Waterval	F	5	BF2	Negative
20/2/85	F9/85	Aquacultura	F	10	BF2	Negative
27/2/85	F11/85	Machado R.T.	F	5	BF2	Negative
27/2/85	F12/85	Misty Hills	F	7	RTG2/FHM	Negative
14/3/85	F15/85	De Kuilen	F	9	RTG2/FHM	Negative
14/3/85	F16/85	Aquacultura	F	15	RTG2/FHM	Negative
14/3/85	F17/85	Maloney's eye	F	5	RTG2/FHM	Negative
15/3/85	F18/85	P. Smith	S	5	RTG2	Negative
15/3/85	F19/85	Waterval	S	1	RTG2	Negative
22/3/85	F21/85	Pleasantways	S	1	BF2	Negative
26/3/85	F22/85	Poortjie	F	1	BF2	Negative
19/4/85	F26/85	P. Smith	F	6	RTG2	Negative
1/5/85	F27/85	Maloney's eye	S	10	RTG2	Negative
28/6/85	F33/85	Waterval	Fry	55	RTG2	Positive
28/6/85	F33/86(A)	Waterval	Fry	50	RTG2/BF2	Positive
28/6/85	F33/85(D)	Waterval	Fry	50	RTG2/BF2	Positive
18/7/85	F35/85	R. Grabie	F	4	RTG2	Negative
1/8/85	F38/85	Waterval	Fry	13	RTG2	Negative
15/8/85	F46/85	Waterval	Fry	20	RTG2	Negative
22/11/85	F60/85	Maloney's eye	F	10	BF2	Negative
26/11/85	F61/85	Waterval	F	15	RTG2	Negative
13/12/85	F67/85	Misty Hills	F	9	RTG2	Negative
7/1/86	F3/86	Dr van Reener	S	4	BF2	Negative
7/1/86	F5/86	Machado R.T.	S	6	RTG2/BF2	Negative
7/1/86	F8/86	Pleasantways	S	2	RTG2/BF2	Negative
8/1/86	F9/86	Waterval	S	20	RTG2/BF2	Negative
8/1/86	F11/86	Pleasantways	S	2	BF2	Negative
9/1/86	F13/86	Waterval	S	6	BF2	Negative
9/1/86	F14/86	K. Combrink	S	2	BF2	Negative
13/1/86	F15/86	J.L.B. Smith	F	6	RTG2/FHM	Negative
12/2/86	F31/86	Jonkershoek	Fry	6	RTG2/BF2	Negative

