**Supplemental Table S1.** Sources of records, date of search, search terms, and number of records retrieved, before de-duplication and screening, obtained from bibliographic databases and other sources.

|  |  |  |  |
| --- | --- | --- | --- |
| **Source** | **Date searched** | **Keyword search** | **No. of records** |
| **Bibliographic databases** |
| Web of Science1 | Aug. 5, 2022 | 3: #1 AND #22: ((((((((((((TS=(swine)) OR TS=(pig)) OR TS=(hog)) OR TS=(boar)) OR TS=(pork)) OR TS=("sus scrofa")) OR TS=("sus domesticus")) OR TS=(barrow)) OR TS=(gilt))) OR TS=(piglet)) OR TS=(sow))1: (((((((TS= ("Japanese encephalitis")) OR TS= ("Japanese b encephalitis")) OR TS=(JEV)) OR TS=(JE)) OR TS= ("summer encephalitis")) OR TS= ("viral encephalitis")) OR TS= ("viral meningitis")) OR TS= ("Russian autumnal encephalitis") | 618 |
| Scopus2  | Aug. 5, 2022 |  TITLE-ABS-KEY ("Japanese encephalitis" OR "Japanese b encephalitis" OR "JEV" OR "je" OR "summer encephalitis" OR "viral encephalitis" OR "viral meningitis" OR "Russian autumnal encephalitis" OR "viral encephalitis") AND (swine OR boar OR hog OR pig OR pork OR "sus scrofa" OR "sus domesticus" OR sow OR piglet OR gilt OR barrow) | 2,545 |
| **Other sources**  |
| USDA Animal and Plant Health Inspection Service3 | Aug. 19-27, 2022 | "Feral swine" "Japanese encephalitis" | 109 |
| Centers for Disease Control and Prevention4 | Sept. 19- 23, 2022 | ALL THIS WORD: Japanese encephalitis ANY OF THESE WORDS: feral wild undomesticated free-range ranging roaming swine pig hog boar pork | 300 |
| USDA Wildlife Services Digital Collection4 | Sept. 1-3, 2022 | 6: “Japanese encephalitis” AND feral AND boar (n = 2)5: “Japanese encephalitis” AND wild AND boar (n = 2)4: “Japanese encephalitis” AND feral AND pig (n = 1)3: “Japanese encephalitis” AND wild AND pig (n = 4)2: “Japanese encephalitis” AND wild AND swine (n = 7)1: “Japanese encephalitis” AND feral AND swine (n = 7) | 23 |
| USDA NIFA Current Research Information System5 | Sept. 9-10, 2022 | "Japanese encephalitis" AND (feral; wild; "free range"; ranging; "free roaming"; game; undomesticated) AND (swine; pig; boar; hog; pork; "sus scrofa") | 25 |
| Hand-search of citations from Vienna Brown6 | Aug. 18, 2022 | Titles were searched for language referring to “Japanese encephalitis virus” and “feral swine” or relevant synonyms | 4 |
| Hand-search of Wildlife Health Australia, 20227 | Aug. 18, 2022 | The reference list was searched for titles referring to “Japanese encephalitis virus” and “feral swine” or relevant synonyms | 14 |

1 TS = Search for topic terms in the following fields within a record. Search in title, abstract, author keywords, and keywords Plus®.

2TITLE-ABS-KEY = Search for topic terms in the title, abstract, and keywords.

3 <https://www.aphis.usda.gov/aphis/home/>

4 Search was performed using the “advanced search” option-fields. Search term string was entered in “Full text Terms” field-option, using “Subfile option” as “(Any)”.

4 <https://nwrc.contentdm.oclc.org/digital/collection/NWRCPubs1>

5 NIFA = National Institute of Food and Agriculture. The assisted search option was utilized, and the “Japanese encephalitis” string was entered into “Full text Terms” and the feral and swine terms into “AND these”.

6 <https://scholar.google.com/citations?hl=en&user=wcfDGWsAAAAJ>

7Wildlife Health Australia. (2022) WHA fact sheet on Japanese encephalitis. Available at: <https://wildlifehealthaustralia.com.au/Incidents/Incident-Information/wildlifehealthaustralia.com.au/Portals/0/ResourceCentre/FactSheets/PublicHealth/Japanese_Encephalitis.pdf>. [August 18, 2022].

**Supplemental Table S2.** Summary and description of key data items. Reference information and study characteristics were captured for all reports. Items related to specific research questions were captured as applicable.

|  |  |  |
| --- | --- | --- |
| **Data category** | **Key item** | **Description** |
| ***Items from all reports*** |
| **Report level** |
| Reference information |  | List of authors, title, journal name, publication date, affiliation, and DOI1  |
| **Study level** |
| Population characteristics | Breed | Reported breeds were extracted as per authors’ description and subsequently categorized by predominant breed or mix. Categories are: Yorkshire (including Yorkshire-predominant crosses), Landrace (including Landrace-predominant crosses), Large White, Berkshire/Landrace, Yorkshire/Landrace, Other (i.e., Black Swayback and white-line crossbreed), Other-research (i.e., Specific pathogen-free pigs with no other breed description, hysterectomy-derived piglets, miniature pigs, and Sinclair miniature pigs with feral background), Unspecified (e.g., “local breeds”, “indigenous”, “imported”), and Feral.  |
| Sex | Reported sex was extracted as male, female, or both (referring to study populations comprised of both sexes). |
| Age | Reported age of swine populations was extracted as reported and subsequently categorized according to the equivalent production stage as per the life cycle of a marketing pig (Pork checkoff and The National Pork Board, 2024). Abortus and stillborn were categorized as fetus, animals between 0 and 3 weeks of age were categorized as nursing, between 3 and 10 weeks of age as weaning, between 10 and 18 weeks as growing, between 18 and 26 as finishing, and greater than 26 weeks as mature.  |
| Study characteristics | Study location  | Information related to the geographical location where the study was conducted (e.g., country, state, province, or city) was extracted as reported by authors. |
| Study type | Studies were classified as: observational (case-control, cohort, cross-sectional studies, and case reports), experimental (studies reporting an intervention or other manipulation of factors), mathematical model (reports describing simulated behavior based on specific values of parameters considered in a mathematical model), or systematic review (review reports describing the use of a systematic and reproducible methodology to appraise relevant literature). |
| Sample size | Reported number of animals per treatment or exposure group. |
| JEV case definition | Criteria used to consider a study unit JEV-positive (i.e., based on clinical signs, diagnostic test(s), either alone or in combination with confirmatory tests, or a combination of these along with virus isolation), JEV genotype isolated (if applicable), and name of diagnostic test(s) used. |
| Study design structure | Exposure to JEV infection was classified as natural or challenge. For challenge exposures, JEV strain, route of administration (or challenge route), and dose of inoculum were extracted when reported. Study animals were deemed to be naturally exposed when JEV infection resulted from a locally circulating virus via an unknown route (i.e., animals were placed outside in an endemic region). All study design structure information was extracted as per authors’ description. |
| **Items related to specific research questions** |
| Transmission routes | Study design structure | Extracted as described above in the “Study design structure” section. |
| Disease process (i.e., organotropism, pathology, and clinical signs) | Route and dose of inoculation | Extracted as described above in the “Study design structure” section.  |
| Strain and genotype | Reported JEV strain and genotype used for the challenge or isolated from naturally infected animals. |
| JEV incubation period | Time period between JEV infection and onset of clinical signs as reported by authors, or calculated by reviewers when enough information was provided (i.e., time of exposure and time of onset of first clinical signs). |
| Days post infection | Reported number of days between infection and detection of virus in tissues, or observation of pathological findings. |
| Clinical signs | Reported clinical signs associated with JEV infection, and proportion of at-risk animals that manifested clinical signs. |
| Tissue infected and viral titer  | Reported organs where JEV was isolated or detected, and corresponding viral titers. |
| Macro- and micro-pathology  | Reported pathological findings associated with JEV infection as described by authors. |
| Diagnostic test used and evaluated | Test name | Name of test being evaluated according to authors’ description. |
| Reference test | Reference test used for comparison according to authors’ description. |
| Sample type | Sample type(s) used for the diagnostic test were extracted as per authors’ description and categorized during the data synthesis process for simplification. * Blood: includes samples reported as blood, serum, umbilical-cord blood, peripheral blood mononuclear cells, and leukocyte pellets.
* Cerebrospinal tissues: includes samples reported as brain, brain stem, basal nuclei, cerebral cortex, cerebral white matter, choroid plexus, cerebrum, cerebellum, cervical spinal cord, frontal lobe, hippocampus, meninges, neocortex, neocortex frontalis, neocortex temporalis, neuroepithelium, olfactory bulb, parietal lobe, sacral spinal cord, spinal cord, spinal ganglia, sciatic nerve, striatum, thalamus, and trigeminal ganglion.
* Testicular samples: includes samples reported as testicular cells, testicular samples, gonad, and swollen testis.
* Abortus: includes samples reported as embryo organs, mummified fetus tissues, abortus, aborted fetuses, placenta, and umbilical cord.
* Intestines: includes samples reported as small intestine, large intestine, colon, duodenum, and jejunum.
* Lymph nodes: includes samples reported as lymph node, cervical lymph node, prescapular lymph node, medial retropharyngeal lymph node, mesenteric lymph node, medial iliac lymph node, and cervical lymph node.
* Semen: includes samples reported as seminal fluid and semen.
* Peripheral nerves: includes samples reported as peripheral nerve and auriculopalpebral nerve.
 |
| Test performance measures | Diagnostic sensitivity and specificity and analytical sensitivity and specificity of the novel test were extracted as reported by authors or calculated by reviewers when reported information allowed. Statistical significance of differences was also extracted when reported. |
| Risk factors | Population characteristics | Extracted as described above in “Population characteristics” section. |
| Environmental and management characteristics of swine operations | Descriptors of risk factors statistically assessed for an association with seroprevalence, seroconversion, antibody titer, or viremia. |
| Viremia | Viral titer value and time of measurement relative to the day of infection, as reported by authors. Statistical significance of differences was also extracted when reported. |
| Seroconversion | Proportion of JEV seronegative animals that developed JEV-antibodies during the study period. Seroconversion was extracted as reported by authors or calculated by reviewers when reported information allowed. Statistical significance of differences was also extracted when reported. |
| Antibody titer | Antibody titer value and time of measurement relative to the day of infection or immunization, as reported by authors. Statistical significance of differences was also extracted when reported. |
| Seroprevalence | Proportion of animals showing evidence of JEV infection (via detection of anti-JEV antibodies or JEV antigen). Seroprevalence was extracted as reported by authors or calculated by reviewers when reported information allowed. Statistical significance of differences was also extracted when reported. |
| Surveillance | Surveillance strategy and outcomes | Surveillance strategy and corresponding measurement of effectiveness (e.g., seroconversion, seroprevalence, morbidity, mortality) were extracted. No studies reported this outcome, therefore no further items were extracted related to this outcome.  |
| Vaccine efficacy | Vaccine information | Reported vaccine name, vaccine strain and genotype, and manufacturer. |
| Route and scheme | Reported vaccination route (e.g., subcutaneous, intranasal, intramuscular), and vaccination scheme as per authors’ description. |
| Challenge strain and genotype | Reported JEV-challenge strain and genotype used to evaluate vaccine efficacy as reported by authors. |
| Efficacy evidence | Outcomes evaluated by the authors to assess vaccine efficacy (e.g., antibody titers, mortality, viremia, viral shedding, clinical signs). Statistical significance of differences was also extracted when applicable and reported. |
| Author’s conclusion | Author’s conclusion regarding the efficacy of the vaccine being evaluated (i.e., efficacious, inconclusive, or not efficacious). |
| Author evidence | Author’s supportive evidence for the conclusion statement regarding vaccine efficacy. |
| Mathematical modelling (Basic reproduction ration (R0)) | Compartments | Reported states considered for all host and vector populations in compartmental models. |
| JEV R0 | Reported basic reproduction ratio (R0) for JEV modeled in swine populations. |
| Route of transmission | Reported route of transmission considered for swine only.  |
| Control strategy evaluated  | List of control strategies evaluated (if applicable). |
| Outcomes | Reported outcomes used by authors to evaluate the respective control strategy. Statistical significance of differences was also extracted when reported. |
| Biosecurity | Biosecurity strategy and outcomes | Application of biosecurity strategies as interventions against JEV introduction in swine operations, and corresponding measurement of effectiveness (e.g., seroconversion) were extracted according to authors’ description. Statistical significance of differences was also extracted when reported. |
| Pork products | Sample type and viral detection | Reported pork-product sample types (processed pork or pork products) associated with any evidence of JEV detection were extracted. No studies reported this outcome, therefore no further items were extracted related to this outcome.  |

1DOI = DigitalObject Identifier (extracted when available).

