3. Department of Health. United Kingdom. Chapter 25: pneumococcal. In: Immunisation against infectious diseases—'The green book'. 2006 [cited 2011 Nov 11]. http://www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/GreenBook/GreenBookGeneralInformation/GreenBookGeneralArticle/fs/en?CONTENT_ID=4097254&chk=isTfGX)

4. Borrow R, Stanford E, Waight P, Helbert M, Balmer P, Warrington R, et al. Serotype-specific immune unresponsiveness to pneumococcal conjugate vaccine following invasive pneumococcal disease. Infect Immun. 2008;76:5305–9. http://dx.doi.org/10.1128/IAI.00796-08

5. Goldblatt D, Southern J, Ashton L, Richmond P, Burbidge P, Tasevska J, et al. Immunogenicity and boosting following a reduced number of doses of a pneumococcal conjugate vaccine in infants and toddlers. Pediatr Infect Dis J. 2006;25:312–9. http://dx.doi.org/10.1097/01.inf.0000207483.60267.e7

6. Örtqvist Å, Henckaerts I, Hedlund J, Poolman J. Non-response to specific serotypes likely cause for failure to 23-valent pneumococcal polysaccharide vaccine in the elderly. Vaccine. 2007;25:2445–50. http://dx.doi.org/10.1016/j.vaccine.2006.09.018

7. Dagan R, Givon-Lavi N, Greenberg D, Fritzell B, Siegrist CA. Nasopharyngeal carriage of Streptococcus pneumoniae shortly before vaccination with a pneumococcal conjugate vaccine causes serotype-specific hyporesponsiveness in early infancy. J Infect Dis. 2010;201:1570–9. http://dx.doi.org/10.1086/652006

8. Väkeväinen M, Soininen A, Lucero M, Nohynek H, Auranen K, Mäkelä PH, et al.; ARIVAC Consortium. Serotype-specific hyporesponsiveness to pneumococcal conjugate vaccine in infants carrying pneumococcus at the time of vaccination. J Pediatr. 2010;157:778–83. http://dx.doi.org/10.1016/j.jpeds.2010.04.071

9. Moscoso M, García E, López R. Pneumococcal biofilms. Int Microbiol. 2009;12:77–85.

Address for correspondence: Shamez Ladhani, Immunisation Department, Centre for Infections, Health Protection Agency, 61 Colindale Ave, London NW9 5EQ, UK; email: shamez.ladhani@hpa.org.uk

African Swine Fever Virus Strain Georgia 2007/1 in *Ornithodoros erraticus* Ticks

To the Editor: African swine fever virus (ASFV) causes a notifiable disease in domestic pigs for which no treatment or vaccine is available, resulting in a mortality rate of ≤100%. In 2007 ASFV was detected in the Caucasus region, first in Georgia and subsequently in Armenia, Azerbaijan, and many parts of Russia, including regions that border other countries in Europe and Asia (*1*).

Most field strains of ASFV can persistently infect *Ornithodoros* ticks, including the species *O. erraticus* in southern Europe (*2*), and ASFV has been isolated from ticks collected >5 years after the last confirmed case in an outbreak (*3*). These ticks can feed on alternative hosts, evade eradication attempts (such as acaricide application and flamethrowers), and survive for up to 15 years (*1*). Although *Ornithodoros* species have been reported in the Caucasus region, their distribution is not well known (*1*). It is also not known if the Georgia 2007/1 ASFV strain responsible for continuing outbreaks in the Caucasus region can replicate in ticks. Thus, we conducted a study to determine whether the Georgia 2007/1 isolate of ASFV can replicate in *Ornithodoros* ticks.

O. erraticus ticks from Alentejo, Portugal (provided by Fernando Boinas, Universidade Técnica de Lisboa in Lisbon, Portugal) were sorted into groups of 10 adults or fifth-instar nymphs, placed into 60-mL containers covered with nylon cloth (16-cm mesh), and maintained at 85% relative humidity and 27°C for 18 months without feeding. Heparinized pig blood containing antibacterial drugs and fungicide (10 μL of streptomycin [10,000 IU/mL], 10 μL of amphotericin B [250 μg/mL], and 5 μL of neomycin [10 mg/mL 0.9% NaCl]/mL of blood) was mixed with the Georgia 2007/1 isolate (*4*) or the OUR T88/1 isolate (*5*) as a positive control to obtain virus titers of 4 $\log_{10}$ or 6 $\log_{10}$ 50% hemadsorbing doses (HAD_{50})/mL blood. These titers were within the observed range in naturally infected pigs (*6*), and thus simulated the field situation.

