Common anti-hemostatic medications increase the severity of uropathogenic *Escherichia coli* sepsis

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Footnote page

The authors have no conflicts of interest to declare.

This work was supported by the University of Sydney Fellowship G197581; NSW Ministry of Health under the NSW Health Early-Mid Career Fellowships Scheme H18/31086; and the Kenyon Family Inflammation Award 2017 (S.H.O.), Kenyon Family Inflammation Award 2019 (E.H.).

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Abstract

Uropathogenic *Escherichia coli* (UPEC) causes urinary tract infections that can result in sepsis. Hemostasis is protective in the pyelonephritis stage of ascending UPEC infection, the role of hemostasis but has not been investigated during sepsis. Here we utilize a zebrafish-UPEC sepsis model to visualize infection-induced coagulation and examine the effects of commonly prescribed anti-hemostatic medications on the infection severity. Treatment of septic zebrafish with warfarin, aspirin, or ticagrelor reduced host survival, while stabilization of clots with aminocaproic acid increased host survival. Our findings provide evidence that commonly prescribed anti-hemostatic medications may worsen the outcome of severe UPEC infection.

Keywords

Hemostasis, coagulation, platelet, Extraintestinal *Escherichia coli*

42 **Background**

43 Uropathogenic *Escherichia coli* (UPEC) is the primary pathogen responsible for urinary tract infection (UTI),
44 which is among the most common bacterial infections worldwide. UPEC most commonly initiates self-
45 limiting cystitis inside the urinary tract, but can ascend the ureters to the kidneys as acute pyelonephritis
46 and potentially progress into sepsis [1, 2].

47

48 Hemostasis plays an evolutionarily conserved roles in both immune control of infections and the
49 pathogenesis of infectious complications from a wide range of pathogens [3, 4]. In the case of UPEC,
50 treatment of rodent UPEC pyelonephritis models with the injectable anticoagulant heparin demonstrates
51 an important role of coagulation in preventing the progression of pyelonephritis to sepsis via an α-
52 hemolysin/renal epithelial cell CD147/Tissue Factor axis [5, 6].

53

54 Elderly patients undergoing long term catheterization are at high risk of UPEC infections. These patients are
55 also likely to be taking anti-hemostatic medications for the management of chronic cardiovascular
56 conditions which might compound their risk and severity of severe UPEC infections. Here, we have used a
57 zebrafish embryo model of UPEC sepsis to study the interaction between commonly prescribed anti-
58 hemostatic medications and the severity of UPEC sepsis [7].

59

60 **Methods**

61 *Zebrafish husbandry*

62 Adult zebrafish were housed at the Centenary Institute (Sydney Local Health District AWC Approval 2017-
63 036). Zebrafish embryos were produced by natural spawning and raised at 28°C in E3 media.

64

65 *Zebrafish lines*

66 Wild type zebrafish are the AB background. Transgenic lines are: *Tg(fabp10a:fgb-EGFP)*^{mi4001} [8], *Tg(-*
67 *6.0itga2b:eGFP)*^{la2} [9].

68

69 *Infection of zebrafish embryos*

70 Aliquots of single cell suspensions of midlog-phase *Escherichia coli* UTI89 carrying the pGI6 plasmid were
71 frozen at -80°C for use in infection experiments. Bacterial aliquots were thawed and diluted with phenol
72 red dye (0.5% w/v). 10-15 nL was injected into the caudal vein or trunk of M-222 (tricaine)-anaesthetized
73 30-48 hpf embryos resulting in a standard infectious dose of approximately 10,000 CFU. Embryos were
74 recovered into E3 and housed at 32°C.

75

76 *Drug treatments*

77 Embryos were treated with vehicle control (DMSO or water as appropriate), 10 µg/ml aspirin, 20 µg/ml
78 tirofiban, or 33 µM warfarin.