**Supplemental Table S3**. Numbers of reports and studies, and corresponding references, per type of study design included in this rapid systematic review.

|  |  |  |
| --- | --- | --- |
| **Study design** | **No. of reports** **(No. of studies)** | **Reference(s)** |
| Experimental    | 99 (162) | Meiklejohn, G., Simpson, T.W., and Stacy, I.B. (1947); Shimizu, T. et al. (1954); Hale, J.H., Lim, K.A., and Colless, D.H. (1957); Gresser, I. et al. (1958); Scherer, W.F., Moyer, J.T., and Izumi, T. (1959); Hurlbut, H.S. (1964); Kodama, K., Sasaki, N., and Inoue, Y.K. (1968); Carey, D.E., Reuben, R., and Myers, R.M. (1969); Ogata, M. et al. (1969); Sazawa, H. et al. (1969a); Sazawa, H. et al. (1969b); Ogata, M. et al. (1970); Ogata, M. et al. (1971); Ueba, N. et al. (1972); Johnsen, D.O. et al. (1974); Fujisaki, Y. et al. (1975); Lee, G.C.Y., Huang, Y.T., and Chang, L.C. (1975); Hayashi, K., Mifune, K., and Shichijo, A. (1976); Maeda, O. et al. (1978); Ueba, N. et al. (1978); Yoshida, I. et al. (1981); Konishi, E., and Yamaoka, M. (1982); Sasaki, O. et al. (1982); Yamaoka, M., Konishi, E., and Matsumura, T. (1982); Konishi, E., and Yamaoka, M. (1983); Yamaoka, M. (1983); Chang, H.-C. et al. (1984a); Chang, H.-C. et al. (1984b); Ohkubo, Y. et al. (1984); Konishi, E. et al. (1992); Cardosa, M.J. et al. (1993); Ilkal, M.A. et al. (1994); Konishi, E. et al. (2000); Williams, D.T. et al. (2001); Nam, J.-H., Chae, S.-L., and Cho, H.-W. (2002); Xinglin, J. et al. (2002); Pyke, A.T. et al. (2004); Xu, G. et al. (2004); Yamada, M. et al. (2004); Yang, D.K. et al. (2004a); Yang, D.K. et al. (2004b); Xinglin, J. et al. (2005); Yang, D.-K. et al. (2006); Fei-Fei, G. et al. (2008); Li, P. et al. (2008); Yamada, M. et al. (2009); Chen, H.-Y. et al. (2010); Fan, J.-M. et al. (2010a); Imoto, J.-I. et al. (2010); Li, Y. et al. (2010); Duong, V. et al. (2011); Dutta, P. et al. (2011); Xu, X.-G. et al. (2011); Liu, H. et al. (2012); Tian, C.J. et al. (2012); Xu, X.-G. et al. (2012); Yang, Z. et al. (2012); Fan, Y.-C. et al. (2013); Rao, P. et al. (2014); Wu, H. et al. (2014); Zeng, Z. et al. (2014); Cha, G.-W. et al. (2015); De Wispelaere, M. et al. (2015); Dhanze, H. et al. (2015); Kolhe, R.P. et al. (2015); Zhang, M. et al. (2015); Hu, L. et al. (2016); Ricklin, M.E. et al. (2016a); Ricklin, M.E. et al. (2016b); Sheng, Z. et al. (2016); García-Nicolás, O. et al. (2017); Wu, X. et al. (2017); Yang, D.-K. et al. (2017); Fan, Y.-C. et al. (2018); Lyons, A.C. et al. (2018); Park, S.L. et al. (2018); Xiao, C. et al. (2018); Xiao, L. et al. (2018); Dhanze, H. et al. (2019); Fan, Y.-C. et al. (2019); Grace, M.R. et al. (2019a); Grace, M.R. et al. (2019b); Pantawane, P.B. et al. (2019); Zheng, B. et al. (2019); Zhou, D. et al. (2019); Chauhan, J. et al. (2020); Dhanze, H. et al. (2020a); Dhanze, H. et al. (2020b); Redant, V. et al. (2020); Wang, X. et al. (2020); Wu, Y. et al. (2020); Young, C.L. et al. (2020); Zhou, Y. et al. (2020); Zhou, D. et al. (2021); Nie, M. et al. (2022); Redant, V. et al. (2022); Xie, S.D. et al. (2022); Yang, L. et al. (2022); Zhang, Y. et al. (2022)  |
| Observational | 135 (180) | Burns, K.F. (1950); Hale, J.H., Lim, K.A., and Colless, D.H. (1957); Scherer, W.F., Moyer, J.T., and Izumi, T. (1959); Scherer, W.F. et al. (1959); Hurlbut, H.S. (1964); Konno, J. et al. (1966); Doi, R. et al. (1967); Konno, J., Endo, K., and Ishida, N. (1967); Unita, T. (1969); Detels, R. et al. (1970); Higgins, D.A. (1970); Simpson, D.I.H. et al. (1970); Yamamoto, H.A. et al. (1970); Yamada, T. et al. (1971); Okuno, T. et al. (1973); Chang, C.P. et al. (1974); Johnsen, D.O. et al. (1974); Van Peenen, P.F.D. et al. (1974); Van Peenen, P.F.D. et al. (1975); Wada, Y., Oda, T., and Mogi, M. (1975); Detels, R. et al. (1976); Hayashi, K., Mifune, K., and Shichijo, A. (1976); Rodrigues, F.M. et al. (1976); Simpson, D.I.H. et al. (1976); Igarashi, A., Morita, K., and Bundo, K. (1981); Kalimuddin, M.D., Narayan, K.G., and Choudhary, S.P. (1982a); Kalimuddin, M.D., Narayan, K.G., and Choudhary, S.P. (1982b); Nandi, A.K. et al. (1982); Igarashi, A. et al. (1983); Yamaoka, M. (1983); Ohkubo, Y., Takashima, I., and Hashimoto, N. (1984); Burke, D.S. et al. (1985a); Burke, D.S. et al. (1985b); Burke, D.S. et al. (1985c); Yamaoka, M., and Konishi, E. (1985); Geevarghese, G. et al. (1987); Gingrich, J.B. et al. (1987); Singh, G., and Rao, T.R. (1988); Takashima, I. et al. (1988); Thein, S., Aung, H., and Sebastian, A.A. (1988); Angami, K. et al. (1989); Geevarghese, G. et al. (1991); Gingrich, J.B. et al. (1992); Peiris, J.S.M. et al. (1992); Cardosa, M.J. et al. (1993); Paul, W.S. et al. (1993); Peiris, J.S.M. et al. (1993); Geevarghese, G. et al. (1994); Makino, Y. et al. (1994); Tadano, M. et al. (1994); Mall, M.P., Kumar, A., and Malik, S.V. (1995); Hanna, J.N. et al. (1996); Oda, K. et al. (1996); Hanna, J.N. et al. (1999); Ratho, R.K., Sethi, S., and Prasad, S.R. (1999); Myint, L. et al. (2000); Chattopadhyay, U.K. (2001); Pyke, A.T. et al. (2001); Chang, K.-J. (2002); See, E. et al. (2002); Xinglin, J. et al. (2002); Ting, S.H.L. et al. (2004); Yang, D.K. et al. (2004c); Yoshida, Y. et al. (2005); Pant, G.R. (2006); Pant, G.R. et al. (2006); Yang, D.-K. et al. (2006); Dutta, P. et al. (2007); Hamano, M. et al. (2007); Nidaira, M. et al. (2007a); Nidaira, M. et al. (2007b); Nitatpattana, N. et al. (2008); Nidaira, M. et al. (2009); Ogawa, H. et al. (2009); Ohno, Y. et al. (2009); Sugiyama, I. et al. (2009); Fan, J.-M. et al. (2010b); Yamanaka, A. et al. (2010); Cao, Q.S. et al. (2011); Duong, V. et al. (2011); Nitatpattana, N. et al. (2011); Obara, M. et al. (2011); Conlan, J.V. et al. (2012); Lindahl, J. et al. (2012); Thakur, K.K. et al. (2012); Yang, D.-K. et al. (2012); Borah, J. et al. (2013); Fan, Y.-C. et al. (2013); Kurane, I. et al. (2013); Lindahl, J.F. et al. (2013); Liu, H. et al. (2013); Teng, M. et al. (2013); Nidaira, M. et al. (2014); Cha, G.-W. et al. (2015); Detha, A., Wuri, D.A., and Santhia, K. (2015); Kolhe, R.P. et al. (2015); Cappelle, J. et al. (2016); Desingu, P.A. et al. (2016); Holt, H.R. et al. (2016); Wu, R. et al. (2016); Yoshikawa, A. et al. (2016); Duong, V. et al. (2017); Kakkar, M. et al. (2017); Zhang, H. et al. (2017a); Zhang, H. et al. (2017b); Baruah, A. et al. (2018); Chai, C. et al. (2018); Di Francesco, J. et al. (2018); Kumar, K. et al. (2018); Ruget, A.-S. et al. (2018); Xiao, C. et al. (2018); Dhanze, H. et al. (2019); Grace, M.R. et al. (2019a); Guo, H.C. et al. (2019); Komiya, T. et al. (2019); Lee, H.S. et al. (2019); Niazmand, M.H. et al. (2019); Yap, G. et al. (2019); Yonemitsu, K. et al. (2019); Zhou, D. et al. (2019); Datey, A. et al. (2020); Dhanze, H. et al. (2020a); Dhanze, H. et al. (2020b); Kumar, H.B.C. et al. (2020a); Kumar, H.B.C. et al. (2020b); Kuwata, R. et al. (2020); Ladreyt, H. et al. (2020); Lee, H.S. et al. (2020); Wang, X. et al. (2020); Chiou, S.-S. et al. (2021); Henriksson, E. et al. (2021); Raut, A.A. et al. (2021); Fan, Y.-C. et al. (2022); Nie, M. et al. (2022); Zhang, Y. et al. (2022)  |
| Mathematical modeling  | 10 (10) | Wada, Y. (1975); Khan, S.U. et al. (2014); De, A. et al. (2016); Riad, M.H. et al. (2017); Diallo, A.O. et al. (2018); Zhao, S. et al. (2018); Kharismawati, H., Fatmawati, and Windarto (2019); Baniya, V., and Keval, R. (2020); Goswami, N.K. (2022); Ladreyt, H., Chevalier, V., and Durand, B. (2022)  |
| Systematic reviews | 5 (5) | Lopez, A.L. et al. (2015); Oliveira, A.R.S. et al. (2017); Oliveira, A.R.S. et al. (2018b); Ladreyt, H. et al. (2019); Suresh, K.P. et al. (2022) |

**Supplemental Table S4.** Pathological findings and organotropic effects of Japanese encephalitis virus (JEV) infection in swine by genotype (GI, GIII, and unknown), including type of exposure (challenge or natural) and strain, population characteristics, system affected, inoculation route and dose, macro- and microscopic pathologic findings, organotropic findings, and respective references.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Exposure (Strain)** | **Population****[breed; sex; age]** | **Inoculation route and dose** | **Macroscopic** **pathological findings** | **Microscopic** **pathological findings** | **Organotropic findings** | **Reference(s)**  |
| ***Genotype I*** |
| **Central nervous system** |
| Challenge(Nakayama) | Large White; Both; Weaning | ID with 106 TCID50 | NR | NR | Brain at 11 dpi:* No detectable viral RNA
 | Ricklin, M.E. et al. (2016a) |
| IV with 106 TCID50 | NR | NR | Brain at 11 dpi:* No detectable viral RNA
 |
| Challenge(JE-91) | Other; NR; Weaning  | IV with 107 TCID50/mL | NR | NR | Nervous tissue (facial nerve, olfactory bulb, olfactory neuroepithelium, optic nerve, piriform cortex, and thalamus) at 3 dpi:* Infectious viral titer ranged from 5.0 × 101 PFU/g to 1.9 × 102 PFU/g
* The olfactory neuroepithelium had the highest viral load of 1.8 × 106 GEQ-TCID50/g