Date	Case No.	Site	Sample	No.	Cells	Results
18/2/86	F33/86	Fisantekraal	Fry	15	RTG2/BF2	Negative
26/2/86	F36/86	Fisantekraal	Fry	19	RTG2/BF2	Negative
27/2/86	F37/86	Waterval	Fry	30	RTG2	Negative
27/2/86	F38/86	P. Smith	F	6	RTG2	Negative
6/3/86	F41/86	Dr van Reener	F	4	BF2	Negative
4/6/86	F66/86	Bushmans R.T.[3]	B.S.	4	BF2	Negative
9/6/86	F69/86	Fisantekraal	Fry	35	RTG2	Negative
19/6/86	F70/86	Waterval	S	2	RTG2	Negative
21/7/86	F85/86	P. Smith	S	1	RTG2	Negative
21/7/86	F86/86	Mountain	S	3	RTG2	Negative
21/7/86	F87/86	P. Smith	S	10	RTG2	Negative
24/8/86	F95/86	Waterval	Fry	50	RTG2	Negative
24/8/86	F96/86	Waterval	Fry	40	BF2	Negative
24/8/86	F97/86	Waterval	Fry	25	RTG2	Negative
28/8/86	F99/86	Machado R.T.	F	8	RTG2	Negative
28/8/86	F100/86	Machado R.T.	F	10	RTG2	Positive
3/9/86	F102/86	Waterval	Fry	110	RTG2/BF2	Negative
3/9/86	F103/86	Waterval	Fry	25	BF2	Negative
3/9/86	F106/86	Mondi Hatchery	Fry	50	RTG2/BF2	Negative
3/9/86	F107/86	Mondi Hatchery	Fry	40	RTG2/BF2	Negative
3/9/86	F108/86	Mondi Hatchery	Fry	65	RTG2/BF2	Negative
3/9/86	F109/86	Mondi Hatchery	Fry	25	RTG2/FHM	Negative
3/9/86	F110/86	Mondi Hatchery	Fry	95	RTG2/FHM	Positive
3/9/86	F111/86	Mondi Hatchery	Fry	5	RTG2/FHM	Negative
3/9/86	F112/86	Mondi Hatchery	Fry	5	BF2/FHM	Positive
3/9/86	F113/86	Mondi Hatchery	Fry	2	BF2/FHM	Negative
3/9/86	F114/86	TPA[4] Lydenburg	Fry	75	BF2/FHM	Negative
3/9/86	F115/86	TPA Lydenburg	Fry	30	RTG2/BF2	Negative
3/9/86	F116/86	TPA Lydenburg	Fry	15	RTG2/BF2	Negative
3/9/86	F117/86	TPA Lydenburg	Fry	30	RTG2/BF2	Negative
3/9/86	F118/86	TPA Lydenburg	Fry	75	RTG2/BF2	Negative
3/9/86	F119/86	Kingfisher	Fry	25	RTG2/BF2	Positive
3/9/86	F120/86	Kingfisher	Fry	25	RTG2/BF2	Negative
3/9/86	F121/86	Kingfisher	Fry	25	RTG2/BF2	Negative
3/9/86	F122/86	Sperwing	Fry	25	RTG2/FHM	Positive
3/9/86	F123/86	Sperwing	Fry	25	RTG2/FHM	Negative
3/9/86	F124/86	Winifred	Fry	25	RTG2/FHM	Negative
3/9/86	F125/86	Katrina's Rust	Fry	30	BF2/FHM	Positive
3/9/86	F126/86	Katrina's Rust	Fry	20	BF2/FHM	Positive
3/9/86	F127/86	Katrina's Rust	Fry	150	BF2/FHM	Positive
3/9/86	F128/86	Fisantekraal	Fry	5	RTG2/BF2	Negative
3/9/86	F129/86	Fisantekraal	Fry	10	RTG2/BF2	Negative
3/9/86	F130/86	Fisantekraal	Fry	15	RTG2/BF2	Positive
24/9/86	F135/86	Fisantekraal	Fry	15	RTG2/BF2	Positive
1/10/86	F136/86	G. Rudert	B.S.	3	RTG2/BF2	Negative
9/11/86	F137/86	Aquaculture	S	4	RTG2/BF2	Negative
9/11/86	F138/86	P. Smith	F	6	RTG2/BF2	Negative
18/11/86	F149/86	Fisantekraal	F	15	RTG2/BF2	Negative

