Supporting Information

Kerr *et al.* The next step in the on-going arms race between myxoma virus and wild rabbits in Australia is a novel disease phenotype.

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Supplementary Text: Clinical syndromes

We observed four broad clinical syndromes (**Table 1**, **Fig. 1** and **Table S1**).

(A). **Typical cutaneous (nodular) myxomatosis**. Large cutaneous primary lesions at the inoculation sites, secondary raised cutaneous lesions on face, ears, legs and body, swollen, closed eyelids with varying levels of mucopurulent discharge, swollen ears, heads and ano-genital regions (**Fig. 1*A.1***). This syndrome was seen with the viruses from the early 1950s: SLS, Ur and KM13. Rabbits infected with SLS were euthanized or died between days 10-14 post-infection (**Fig 1*B***). Temperatures typically rose from days 4-6 and stayed elevated often falling below normal shortly before euthanasia (**Fig. S6B**).

(B) **Acute collapse syndrome**. Rabbits became moribund over a few hours and died between days 10-15 with only minor signs of typical myxomatosis (**Fig. 1*A.2*** and **Fig. 1*B***). Viral inoculation site was poorly differentiated from the surrounding skin and different to the raised inflamed primary lesion seen in the cutaneous disease (**Fig. S*3A***). Secondary cutaneous lesions were absent. Massive pulmonary oedema (**Fig. 1*C***), often with haemorrhage (**Fig. S3*D***) or severe swelling and haemorrhage of one hind leg was typical (**Fig. 1*D***), as well as pale and swollen liver (**Fig. S3*E***), subcutaneous oedema (**Fig. S3*B***), and bleeding from minor injuries (**Fig. S3*C***). This syndrome was seen in rabbits infected with all but two of the viruses from the 1990s. The phenotype is strikingly distinct from that seen with the progenitor SLS virus or those from the early radiation (1). One virus, Meby, which had evolved separately for more 20 years from the other viruses analysed here, had an intermediate phenotype with some acute collapse but elements of the cutaneous form.

(C) **Prolonged survival with progressive "amyxomatosis" myxomatosis**. This was seen in animals infected with viruses from the 1990s that survived longer than 14-16 days (**Fig. 1*B***). Delineation of the primary lesion at the inoculation site did not occur, and secondary cutaneous lesions were rare and only developed very late in the course of the disease. This disease syndrome resembled the amyxomatous phenotype described in some European MYXV isolates (2-5).

(D) **Attenuated cutaneous myxomatosis**. Rapid development of mild symptoms such as raised primary lesion, mild eyelid and ear swelling, early high fever, and small secondary lesions on ears and eyelids. Recovery occurred 10-15 days after infection (**Fig. 1*A4*** and **Fig. 1*B***). This syndrome developed in rabbits infected with the modern grade 5 OB3 Y317 and in one rabbit that survived infection with the Meby strain.

Supplementary Text: Additional bacteriology background and results

Although secondary infection with gram-negative bacteria such as *Pasturella multocida* or *Bordetella bronchiseptica* is common in wild rabbits and domestic rabbits infected with virulent MYXV such as SLS, infection is normally limited to the upper respiratory tract and conjunctivae; internal organs are normally sterile (6, 7) and treatment with antibiotics makes no difference to the outcome of the MYXV infection (8). In rabbits infected with more attenuated viruses, secondary bacterial pneumonia is not uncommon. Indeed, secondary bacteria were critical to the apparent virulence of so called amyxomatous strains of MYXV isolated from domestic rabbit farms and tested in Belgium (3, 4).

Nasal swabs were cultured from a sample of 31 rabbits on arrival in the PSU facility, 10 days prior to infection; only 1 was found positive for *Staphylococcus aureus*, which was sensitive to enrofloxacin. At day of infection (day 0), nasal swabs were collected and cultured from all 48 rabbits and all were negative for *S. aureus.* At day 10 all 48 rabbits were again swabbed and samples cultured: all rabbits in the control groups had mixed growth but were negative for *S. aureus*. Rabbits in the enrofloxacin treated groups either had no growth on culture (13 rabbits) or had scanty to moderate growth of coagulase negative *Staphylococcus sp* (11 rabbits). Swabs collected at autopsy from the bronchus of 3 control rabbits grew *S. aureus*, *Enterococcus* and *Pseudomonas aeruginosa* as well as *E. coli*. It is likely that some of these reflected background faecal contamination.

Supplementary Text: Additional materials and methods

Rabbits

Male, outbred New Zealand White laboratory rabbits (*Oryctolagus cuniculus*) 4 months of age were purchased from Harlan Laboratories (USA). The rabbits were specific pathogen free for a large number of pathogens and internal and external parasites. Crucially for the purposes of this study they were free of *Bordetella bronchiseptica* and *Pasteurella multocida*, the common causes of secondary disease in rabbits with myxomatosis.

Outcomes of infection with viruses such as SLS, Ur and BRK were similar to previous work in outbred crossbred laboratory rabbits undertaken in Australia (9, 10) and to the work originally done by Fenner with crossbred laboratory rabbits and New Zealand whites. All procedures were approved by The Pennsylvania State University Institutional Animal Care and Use Committee – permit numbers: 33615, 42748, 46919.

Virulence phenotyping

Two separate trials were conducted to examine virus phenotypes. Trial 1 consisted of 8 viruses and trial 2 consisted of 10 viruses; two viruses, BD23 and BRK 12/2/93, were tested in both trials (**Table 1**). A total of 100 pfu of virus in 100 µl of PBS was injected intradermally into the rump on the right hand side of each of 6 rabbits. Each rabbit was examined daily and, from d10 post-infection monitored twice daily. Food and water intake were monitored. Examination included rectal temperature, body weight, primary cutaneous lesion size and shape at the inoculation site and secondary cutaneous lesion distribution plus semi quantitative scoring on a 0 to 3 scale for clinical parameters including: demeanour, eyelid swelling, ear swelling, anogenital swelling, scrotal oedema, blepharoconjunctivitis, nasal discharge and respiratory difficulty.

In trial 1, rabbits were biopsied at the primary inoculation site at 5 day intervals using a disposable dermal punch biopsy and local anaesthesia with bupivacaine and samples used to measure virus loads by qPCR (**Figure S7**) (5). In trial 2, rabbits were blood sampled for haematology at days 0 and 10. We did not collect biopsies in the second trial to determine whether the biopsy procedure was responsible for bacterial infections (although it was not an issue with SLS, UR, or KM13 infected rabbits in trial 1). Dead and euthanized animals were autopsied as soon as possible after death and tissue samples collected for histology. Survival times for euthanized rabbits were inferred as described below. Rabbits that died overnight were autopsied but not further sampled because of autolysis and potential bacterial contamination of samples. The results for the two viruses in both trials, BD23 and BRK 12/2/93, gave similar results.

Antibacterial treatments

If bacterial sepsis was critical in the pathogenesis of the disease then limiting the bacterial infection should extend survival times. To test this hypothesis, groups of 12 rabbits were each infected with SLS (1950 progenitor virus; grade 1) or three of the viral strains from the 1990s that caused the acute mortality syndrome, BRK 4/93 (1993; grade 1), BD23 (1999; grade 2) and WS6 1071(1995; grade 3). In each group, six rabbits were treated with the broad-spectrum antibacterial enrofloxacin from day 5 to day 16 after infection at a dose of 26.25 mg twice daily. The remaining 6 rabbits were treated with PBS as a control.

Bacteriological monitoring was done on nasal swabs at days -10, 0 and 10. Rabbits were monitored twice daily and all dead and euthanized rabbits autopsied and sampled for analysis. Animals that died overnight were autopsied but not further sampled.

Defining endpoints for euthanasia

Fenner's original experiments used death as an endpoint. Because this is no longer ethically acceptable, we defined endpoints for euthanasia based on detailed clinical examination. Rabbits were euthanized based on the degree of clinical severity using respiratory difficulty, depression, inanition, reluctance to move, weakness on handling, weight loss and failure to eat or drink as indicators; any rabbit exhibiting pain or with a subnormal temperature was immediately euthanized. This means our virulence grades are necessarily inferred. For rabbits that were moribund or recently dead, no correction was made to survival times. This involved all rabbits with acute collapse syndrome because onset is very acute and death rapid. For rabbits that were euthanized, survival times were estimated based on the degree of severity of clinical signs and rapidity of change and corrections of 12 to 48 hours added to the time of euthanasia. This becomes a difficult judgement call only for longer surviving rabbits where somewhat subjective welfare-based decisions are unavoidable. Because we were trying to minimize animal suffering, we may have euthanized some of these animals early and so will underestimate average survival times. Our conclusions are unaffected if we underestimated survival times for long-surviving rabbits. For rabbits found dead, time of death was taken as the midpoint between observations. Average survival times (AST) were normalized from inferred survival times transformed using $\log_{10}$(ST-8) and the average survival time back-transformed; where necessary, survivors were allocated a ST of 60 days (1). Some rabbits with prolonged clinical courses (clinical syndrome C, see **Supplementary Text** above) had to be euthanized under our Institutional Animal Care and Use procedures but may have survived. This possible over-estimate of the lethality of a small group of rabbits with syndrome C does not affect our conclusions.