Cerebellum, thalamus, frontal and temporal lobes at 3 dpi: * Average viral loads > 103 GEQ-TCID50/g
* Sciatic nerve had the lowest mean viral titer of 4.4 × 101GEQ-TCID50/g
 | Park, S.L. et al. (2018) |
| Challenge(YL2009-4) | Other (research); NR; Weaning | SC with 107 FFU  | NR | Brain (cerebrum):* Multifocal gliosis and mononuclear perivascular cuffs
 | Brain at 8 dpi: * 104.5 to 105.8 copies/g of viral RNA
 | Fan, Y.-C. et al. (2018) |
| Observational | NR;NR; Nursing | NA | Brain: * Multifocal hemorrhage
 | NR | Brain: * Neurons were positive for JEV antigen via immunohistochemistry
 | Cao, Q.S. et al. (2011) |
| **Lymphatic system** |
| Challenge(Nakayama) | Large White; Both; Weaning | ID with 106 TCID50 | NR | NR | Lymph nodes, bone marrow, spleen, and thymus:* Viral RNA detected from 3-11 dpi

Tonsil: * Viral RNA detected from 3-11 dpi and at 25 dpi
* Tonsils of all infected pigs were positive for viral isolation
 | Ricklin, M.E. et al. (2016a) |
| IV with 106 TCID50 | NR | NR |
| Challenge(JE-91)  | Other; NR; Weaning | IV with 107 TCID50/mL | NR | NR | Spleen: * 9.0 × 102 PFU/g of infectious viral titer
* 9.7 × 103 GEQ-TCID50/g of viral RNA titer

Mesenteric lymph nodes:* 3.1 × 103 PFU/g of infectious viral titer
* 7.6 × 103 GEQ-TCID50/g of viral RNA titer

Tonsil (1 animal): * 7.3 × 103 PFU/g of infectious viral titer
* 2.6 × 104 GEQ-TCID50/g of viral RNA titer
 | Park, S.L. et al. (2018)  |
| Challenge(YL2009-4) | Other (research); NR; NR | SC with 107 FFU  | NR | NR | Tonsil at 8 dpi:* 105.6-107.2 copies/g of viral RNA

Lymph nodes at 8 dpi:* 104.2-107.0 copies/g of viral RNA
 | Fan, Y.-C. et al. (2018) |
| **Multiple systems (Digestive, urinary, and/or musculoskeletal)** |
| Challenge(Nakayama) | Large White; Both; Weaning | ID with 106 TCID50 | NR | NR | Ileum, kidney, liver, and skeletal muscle:* Viral RNA detected from 3-11 dpi
 | Ricklin, M.E. et al. (2016a) |
| IV with 106 TCID50 | NR | NR |
| **Reproductive system** |
| Observational | NR; Both;NR | NA | NR | NR | Aborted fetuses and testicular fluid samples were positive for JEV using RT-AAA and RT-qPCR methods | Nie, M. et al. (2022) |
| **Exposure (Strain)** | **Population****[breed; sex; age]** | **Inoculation route and dose** | **Macroscopic** **pathological findings** | **Microscopic** **pathological findings** | **Organotropic findings** | **Reference(s)**  |
| ***Genotype III*** |
| **Central nervous system** |
| Challenge(Fuji) | NR;NR; NR | IC with 106.3 TCID50  | NR | NR | Brain:* 5.5 log TCID50/g or mL

Spinal cord: * 3.2 log TCID50/g or mL
 | Sazawa, H. et al. (1969a) |
| Challenge(Nakayama) | Landrace; NR; Weaning | ID with 105 TCID50 | NR | NR | Brainstem:* 9/18 samples were positive

Cerebellum: * 10/18 samples were positive

Thalamus: * 10/18 samples were positive
* Highest median viral load

Olfactory bulb: * 11/18 samples were positive
* Highest median viral load

Cerebrum: * 12/18 samples were positive
 | Redant, V. et al. (2020) |
| IN with 105 TCID50 | NR | NR | Trigeminal ganglion:* 1/15 samples were positive

Brainstem:* 4/15 samples were positive

Cerebellum: * 9/15 samples were positive

Thalamus: * 7/15 samples were positive
* Highest median viral load

Olfactory bulb: * 11/15 samples were positive
* Highest median viral load
 |
| Challenge(Fuji) | NR; NR; NR | IN with 107 TCID50 | NR | NR | Brain: * 5.7 log TCID50/g or mL

Spinal cord: * Negative
 | Sazawa, H. et al. (1969a) |
| Challenge(Nakayama) |  Large White; Both; Weaning | Exposure to animals challenged ID and IV with a total dose of 107 TCID50 | NR | Typical microscopic lesions of viral meningoencephalomyelitis were present regardless of the mode of infectionBrain: * Multifocal perivascular mononuclear cuffs, often affecting the gray matter more than the white matter
* Multifocal glial nodules
* Neuronal degeneration and necrosis with small numbers of neutrophils
* Mild multifocal lymphohistiocytic meningitis
 | Brain stem, olfactory bulb, neocortex, thalamus, and basal nuclei:* Up to 103-104 RNA U/g with significant differences between the neocortex and olfactory bulb and the neo cortex and brain stem
 | Ricklin, M.E. et al. (2016a) |
| ID and IV with a total dose of 107 TCID50 | NR | CNS, meninges, choroid plexus, spinal cord: * Little to no viral RNA was detected
 | Ricklin, M.E. et al. (2016a); Ricklin, M.E. et al. (2016b) |
| ORN with 101 or 102 TCID50 | NR | Thalamus and basal nuclei: * 1,000-10,000 RNA U/g-1
* Levels were similar in the low and middle doses
 | Ricklin, M.E. et al. (2016a) |
| ORN with 103 TCID50 | NR |
| ORN with 105 TCID50 | NR |
| ORN with 107 TCID50 | NR |
| Challenge (Nakayama) | Landrace; NR;Weaning | ORN with 103 TCID50 | NR | NR | Neocortex: * 10-1-103.5 U/mL-1 of viral RNA

Thalamus: * 10-0.5-104 U/mL-1 of viral RNA

Striatum: * 10-1-104 U/mL-1 of viral RNA

Brain stem: * 100-103 U/mL-1 of viral RNA

Olfactory bulb: * 10-1-102 U/mL-1 of viral RNA
 | García-Nicolás, O. et al. (2017) |
| Challenge(Fuji) | NR; NR; NR | SC with 107 TCID50 | NR | NR | Brain and spinal cord: * No virus was isolated
 | Sazawa, H. et al. (1969a) |
| Challenge(CH1392) |  Other (research); NR; NR | SC with 107 FFU | NR | Brain:* Multifocal gliosis
* Mononuclear perivascular cuffs
 | Brain at 8 dpi: * 104.5 to 105.8 copies/g of viral RNA
 | Fan, Y.-C. et al. (2018) |
| Challenge(SA14-14-2;rA66G) | Large White; Both; Weaning | 2 mL of 2 × 107 TCID50 | NR | Cerebral cortex and thalamus:* Mononuclear perivascular cuff
* Multifocal gliosis
* Neuronal degeneration
* Necrosis with focal neuronophagia and satellitosis (thalamus)
 | Cerebral cortex, cerebellum, thalamus, and spinal cord:* JEV antigen-positive neurons were present
 | Xie, S.D. et al. (2022) |
| **Lymphatic system** |
| Challenge(Fuji) | NR; NR; NR | IC with 106.3 TCID50 | NR | NR | Lymph node:* 3.7 log TCID50/g or mL

Spleen: * 4.5 log TCID50/g or mL
 | Sazawa, H. et al. (1969a) |
| Challenge(Nakayama) | Landrace; NR; Weaning | ID with 105 TCID50 | NR | Tonsil:* From 3-10 dpi, OAS1 and IFNB MRNA upregulation ranged from 2-15-fold and 2-7-fold, respectively
* By 21 dpi, OAS1 and IFNB MRNA levels dropped to the levels observed in control pigs
* From 3 until 21 dpi, there was a significant reduction in the frequency of CD4+CD8+ double-positive T cells
* At 3 and 5 dpi, CD4+ and CD8+ single positive T cells were significantly reduced
* At 7 dpi, the frequency of CD14+ monocytes increased and subsequently decreased in frequency from 10 dpi to 21 dpi
 | Tonsil: * Up to 106 TCID50/g viral RNA
* Persisted until 21 dpi
 | Redant, V. et al. (2022) |
| Challenge(Nakayama) | Large White; Both; Weaning | Exposure to animals challenged ID and IV with a total dose of 107 TCID50 | NR | NR | Lymph nodes:* Up to 103 RNA U/g

Tonsils:* 1.4 × 104 to 6.6 ×104 RNA U/g
* >5 × 101 to 6.81 × 103 TCID50 per g
 | Ricklin, M.E. et al. (2016a) |
| ID and IV with a total dose of 107 TCID50 | NR | Tonsil: * Lymphoid hyperplasia
 | Lymph nodes, spleen, bone marrow, and thymus: * 10-1,000 viral RNA U/g

Tonsil: * 5 × 105 RNA U/g, no reduction in viral RNA load at 11 dpi
* Live virus was detected in all tonsils obtained from infected pigs until 11 dpi with viral loads of up to 3 × 105 TCID50/g

Viral RNA in all examined tissues of some animals persisted up to 11 dpi  | Ricklin, M.E. et al. (2016b) |
| Challenge(Nakayama) | Landrace; NR; Weaning | IN with 105 TCID50 | NR | NR | Pre-scapular lymph nodes and spleen from 5 dpi (peak viremia) to end of experiment: * Viral RNA was detected but no infectious virus was detected via viral titration
 | Redant, V. et al. (2020) |
| Challenge(Fuji) | NR; NR; NR | IN with 107 TCID50  | NR | NR | Lymph node: * Negative for virus isolation

Spleen: * 4.3 log TCID50/g or mL
 | Sazawa, H. et al. (1969a) |
| Challenge(Nakayama) | Large White; Both; Weaning | ORN with 101 or 102 TCID50 | NR | NR | Lymph nodes at 10 dpi:* 100-1,000 RNA U/g-1

Tonsils:* At 10 dpi, 100-100,000 RNA U/g-1
* At 21 dpi, 103-104 U/g-1
 | Ricklin, M.E. et al. (2016a) |
| ORN with 103 TCID50 | NR | NR |
| ORN with 105 TCID50 | NR | NR |
| ORN with 107 TCID50 | NR | NR |
| Challenge (Nakayama) | Landrace; NR; Weaning | ORN with 103 TCID50  | NR | NR | Lymph node:* Viral RNA load was 100-102

Tonsils: * Viral RNA load 10-0.5-105
 | García-Nicolás, O. et al. (2017) |
| Challenge(Fuji) | NR; NR; NR | SC with 107 TCID50  | NR | NR | Lymph node:* 3.5 log TCID50/g or mL

Spleen:* 4.3 log TCID50/g or mL
 | Sazawa, H. et al. (1969a) |
| Challenge(CH1392) | Other (research); NR; NR | SC with 107 FFU  | NR | NR | Tonsils at 8 dpi:* 105.6 to 107.2 copies/g of viral RNA

Lymph nodes at 8 dpi:* 104.2 to 107 copies/g of viral RNA
 | Fan, Y.-C. et al. (2018) |
| Challenge(SA14-14-2) | Large White; Both; Weaning | 2 mL of 2 × 107 TCID50 | NR | Palatine tonsils: * JEV antigen-positive cells were mainly concentrated in the follicles
 | NR | Xie, S.D. et al. (2022) |
| **Multiple systems (Respiratory, digestive, vascular, urinary, and musculoskeletal)** |
| Challenge(Fuji) | NR; NR; NR | IC with 106.3 TCID50 | NR | NR | Lung:* 2.7 log TCID50/g or mL