Ticks were fed infected blood by using a Hemotek membrane-feeding system (Discovery Workshops, Accrington, UK). Meal reservoirs were covered with stretched Parafilm that was wiped with a thin film of uninfected blood to encourage feeding. Pots of ticks were placed on the membrane and allowed to feed for 20 minutes.

Immediately after and 3, 6, 9, and 12 weeks after feeding, 10 ticks from each feeding group were killed by freezing in dry ice. After being washed with a detergent solution and phosphate-buffered saline, ticks were placed individually in tubes with 200 μL of RPMI medium (Sigma-Aldrich Company Ltd., Gillingham, UK), a 3 mm-diameter stainless steel ball (Dejay Distribution Ltd., Launceston, UK), and 1-mm silicon carbide particles (Stratech Scientific Ltd, Newmarket, UK). They were then homogenized by shaking for 5 cycles of 3 minutes at 25-Hz frequency using a TissueLyser (QIAGEN, Valencia, CA, USA). To complete a 1-mL volume, 800 μL of RPMI medium was added to the tubes after centrifuging 2× for 30 seconds at 2,000 rpm. Supernatants were transferred to fresh tubes and centrifuged for 5 minutes at 1,000 × g.

Virus titers were estimated on porcine bone marrow cells (*7*) and expressed as $\log_{10}$ HAD_{50} per tick. Previous studies suggest that it takes 3–4 weeks for ticks to completely digest and clear ingested blood and that virus isolated after this period is due to viral replication (*5,6*). A general
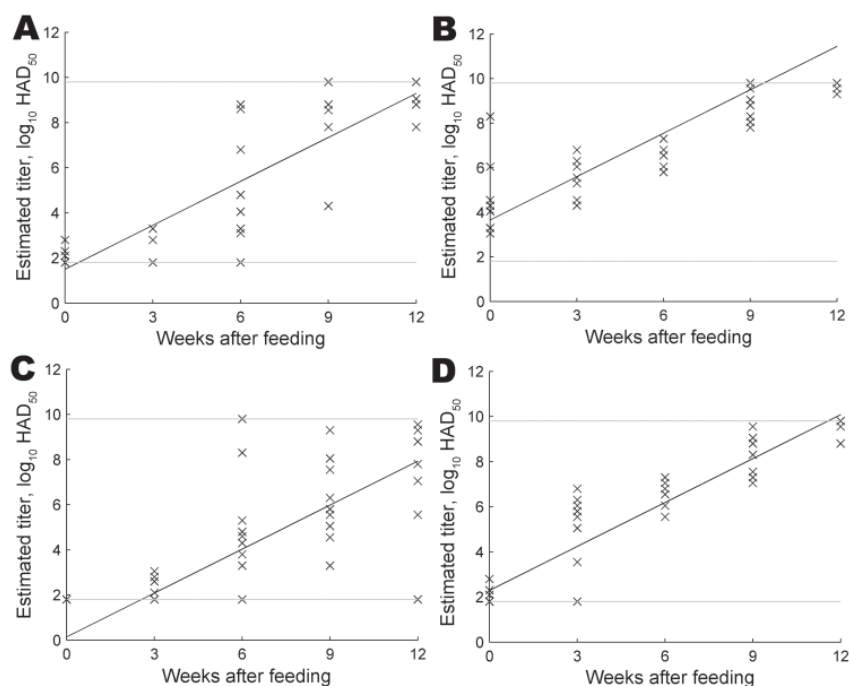
Figure. Predicted regression for each isolate–dose combination is shown. A) Ticks fed on African swine fever virus (ASFV) strain OUR T88/1 at 4 $\log_{10}$ 50% hemadsorbing doses (HAD$_{50}$)/mL. B) Ticks fed on ASFV strain OUR T88/1 at 6 $\log_{10}$ HAD$_{50}$/mL. C) Ticks fed on ASFV strain Georgia 2007/1 at 4 $\log_{10}$ HAD$_{50}$/mL. D) Ticks fed on ASFV strain Georgia 2007/1 at 6 $\log_{10}$ HAD$_{50}$/mL. Crosses indicate experimental results, and solid line indicates model prediction. Dashed horizontal lines show the limits of the tissue culture sensitivity (lower limit 1.8 $\log_{10}$ HAD$_{50}$ and upper limit 9.8 $\log_{10}$ HAD$_{50}$).

linear model, fitted via maximum likelihood, was used to assess the effects of isolate, dose, time after feeding, and interaction between isolate and time after feeding on the viral titer in the tick. Confidence intervals were calculated by profile likelihood.