Bibliography

1. Fenner F & Marshall ID (1957) A comparison of the virulence for European rabbits (*Oryctolagus cuniculus*) of strains of myxoma virus recovered in the field in Australia, Europe and America. *J Hyg (Camb)* 55:149-191.
2. Joubert L, Duclos P, & Toaillen P (1982) La myxomatose des garennes dans le sud-est. La myxomatose amyxomateuse. *Rev Méd Vét* 133:739-753.
3. Marlier D*, et al.* (2000) Study of the virulence of five strains of amyxomatous myxoma virus in crossbred New Zealand White/Californian conventional rabbits, with evidence of long-term testicular infection in recovered animals. *J Comp Path* 122:101-113.
4. Marlier D, Cassart D, Boucrat-Baralon C, Coignoul F, & Vindevogel H (1999) Experimental infection of specific pathogen-free New Zealand white rabbits with five strains of amyxomatous myxoma virus. *J Comp Path* 121:369-384.
5. Kerr PJ*, et al.* (2017) Genomic and Phenotypic characterization of myxoma virus from Great Britain reveals multiple evolutionary pathways distinct from those in Australia. *PLoS Pathog* 13:e1006252.
6. Hobbs JR (1928) Studies of the nature of infectious myxoma of rabbits. *Am J Hyg* 8:800-839.
7. Rivers TM (1930) Infectious myxomatosis of rabbits: observations on the pathological changes induced by virus myxomatosis (Sanarelli). *J Exp Med* 51:965-975.
8. McKercher DG (1952) Infectious myxomatosis. I. Vaccination. II. Antibiotic therapy. *American J Vet Res* 13:425-429.
9. Best SM & Kerr PJ (2000) Coevolution of host and virus: the pathogenesis of virulent and attenuated strains of myxoma virus in resistant and susceptible European rabbits. *Virology* 267:36-48.
10. Saint KM, French N, & Kerr P (2001) Genetic variation in Australian isolates of myxoma virus: an evolutionary and epidemiological study. *Arch Virol* 146:1105-1123.
11. Upton C, Macen JL, Schreiber M, & McFadden G (1991) Myxoma virus expresses a secreted protein with homology to the tumour necrosis factor receptor gene family that contributes to viral virulence. *Virology* 184:370-382.
12. Macen J, L., Upton C, Nation N, & McFadden G (1993) Serp 1, a secreted proteinase inhibitor encoded by myxoma virus is a secreted glycoprotein that interferes with inflammation. *Virology* 195:348-363.
13. Mossman K, Fong Lee S, Barry M, Boshkov L, & McFadden G (1996) Disruption of M-T5, a novel myxoma virus gene member of the poxvirus host range superfamily, results in dramatic attenuation of myxomatosis in infected European rabbits. *J Virol* 70:4394-4410.
14. Liu J, Wennier S, Zhang L, & McFadden G (2011) M062 is a host range factor essential for myxoma virus pathogenesis and functions as an antagonist of host SAMD9 in human cells. *J Virol* 85:3270–3282.
15. Barrett JW*, et al.* (2007) Myxoma virus M063R is a host range gene essential for virus replication in rabbit cells. *Virology* 361:123-132.
16. Blanié S, Mortier J, Delverdier M, Bertagnoli S, & Camus-Bouclainville C (2009) M148R and M149R are two virulence factors for myxoma virus pathogenesis in the European rabbit. *Vet Res* 40:11.
17. Messud-Petit F*, et al.* (1998) Serp 2, an inhibitor of the interleukin-1b-converting enzyme, is critical in the pathobiology of myxoma virus. *J Virol* 72:7830-7839.
18. Guindon S*, et al.* (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* 59:307-321.

Table S1. Clinical and autopsy summary

Virus Year isolated Virulence grade	**Clinical disease**	**Autopsy findings**
SLS 1950 1	Severe cutaneous myxomatosis; eyelids swollen closed; ears swollen and drooping; moderate to severe ano-genital swelling; scrotal oedema; large, raised, well defined primary lesion; secondary lesions over body, legs, ears, head; obstruction of upper respiratory tract; loss of body weight; depression.	Very enlarged lymph nodes and spleen; lungs generally grossly normal; 1/6 had excessive bloody fluid in the pericardial sac. 2/6 had some white scarring on the liver surface. Very large, well differentiated primary lesion. No bacteria seen in histological sections.
KM13 1952 5	Cutaneous myxomatosis; onset somewhat delayed and clinical signs more severe than Ur but variation within the group; some rabbits had complete eye closure; one rabbit euthanized and two were seriously ill at the termination of the trial at day 32.	Several rabbits had pneumonia at autopsy and excess abdominal fluid – no bacteria or cells in abdominal fluid.
Ur 1953 5	Cutaneous myxomatosis; rapid onset; large raised primary lesion; swollen eyelids and ears; eyelids not completely closed; swollen head; some mucoid to mucopurulent ocular and nasal discharge; large numbers of secondary lesions on head, ears, eyelids, legs, feet, body; scrotal oedema. Eyelids becoming granulomatous. Considerable variation within the group. Rabbits began to recover between d12-15 but many had elevated temperatures. No deaths.	2 rabbits had evidence of pneumonia at autopsy at day 32; bacterial cultures were negative.
BRK 4/93 1993 1	Moderate clinical signs at day 10: eyelids 1/2 to 2/3 closed; ears mildly to moderately swollen; heads swollen; all died acutely between d10-12.	Pulmonary oedema/haemorrhage; hind leg oedema/haemorrhage; haemorrhages over intestinal walls, thymus. Bacteria in LNs, spleen; liver, lungs.

BD44 1999 1	Slow onset of relatively mild myxomatosis; day 11: eyelids 2/3 closed; ears swollen; mild to moderate anogenital swelling; rabbits quiet; 4/6 acute deaths between days 11-12 and the remaining acute deaths by d15.	Pulmonary oedema/haemorrhage; enlarged popliteal LNs and spleens; liver pale some white spots on surface; haemorrhages in intestinal walls; oedema in legs; poorly differentiated primaries 3-4mm thick. Bacteria in LN, spleen, liver, lung.
SWH 9/92 1992 1	Relatively rapid development of moderate clinical signs. At day 10 swollen heads, moderately swollen eyelids and ears and quiet; 4/6 died acutely at day 10-11; remaining 2 developed more typical myxomatosis and were euthanized at day 16 and day 20.	Acute deaths: hind leg oedema/haemorrhage; pulmonary oedema/haemorrhage; haemorrhages over intestinal walls and thymus. Bacteria in LNs, lung, liver, kidney.
BD23 1999 1/2	Relatively slow onset of mild myxomatosis with slightly swollen eyelids and ears at d10. Acute deaths between day 10-13 in 11/12 rabbits – remaining rabbit developed typical myxomatosis; secondary lesions just starting on ear tips at d22. Almost no visible primary lesions.	Pulmonary oedema/haemorrhage; enlarged black spleens; livers often had white spots over the surface or were pale and swollen. Lymph nodes generally small; 2/12 had swollen, oedematous, haemorrhagic hind leg(s). Primary lesions undifferentiated and flat 2-3 mm thick on cut section. Histology: necrotic foci in liver. Bacteria in lungs, lymph node, spleen, liver.
BRK 12/2/93 1993 1/2	Swollen ears and swollen heads. 11/12 rabbits died acutely between days11-14. Remaining rabbit developed more typical myxomatosis but with no secondary lesions.	Pulmonary oedema/haemorrhage; hind leg oedema/haemorrhage; haemorrhages over intestinal walls and thymus; coccoid bacteria in lung, liver, kidney, LNs. Late death had bacterial pneumonia; *Staph aureus* cultured from pericardial fluid.
WS6 346 1995 2	Relatively slow onset of mild myxomatosis with 3/6 acute deaths between days 12-13.5; remaining rabbits developed more typical myxomatosis but without secondary lesions.	Pulmonary oedema/haemorrhage; hind leg oedema/haemorrhage; some haemorrhage in intestinal/stomach walls and mesentery. Primaries poorly differentiated 2-3mm thick on cut section.
SWH 8/2/93 1993 2	Relatively mild clinical signs at day 10; 4/6 acute deaths between days 11-14.5; remaining 2 developed more typical myxomatosis but without secondary lesions and died	Acute deaths- pulmonary oedema/haemorrhage; hind leg oedema/haemorrhage; 2 later deaths had pulmonary oedema and pericarditis – cytology showed