Liver:* <1.7 log TCID50/g or mL

Kidney:* 2.2 log TCID50/g or mL
 | Sazawa, H. et al. (1969a) |
| Challenge(Nakayama) | Landrace; NR; Weaning | ID with 105 TCID50 | NR | NR | Liver and kidney: * 102-103 TCID50/g tissue
* No viral RNA was detected at 10 and 14 dpi
 | Redant, V. et al. (2020) |
| IN with 105 TCID50 | NR | NR |
| Challenge(Nakayama) | Large White; Both; Weaning | Exposure to animals challenged ID and IV with a total dose of 107 TCID50 | NR | NR | Trachea and nasal cavity:* Low to no viral RNA detected

Ileum:* Between 102 and 104 RNA U/g

Jejunum:* Not positive for viral RNA
 | Ricklin, M.E. et al. (2016a) |
| IV and ID with a total dose of 107 TCID50 | Mild peritoneal effusion was present in 5/12 animals at 5 and 7 dpi | Other non-CNS tissues had no histologic changes | Distal ileum at 3 dpi: * 10-1,000 RNA U/g and persisted up to 11 dpi in some pigs

Liver and kidney: * Viral RNA loads of 10-1,000 RNA U/g, up to 11 dpi in some animals

Skeletal muscle: * <10 RNA U/g or negative

Peripheral blood: * 10 RNA U/mL at 3 dpi
 | Ricklin, M.E. et al. (2016b) |
| Challenge(Fuji) | NR; NR; NR | IN with 107 TCID50  | NR | NR | Lung, liver, and kidney:* <1.7 log TCID50/g or mL
 | Sazawa, H. et al. (1969a) |
| Challenge(Nakayama) | Large White; Both; Weaning | ORN with 101, 102, 103, 105, or 107 TCID50  | NA | NA | Ileum at 10 dpi in lowest-dose-infected pigs: * 100-1,000 U/g of viral RNA

Urine: * 0.5 RNA U/mL-1 (1 animal)
 | Ricklin, M.E. et al. (2016a) |
| Challenge (Nakayama) | Landrace; NR; Weaning | ORN with 103TCID50 | NR | NR | Ileum: * 102-104 viral RNA load

Jejunum: 10-1-102 viral RNA load  | García-Nicolás, O. et al. (2017) |
| Challenge(Fuji) | NR; NR; NR | SC with 107 TCID50  | NR | NR | Lung:* 2.2 log TCID50/g or mL

Liver:* 2.0 log TCID50/g or mL

Kidney:* 2.3 log TCID50/g or mL
 | Sazawa, H. et al. (1969a) |
| **Reproductive system** |
| Challenge | Large White; Male; Finishing | 108 PFU | NR | Seminiferous tubules: * Dissociation and disorganization of spermatogenic and Sertoli cells
* Interstitial hemorrhage
* Perivascular inflammation
 | Spermatogenic and Sertoli cells:* Positive for JEV E antigen
 | Zheng, B. et al. (2019) |
| Challenge(Kanagawa) | Yorkshire; Female; NR | 5 mL of JEV (inoculum size = 106.5, 109.3, or 109.5 IC mouse LD50) 36 to 97 days after copulation | Mummification of two of the fetuses | NR | No virus was isolated from healthy fetuses | Shimizu, T. et al. (1954) |
| 5 mL (inoculum size = 106, 107.2, or 108.5 IC mouse LD50) 40 to 81 days after copulation | Fetuses were either normal, mummified, or alive with hydrocephalus and subcutaneous edema | Fetal brain: * Perivascular cuff and diffuse gliosis
 | JEV was isolated from all fetuses. No virus was detected from organs of the dams |
| Challenge (JaOH 0566) | Yorkshire/ Landrace; Female; Mature | 107.9 PFU | Embryos had petechia on the head, hips, and umbilicus | NR | Virus was isolated from the placenta | Yoshida, I. et al. (1981) |
| Natural -Experimental | NR;NR;NR | NA | NR | NR | Virus isolated from fetal cerebrospinal fluid in PK15 cells; cytopathic effects were observed  | Fan, J.M. et al. (2010a) |
|   Observational | NR; Both; NR | NA | NR | NR | Virus isolated from fetal cerebrospinal fluid in BHK-21 cells: 5/37 samples had cytopathic effects after three blind passages | Teng, M. et al. (2013) |
| NA | NR | NR | Virus isolated from seminal fluid in BHK-21 cells: 2/12 samples had cytopathic effects after three blind passages |
| Observational | Landrace; Female;NR | NA | Brain of stillborn piglet:* Hydranencephaly
* Lissencephaly
* Lateral ventricular dilation

Lymph nodes of stillborn piglet:* Severe congestion
 | Brain of stillborn piglet: * Neuropilar edema, congestion, and microhemorrhages
* Neuronal degeneration
* Focal to diffuse gliosis
* Occasional mononuclear perivascular cuffs in the subependymal region
* Immunolabelling of intracytoplasmic viral antigen in neurons in the cerebrum
 | Brain of stillborn piglet: * Viral nucleic acids were detected in seven stillborn piglets from two sows
 | Desingu, P.A. et al. (2016) |
| **Exposure****(Strain)** | **Population [breed; sex;****age]** | **Inoculation route and dose** | **Macroscopic** **pathological findings** | **Microscopic** **pathological findings** | **Organotropic findings** | **Reference(s)** |
| ***Unknown genotype*** |
| **Central nervous system** |
| Challenge(NR) | NR;NR; Finishing | IV 1 mL of 1-2 dilution of a fresh mouse brain suspension (107) | 1 pig: diffuse hyperemia and cerebellar necrosisTissues from other pigs were grossly unremarkable | One of the pigs had cisternal fluid leukocytosis | There was no detectable virus in mice that were inoculated with brain suspensions of infected pigs | Meiklejohn, G. et al. (1947) |
| Challenge(IB2001) | NR;NR; Weaning | IV with 106 TCID50 | Brain: * No gross findings were observed at 3 dpi
* Diffuse edema at 7 dpi
 | The cerebrum, midbrain, and cerebellum had mild meningitisBrain, cerebrum: * Multifocal gliosis, mononuclear perivascular cuffs, neuronal necrosis and neuronophagia at 3 and 7 dpi
* Lesions were present in the gray matter at 3 dpi, and more severely affected both the gray and white matter at 7 dpi

Cerebellum, spinal cord, trigeminal ganglia:* No microscopic changes were present at 3 dpi and 7 dpi
 | Cortex of frontal and temporal lobes and thalamic gray matter: * JEV positive neurons were present at 3 dpi, but not at 7 dpi

White matter, choroid plexus, ependyma, and glial cells: * Negative for JEV antigen
 | Yamada, M. et al. (2004) |
| Challenge(IB2001) | NR; NR; Weaning | IN with 106 TCID50 | 3, 5, 7, and 14 dpi: No significant gross lesions were present in any of the piglets at 3, 5, 7, 15 dpi or at necropsy | Nasal epithelium:* Multiple necrotic foci admixed with lymphocytes at 3 dpi

Olfactory bulb: * Mild neuronal degeneration or necrosis at 5 and 7 dpi
* Multifocal glial nodules at 5, 7, and 14 dpi
* Mononuclear perivascular cuffs were present in the cortex adjacent to the olfactory bulb at 14 dpi

Pyriform cortex:* Multifocal gliosis, mononuclear perivascular cuffs, neuronal necrosis and neuronophagia at 7 dpi
 | Olfactory bulb, granular layer:* JEV antigens were detected in the cytoplasm and neuronal processes of the nerve cells at 3 and 5 dpi

Pyriform cortex and occasionally in the amygdala nucleus:* Neuronal cytoplasm was positive for JEV antigen at 5 dpi and corresponded to the distribution of brain lesions

Nasal or olfactory epithelium, meninges, vascular endothelium, cerebral white matter, choroid plexus, ependyma, and glial cells:* No JEV antigens were detected at 7 and 14 dpi
 | Yamada, M. et al. (2009) |
| Challenge(AS-6) | NR; NR; Weaning | 5 mL of 106 TCID50/mL | Brain at 3 dpi and 7 dpi: * Edema
 | Cerebrum, cerebellum, thalamus, midbrain, pons, medulla oblongata, and spinal cord:* Non-suppurative encephalitis at 3 and 7 dpi

Immunohistochemistry: * There were strongly positive intracytoplasmic JEV antigens in the neurons in the cortex of the frontal and temporal lobes and in the gray matter of the thalamus and midbrain. Similar changes were present in the large neurons of the spinal cord in one pig.
 | NR | Yamada, M. et al. (2004) |
| Challenge(NJ2008) | Large White; Both; Weaning | 2 mL of 2 x 107 TCID50 | NR | Cerebral cortex and thalamus:* Mononuclear perivascular cuff
* Multifocal gliosis
* Neuronal degeneration
* Necrosis with focal neuronophagia and satellitosis (thalamus)

Cerebral cortex, cerebellum, thalamus, and spinal cord: * Cells were positive for anti-NS1 or anti-NS3 JEV antigen
 | NR | Xie, S.D. et al. (2022) |
| Challenge(Sagara) | NR; NR; NR | IC with 104.5 TCID50 | NR | NR | Brain:* 2.2 log TCID50/g or mL to 4.7 log TCID50/g or mL

Spinal cord: * Negative to positive
 | Sazawa, H. et al. (1969a) |
| IC with 105.2 TCID50 | NR | NR | Brain and spinal cord: * Negative to <1.6 log TCID50/g or mL
 |
| SC with 105 TCID50 | NR | NR | Brain:* 3.0 log TCID50/g or mL

Spinal cord:* 2.7 log TCID50/g or mL
 |
| SC with 105.2 TCID50 | NR | NR | Brain:* <1.8 to 2.5 log TCID50/g or mL

Spinal cord:* <1.7 to 3.0 log TCID50/g or mL
 |
| Natural -Experimental | NR; NR; NR | NA | NR | NR | RT-PCR detected JEV antigen from 1 brain sample | Zhang, Y. et al. (2022) |
| Observational | NR; NR; Nursing | NA | Brain and spinal cord:* Congestion of blood vessels within the meninges of the brain and spinal cord
* Diffuse severe edema
 | Cerebral cortex, basal ganglia, and brain stem (gray and white matter):* Multifocal neuronal degeneration, necrosis, and neuronophagia
* Mononuclear perivascular cuffs
* Multifocal gliosis
 | NR | Burns, K.F. (1950) |
| **Lymphatic system** |
| Challenge(Sagara) | NR; NR; NR | IC with 104.5 TCID50 | NR | NR | Lymph node:* <1.7 to 3.0 log TCID50/g or mL

Spleen:* Negative to 2.5 log TCID50/g or mL
 | Sazawa, H. et al. (1969a) |
| IN with 105.2 TCID50 | NR | NR | Lymph node:* Negative to 2.0 log TCID50/g or mL

Spleen: * <1.7 to 2.6 log TCID50/g or mL
 |
| SC with 105 TCID50 | NR | NR | Lymph node:* 2.5 log TCID50/g or mL

Spleen:* 2.8 log TCID50/g or mL
 |
| SC with 105.2 TCID50 | NR | NR | Lymph node:* <1.7 to 3.7 log TCID50/g or mL

Spleen:* <1.6 to 4.3 log TCID50/g or mL
 |
| Challenge(NJ2008; rA66G) |  Large White; Both; Weaning | 2 mL of 2 × 107 TCID50  | NR | Palatine tonsils:* JEV antigen-positive cells were mainly concentrated in the follicles
* There was a significant reduction in MHC II molecule in virus-infected tonsils
 | NR | Xie, S.D. et al. (2022) |
| Natural -Experimental | NR; NR; NR | NA | NR | NR | RT-PCR detected JEV antigen from 1 spleen sample  | Zhang, Y. et al. (2022) |
| Observational | NR; NR; Nursing | NA | Thoracic and mesenteric lymph nodes:* Lymphadenomegaly
* Multifocal splenic necrosis
 | NR | NR | Burns, K.F. (1950) |
| **Multiple systems (Respiratory, digestive, and urinary)** |
| Challenge(Sagara) | NR; NR; NR | IC with 104.5 TCID50 | NR | NR | Lung:* 2.0 to 2.5 log TCID50/g or mL

Liver:* Negative to 3.2 log TCID50/g or mL

Kidney: * <1.7 to 2.3 log TCID50/g or mL
 | Sazawa, H. et al. (1969a) |
| IN with 105.2 TCID50 | NR | NR | Lung:* Negative to 3.2 log TCID50/g or mL

Liver:* Negative to 2.5 log TCID50/g or mL

Kidney: * <1.6 to 2.0 log TCID50/g or mL
 |
| SC with 105 TCID50 | NR | NR | Lung: * Positive

Liver:* <1.8 log TCID50/g or mL

Kidney:* 4.0 log TCID50/g or mL
 |
| SC with 105.2 TCID50 | NR | NR | Lung:* <1.8 to 3.3 log TCID50/g or mL

Liver: * Negative to 4.7 log TCID50/g or mL

Kidney:* 2.0 to 4.2 log TCID50/g or mL
 |
| Observational | NR; NR; Nursing | NA | Abdomen/digestive:* Abundant pleural and peritoneal serosanguinous effusion

Other organs:* Subcutaneous edema
* Multifocal hepatic necrosis
* Petechial hemorrhages of serosal membranes
 | NR | NR | Burns, K.F. (1950) |
| **Reproductive system** |
| Challenge(AS-6) | Landrace; Female; NR | 105.5 TCID50 | Placenta:* Hyperemia
* Hemorrhage
* Necrosis

Fetuses:* Mummification
* Hyperemia
* Hemorrhage
 | NR | NR | Fujisaki, Y. et al. (1975) |
| Observational | NR; Female; Mature | NA | Aborted fetuses | NR | Virus isolated from aborted fetuses | Takashima, I. et al. (1988) |

dpi = Days post-infection; FFU = Focus-forming units; NA = Not applicable/available; NR = Not reported; PFU = Plaque-forming units; Sex: Both = female and male.