Date	Case	Site	Sample	No	Cells	Results
1/12/86	F152/86	Mountain	S	40	RTG2/BF2	Negative
4/12/86	F153/86	La Rochelle	F	30	RTG2/BF2	Negative
4/12/86	F154/86	La Rochelle	S	150	RTG2/BF2	Negative
17/12/86	F155/86	Maloney's eye	F	10	RTG2/BF2	Negative
18/12/86	F156/86	Maloney s eye	F	5	RTG2/BF2	Negative
6/1/87	F1/87	Aquacultura	S	34	RTG2/BF2	Negative
8/1/87	F2/87	P. Smith	F	6	RTG2/BF2	Negative
14/1/87	F4/87	Zimbabwe	Fry	15	RTG2/BF2	Negative
27/1/87	F15/87	Zimbabwe	Fry	10	RTG2/BF2	Negative
29/1/87	F18/87	Waterval	S	15	RTG2/BF2	Negative
6/2/87	F26/87	Waterval	Fry	70	RTG2/BF2	Negative
6/2/87	F28/87	Waterval	S	3	RTG2/BF2	Negative
6/2/87	F32/87	Waterval	W.F.	3	RTG2/BF2	Negative
6/2/87	F33/87	Waterval	S	2	RTG2/BF2	Negative
6/2/87	F34/87	Waterval	S	3	RTG2/BF2	Negative
13/2/87	F39/87	Waterval	F	5	RTG2/BF2	Negative
17/2/87	F41/87	Maloney's eye	Fry	30	RTG2/BF2	Negative
23/2/87	F43/87	Maloney's eye	S	5	RTG2/BF2	Negative
23/2/87	F44/87	Maloney's eye	Fry	30	RTG2/BF2	Negative
25/2/87	F45/87	Waterval	Fry	45	RTG2/BF2	Negative
23/3/87	F57/87	Waterval	S	15	RTG2/BF2	Negative
2/4/87	F63/87	W.J. Snyman	F	45	RTG2/BF2	Negative
3/4/87	F64/87	Waterval	Fry	50	RTG2/BF2	Negative
3/4/87	F65/87	Waterval	F	1	RTG2/BF2	Negative
23/4/87	F75/87	Machado R.T.	S	4	RTG2/BF2	Negative
23/4/87	F76/87	Machado R.T.	S	5	RTG2/BF2	Negative
23/4/87	F82/87	Waterval	Fry	25	RTG2/BF2	Negative
13/5/87	F85/87	Fisantekraal	S	5	RTG2/BF2	Negative
13/5/87	F86/87	Fisantekraal	S	5	RTG2/BF2	Negative
21/5/87	F89/87	Piri	Fry	25	RTG2/BF2	Negative
25/5/87	F90/87	Waterval	S	5	RTG2/BF2	Negative
6/7/87	F103/87	Maloney's eye	S	4	RTG2/BF2	Negative
16/7/87	F112/87	Dewdale	B.S.	3	RTG2/BF2	Negative
16/7/87	F118/87	NPB Kamberg	F	85	RTG2/BF2	Negative
23/7/87	F121/87	Elandspad	S	25	RTG2/BF2	Negative
24/7/87	F122/87	Maloney's eye	S	5	RTG2/BF2	Negative
3/8/87	F124/87	Fisantekraal	S	4	RTG2/BF2	Negative
3/8/87	F129/87	Waterval	Fry	60	RTG2/BF2	Negative
3/8/87	F130/87	Waterval	Fry	60	RTG2/BF2	Negative
3/8/87	F131/87	Waterval	Fry	100	RTG2/BF2	Negative
6/8/87	F136/87	Machado R.T.	Fry	100	RTG2/BF2	Negative
21/8/87	F142/87	RVL[5] Allerton	S	15	RTG2/BF2	Negative
24/8/87	F143/87	Elandspad	Fry	200	RTG2/BF2	Negative

1 Searun Rainbow Trout Farm 2 Machado Rainbow Trout Farm
3 Bushmans River Trout Farm 4 Transvaal Provincial Administration
5 Regional Veterinary Laboratory 6 Slaughter fish
7 Fingerling

Appendix 3. List of all localities from which viscera of asymptomatic fish were collected for virus isolation from January 1983 to the end of August 1987.