79

80 *Imaging*

81 Imaging was carried out on embryos anaesthetized in M-222 (Tricaine) mounted in 0.75% low melting point
82 agarose on a Deltavision Elite fluorescence microscope. Editing was carried out with Image J Software
83 Version 1.51j.

84

85 *Statistics*

86 Survival analyses were performed by Log-rank tests in GraphPad Prism. Fluorescent pixel count analyses
87 were performed by T-test in GraphPad Prism.

88

89 **Results**

90 *Coagulation is targeted to bloodborne UPEC in zebrafish embryos*

91 UPEC infection-induced clotting has observed in mouse explants [5, 6]. We infected *Tg(fabp10a:fgb-*
92 *EGFP)*^{mi4001} zebrafish embryos, where clots can be visualized by GFP fluorescence, with UTI89 UPEC carrying
93 the pGI6 plasmid, allowing visualization of red fluorescent UPEC, and performed timelapse microscopy. We
94 observed the progressive formation of both arterial and venous clots in infected embryos (Figure 1A).

95

96 *Warfarin increases the severity of UPEC sepsis in zebrafish embryos*

97 Inhibition of clotting with heparin has been reported to worsen UPEC infection in mice [5, 6]. We sought to
98 determine if commonly prescribed anti-coagulants had a similar effect in our zebrafish model using
99 warfarin, a vitamin K antagonist and the most commonly prescribed anti-coagulant.

100

101 We have previously demonstrated that warfarin reduced mycobacterial infection-induced coagulation in
102 zebrafish embryos [4], here we demonstrate that warfarin reduced clot formation at a sterile wound at the
103 embryo stage used in our UPEC infection experiments (Figure 1B). Treatment with warfarin decreased
104 embryo survival following systemic UPEC infection (Figure 1C). Conversely, clot stabilization with
105 aminocaproic acid increased embryo survival following systemic UPEC infection (Figure 1D).

106

107 *Zebrafish thrombocytes interact with UPEC*

108 Clotting and thrombosis are coordinated during infection-induced hemostasis [3, 4]. To determine if
109 zebrafish thrombocytes could play a role in UPEC sepsis, we infected *Tg(-6.0itga2b:eGFP)*^{la2} embryos, where
110 thrombocytes can be visualized by GFP expression, with UPEC- pGI6. We observed long lasting interactions
111 between zebrafish thrombocytes and clumps of UPEC but were unable to determine if the bacteria were
112 extracellular or intracellular (Figure 2A).

113

114 *Aspirin and ticagrelor increase the severity of UPEC sepsis in zebrafish embryos*

115 Having shown a negative effect of inhibiting clotting, we next investigated the effect of commonly
116 prescribed anti-platelet medications on systemic UPEC infection. Aspirin and ticagrelor reduced platelet
117 aggregation to a sterile wound (Figure 2B). Inhibition of platelets with either aspirin or ticagrelor reduced
118 survival of infected embryos (Figure 2C and 2D).

119

120 **Discussion**

121 We have found that common anti-hemostatic medications worsen the survival of UPEC-infected zebrafish.
122 Anti-hemostatic drug use is highly prevalent in the elderly as a preventative measure against heart attack