Challenge route: IC = Intracerebral; ID = Intradermal; IN = Intranasal; IV = Intravenous; ORN = Oronasal; SC = Subcutaneous.

Comments pertaining to Ricklin et al. (2016a): The IV/ID infected group was analyzed 6 and 10 days after the onset of viremia (taken as a measure due to the unknown infection time and different incubation periods of the other groups). Tissue from pigs infected by contact at 4, 6, and 7 days after the onset of viremia. Tissue from oronasally infected animals was 7, 8, and 9 days after the onset of viremia.

**Supplemental Table S5.** Diagnostic tests used for detection of Japanese encephalitis virus (JEV) in swine across all reports, including test name, description, and sample type, organized by diagnostic test method categorization as per the World Organisation of Animal Health (WOAH) list of approved tests for detection of JEV.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Test method1** | **Test name** | **Test variation** | **Sample type** | **Reference(s)** |
| **Detection of virus**  |
| Virus isolation  | Virus isolation |  | Abortus, blood, bone marrow, cerebrospinal tissue, intestine, kidney, liver, lymph node, oro-nasal swab, peripheral nerve, semen, skeletal muscle, spleen, thymus, tonsil  | Hale, J.H., Lim, K.A., and Colless, D.H. (1957); Unita, T. (1969); Igarashi, A., Morita, K., and Bundo, K. (1981); Takashima, I. et al. (1988); Pyke, A.T. et al. (2001); Yang, D.K. et al. (2004b,c); Yoshida, Y. et al. (2005); Nitatpattana, N. et al. (2008); Fan, J.-M. et al. (2010b); Cao, Q.S. et al. (2011); Obara, M. et al. (2011); Liu, H. et al. (2013); Teng, M. et al. (2013); Ricklin, M.E. et al. (2016b); Duong, V. et al. (2017); Kuwata, R. et al. (2020); Wang, X. et al. (2020); Zhang, Y. et al. (2022) |
| Plaque assay | Plaque assay in porcine kidney cells | Blood | Kodama, K., Sasaki, N., and Inoue, Y.K. (1968); Sazawa, H. et al. (1969a) |
| Plaque assay in Vero cells | Blood, cerebrospinal tissue, eye, heart, intestine, kidney, liver, lung, lymph node, nasal turbinate, olfactory neuroepithelium, oro-nasal swab, peripheral nerve, skeletal muscle, spleen, tonsil, trachea  | Yang, D.-K. et al. (2012); Park, S.L. et al. (2018) |
| Testicular sample | Zheng, B. et al. (2019) |
| Micro-plaque assay | Blood | Fan, Y.-C. et al. (2018) |
| Antigen detection | Micro-antigen focus assay |  | Blood | Fan, Y.-C. et al. (2019) |
| Immunofluorescence antibody test |  | Cerebrospinal tissue, testicular sample, tonsil  | Nitatpattana, N. et al. (2008); Cao, Q.S. et al. (2011); Zheng, B. et al. (2019); Xie, S.D. et al. (2022)  |
| Fluorescent antibody staining |  | Abortus  | Yoshida, I. et al. (1981) |
| Rapid immunochromatographic strip |  | Abortus, cerebrospinal tissue, spleen | Li, Y. et al. (2010) |
| Immunohistochemistry |  | Cerebrospinal tissue, eye, heart, intestine, kidney, liver, lung, lymph node, nasal cavity, skeletal muscle, spleen, stomach, testicular sample, tonsil, trachea  | Yamada, M. et al. (2009); Cao, Q.S. et al. (2011); Zheng, B. et al. (2019); Xie, S.D. et al. (2022)  |
| Antigen-capture enzyme-linked immunoassay (sandwich ELISA) |  | Blood | Fan, Y.-C. et al. (2018) |
| Molecular tests | Real-time reverse transcriptase polymerase chain reaction (Real-time RT-PCR)  |  | Abortus, blood, cerebrospinal tissue, lymph node, tonsil  | Pyke, A.T. et al. (2001); Pyke, A.T. et al. (2004); Liu, H. et al. (2012); Nidaira, M. et al. (2014); Dhanze, H. et al. (2015); Duong, V. et al. (2017); Kakkar, M. et al. (2017); Di Francesco, J. et al. (2018); Fan, Y.-C. et al. (2018); Yap, G. et al. (2019); Wang, X. et al. (2020); Zhou, Y. et al. (2020)  |
| Real-time Reverse transcriptase quantitative polymerase chain reaction (Real-time RT-qPCR) | Bone marrow, cerebrospinal tissue, ileum, kidney, liver, lymph node, peripheral nerve, skeletal muscle, spleen, thymus, tonsil  | Ricklin, M.E. et al. (2016a,b)  |
| Real-time TaqMan one-step Reverse transcriptase polymerase chain reaction (Real-time TaqMan one-step RT-PCR)  | Abortus, blood, cerebrospinal fluid, semen, visceral tissue  | Pyke, A.T. et al. (2004); Yang, D.K. et al. (2004b); Wu, X. et al. (2017) |
| Real-time TaqMan two-step Reverse transcriptase polymerase chain reaction (Real-time TaqMan two-step RT-PCR)  | Blood | Pantawane, P.B. et al. (2019) |
| Duplex real-time TaqMan reverse transcriptase quantitative polymerase chain reaction (Duplex real-time TaqMan RT-qPCR) | Abortus, blood, cerebrospinal tissue, kidney, liver  | Wang, X. et al. (2020); Zhang, Y. et al. (2022) |
| Reverse transcriptase polymerase chain reaction (RT-PCR) |  | Abortus, blood, cerebrospinal tissue, culture fluid infected with pig sample, kidney, liver, lung, lymph node, spleen  | Pyke, A.T. et al. (2001); Williams, D.T. et al. (2001); Yang, D.K. et al. (2004b,c); Yoshida, Y. et al. (2005); Nitatpattana, N. et al. (2008); Nidaira, M. et al. (2009); Obara, M. et al. (2011); Liu, H. et al. (2012); Xu, X.-G. et al. (2012); Liu, H. et al. (2013); Yoshikawa, A. et al. (2016); Duong, V. et al. (2017); Zhang, H. et al. (2017b); Pantawane, P.B. et al. (2019); Wang, X. et al. (2020); Kuwata, R. et al. (2020); Zhang, Y. et al. (2022) |
| RT-polymerase chain reaction. These papers do not define meaning of “RT” | Abortus, blood, cerebrospinal fluid, cerebrospinal tissue, lung, lymph node, post-mortem tissue semen, spleen, tonsil, visceral tissue  | Fan, J.-M. et al. (2010a,b); Li, Y. et al. (2010); Cao, Q.S. et al. (2011); Tian, C.J. et al. (2012); Teng, M. et al. (2013); Desingu, P.A. et al. (2016); Hu, L. et al. (2016); Wu, R. et al. (2016); Guo, H.C. et al. (2019); Datey, A. et al. (2020); Raut, A.A. et al. (2021); Zhou, D. et al. (2021)  |
| Reverse transcriptase droplet digital polymerase chain reaction (RT-DDPCR) | Abortus, blood, cerebrospinal fluid, semen, visceral tissue  | Wu, X. et al. (2017) |
| Single reverse transcriptase polymerase chain reaction | Abortus, heart, intestine, kidney, liver, lung, lymph node, semen, spleen, testicular sample, tonsil  | Chen, H.-Y. et al. (2010); Zeng, Z. et al. (2014); Zhang, M. et al. (2015)  |
| Multiplex reverse transcriptase polymerase chain reaction (Multiplex RT-PCR) | Abortus, blood, diarrhea sample, heart, intestine, kidney, liver, lung, lymph node, oro-nasal secretion from chewable rope, “principal-organs”, spleen, testicular sample, tonsil  | Ogawa, H. et al. (2009); Chen, H.-Y. et al. (2010); Chiou, S.-S. et al. (2021); Fan, Y.-C. et al. (2022) |
| TaqMan one-step reverse transcriptase quantitative polymerase chain reaction | Blood, cerebrospinal tissue, eye, heart, intestine, kidney, liver, lung, lymph node, nasal turbinate, olfactory neuroepithelium, oro-nasal swab, peripheral nerve, skeletal muscle, spleen, tonsil, trachea  | Park, S.L. et al. (2018); Zhang, Y. et al. (2022) |
| Duplex TaqMan real-time reverse transcriptase quantitative polymerase chain reaction | Abortus, blood, cerebrospinal tissue, kidney, liver, spleen | Wang, X. et al. (2020); Zhang, Y. et al. (2022) |
| TaqMan real-time one-step reverse transcription polymerase chain reaction | Abortus, blood, cerebrospinal fluid, semen, visceral tissue  | Pyke, A.T. et al. (2004); Yang, D.K. et al. (2004b); Wu, X. et al. (2017) |
| Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) | Abortus, blood, bone marrow, cerebrospinal fluid, cerebrospinal tissue, intestine, kidney, liver, lymph node, nasal cavity, nasal epithelium, olfactory neuroepithelium, oral fluid, oro-nasal swab, peripheral nerve, skeletal muscle, skin, spleen, testicular sample, thymus, tonsil, trachea, visceral tissue | Cappelle, J. et al. (2016); Ricklin, M.E. et al. (2016b); García-Nicolás, O. et al. (2017); Lyons, A.C. et al. (2018); Redant, V. et al. (2020); Young, C.L. et al. (2020); Zhou, D. et al. (2021); Nie, M. et al. (2022); Redant, V. et al. (2022); Xie, S.D. et al. (2022) |
| Fluorescent reverse transcription recombinase-aided amplification (Fluorescent RT-RAA) | Abortus, testicular sample | Nie, M. et al. (2022) |
| Reverse transcription loop-mediated isothermal amplification assay (RT-LAMP) | Blood | Liu, H. et al. (2012); Tian, C.J. et al. (2012) |
| Accelerated reverse transcription loop-mediated isothermal amplification assay (Accelerated RT-LAMP) | Blood | Tian, C.J. et al. (2012) |
| Real-time polymerase chain reaction (Real-time PCR) | Singleplex real-time polymerase chain reaction | Blood, kidney, liver, lung, lymph node  | Wu, H. et al. (2014); Komiya, T. et al. (2019)  |
| Multiplex real-time polymerase chain reaction | Blood, kidney, liver, lung, lymph node  | Wu, H. et al. (2014) |
| EvaGreen-based multiplex real-time polymerase chain reaction (EG-MPCR) | Blood, kidney, liver, lung, lymph node  | Rao, P. et al. (2014) |
| Real-time nucleic acid sequence-based amplification | Abortus, blood, cerebrospinal fluid, semen, visceral tissue | Zhou, D. et al. (2021) |
| Polymerase chain reaction (PCR) | Conventional polymerase chain reaction | Abortus, blood, cerebrospinal fluid, kidney, liver, lung, lymph node, semen, spleen, tonsil, visceral tissue | Rao, P. et al. (2014); Cappelle, J. et al. (2016); Hu, L. et al. (2016); Kakkar, M. et al. (2017); Di Francesco, J. et al. (2018); Xiao, L. et al. (2018)  |
| Multiplex polymerase chain reaction (Multiplex PCR) | Abortus, blood, kidney, liver, lung, lymph node, semen, spleen, tonsil  | Xu, X.-G. et al. (2012); Zeng, Z. et al. (2014); Zhang, M. et al. (2015); Hu, L. et al. (2016) |