Results showed that the Georgia 2007/1 strain can replicate in the *O. erraticus* tick. We recovered virus titers of <1.8 to >9.8 $\log_{10}$ HAD$_{50}$ per tick. Ticks that fed on blood containing 6 $\log_{10}$ HAD$_{50}$ ASFV on

average had virus titers 2.15 $\log_{10}$ HAD$_{50}$ higher than those for ticks that fed on blood containing 4 $\log_{10}$ HAD$_{50}$/mL. Over time, the average titer for both isolates increased at an estimated rate of 0.65 $\log_{10}$ HAD$_{50}$/week, indicating replication. Statistical analysis suggested that immediately after feeding, ticks fed on the Georgia 2007/1 strain contained 1.36 $\log_{10}$ HAD$_{50}$ less virus than those fed on the OUR T88/1 isolate, but we detected no statistically significant difference in the replication rates of

the 2 isolates. Parameter estimates are shown in the Table and the model fit is shown in the Figure.

The whole-tick titers reported in this study are consistent with those from previous studies (*5,6*). However, ≥9 weeks after the ticks fed, we observed higher titers than those reported (*5*), and many results showed ≥9.8 $\log_{10}$ HAD$_{50}$ per tick. The TissueLyser may have been more effective at releasing ASFV from tick tissues than previously used methods. Our results suggest that virus replication within the ticks began by 3 weeks after feeding on infected blood; this timing is consistent with that in previous studies (*5,8,9*).

We observed high viral titers for ≥12 weeks after infection. Previous studies showed that ASFV can persist at high titers for 20 weeks after infection (*10*). We demonstrated that ASFV Georgia 2007/1 isolate can replicate efficiently in ticks. This finding highlights the importance of clarifying the distribution of *Ornithodoros* species ticks in the Russian Federation and Caucasus region and the relationship of these ticks to species susceptible to ASFV.

Table. General linear model of the effects of different parameters on the titer of ASFV in experimentally infected *Ornithodoros erraticus* ticks*

Parameter	Maximum likelihood estimator (95% CI)
Constant	1.4985 (0.7084 to 2.2610)†
ASFV strain	−1.3620 (−2.4007 to −0.3482)†
Dose	2.1538 (1.5889 to 2.7316)†
Time after feeding (effect per week)	0.6494 (0.5546 to 0.7481)†
Isolate–time interaction	−0.0025 (−0.1400 to 0.1363)‡

*Ticks were fed pig blood with 4 $\log_{10}$ or 6 $\log_{10}$ HAD$_{50}$/mL ASFV strain Georgia 2007/1 or strain OURT88/1. ASFV, African swine fever virus; HAD$_{50}$, 50% hemadsorbing doses.
†Statistically significant, p<0.01.
‡Not significant (p>0.05).

**Adriana V. Diaz,
Christopher L. Netherton,
Linda K. Dixon,
and Anthony J. Wilson**

Author affiliations: The Royal Veterinary College, London, United Kingdom (A.V. Diaz); and Institute for Animal Health, Pirbright, Surrey, United Kingdom (A.V. Diaz, C.L. Netherton, L.K. Dixon, A.J. Wilson)

References

1. European Food Safety Authority. EFSA Panel on Animal Health and Welfare; scientific opinion on African swine fever. EFSA Journal. 2010;8:1556. http://dx.doi.org/10.2903/j.efsa.20101556
2. Sanchez-Botija C. Reservoirs of ASFV: a study of the ASFV in arthropods by means of the haemadsorption test. Bull Off Int Epizoot. 1963;60:895–9.
3. Boinas FS, Wilson AJ, Hutchings GH, Martins C, Dixon LJ. The persistence of African swine fever virus in field-infected Ornithodoros erraticus during the ASF endemic period in Portugal. PLoS ONE. 2011;6:e20383. http://dx.doi.org/10.1371/journal.pone.0020383
4. Rowlands RJ, Michaud V, Heath L, Hutchings G, Oura C, Vosloo W, et al. African swine fever virus isolate, Georgia, 2007. Emerg Infect Dis. 2008;14:1870–4. http://dx.doi.org/10.3201/eid1412.080591
5. Rowlands RJ, Duarte MM, Boinas F, Hutchings G, Dixon LK. The CD2v protein enhances African swine fever virus replication in the tick vector, Ornithodoros erraticus. Virology. 2009;393:319–28. http://dx.doi.org/10.1016/j.virol.2009.07.040
6. Basto AP, Nix RJ, Boinas F, Mencles S, Silva MJ, Cartaxeiro C, et al. Kinetics of African swine fever virus infection in Ornithodoros erraticus ticks. J Gen Virol. 2006;87:1863–71. http://dx.doi.org/10.1099/vir.0.81765-0
7. Malmquist WA, Hay D. Hemadsorption and cytopathic effect produced by ASFV in swine bone marrow and buffy coat cultures. Am J Vet Res. 1960;21:104–8.
8. Kleiboeker SB, Burrage TG, Scoles GA, Fish D, Rock DL. African swine fever virus infection in the argasid host, Ornithodoros porcinus porcinus. J Virol. 1998;72:1711–24.
9. Kleiboeker SB, Scoles GA, Burrage TG, Sur JH. African swine fever virus replication in the midgut epithelium is required for infection of Ornithodoros ticks. J Virol. 1999;73:8587–98.
10. Basto AP, Portugal RS, Nix RJ, Cartaxeiro C, Boinas F, Dixon LK, et al. Development of a nested PCR and its internal control for the detection of African swine fever virus (ASFV) in Ornithodoros erraticus. Arch Virol. 2006;151:819–26. http://dx.doi.org/10.1007/s00705-005-0654-2