	at day 18 and day 20.	bacteria & neutrophils. *Staph aureus* cultured from pericardial fluid.
SWH 805 1993 2	Very swollen heads and ears but only moderate eyelid swelling at day 12. 4/6 acute deaths day 12-13 with another day 15; final rabbit euthanized at day 28.	Acute deaths- pulmonary oedema/haemorrhage; hind leg oedema/haemorrhage; spleens and popliteal LNs very enlarged; primaries undifferentiated 2-3 mm thick on cut section.
BRK 897 1995 3	Relatively rapid development of swollen heads and ears; 4/6 acute deaths between days 12-15; remaining 2 rabbits developed more typical myxomatosis but without secondary lesions and may have survived.	Acute deaths- pulmonary oedema/haemorrhage; oedema/haemorrhage in hind legs. Histology: bacteria in popliteal LNs, spleen, lungs, liver, cardiac muscle.
WS6 1071 1995 3	Relatively slow onset; swollen ears, eyelids and heads at day 12 with progression to more typical myxomatosis but without secondary lesions and deaths from day 12-28.2 acute deaths d12 and 15.	Acute deaths- pulmonary oedema/haemorrhage +/- hind leg oedema/haemorrhage. Large numbers of bacteria in tissues. Later deaths: myxomatosis often with evidence of pneumonia.
SWH1209 1996 3	Relatively mild clinical signs at d10; 2/6 acute deaths at days 13-14; remaining rabbits developed myxomatosis with the longest survivor developing some small secondary lesions at 28 days.	Acute deaths: hind leg oedema/haemorrhage; some pulmonary oedema/haemorrhage. Later deaths- pneumonia, hepatic necrosis.
Meby 1991 3	Development of cutaneous type myxomatosis by d10. 1/6 died acutely at day 11 with pulmonary oedema/haemorrhage and 3 over the next 5 days 2 of which had pulmonary oedema/haemorrhage while the last had haemorrhagic pneumonia with bacteria and acute inflammation; the 5th was euthanized at d22 with pneumonia but the last was clearly recovering from a mild cutaneous infection at this point.	Acute deaths: pulmonary oedema/haemorrhage; oedema/haemorrhage in hind leg; haemorrhages over intestinal walls and Peyer's patches. Bacteria + inflammation in lung d16. Primary lesions were more typical of cutaneous myxomatosis but only 2 rabbits developed secondary lesions.
OB3 Y317	Rapid development of raised primary lesion and high	All rabbits recovered uneventfully.

1994 5	temperatures; mild eyelid and ear swelling with discrete small secondaries – these started to scab by day 9; 4/6 developed nasal discharge/snuffles by day 10 but primary scabbing at this time; day 13 all bright and alert; 1/6 still had an upper respiratory infection.	

Table S2 Mutations present in all modern viruses

Gene	Mutation	Protein function
M002L/R	A226V	TNF inhibition/antiapoptosis
M003.1L/R	A37V	Homology to VACV B15R (secreted IL-1β receptor)
M005L/R	R434W	Antiapoptosis/E3 ubiquitin ligase
M005L/R	S209Y	Antiapoptosis/E3 ubiquitin ligase
M009L	T insert ORF disrupted	Putative E3 ubiquitin ligase
M014L	V175I	Putative E3 ubiquitin ligase
M014L	G122W	Putative E3 ubiquitin ligase
M015L	V85A	Ribonucleotide reductase small subunit
M017L	E71K	unknown
M025L	**M11I**	**Homology to VACV F16L**
M045L	D263N	Protease involved in morphogenesis
M052L	S29N	Core protein
M057L	L90V	VACV L3L orthologue; core protein
M062R	**K142T**	**Host-range (essential for rabbit cells); binds human SAMD9**
M063R	S195C	Host-range (essential for rabbit cells)
M065R	T98M	Poly A pol regulatory subunit
M083L	C insert corrects [1]ORF	Inactive carbonic anhydrase homologue/ virion protein
M121R	**S21F**	**VACV A33 orthologue; [2]EV assembly**
M134R	S84P	Variola B22R orthologue
M148R	L383F	E3 ubiquitin ligase
M151R	R173G	Serp 2 (serine protease inhibitor)
M154L	**Y53C**	**VACV M2L orthologue (NF-κB inhibition)**

1. SLS, KM13 and Ur all have the M083L ORF disrupted due to a single nt deletion. This inserted nt corrects the ORF in all modern viruses

2. Extracellular enveloped virus

Shaded mutations are in genes with virulence functions demonstrated in knock-out virus (11-17).

Mutations in bold are not present in Meby.

Figure S1. Maximum likelihood phylogenetic tree of Australian viruses and their year of isolation. The tree was inferred using the GTR+I+Γ model of nucleotide substitution available in the PhyML package (18) Those viruses phenotyped in this study are shown in bold. Branch lengths are scaled according to the number of nucleotide substitutions per site, and bootstrap support values are shown for key nodes. The tree is rooted on the progenitor virus SLS.

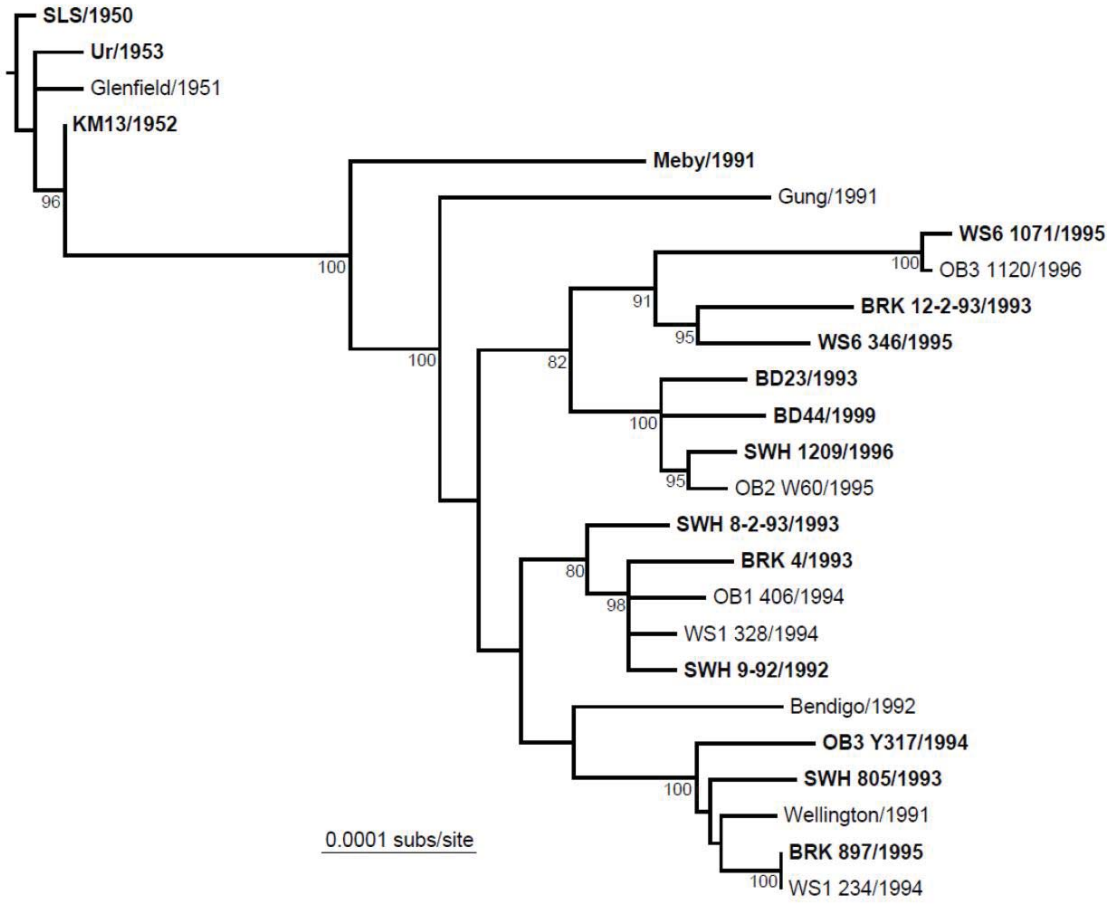
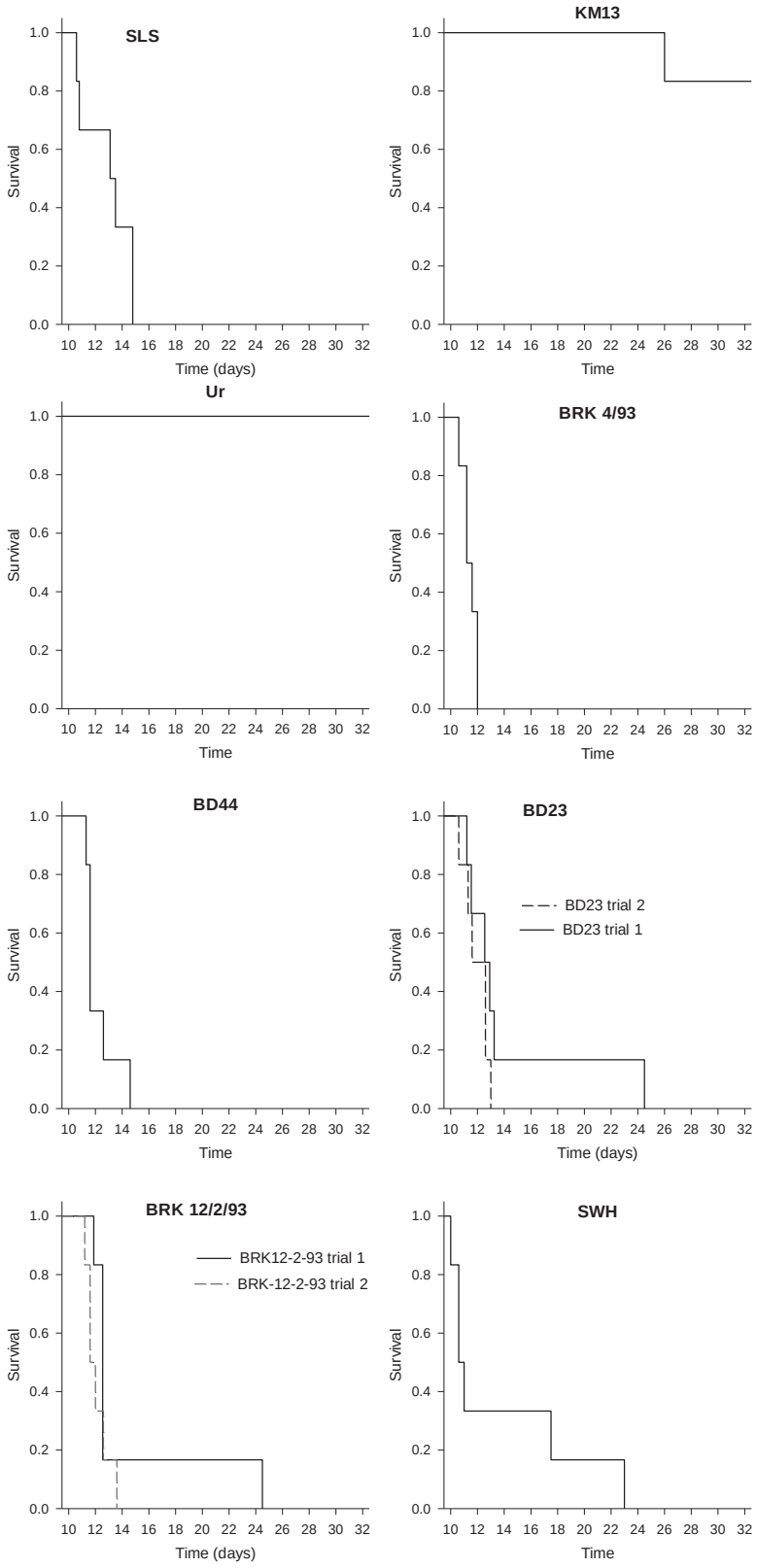