Date	Site	Age	Sample	Number	Cells	Results
5/6/85	Waterval	Fry	W[4]	150	BF2	Negative
5/6/85	Waterval	S[5]	V[3]	150	BF2	Negative
5/6/85	Aquacultura	S	V	150	BF2	Negative
5/6/85	Aquacultura	S	V	30	BF2	Negative
5/6/85	Pleasantways	B.S.[1]	V	15	BF2	Negative
6/6/85	La Rochelle	S	V	150	BF2	Negative
6/6/85	La Rochelle	F[2]	V	50	BF2	Negative
6/6/85	Mondi Hatchery	Fry	W	50	BF2	Negative
21/1/86	La Rochelle	S	V	115	RTG2/BF2	Negative
21/1/86	Aquacultura	S	V	150	RTG2/BF2	Negative
21/1/86	Waterval	S	V	150	RTG2/BF2	Negative
21/1/86	Pleasantways	F	V	90	RTG2/BF2	Negative
21/1/86	Pleasantways	S	V	5	RTG2/BF2	Negative
21/1/86	S. Gifford	F	V	85	RTG2/BF2	Negative
21/1/86	S. Gifford	S	V	25	RTG2/BF2	Negative
26/2/86	P. Smith	S	V	150	RTG2/BF2	Negative
3/6/86	Bushmans R.T.[6]	F	W	30	RTG2/BF2	Negative
3/6/86	Bushmans R.T.	S	V	126	RTG2/BF2	Negative
3/6/86	Rainbow Lakes	B.S.	V	7	RTG2/BF2	Negative
4/6/86	P. Smith	S	V	40	RTG2/BF2	Negative
9/6/86	Fisantekraal	S	V	150	RTG2/BF2	Positive
16/7/86	Mountain	S	V	130	RTG2/BF2	Negative
16/7/86	La Rochelle	S	V	120	RTG2/BF2	Positive
16/7/86	P. Smith	S	V	115	RTG2/BF2	Negative
17/7/86	TPA[7] Lydenburg	Fry	W	140	RTG2/BF2	Positive
17/7/86	de Kuilen	Fry	W	160	RTG2/BF2	Negative
18/7/86	Pleasantways	Fry	W	25	RTG2/BF2	Negative
19/7/86	Aquacultura	S	V	150	RTG2/BF2	Negative
5/8/86	Waterval	Fry	W	85	RTG2/BF2	Negative
11/9/86	Bushmans R.T.	Fry	W	495	RTG2/BF2	Negative
29/1/87	Driehoek	S	V	150	RTG2/BF2	Negative
29/1/87	Waterval	B.S.	V	150	RTG2/BF2	Negative
29/1/87	Katrina's Rust	S	V	150	RTG2/BF2	Negative
29/1/87	Aquacultura	S	V	150	RTG2/BF2	Negative
6/2/87	La Rochelle	F	V	150	RTG2/BF2	Negative
6/2/87	La Rochelle	B.S.	V	150	RTG2/BF2	Negative
6/2/87	TPA Lydenburg	F	V	150	RTG2/BF2	Negative
5/3/87	de Kuilen	B.S	V	150	RTG2/BF2	Negative
5/3/87	Lydenburg	B.S.	V	150	RTG2/BF2	Negative
5/3/87	de Kuilen	B.S.	V	150	RTG2/BF2	Negative
5/3/87	Pleasantways	B.S.	V	144	RTG2/BF2	Negative
5/3/87	P. Smith	S	V	150	RTG2/BF2	Negative
5/3/87	Mountain	B.S.	V	50	RTG2/BF2	Negative
3/4/87	L. T. Beck[8]	F	V	50	RTG2/BF2	Negative
3/4/87	L. T. Beck	F	V	150	RTG2/BF2	Negative

Date	Site	Age	Sample	No	Cells	Results
23/4/87	Kingfisher	F	V	150	RTG2/BF2	Negative
23/4/87	Schoonspruit	Fry	W	150	RTG2/BF2	Negative
23/4/87	Sperwing	Fry	W	150	RTG2/BF2	Negative
23/4/87	Sperwing	F	V	25	RTG2/BF2	Negative
23/4/87	Sperwing	F	V	125	RTG2/BF2	Negative
18/6/87	Katrina's Rust	F	V	10	RTG2/BF2	Negative
24/6/87	La Rochelle	F	V	150	RTG2/BF2	Negative
13/7/87	Qwa Qwa	F	V	20	RTG2/BF2	Negative
16/7/87	Waterval	Fry	V	500	RTG2/BF2	Negative
16/7/87	P. Doyle	F	V	150	RTG2/BF2	Negative
29/7/87	Aquacultura	S	V	150	RTG2/BF2	Negative
29/7/87	Waterval	S	V	50	RTG2/BF2	Negative
29/7/87	Waterval	S	V	150	RTG2/BF2	Negative
13/8/87	Rainbow Lakes	B.S.	V	21	RTG2/BF2	Negative

1 = Brood stock
2 = Fingerling
3 = Viscera (Liver, spleen & kidney)
4 = Whole fish (Fry too small to eviscerate)
5 = Slaughter fish (and less than 1 year old)
6 = Bushmans R.T. = Bushmans River Trout Farm
7 = Transvaal Provincial Administration
8 = Little Trout Beck

Appendix 4. List of all localities from which ovarian fluid samples from asymptomatic fish were collected for virus isolation from January 1983 to the end of August 1987