Address for correspondence: Anthony J. Wilson, Institute for Animal Health, Pirbright Laboratory, Ash Rd, Pirbright, Surrey GU24 0NF, UK; email: anthony.wilson@iah.ac.uk

Apparent Triclabendazole-Resistant Human *Fasciola hepatica* Infection, the Netherlands

To the Editor: In December 2007, a 71-year-old sheep farmer sought care with a 4-month history of intermittent right upper quadrant pain, night sweats, anorexia, and a 5-kg weight loss. His medical history was unremarkable, and he had not traveled outside the Netherlands for ≈30 years. Physical examination revealed no abnormalities.

Blood tests showed an elevated erythrocyte sedimentation rate of 35 mm/h (reference 1–15 mm/h), normocytic anemia (hemoglobin 7.0 mmol/L [reference 8.5–11 mmol/L]), and eosinophilia (2.5×10^9 cells/L [reference $0.0–0.5 \times 10^9$ cells/L]). Levels of alkaline phosphatase, γ-glutamyl transferase, and alanine aminotransferase were elevated (146 U/L [reference 10–120 U/L], 143 U/L [reference 5–50 IU/L], and 54 U/L [reference 0–45 U/L], respectively). Levels of bilirubin and aspartate aminotransferase were normal. Computed tomography of the liver showed several irregularly shaped low-attenuating lesions ranging in size from 1 to 4 cm. High titers of IgG (640 [cutoff 40], determined by enzyme immunoassay) against *Fasciola hepatica* were detected. Subsequently, *F. hepatica* eggs were detected in fecal samples.

The patient, who spontaneously had become asymptomatic shortly after seeking care, was treated unsuccessfully with the benzimidazole derivative triclabendazole (TCBZ) on 3 separate occasions during the next 2 years. He was first treated with a single dose of 10 mg/kg TCBZ (Fasinex suspension; Novartis Animal Health Ltd., Surrey, UK), then with 2 doses 24 hours apart, and on the last occasion with 2 doses of TCBZ (Egaten; Sipharm Sisseln AG, Sisseln, Switzerland) 10 mg/kg 12 hours apart; the last 4 treatments were taken with food. Feces remained positive for *F. hepatica* eggs after each treatment. IgG titers remained positive (320, by enzyme immunoassay), and flukes could be visualized by ultrasound in the gallbladder and common bile duct (Figure). Thereafter, the patient was treated with nitazoxanide (500 mg 2×/d for 7 days); however, fecal samples remained positive for *F. hepatica* eggs. Lastly, after recent experiments of a combination therapy in a rat model (*1*), we treated the patient with TCBZ (Egaten, 10 mg/kg) combined with ketoconazol 10 mg/kg taken with food. Still, his feces remained positive for *F. hepatica* eggs.

Fascioliasis is a zoonotic disease caused by the foodborne trematode *F. hepatica* or *F. gigantica*, which has a complex life cycle and mainly affects sheep and cattle (*2*). Eggs of the adult worms (2–4 cm) that live in the bile ducts of the final host are excreted in the feces and develop into larvae (miracidia) in water. The miracidia then penetrate, and further develop in, snails of the family Lymnaeidae. Free-swimming cercariae exit the snail and attach to aquatic vegetation, where they encyst as metacercariae. After