Figure S2. Kaplan-Meier plots of virus phenotypes. Survival curves were measured in two trials, as detailed in Table 1; virulence phenotypes for two strains, BD23 and BRK 12/2/93, were measured in both trials.



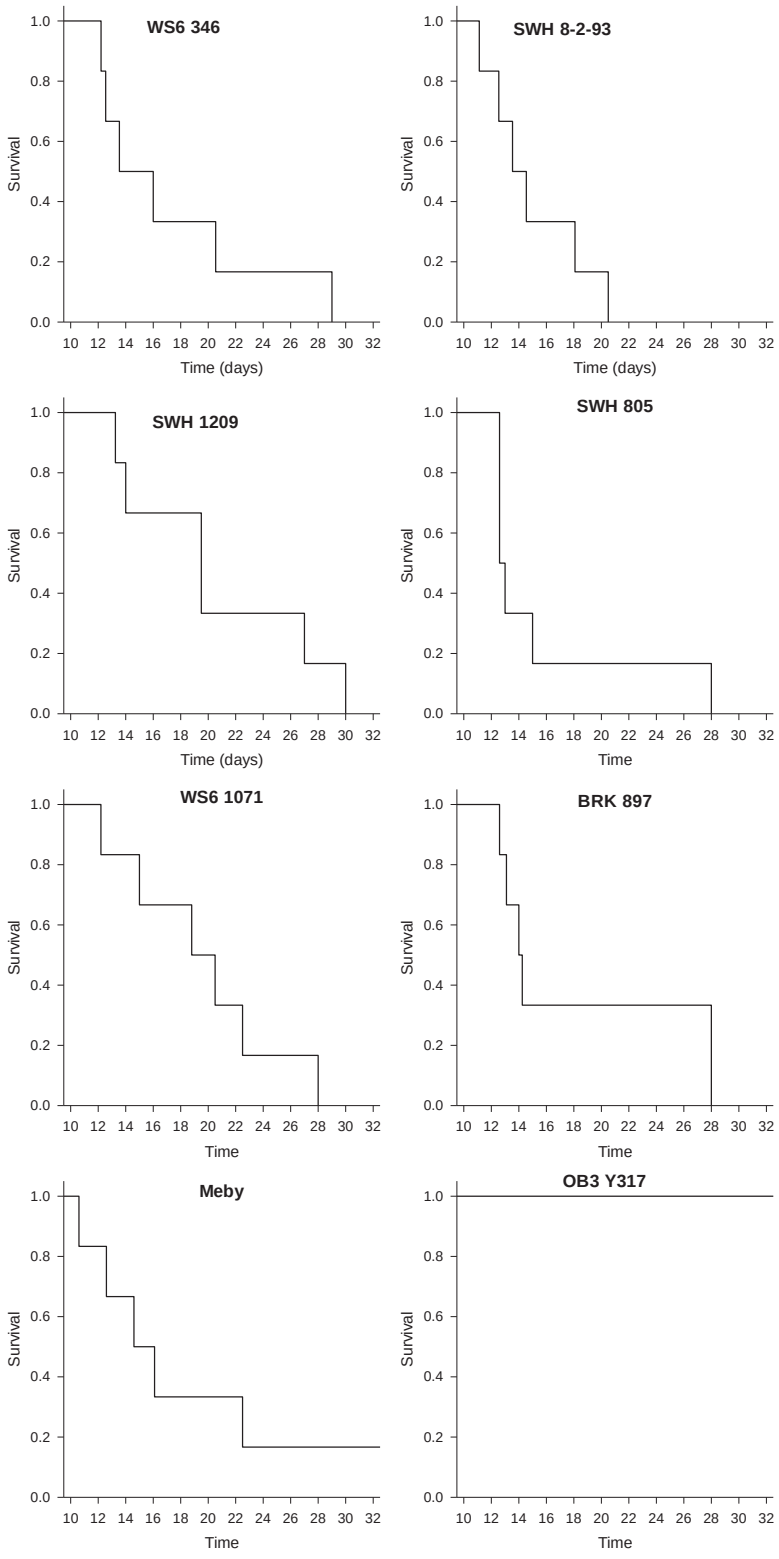

WS6 346
SWH 8-2-93
SWH 1209
SWH 805
WS6 1071
BRK 897
Meby
OB3 Y317
Survival
Time (days)
Time
0.0
0.2
0.4
0.6
0.8
1.0
10 12 14 16 18 20 22 24 26 28 30 32

Figure S3. **Pathology of primary lesions and other tissues.** A. Typical primary lesions of a rabbit infected with SLS and a rabbit infected with BD23. Histological images show the vesicular degeneration (arrows) of the epidermis and swelling and disruption of the dermis in the SLS primary, whereas the BD23 primary has little or no disruption to the epidermal layer beyond some hypertrophy, hyperplasia and degeneration of the epidermal cells with minimal disruption of the underlying dermis (scale bar = 100 μm). B. Subcutaneous oedema of the hind leg (arrow). C. Spontaneous haemorrhage in the ear. D. Haemorrhage and oedema in lung. E. Swollen pale liver.