123 and stroke. Alongside this, the risk of urinary tract infections also increases from middle to old age and
124 there is elevated risk of recurrent urinary tract infections in aged care settings [10]. Our data illustrates a
125 negative interaction between preventative anti-hemostatic medication usage and the severity of UPEC
126 sepsis.
127
128 Our results add to a growing body of literature that hemostasis, and specifically clotting, is crucial to the
129 early containment of blood born UPEC [5, 6]. Clinical trials of anticoagulants to treat sepsis-induced
130 coagulopathy have delivered inconsistent results, however an emerging theme is that early coagulation is
131 host protective while late coagulation drives pathology [11]. Subsequently, administration of
132 anticoagulants at early stage can be detrimental to the clearance of pathogen by the native immune system
133 [12]. Additionally, there is little evidence on the beneficial effect of anticoagulants on the overall
134 population, however, is beneficial for critically ill subgroups such as sepsis-induced disseminated
135 intravascular coagulation [11, 13].
136
137 The negative effects of the antiplatelet drugs aspirin and ticagrelor demonstrate a host-protective role of
138 platelets during UPEC sepsis. Degranulation of activated platelets releases important inflammatory
139 mediators such as antimicrobial proteins, cytokines, and ADP/ATP which can directly kill pathogens and
140 activate cellular immunity [14]. Clinical trials with antiplatelets, especially aspirin, have demonstrated an
141 improvement in mortality of severe septic patients which, similar to anti-coagulant therapy, may reflect an
142 effect of timing [15].
143
144 Future studies should investigate the interaction between hemostasis and the natural course of UPEC
145 infection in both animal models and by retrospective clinical chart review. The potential for interaction
146 between aspirin and warfarin, the two most commonly prescribed anti-hemostatic medications, with
147 catherization-associated UPEC infections involves a significant proportion of aged care patients and may
148 represent an important cause of excess morbidity.
149

Funding

This work was supported by the University of Sydney Fellowship [grant number G197581]; NSW Ministry of Health under the NSW Health Early-Mid Career Fellowships Scheme [grant number H18/31086]; Centenary Institute Booster Grant to E.H.; and the Kenyon Family Inflammation Award 2017 to S.H.O., Kenyon Family Inflammation Award 2019 to E.H..

Acknowledgements

We thank Associate Professor Iain Duggin for the UPEC strain and plasmid; Dr Angela Kurz and Sydney Cytometry for assistance with imaging equipment; and Dr Vivien Chen for helpful discussion of results.

Author contributions

E.H., and S.H.O designed the experiments. V.T., E.H., and S.H.O performed the experiments. V.T., E.H., and S.H.O wrote the paper. E.H., W.J.B., and S.H.O. supervised the project.

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196

197 Figure Legends

198 Figure 1: Clotting is host-protective against systemic UPEC infection.

199 (A) Intravital microscopy of green fibrin deposition around UPEC pGI6 (red) in a *Tg(fabp10a:fgb-EGFP)*^{mi4001}

200 embryo. Filled arrowhead indicates site of arterial clot formation, hollow arrowhead indicates site of

201 venous clot formation. Scale bar indicates 10 µm. (B) Fibrin-GFP fluorescent area 2 hours post wounding.

202 (C) Survival of UPEC-infected embryos treated with warfarin. n>=18 per group, representative of two

203 biological replicates. (D) Survival of UPEC-infected embryos treated with warfarin or ACA. n>=20 per group,

204 representative of two biological replicates.

205

206 Figure 2: Platelet activation is host-protective against systemic UPEC infection.

207 (A) Intravital microscopy of green thrombocytes interacting with UPEC pGI6 (red) in a *Tg(-6.0itga2b:eGFP)*^{la2}

208 embryo. Filled arrowhead indicates first thrombocyte that interacts with clump of UPEC for 30 minutes,

209 hollow arrowhead indicates a second thrombocyte that interacts with the same clump of UPEC for 21

210 minutes. Unindicated green cells are thrombocytes in circulation. t0 is approximately 1 hour post infection.

211 Scale bar indicates 10 µm. (B) CD41 (thrombocyte) fluorescent area at 2 hours post wounding. (C) Survival

212 of UPEC-infected embryos treated with aspirin. n>=25 per group, representative of two biological