| Suspension array system for multiplex polymerase chain reaction | Abortus, blood, cerebrospinal fluid, semen, visceral tissue  | Xiao, L. et al. (2018) |
| Multiplex ligation-dependent probe amplification (MLPA) |  | Blood  | Zhou, Y. et al. (2020) |
| Other | Circulating virus titrated in mice |   | Abortus, blood | Meiklejohn, G., Simpson, T.W., and Stacy, I.B. (1947); Shimizu, T. et al. (1954); Scherer, W.F., Moyer, J.T., and Izumi, T. (1959); Komada, K., Sasaki, N., and Inoue, Y.K. (1968); Carey, D.E., Reuben, R., and Myers, R.M. (1969); Lee, G.C.Y., Huang, Y.T., and Chang, L.C. (1975); Fujisaki, Y. et al. (1975); Yoshida, I. et al. (1981); Ilkal, M.A. et al. (1994);Williams, D.T. et al. (2001) |
| **Antibody detection** |
| Complement fixation test (CFT) | Complement fixation test (CFT) |  | Blood, mouse brain infected with pig sample | Meiklejohn, G., Simpson, T.W., and Stacy, I.B. (1947); Burns, K.F. (1950); Scherer, W.F. et al. (1959); Scherer, W.F., Moyer, J.T., and Izumi, T. (1959); Carey, D.E., Reuben, R., and Myers, R.M. (1969); Okuno, T. et al. (1973); Ueba, N. et al. (1978); Kalimuddin, M.D., Narayan, K.G., and Choudhary, S.P. (1982a,b); Paul, W.S. et al. (1993); Ratho, R.K., Sethi, S., and Prasad, S.R. (1999)  |
| Enzyme-linked immunoassays (ELISA) | Enzyme-linked immunoassay (ELISA) |  | Blood  | Konishi, E., and Yamaoka, M. (1982); Yamaoka, M., Konishi, E., and Matsumura, T. (1982); Konishi, E., and Yamaoka, M. (1983); Igarashi, A. et al. (1983); Yamaoka, M. (1983); Chang, H.-C. et al. (1984b); Ohkubo, Y. et al. (1984); Ohkubo, Y., Takashima, I., and Hashimoto, N. (1984); Burke, D.S. et al. (1985b); Yamaoka, M., and Konishi, E. (1985); Gingrich, J.B. et al. (1987); Cardosa, M.J. et al. (1993); Paul, W.S. et al. (1993); Makino, Y. et al. (1994); Tadano, M. et al. (1994); Hamano, M. et al. (2007); Nidaira, M. et al. (2007a); Fei-Fei, G. et al. (2008); Fan, J.-M. et al. (2010b); Nitatpattana, N. et al. (2011); Xu, X.-G. et al. (2011); Conlan, J.V. et al. (2012); Lindahl, J. et al. (2012); Lindahl, J.F. et al. (2013); Cappelle, J. et al. (2016); Holt, H.R. et al. (2016); Yoshikawa, A. et al. (2016); Sheng, Z. et al. (2016); Ricklin, M.E. et al. (2016b); Kakkar, M. et al. (2017); Zhang, H. et al. (2017a,b); Kumar, K. et al. (2018); Ruget, A.-S. et al. (2018); Xiao, C. et al. (2018); Komiya, T. et al. (2019); Guo, H.C. et al. (2019); Yonemitsu, K. et al. (2019); Lee, H.S. et al. (2019); Lee, H.S. et al. (2020); Wu, Y. et al. (2020); Dhanze, H. et al. (2020a,b); Kumar, H.B.C. et al. (2020a,b); Henriksson, E. et al. (2021); Yang, L. et al. (2022)  |
| Indirect enzyme-linked immunoassay (Indirect ELISA) | Blood | Chattopadhyay, U.K. (2001); Xinglin, J. et al. (2005); Yang, D.-K. et al. (2006); Fan, J.-M. et al. (2010b); Duong, V. et al. (2011); De Wispelaere, M. et al. (2015); Kolhe, R.P. et al. (2015); Di Francesco, J. et al. (2018); Dhanze, H. et al. (2019); Grace, M.R. et al. (2019b); Zhou, D. et al. (2019); Dhanze, H. et al. (2020b); Chauhan, J. et al. (2020); Yang, D.-K. et al. (2012)  |
| Blocking enzyme-linked immunoassay (B-ELISA) | Blood | Williams, D.T. et al. (2001); Zhou, D. et al. (2019)  |
| Sandwich enzyme-linked immunoassay (S-ELISA) | Blood | Chang, H.-C. et al. (1984a); Burke, D.S. et al. (1985a); Gingrich, J.B. et al. (1992); Xiao, C. et al. (2018)  |
| Competitive enzyme-linked immunoassay (C-ELISA) | Blood | Pant, G.R. (2006); Pant, G.R. et al. (2006); Thakur, K.K. et al. (2012); Lindahl, J.F. et al. (2013); Detha, A. et al. (2015) |
| Hemagglutination inhibition test (HI) | Hemagglutination inhibition test (HI) |  | Blood, or NR  | Gresser, I. et al. (1958); Scherer, W.F., Moyer, J.T., and Izumi, T. (1959); Scherer, W.F. et al. (1959); Konno, J. et al. (1966); Konno, J., Endo, K., and Ishida, N. (1967); Doi, R. et al. (1967); Kodama, K., Sasaki, N., and Inoue, Y.K. (1968); Unita, T. (1969); Carey, D.E., Reuben, R., and Myers, R.M. (1969); Sazawa, H. et al. (1969a,b); Ogata, M. et al. (1969); Higgins, D.A. (1970); Detels, R. et al. (1970); Yamamoto, H.A. et al. (1970); Ogata, M. et al. (1970); Ogata, M. et al. (1971); Yamada, T. et al. (1971); Ueba, N. et al. (1972); Okuno, T. et al. (1973); Chang, C.P. et al. (1974); Johnsen, D.O. et al. (1974); Fujisaki, Y. et al. (1975); Van Peenen, P.F.D. et al. (1974); Lee, G.C.Y., Huang, Y.T., and Chang, L.C. (1975); Van Peenen, P.F.D. et al. (1975); Wada, Y., Oda, T., and Mogi, M. (1975); Rodrigues, F.M. et al. (1976); Simpson, D.I.H. et al. (1976); Detels, R. et al. (1976); Ueba, N. et al. (1978); Maeda, O. et al. (1978); Yoshida, I. et al. (1981); Igarashi, A., Morita, K., and Bundo, K. (1981); Yamaoka, M., Konishi, E., and Matsumura, T. (1982); Konishi, E., and Yamaoka, M. (1982); Sasaki, O. et al. (1982); Kalimuddin, M.D., Narayan, K.G., and Choudhary, S.P. (1982a,b); Nandi, A.K. et al. (1982); Yamaoka, M. (1983); Konishi, E., and Yamaoka, M. (1983); Igarashi, A. et al. (1983); Chang, H.-C. et al. (1984a,b); Ohkubo, Y. et al. (1984); Ohkubo, Y., Takashima, I., and Hashimoto, N. (1984); Burke, D.S. et al. (1985a,b,c); Gingrich, J.B. et al. (1987); Takashima, I. et al. (1988); Thein, S., Aung, H., and Sebastian, A.A. (1988); Singh, G., and Rao, T.R. (1988); Takashima, I. et al. (1988); Angami, K. et al. (1989); Geevarghese, G. et al. (1991); Gingrich, J.B. et al. (1992); Konishi, E. et al. (1992); Cardosa, M.J. et al. (1993); Ilkal, M.A. et al. (1994); Geevarghese, G. et al. (1994); Tadano, M. et al. (1994); Mall, M.P. et al. (1995); Hanna, J.N. et al. (1996); Oda, K. et al. (1996); Hanna, J.N. et al. (1996); Hanna, J.N. et al. (1999); Ratho, R.K., Sethi, S., and Prasad, S.R. (1999); Konishi, E. et al. (2000); Myint, L. et al. (2000); Pyke, A.T. et al. (2001); Xinglin, J. et al. (2002); Chang, K.-J. (2002); Nam, J.-H., Chae, S.-L., and Cho, H.-W. (2002); See, E. et al. (2002); Yang, D.K. et al. (2004a); Xinglin, J. et al. (2005); Yang, D.-K. et al. (2006); Nidaira, M. et al. (2007a,b); Nidaira, M. et al. (2009); Sugiyama, I. et al. (2009); Yamanaka, A. et al. (2010); Imoto, J.-I. et al. (2010); Duong, V. et al. (2011); Dutta, P. et al. (2011); Conlan, J.V. et al. (2012); Borah, J. et al. (2013); Kurane, I. et al. (2013); Nidaira, M. et al. (2014); Kolhe, R.P. et al. (2015); Yang, D.-K. et al. (2017); Baruah, A. et al. (2018); Niazmand, M.H. et al. (2019); Ladreyt, H. et al. (2020); Raut, A.A. et al. (2021) |
| Neutralization tests (NT) | Virus neutralization test |  | Blood, or NR | Meiklejohn, G., Simpson, T.W., and Stacy, I.B. (1947); Shimizu, T. et al. (1954); Gresser, I. et al. (1958); Scherer, W.F. et al. (1959); Scherer, W.F., Moyer, J.T., and Izumi, T. (1959); Ogata, M. et al. (1970); Ueba, N. et al. (1978); Burns, K.F. (1950) Hale, J.H., Lim, K.A., and Colless, D.H. (1957); Hurlbut, H.S. (1964); Komada, K., Sasaki, N., and Inoue, Y.K. (1968); Sazawa, H. et al. (1969b); Carey, D.E., Reuben, R., and Myers, R.M. (1969); Simpson, D.I.H. et al. (1970); Fujisaki, Y. et al. (1975); Rodrigues, F.M. et al. (1976); Simpson, D.I.H. et al. (1976); Nandi, A.K. et al. (1982); Sasaki, O. et al. (1982); Singh, G., and Rao, T.R. (1988); Thein, S., Aung, H., and Sebastian, A.A. (1988); Angami, K. et al. (1989); Geevarghese, G. et al. (1991); Konishi, E. et al. (1992); Paul, W.S. et al. (1993); Geevarghese, G. et al. (1994); Oda, K. et al. (1996); Konishi, E. et al. (2000); Williams, D.T. et al. (2001); See, E. et al. (2002); Ting, S.H.L. et al. (2004); Yang, D.-K. et al. (2006); Fei-Fei, G. et al. (2008); Ohno, Y. et al. (2009); Fan, J.-M. et al. (2010b); Xu, X.-G. et al. (2011); Dutta, P. et al. (2011); Tian, C.J. et al. (2012); Yang, D.-K. et al. (2012); Kolhe, R.P. et al. (2015); Yang, D.-K. et al. (2017); Baruah, A. et al. (2018); Ruget, A.-S. et al. (2018); Dhanze, H. et al. (2019); Grace, M.R. (2019a); Redant, V. et al. (2020); Dhanze, H. et al. (2020b); Redant, V. et al. (2022)  |
| Plaque reduction neutralization test (PRNT) |  | Blood | Johnsen, D.O. et al. (1974); Detels, R. et al. (1976); Gingrich, J.B. et al. (1992); Peiris, J.S.M. et al. (1992); Cardosa, M.J. et al. (1993); Peiris, J.S.M. et al. (1993); Hanna, J.N. et al. (1996); Pyke, A.T. et al. (2001); Nam, J.-H., Chae, S.-L., and Cho, H.-W. (2002); Pant, G.R. et al. (2006); Hamano, M. et al. (2007); Li, P. et al. (2008); Imoto, J.-I. et al. (2010); Xu, X.-G. et al. (2011); Yang, D.-K. et al. (2012); Fan, Y.-C. et al. (2013); De Wispelaere, M. et al. (2015); Sheng, Z. et al. (2016); Ricklin, M.E. et al. (2016b); Yang, D.-K. et al. (2017); Park, S.L. et al. (2018); Komiya, T. et al. (2019); Yap, G. et al. (2019); Young, C.L. et al. (2020); Redant, V. et al. (2022); Yang, L. et al. (2022)  |
| Focus reduction neutralization test (FRNT)  |  | Blood, cerebrospinal tissue, lymph node, oro-nasal swab, tonsil  | De Wispelaere, M. et al. (2015); García-Nicolás, O. et al. (2017); Nidaira, M. et al. (2007b); Fan, Y.-C. et al. (2018); Ladreyt, H. et al. (2020); Fan, Y.-C. et al. (2022) |
| Other | Cytopathic effect plaque method |  | Blood | Ogata, M. et al. (1969) |
| Latex agglutination test |  | Blood | Xinglin, J. et al. (2002); Xu, G. et al. (2004); Grace, M.R. et al. (2019a,b) |
| Dot enzyme immunoassay (DEIA) |  | Blood | Cardosa, M.J. et al. (1993) |
| Immunohistochemistry |  | Cerebrospinal tissue  | Yamada, M. et al. (2004); Redant, V. et al. (2020)  |
| Immunofluorescence antibody test |  | Blood | Takashima, I. et al. (1988); Cha, G.-W. et al. (2015); Yap, G. et al. (2019)  |