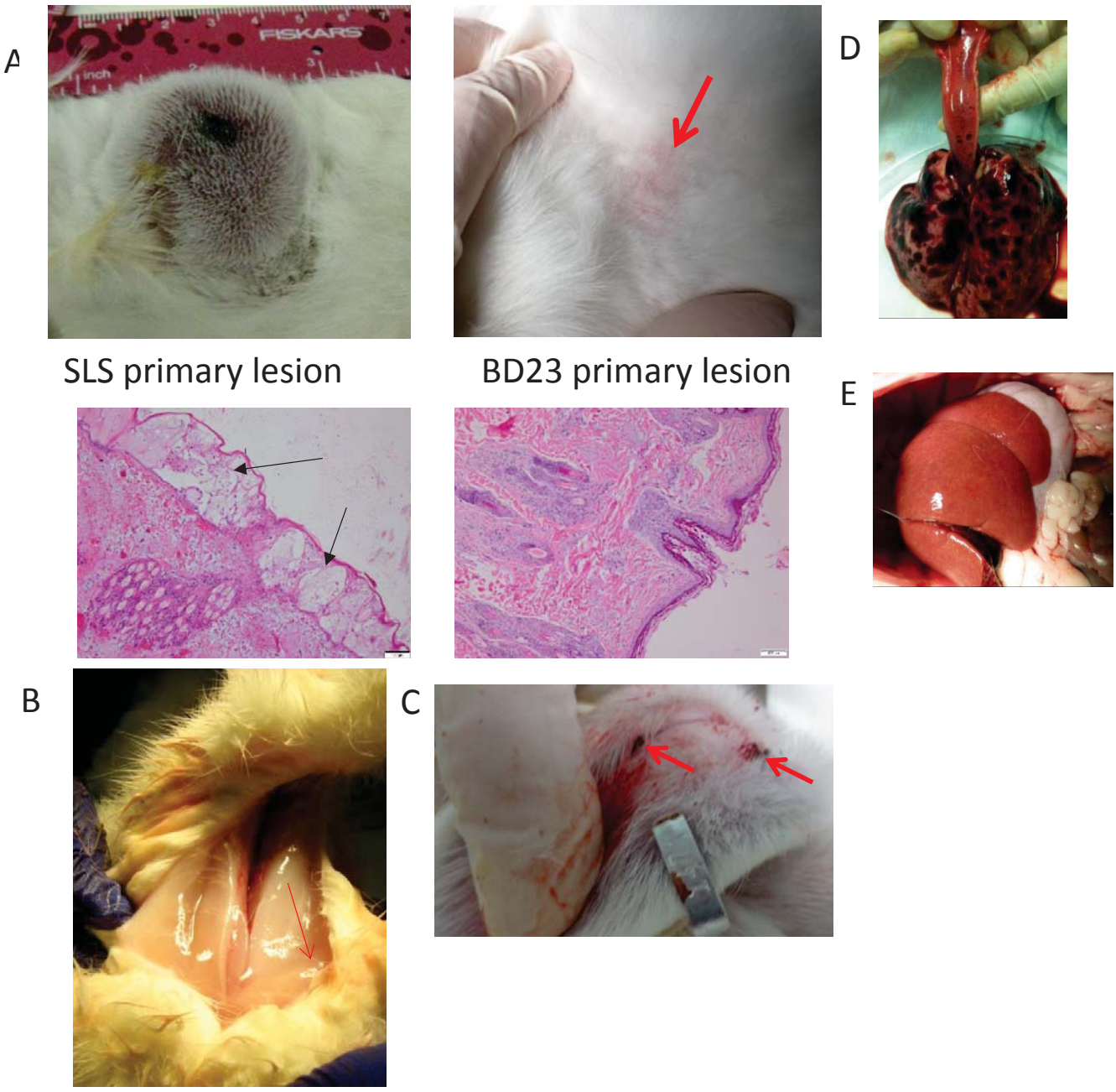


Figure S4. Histology of acute collapse: bacterial invasion and lack of cellular immune response. A. Spleen showing loss of lymphocytes from white pulp and large aggregates of bacteria (arrows) scale bar = 100 μm. B. Bacteria in heart muscle (scale bar = 20 μm. C. liver, sinusoids packed with bacteria (arrows) and surrounding cell death (scale bar = 20 μm). D. kidney with large numbers of bacteria in glomerular capillaries (arrow) (scale bar = 20 μm). E. Lymphocyte death, haemorrhage and bacterial invasion in gut lymphoid tissue. White arrows indicate lymphoid follicles full of red blood cells and necrotic lymphocytes; red arrows indicate examples of bacteria (scale bar = 200 μm).

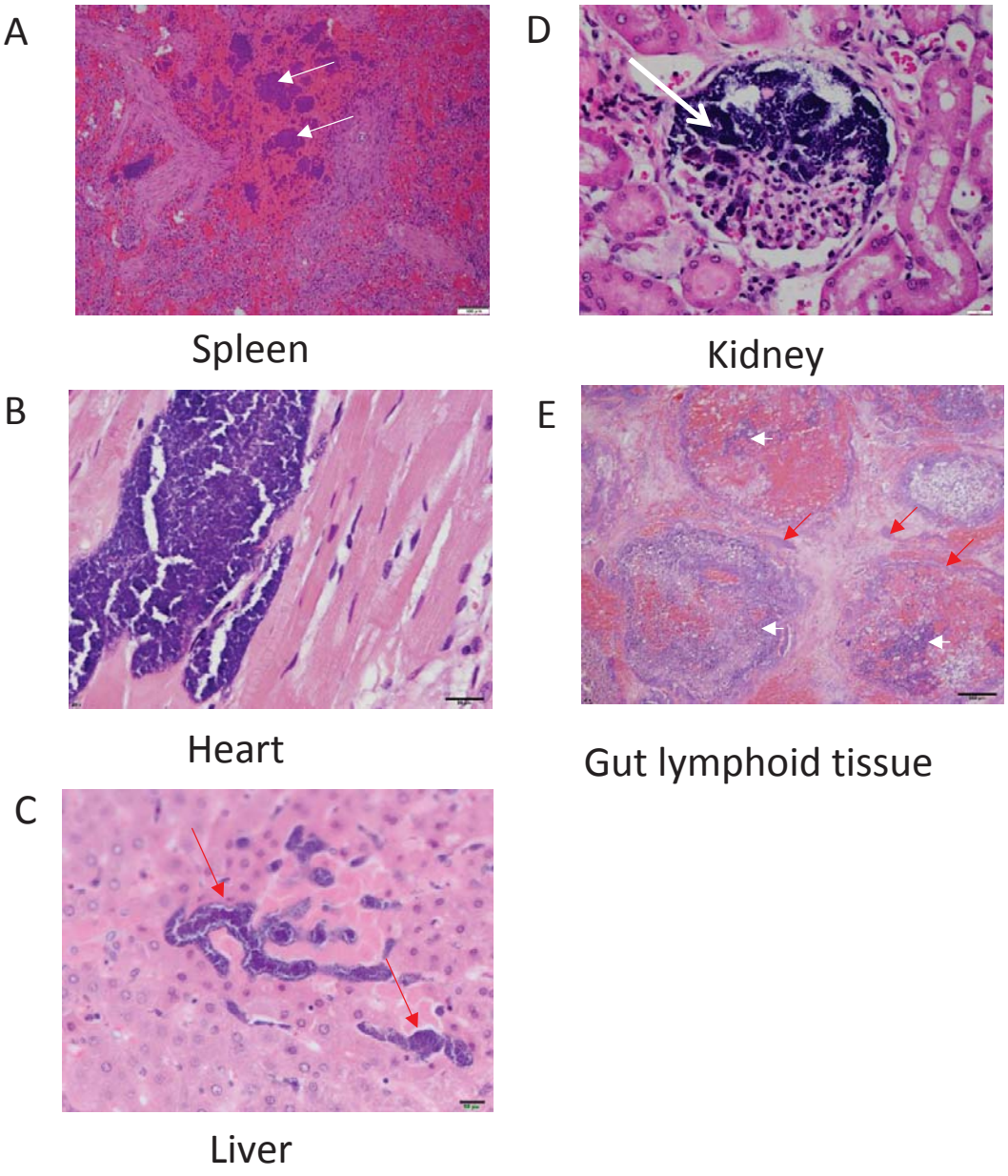


Figure S5. Peripheral blood lymphocyte (left panel) and neutrophil (right panel) counts at days 0 and 10 post-infection. Plots show number for each individual at day 0 (blue) and day 10 (red) (vertical lines show day 10) for each virus infection.

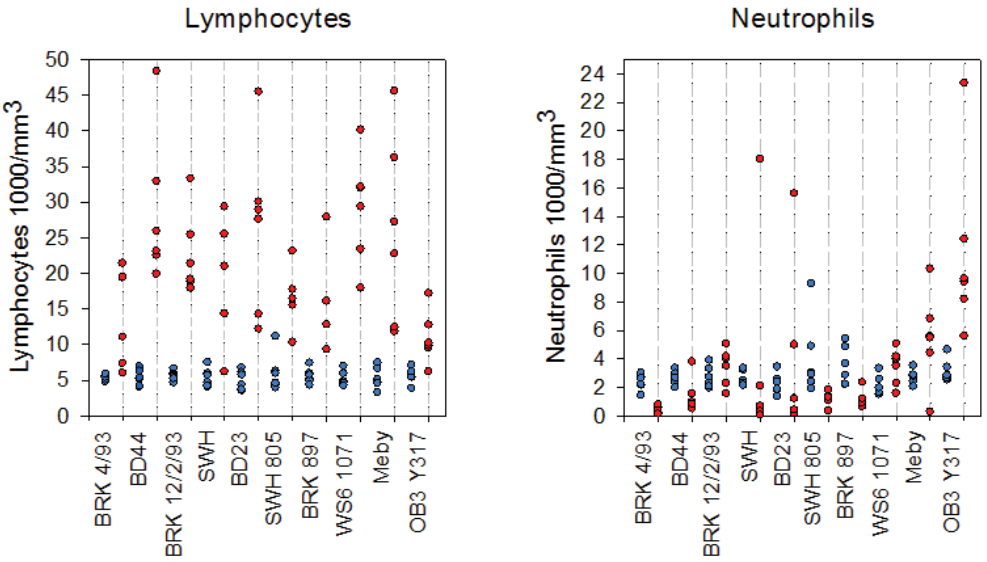


Figure S6A. Correlation of peripheral blood neutrophil count with survival time. Regression line for ln d10 neutrophil titre against survival time (ST) transformed as $\log_{10}$(ST-8) for all rabbits in Trial 2 (see Table 1).