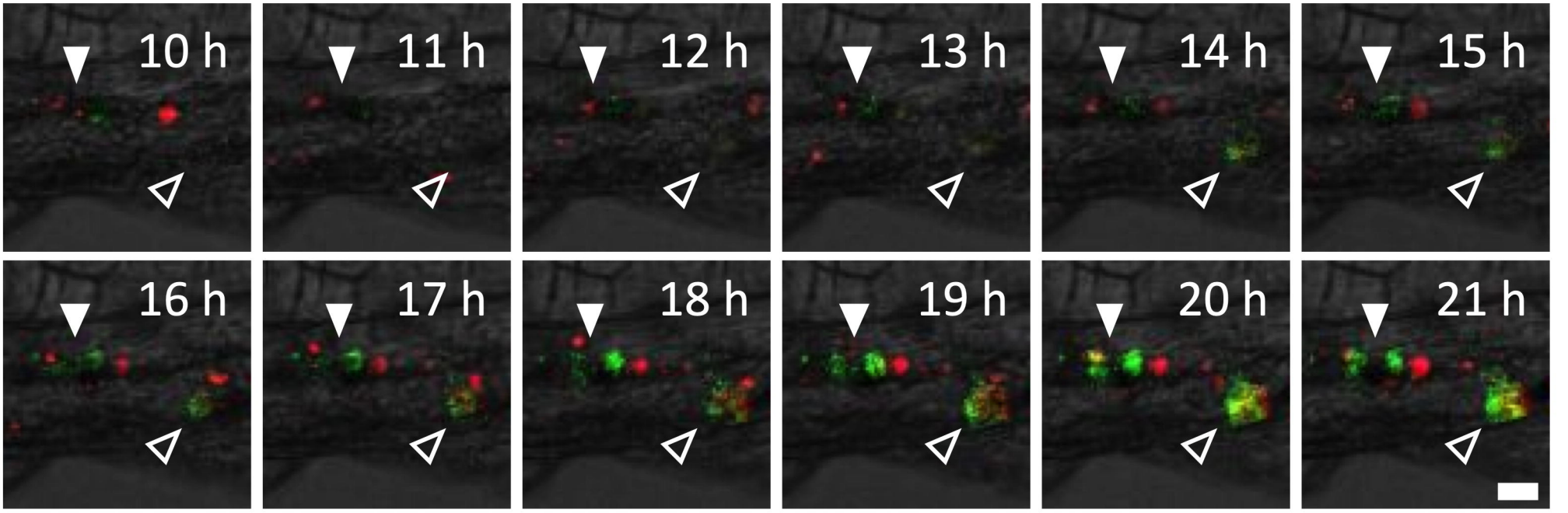
213 replicates. (D) Survival of UPEC-infected embryos treated with ticagrelor. n=25 per group, representative of

214 two biological replicates.

215

A

Tg(fabp10a:fgb-EGFP)^{mi4001} UPEC-pGI6



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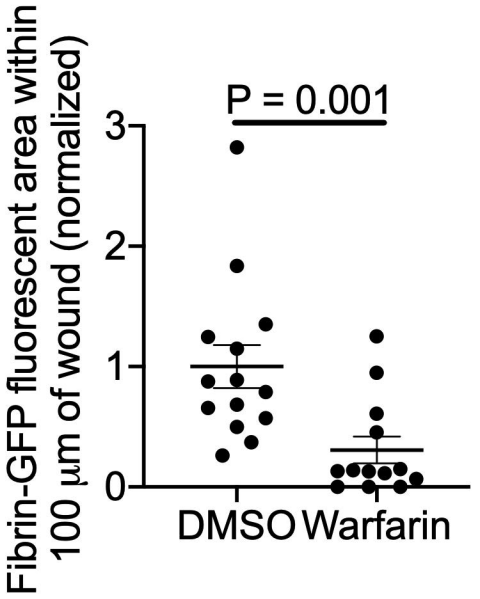