Date	Site	Number	Cells	Results
5 6/85	Pleasantways	215	BF2	Negative
7/6/85	de Kuilen	150	BF2	Negative
4/7/85	Bushmans R.T.[1]	45	BF2	Negative
19/7/85	Rainbow Lakes	110	BF2	Negative
2/3/86	Bushmans R.T.	85	RTG2/BF2	Negative
3/5/86	Fisantekraal	35	RTG2/BF2	Negative
3/6/86	Bushmans R.T.	85	RTG2/BF2	Negative
3/6/86	Rainbow Lakes	30	RTG2/BF2	Negative
5/6/86	Pleasantways	35	RTG2/BF2	Negative
7/6/86	de Kuilen	150	RTG2/BF2	Negative
9/6/86	Fisantekraal	45	RTG2/BF2	Negative
27/6/86	Rainbow Lakes	130	RTG2/BF2	Negative
4/7/86	Bushmans R.T.	50	RTG2/BF2	Negative
16/7/86	La Rochelle	10	RTG2/BF2	Negative
18/7/86	Pleasantways	120	RTG2/BF2	Negative
19/7/86	Rainbow Lakes	180	RTG2/BF2	Negative
24/7/86	Aquacultura	165	RTG2/BF2	Positive
3/8/86	Waterval	75	RTG2/BF2	Negative
3/9/86	Pleasantways	20	RTG2/BF2	Negative
3/9/86	Driehoek	90	RTG2/BF2	Negative
12/6/87	de Kuilen	150	RTG2/BF2	Negative
12/6/87	Katrina's Rust	40	RTG2/BF2	Negative
17/6/87	Gwa Gwa	160	RTG2/BF2	Negative
18/6/87	Katrina's Rust	45	RTG2/BF2	Negative
18/6/87	Pleasantways	25	RTG2/BF2	Negative
13/7/87	Pleasantways	45	RTG2/BF2	Negative
13/7/87	TPA Lydenburg	60	RTG2/BF2	Negative
13/7/87	TPA Lydenburg	65	RTG2/BF2	Negative
13/7/87	Pleasantways	30	RTG2/BF2	Negative
16/7/87	Blyde River	20	RTG2/BF2	Negative
16/7/87	TPA[2] Lydenburg	85	RTG2/BF2	Negative
16/7/87	Pleasantways	110	RTG2/BF2	Negative
16/7/87	Bushmans R.T.	160	RTG2/BF2	Negative
23/7/87	Elandspad	40	RTG2/BF2	Negative
3/8/87	Fisantekraal	10	RTG2/BF2	Negative
3/8/87	Blyde River	22	RTG2/BF2	Negative

1 = Bushmans River Trout Farm
2 = Transvaal Provincial Administration

Appendix 5. List of all samples of ova, both imported and local, examined for the presence of viruses from January 1983 to the end of August 1987.

Date	Case No	Source	No	Cells	Results
11/7/83	F18/83	de Kuilen	1000	RTG2	Negative
14/2/85	F9/85	U.S.A.	200	RTG2	Negative
6/3/85	F13/85	U.S.A.	200	RTG2/BF2	Negative
2/11/85	F56/85	U.S.A.	200	RTG2/BF2	Positive
3/1/86	F1/86	U.S.A.	200	RTG2/BF2	Negative
10/1/86	F10/86	England	200	RTG2/BF2	Negative
9/4/86	F51/86	England	200	RTG2/BF2	Negative
29/4/86	F55/86	England	200	BF2	Negative
1/5/86	F56/86	Denmark	200	RTG2/BF2	Negative
3/5/86	F57/86	U.S.A.	200	RTG2/BF2	Negative
3/5/86	F59/86	U.S.A.	200	RTG2/BF2	Negative
18/11/86	F150/86	England	200	RTG2/BF2	Negative
15/1/87	F5/87	Denmark	200	RTG2/BF2	Negative
16/1/87	F9/87	Denmark	200	RTG2/BF2	Negative
27/1/87	F17/87	England	200	RTG2/BF2	Negative
6/2/87	F27/87	Denmark	200	RTG2/BF2	Negative
10/3/87	F53/87	England	200	RTG2/BF2	Negative
12/3/87	F54/87	England	200	RTG2/BF2	Negative
3/4/87	F66/87	Denmark	200	RTG2/BF2	Negative
3/4/87	F67/87	Denmark	200	RTG2/BF2	Negative
16/4/87	F74/87	Denmark	200	RTG2/BF2	Negative
3/8/87	F132/87	Waterval	200	RTG2/BF2	Negative

Author Bragg Robert Richard
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