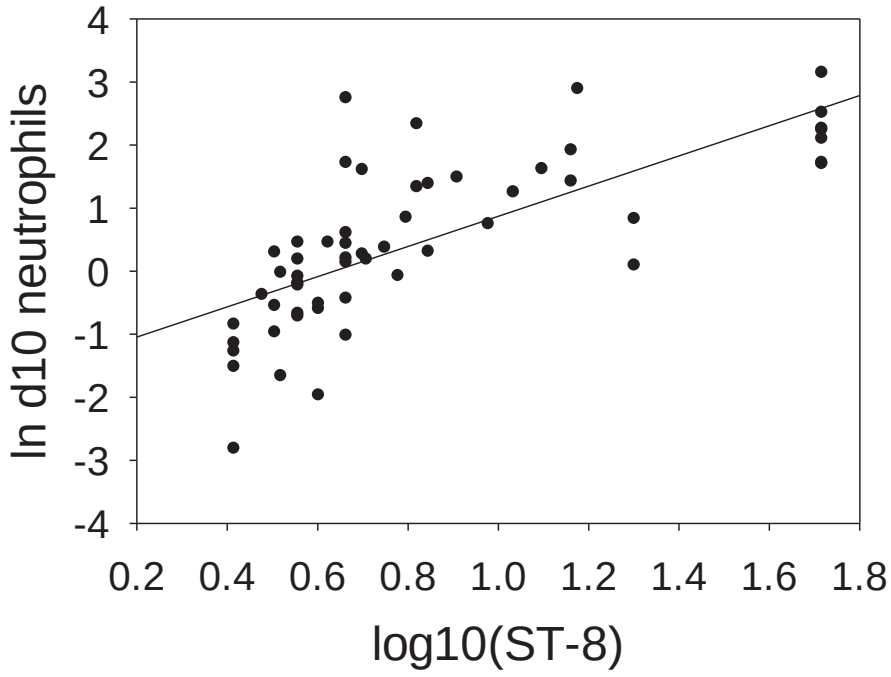


Figure S6B. Daily rectal temperatures in rabbits infected with SLS, BRK 4/93, SWH 805 and OB3 Y317. Rectal temperatures for each surviving rabbit for the survival plots in Figure 1 are shown.

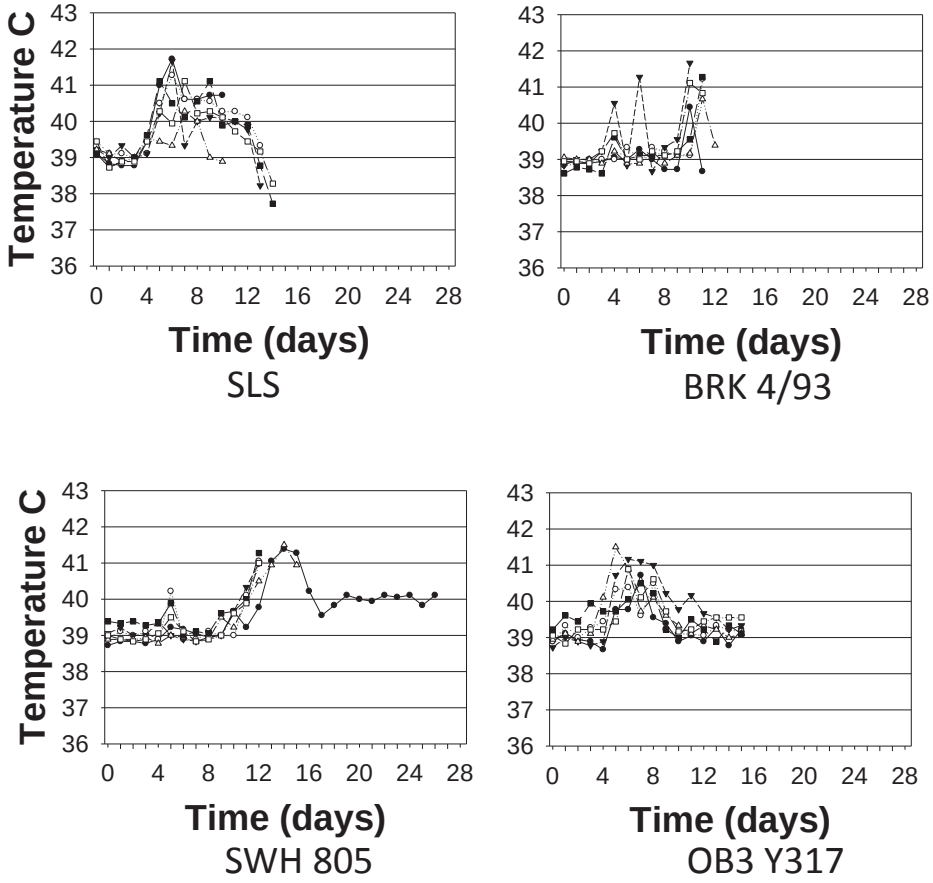


Figure S7. Genome copy number in the primary lesion at the inoculation site. Scatter plot showing genome copy number measured by QPCR. Up to 3 rabbits were biopsied at each time point depending on how many were surviving.

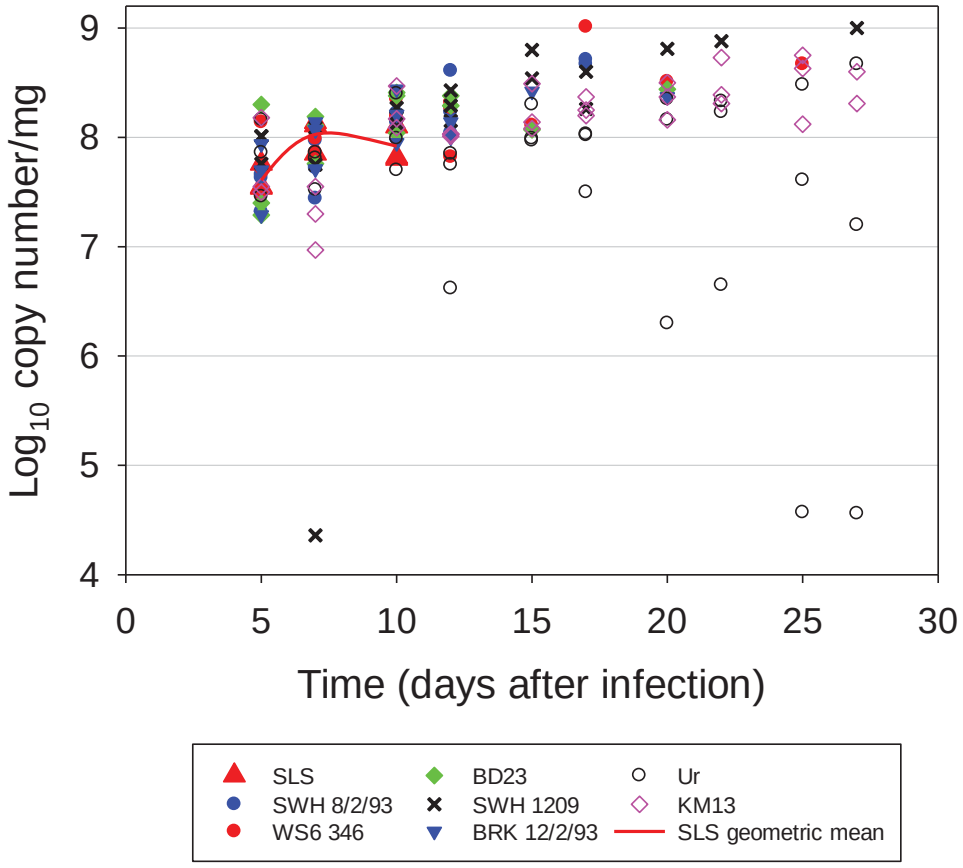
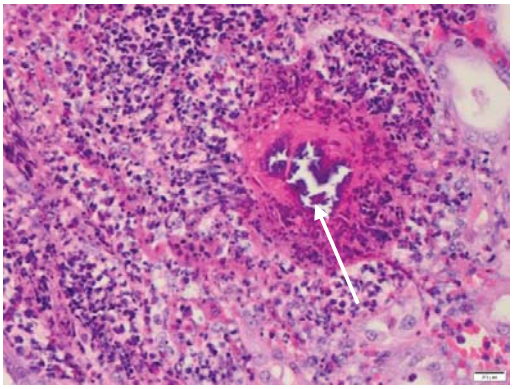
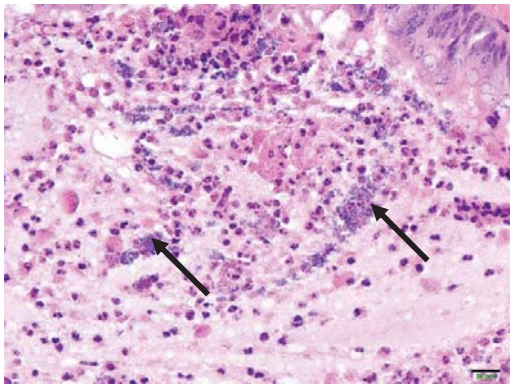


Figure S8. Inflammatory response in prolonged infections. A. Kidney d22 Meby (1991); acute inflammation around a vein with bacteria in the lumen (arrowed) (scale bar = 20 μm). B. Lung d19 SWH 1209 (1996); acute inflammation around bacteria (arrowed) (scale bar = 20 μm). C. Pneumonia and acute inflammation with masses of neutrophils in interstitial tissue and bronchioles KM13 (1952) d32 (scale bar = 100 μm).