NR = Not reported.

1Classified according to WOAH approved tests list, available at: <https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/A_summry.htm> (Part 3, Section 3.1, Chapter 3.1.10).

**Supplemental Table S6a.**  Diagnostic and analytical sensitivity and specificity of diagnostic tests evaluated for the detection of Japanese encephalitis virus (JEV) and JEV antibodies.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Test name** | **Sample type** | **Species evaluated for cross-reactivity** | **Cross-reactivity to *Flavivirus spp.*** | **Analytical sensitivity** | **Reference test** | **Diagnostic sensitivity** | **Diagnostic specificity** | **Comments** | **Reference(s)** |
| ***Detection of virus*** |
| **Antigen detection** |
| Immunochromato-graphic strip test | Mummified fetal tissue, spleen, and tissue homogenate of brain | FMDV, PCV-2, PRRSv, PRV, SFV  | None evaluated | 2.5x105 PFU | RT-PCR | 85.7% | 99.3% | Reviewer calculated from Table 3 | Li, Y. et al. (2010) |
| Immunochromato-graphic | Serum | NR | NA | NR | IgG IFA | 84.8% (95% CI: 78.3 – 89.6) | 97.8% (95% CI: 93.3 – 99.5) |  | Cha, G.-W. et al. (2015) |
| **Molecular tests** |
| Bio-Plex 200 | Abortus, blood, cerebro-spinal fluid, semen, and visceral tissue | FMDV, *H. parasuis*, TGEV, PEDV,*P. multocida*, *Salmonella* | None evaluated | 103 copies/µL when all 7 templates were present | PCR/RT-PCR | 62.5% | NR | Reviewer calculated from Table 1 | Xiao, L. et al. (2018) |
| EvaGreen-based multiplex real-time PCR | Serum and tissue (kidney, liver, lung, and lymph node) | PBOV, PCV-1, PEDV, TGEV | NR | 100 copies/μL | PCR | See comments | See comments | No samples tested positive for JEV | Rao, P. et al. (2014) |
| GenomeLab Gene Expression Profiler analyser-based multiplex PCR | Aborted fetus and tissue homogenate of kidney, lung, lymph node, and spleen | PRRSv-NA, CSFV, PCV-2, SIV (subtype H1 and H3), PPV, PRV | None evaluated | 100 copies/μL  | Single RT-PCR/real-time PCR | 100.0% | 100.0% | Reviewer calculated from Table 3 | Zhang, M. et al. (2015) |
| Multiplex ligation-dependent probe amplification | Umbilical cord blood | CSFV, PCV-2,PPV, PRRSV, PRV | None evaluated | 8 copies/ assay | Real-time PCR | See comments | See comments | No samples tested positive for JEV with either test | Zhou, Y. et al. (2020) |
| Multiplex real-time PCR | Serum and tissue (kidney, liver, lung, and lymph node) | BVDV, PBOV, PCV-1, PEDV, TGEV | None evaluated | 100 copies/μL | Singleplex real-time PCR | See comments | See comments | No samples tested positive for JEV – See test comparison in Table 3 | Wu, H. et al. (2014) |
| Multiplex RT-PCR | Aborted fetus, colon, duodenum, gonad, heart, jejunum, kidney, liver, lung, lymph node, spleen, and tonsil | PRRS, SIV | None evaluated | 10-5 ng | Virus isolation | 66.7% | NR |  | Chen, H.-Y. et al. (2010) |
| Reverse Transcriptase loop-mediated isothermal amplification (RT-LAMP) | Blood  | PCV, PRRSv, CSFV, BVDV, SHEV, CHIKV, YFV, SINV | None evaluated | 2.57 copies/μL (GI) and 2.34 copies/μL (GIII) | RT-PCR | See comments | See comments | See Table 4 for more detailed information  | Liu, H. et al. (2012) |
| Real time RT-PCR | See comments | See comments |
| Reverse transcription loop-mediated isothermal amplification (RT-LAMP) | Serum | CSFV, PCV-2, PRV, PRRSv, PPV | None evaluated | 8.13 PFU/ml | RT-PCR | 87.0% | 99.0% |  | Tian, C.J. et al. (2012) |
| Real time RT-PCR | Blood | CSFV, **WNV** | No | 12 copies/μL | NA | NA | NA | Real time RT-PCR was developed by authors and used as reference for RT-LAMP and RT-PCR | Dhanze, H. et al. (2015) |
| Reverse Transcriptase loop-mediated isothermal amplification (RT-LAMP) | Blood | CSFV, **WNV** | No | 12 copies/μL | Real time RT-PCR | 100.0% | 100.0% |
| RT-PCR | Blood | CSFV, **WNV** | No | 1.2x105 copies/μL | Real time RT-PCR | 25.0% | 100.0% |
| ***Antibody detection*** |
| **Enzyme-linked immunoassays (ELISA)** |
| Blocking ELISA (bELISA) | Serum | CSFV, PCV-2, PPV, PRRSV, PRV | None evaluated | 1:80 dilution was above the 34.03% percentage of inhibition | Indirect ELISA | See comment | See comment | See Table 2 and text for specifics on diagnostic sensitivity and specificity | Zhou, D. et al. (2019) |
| Biotin-labeled protein-A ELISA (BLPA-ELISA) | Serum | NR | NA | NR | HI | 98.1% | 98.5% |  | Chang, H.-C. et al. (1984b) |
| Biotin-labeled antigen sandwich enzyme-linked immunosorbent assay (BLA-S-ELISA) | Serum | NR | NA | NR | HI and labeled avidin-biotin (LAB)-ELISA IgM | See comments | See comments | Antibody titers at multiple time points post inoculation with JE were compared between tests (Figure 2) | Chang, H.-C. et al. (1984a) |
| ELISA Aceton-ether (AE) zonal antigen | Serum | NR | NA | NR | HI  | 91.7% | 97.2% | Reviewer calculated from Table 1 | Ohkubo, Y. et al. (1984a) |
| ELISA sucrose-aceton (SA) antigen | Serum | NR | NA | NR | HI  | 100.0% | 16.9% |
| ELISA-IgG | Serum | NR | NA | NR | HI  | 93.3% | 100.0% | Reviewer calculated from Table 2 | Yamaoka, M. (1983) |
| ELISA-IgM | Serum | NR | NA | NR | HI | 82.9% | 93.7% | Reviewer calculated from Table 2. Note on calculation: samples that were noted as “Doubtful” were combined with the “Positives” | Yamaoka, M., Konishi, E., and Matsumura, T. (1982)  |
| Indirect ELISA | Serum | AR, *Br. suis*, chlamydia, PRRS, PRV, PPV  | None evaluated | NR | HI | 83.6% | 89.5% | Reviewer calculated from Table 2 | Xinglin, J. et al. (2005) |
| Indirect ELISA | Serum | NR | NA | NR | HI | 94.3% | 81.5% | Reviewer calculated from Table 1 | Yang, D.-K. et al. (2006) |
| Serum neutraliza-tion | 93.7% | 81.0% |
| Indirect ELISA  | Serum | NR | NA | NR | HI | 92.2% | 91.8% | Reviewer calculated from Table 1 | Yang, D.-K. et al. (2017) |
| PRNT90 | 98.6% | 95.0% |
| VNT | 98.7% | 95.0% |
| Indirect ELISA IgG | Serum | **WNV** | Yes | NR | VNT | 82.8% | 78.9% | Reviewer calculated from Table 2 | Kohle, R.P. et al. (2015) |
| Indirect ELISA IgM | Serum | **WNV** | Yes | NR | VNT | 62.8% | 81.6% |
| Recombinant non-structural (NS1) protein-based dipstick IgG ELISA | Serum | NR | NA | NR | Indirect IgG ELISA | 100.0% | 92.9% | Reviewer calculated from Table 1 | Chauhan, J. et al. (2020) |
| Recombinant non-structural 1 (NS1) protein based indirect IgG ELISA | Serum | NR | NA | Optimal conditions were an antigen concentra-tion of 10 μg/ml and 1:200 dilution of the hyper-immune serum | VNT | 91.0% | 97.0% |  | Dhanze, H. et al. (2019) |
| Recombinant non-structural (NS1) protein based indirect IgM ELISA | Serum | NR | NA | NR | VNT | 90.6% | 81.8% | Reviewer calculated from Table 2 | Dhanze, H. et al. (2020b) |
| IgM ELISA | 95.3% | 98.6% | Reviewer calculated from Table 3 |
| ***Other*** |
| Lateral flow assay – format I | Serum | NR | NA | Dilution of 1:100 | Indirect IgG ELISA | See comments | See comments | Authors determined this test was not suitable for JEV antibody screening | Dhanze, H. et al. (2020a) |
| Lateral flow assay – format II | Serum | NR | NA | Dilution of 1:51,200 | Indirect IgG ELISA | 55.7% | 100.0% | 100% sensitivity and specificity reported in subset of samples collected during monsoon and post-monsoon season |
| Lateral flow assay – format III | Serum | NR | NA | NR | Lateral flow assay – format II | 25.6% | 100.0% |  |
| Latex agglutination test | Serum | AR, *Br. suis*, swine chlamy-diosis, PPV | None evaluated | Most sensitive at 37°C and the agglutina-tion titer was at a 1:32 dilution using an antigen dilution of 1:32 | HI | 91.7% | NR |  | Xinglin, J. et al. (2002) |
| Latex agglutination test | Serum | NR | NA | NR | IgG ELISA | 80.2% | 95.2% |  | Grace, M.R. et al. (2019b) |
| Latex agglutination test | Serum | NR | NA | 750 µg/ml of protein with hyper-immune serum in 1:16 dilution | VNT | 82.2% | 87.8% | Reviewer calculated from Tables 2 and 3 | Grace, M.R. et al. (2019a) |

NA = Not applicable; NR = Not reported.

Test names: ELISA = Enzyme-linked immunosorbent assay; HI = Haemagglutination inhibition; IFA = Immunofluorescence assay; PCR = Polymerase chain reaction; PRNT = Plaque reduction neutralization test; qPCR = Quantitative-polymerase chain reaction; RT-PCR = Reverse-transcriptase polymerase chain reaction; VNT = Virus neutralization test.