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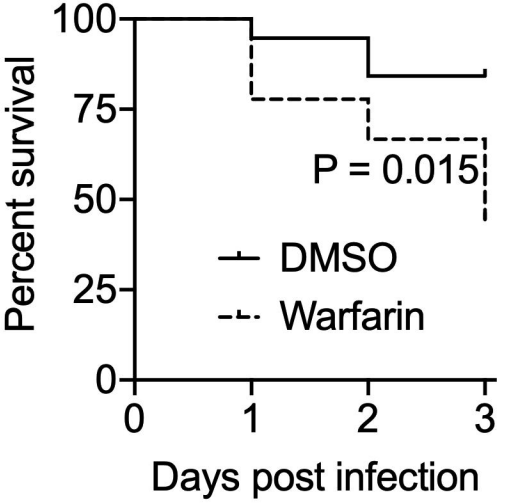

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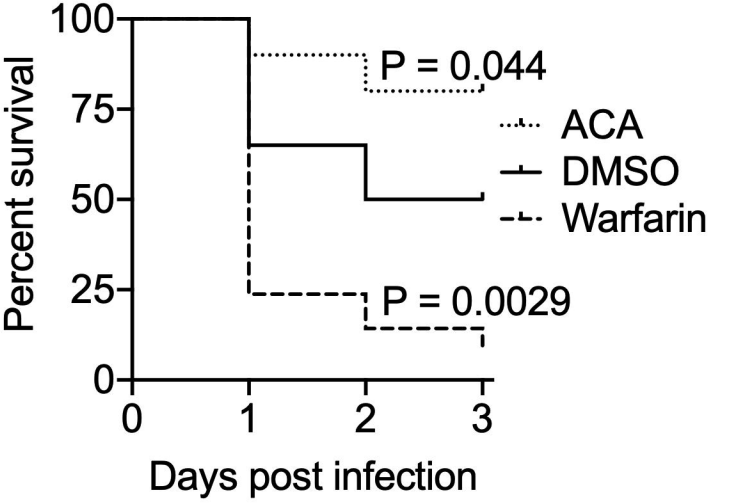
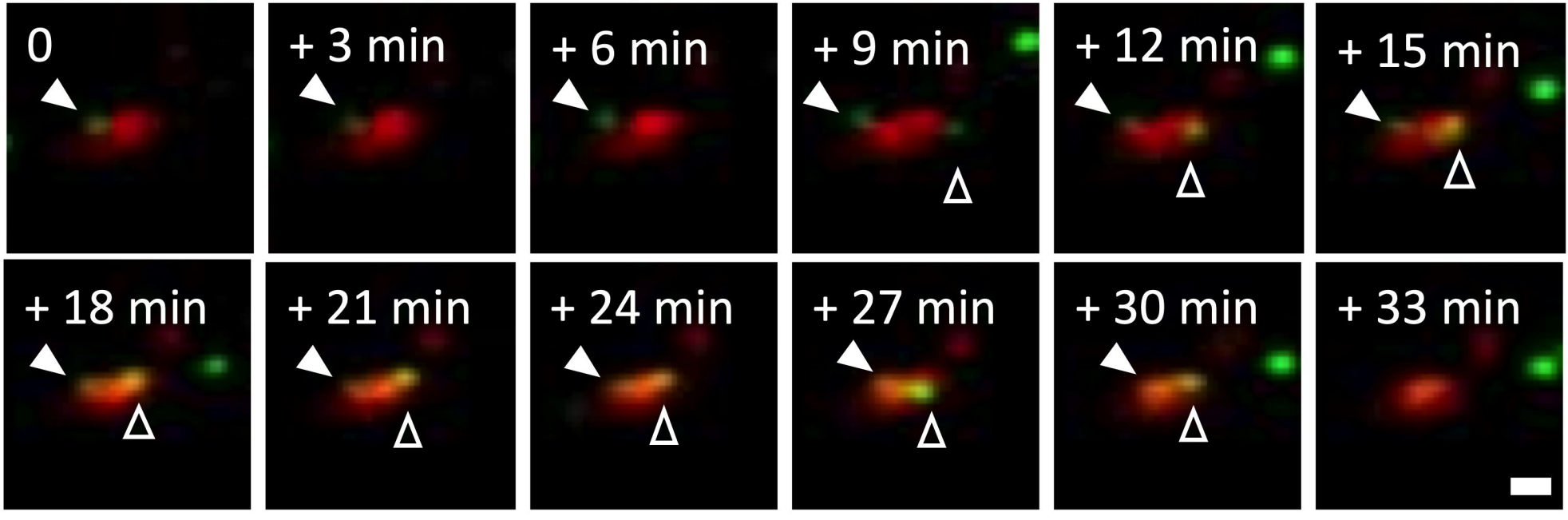