A



B



C

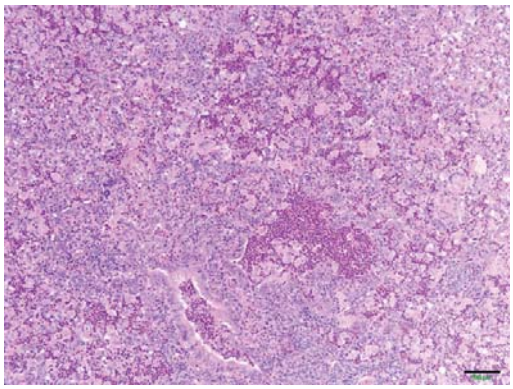


Figure S9. Weight gain due to oedema for rabbits infected with SLS (1950 grade 1 progenitor), BRK 4/93 (1993 acute) or OB3 Y317 (1994 grade 5). Percentage difference in body weight for individual rabbits at day 8 and day 10 after infection compared to day 0.

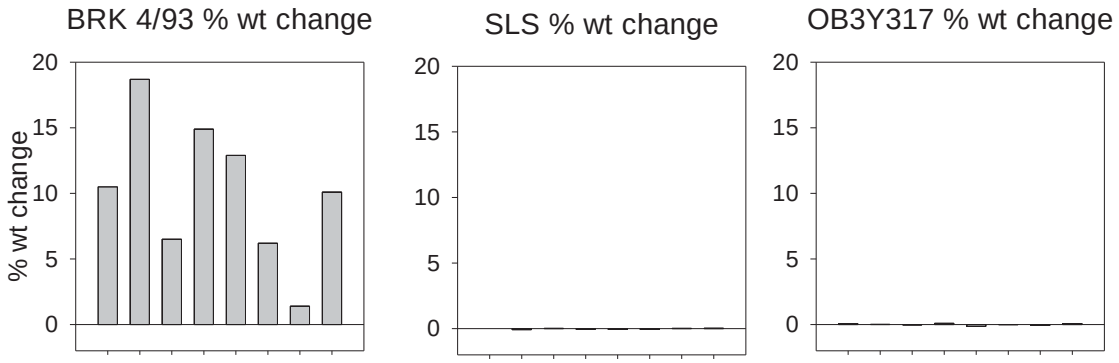


Figure S10. Peripheral blood lymphocyte and neutrophil counts for rabbits infected with SLS (1950 grade 1 progenitor), BRK 4/93 (1993 acute) or OB3 Y317 (1994 grade 5). Plotted points are at days 0, 4, 8 and 10 post- infection. All 12 rabbits in each group were bled at day 0, at the other time points 4 rabbits were bled.

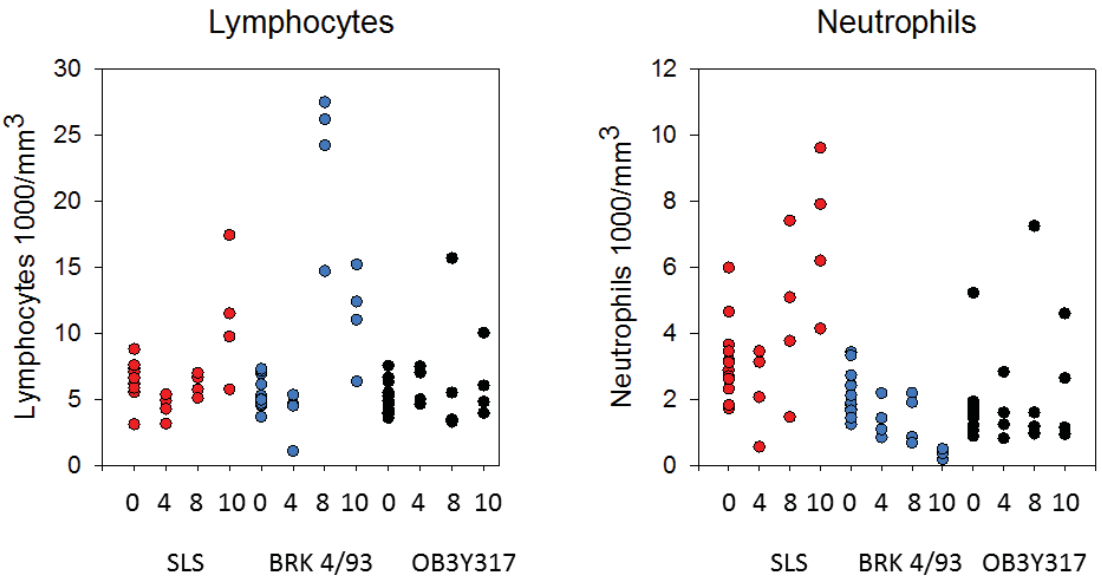
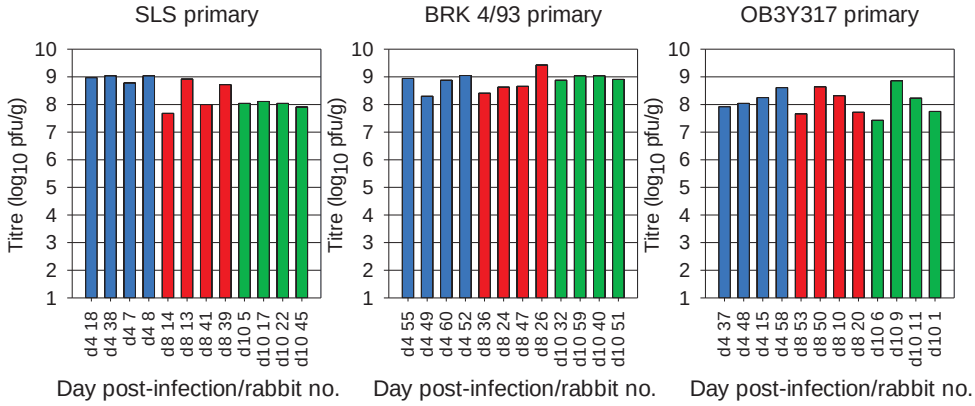


Figure S11. Viral titres in various locations in rabbits infected with SLS (1950 grade 1 progenitor), BRK 4/93 (1993 acute) or OB3 Y317 (1994 grade 5). Log 10 pfu/g virus titres are shown for A. primary lesion. B. draining lymph node. C. contralateral lymph node. D. spleen. E. Lung. F. Liver. G. Brain. Time points are days 4 (blue bars), 8 (red bars) and 10 (green bars); 4 rabbits were killed at each time point. Brain titres are shown for day 10 only. Virus was not detected in the liver of rabbits infected with OB3 Y317.

A



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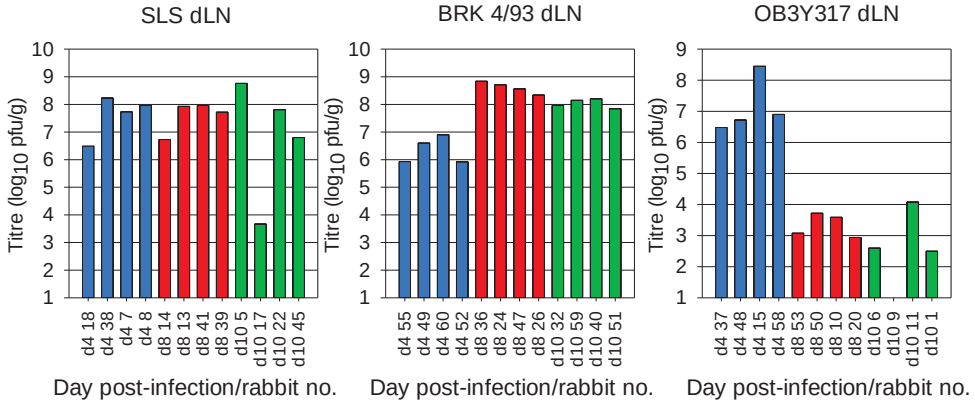

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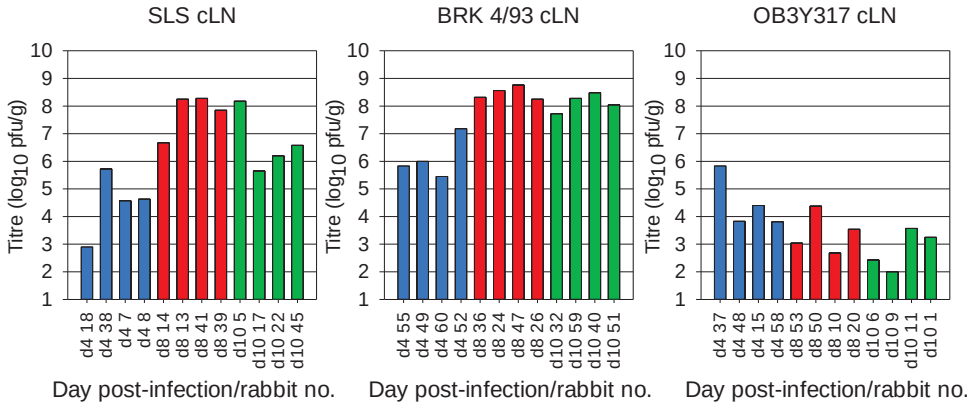