A

Tg(-6.0itga2b:eGFP)^{la2} UPEC-pGI6



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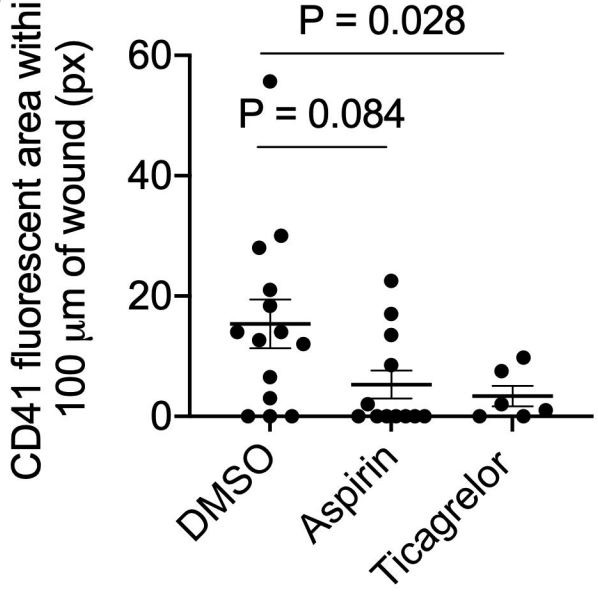

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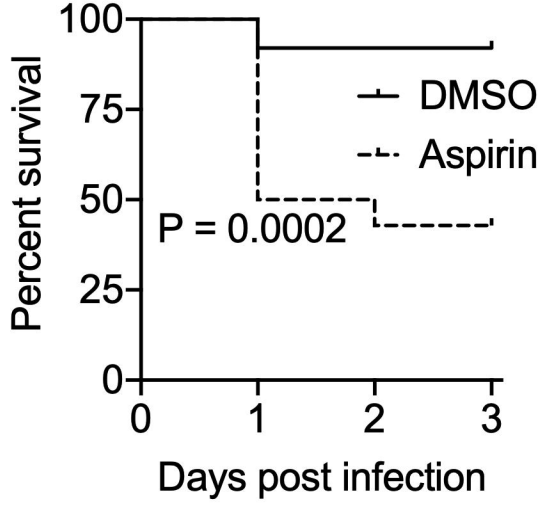
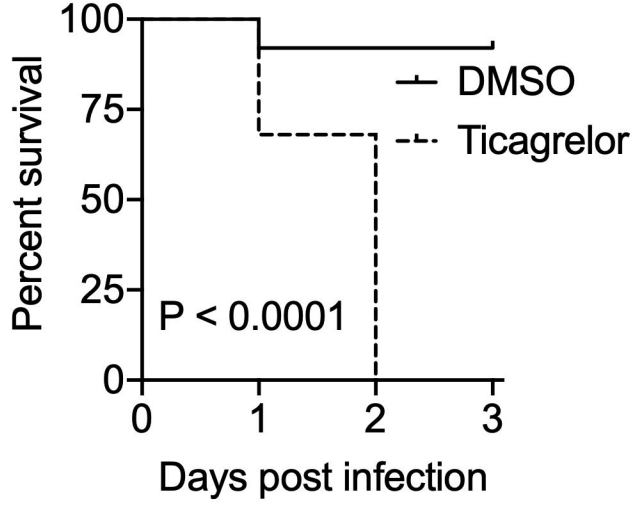
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