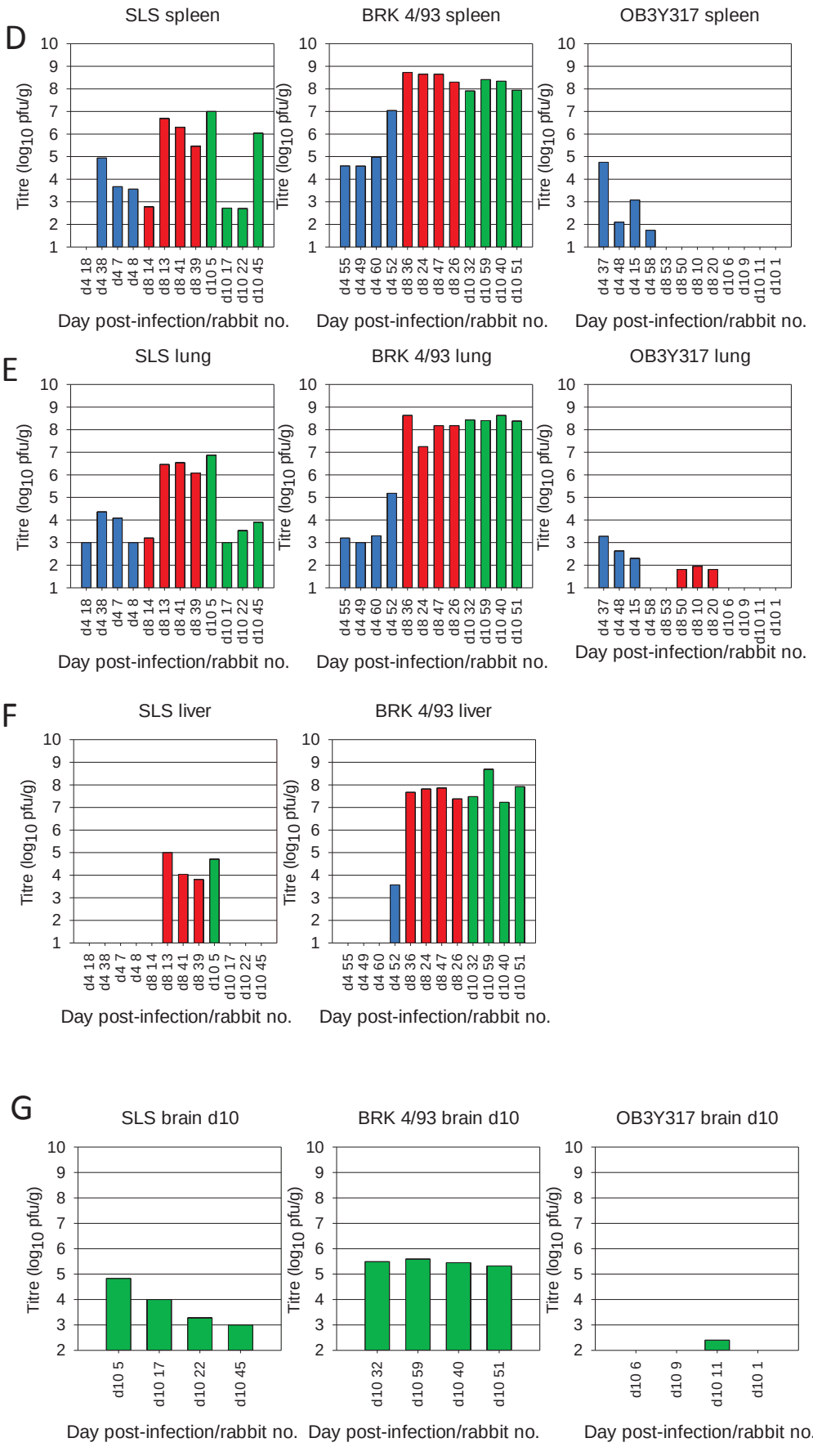
D
SLS spleen
BRK 4/93 spleen
OB3Y317 spleen
Titre (log10 pfu/g)
Day post-infection/rabbit no.
E
SLS lung
BRK 4/93 lung
OB3Y317 lung
F
SLS liver
BRK 4/93 liver
G
SLS brain d10
BRK 4/93 brain d10
OB3Y317 brain d10

Figure S12. Histology of lymph node and testis of rabbits infected with SLS (1950 grade 1 progenitor) and BRK 4/93 (1993 acute) at day 10. A. Draining lymph node BRK showing loss of lymphocytes and empty space (scale bar = 100 μm). B. HP of A (scale bar = 20 μm). C. SLS draining LN partial loss of lymphocytes and influx of neutrophils with proliferation of stromal cells (scale bar = 100 μm). D. HP of C (scale bar = 20 μm). E. SLS primary lesion with massive disruption of dermis (scale bar = 100 μm). F. BRK primary lesion (scale bar = 100 μm). G BRK testis early degeneration of seminiferous epithelium but no inflammatory response (scale bar = 100 μm). H. SLS testis (scale bar = 100 μm); arrow indicates acute inflammatory cells.

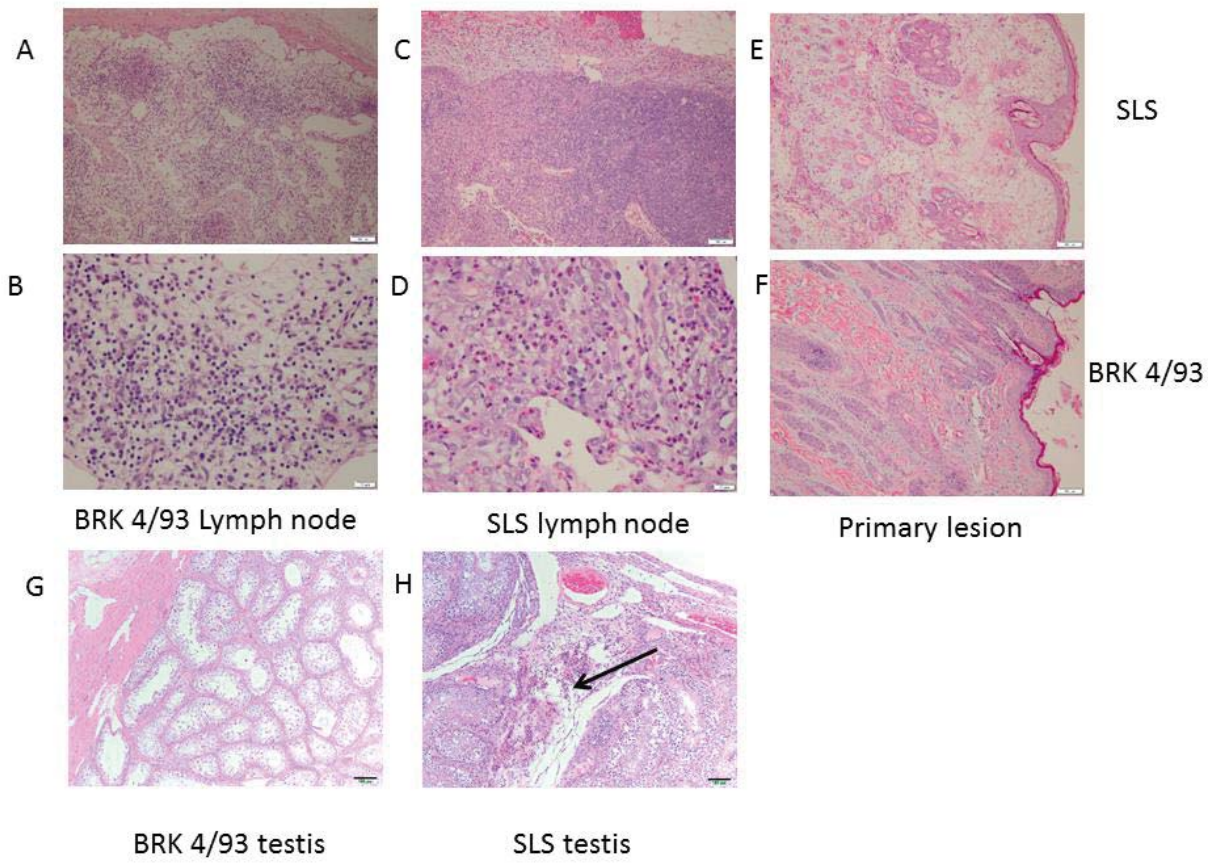

Figure S13. Daily rectal temperatures for rabbits treated with enrofloxacin or control treated rabbits. Temperatures are shown for all surviving rabbits infected with SLS, BRK 4/93, BD23 or WS6 1071 and either treated with enrofloxacin from d4 to d16 or with control PBS. Arrows indicate d16.