Species/virus names: AR = Atrophic rhinitis; ASFV = African swine fever virus; *Br. suis = Brucella suis*; BVDV = Bovine viral diarrhea virus; CHIKV = Chikungunya virus; CSFV = Classical Swine Fever virus; DENV = Dengue virus; *E. coli = Escherichia coli*; FMDV = Foot and Mouth disease virus; GETV = Getah virus; *H. parasuis = Haemophilus parasuis*; KUNV = Kunjin virus; MVEV = Murray Valley encephalitis virus; PBOV = Porcine bocavirus; PCV-1 = Porcine circovirus type 1; PCV-2 = Porcine circovirus type 2; PEDV = Porcine epidemic diarrhea virus; PK15 cells = Pig kidney cells; *P. multocida = Pasteurella multocida*; PPV = Porcine parvovirus; PRRSv = Porcine reproductive and respiratory syndrome virus; PRV = Pseudorabies virus; SHEV = Swine hepatitis E virus; SINV = Sindbis virus; SIV = Swine Influenza virus; TGEV = Transmissible gastroenteritis virus; WNV = West Nile virus; YFV = Yellow Fever virus; ZIKV = Zika virus.

**Supplemental Table S6b.** Antigen detection and molecular diagnostic tests for detection of Japanese encephalitis virus (JEV) that were evaluated against a reference test, organized alphabetically by test name and publication year, that only reported analytical sensitivity and specificity.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Test name** | **Sample type** | **Species evaluated for cross-reactivity** | **Cross-reactivity to *Flavivirus spp.*** | **Analytical sensitivity** | **Reference test** | **Diagnostic sensitivity** | **Diagnostic specificity** | **Comments** | **Reference(s)** |
| **Antigen detection** |
| Protein biochip array | Serum | CSFV, PPV, PRRSV | None evaluated | SNR value (median signal intensity to median background intensity) > 3.38 | ELISA | NR | NR | Table 3 reports positive and negative counts | Wu, Y. et al. (2020) |
| **Molecular tests** |
| Multiplex PCR | Serum and tissue (aborted fetus, lung, lymph node, and spleen)  | BVDV, *E. coli*, PCV-1, PK15 cells, TGEV | None evaluated | 450 pg | Single PCR | NR | NR |  | Xu, X.-G. et al. (2012) |
| Multiplex PCR | Kidney, liver, lung, lymph node, spleen, and tonsil | SIV, *E. coli* | None evaluated | 46 pg | Single RT- PCR | NR | NR | Authors tested clinical samples, but did not report any information from the reference test | Zeng, Z. et al. (2014) |
| Real-time nucleic acid sequence-based amplification (RT-NASBA) | Abortus, blood, cerebrospinal fluid, and visceral tissue  | CSFV, PCV-2, PPV, PRRSV, PRV  | None evaluated | 6 copies/ reaction | RT-PCR  | NR | NR | Positive rate reported in Table 1 | Zhou, D. et al. (2021) |
| RT-qPCR | NR | NR |
| Reverse transcriptase – digital droplet polymerase chain reactions (RT-ddPCR) | Abortus, blood, cerebrospinal fluid, semen, and visceral tissue | CSFV, **DENV**, PRRSv, high pathogenic PRRSv, **WNV**, **YFV** | No | 2 copies/20 μL | Real-time TaqMan RT-PCR | NR | NR |  | Wu, X. et al. (2017) |
| Reverse transcription recombinase‑aided amplification (RT‑RAA) | Serum | CSFV, GETV, PEDV, PRRSV, TGEV | None evaluated | 5.5 copies/µL | RT-qPCR | NR | NR |  | Nie, M. et al. (2022) |
| TaqMan real-time RT-PCR | Blood | **WNV** | No | 2.8 copies/ reaction | RT-PCR | NR | NR |  | Pantawane, P.B. et al. (2019) |
| Duplex TaqMan RT-qPCR | Blood and tissue (brain, kidney, liver, and spleen) | ASFV, CSFV, **DENV**, PCV-2, PPV, PRRSV, **WNV**, **ZIKV**  | No | 10 genomic copies | RT-PCR  | NR | NR |  | Wang, X. et al. (2020) |
| Virus isolation | NR | NR |  |
| DNA sequencing | NR | NR |  |
| Duplex TaqMan RT-qPCR | Blood and tissue (brain, kidney, and liver)  | ASFV, CSFV, PPV, PRRSV, PRV | None evaluated | 1,000 genomic copies | RT-PCR | NR | NR |  | Zhang, Y. et al. (2022) |
| Virus isolation | NR | NR |

NR = Not reported.

Test names: ELISA = Enzyme-linked immunosorbent assay; PCR = Polymerase chain reaction; qPCR = Quantitative-polymerase chain reaction; RT-PCR = Reverse-transcriptase polymerase chain reaction; RT-qPCR = Reverse-transcriptase quantitative polymerase chain reaction.

Species names: ASFV = African swine fever virus; BVDV = Bovine viral diarrhea virus; CSFV = Classical Swine Fever virus; DENV = Dengue virus; *E. coli = Escherichia coli*; GETV = Getah virus; PCV-1 = Porcine circovirus type 1; PCV-2 = Porcine circovirus type 2; PEDV = Porcine epidemic diarrhea virus; PK15 cells = Pig kidney cells; PPV = Porcine parvovirus; PRRSv = Porcine reproductive and respiratory syndrome virus; PRV = Pseudorabies virus; SIV = Swine Influenza virus; TGEV = Transmissible gastroenteritis virus; WNV = West Nile virus; YFV = Yellow Fever virus; ZIKV = Zika virus.

**Supplemental Table S7.** Vaccine information, including strain and manufacturer, study design and challenge strain, population and sample size of vaccinated swine, author conclusion about vaccine efficacy (efficacious, not efficacious, and inconclusive), evidence used to determine efficacy, and record information from vaccine efficacy studies for domestic swine organized by vaccine type (live attenuated, killed, multiple type comparisons, and other) and year of publication within vaccine type. Superscripts denote study specific information within the applicable row.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Vaccine information** | **Study design and challenge strain (Genotype)** | **Population** **[breed; sex; age] (sample size vaccinated)** | **Author conclusion** | **Efficacy evidence** | **Author evidence** | **Reference(s)** |
| **Live attenuated** |
| S’ strain alone (author produced) and a mixture of S’ strain and E- strain of the GP series of the hog cholera virus (supplied by Dr. Kumagi)a | Vaccinate & challenge with Sagara | Other (research); NR; Nursing (n = 3)  | Efficacious | * Antibody titers
* Clinical signs
* Viremia
 | * Vaccinated piglets showed no clinical symptoms or viremia after challenge
 | Sazawa, H. et al. (1969b)a; Sazawa, H. et al. (1969a)b |
| NR; NR; Weaning  (n = 2)  |
| S’ strain (author produced)a,b | Other (research); NR; Nursing (n = 1a; n = 6b)  |
| NR; NR; Weaning  (n = 2a; n = 11b)  |
| Strain TWN-21 (author produced) | Vaccinate & natural exposure  | NR; Female; Mature  (n = 12) | Efficacious | * Antibody titers
* Viremia
 | * Vaccinated pigs had higher antibody titers than control pigs after the JE epidemic (12 weeks after the start of the experiment)
* Viremia was detected in 6/10 controls while all vaccinated sows were negative
 | Lee, G.C.-Y., Huang, Y.-T., and Chang, L.-C. (1975) |
| S- strain (author produced) | Vaccinate & challenge with AS-6 | Other (research); NR; Growing (n = 2) | Inconclusive | * Antibody titers
* Viremia
 | * Viremia was not detected in the pig challenged at eight weeks post vaccination
* Viremia was detected in the pig challenged 11 weeks post vaccination
 | Fujisaki, Y. et al. (1975) |
| Landrace; NR; Finishing (n = 2) | * Viremia was not detected in the pig challenged three weeks post vaccination
* Viremia was detected in the pig challenged seven weeks post vaccination
 |
| Landrace; Female; Mature (n = 2)  | Efficacious | * Antibody titers
* Clinical signs
* Vertical transmission
* Viremia
 | * No abnormal pathology was found in the placenta or fetuses of the immunized sows, but control sows (n = 2) had hyperemia, hemorrhage, and necrosis in the placenta as well as mummified fetuses
* Viremia was not found in the immunized sows, but was seen in control sows
 |
| Vaccinate, booster, & challenge with AS-6 | Landrace; NR; Finishing (n = 2)  | Efficacious | * Antibody titers
* Viremia
 | * Viremia was not detected in vaccinated pigs post challenge
 |
| Commercially available lyophilized vaccine (Lot L-4) produced in monkey kidney cell culture (Kanonji Institute, The Research Foundation for Microbial Diseases of Osaka University, Kanonji, Kagawa) | Vaccinate & natural exposure  | Yorkshire/Landrace; NR; Finisher (n = 5)  | Efficacious | * Antibody titers
* Viremia
* Virus isolation
 | * Antibody titers in vaccinated pigs were lower than the unvaccinated group
* No virus was isolated in the vaccinated group
 | Ueba, N. et al. (1978) |
| Strain M-PK/L (Biken Laboratories, Japan) | Vaccinate & challenge with JaGAr-01 (GIII) | NR; NR; Weaning  (n = NR)  | Efficacious | * Antibody titers
* Transmission to mosquitoes
* Viremia
 | Vaccinated pigs:* Developed circulating antibodies
* Were unable to transmit virus to mosquitoes fed on their skin
* Did not develop viremia after challenge
 | Sasaki, O. et al. (1982) |
| at222 GIII (Kaohsiung Biological Product Co., Ltd.) | Vaccinate, booster, & natural exposure | NR; NR; Weaning (n = 20) | Inconclusive | * Antibody titers
* Cross-protectivity to GI
 | * 95% of weaned piglets were seropositive (PRNT50 ≥ 10) against the at222 strain after two doses of the vaccine, whereas only 10% of piglets were seropositive against the GI T2009-1 strain
 | Fan, Y.-C. et al. (2013) |
| **Killed** |
| **Vaccine information** | **Study design and challenge strain (Genotype)** | **Population** **[breed; sex; age] (sample size vaccinated)** | **Author conclusion** | **Efficacy evidence** | **Author evidence** | **Reference(s)** |
| Formalin inactivated JE vaccine for animal use | Vaccinate, booster, and natural exposure | NR; NR; Grower to Mature (n = 2c; n = 3d)  | Efficacious | * Antibody titersc,d
* Viremiac
 | * Not all of the vaccinated pigs developed viremia
 | Ogata, M. et al. (1969)c; Ogata, M. et al. (1970)d |
| Formalin inactivated JE vaccine for animal use plus Freund’s complete adjuvant (Difco Lab) | NR; NR; Grower to Mature (n = 3c,d)  | * HI and neutralizing antibody titers were higher than in pigs vaccinated with only the vaccinec,d
* Not all of the vaccinated pigs developed viremiac
 |
| "High Titer Killed Vaccine" for veterinarian use (Kaketsuken Lot. 23) | Vaccinate (x4) and natural exposure | Yorkshire and Landrace; Both; Finishing (n ≅ 60) | Inconclusive | * Antibody titers
* Viremia
* Virus isolation
 | * Average HI antibody titers were lower in the vaccinated group compared to the unvaccinated
* Viremia was detected in all unvaccinated pigs and in 45% of vaccinated pigs, but was of shorter duration
* The rate of virus isolation was higher in mosquitoes collected near the vaccinated group pen compared to the unvaccinated pen
 | Ueba, N. et al. (1972) |
| **Multiple type comparisons** |
| **Vaccine information** | **Study design and challenge strain (Genotype)** | **Population** **[breed; sex; age] (sample size vaccinated)** | **Author conclusion** | **Efficacy evidence** | **Author evidence** | **Reference(s)** |
| Live attenuated M mutant strain (author produced) | Vaccinate & challenge with Furumoto | Other (research); NR; Nursing (n = 4)  | Efficacious | * Antibody titers
* Clinical signs
* Viremia
 | * Pigs inoculated with a dose of 107 (n = 2) or 108 TCD50 (n = 1) produced antibodies after vaccination and did not show abnormal clinical signs or viremia after the challenge. The pig vaccinated with the lowest dose (102 TCD50) did not produce antibodies after vaccination and showed abnormal clinical signs and viremia after the challenge
 | Kodama, K., Sasaki, N., and Inoue, Y.K. (1968) |
| Formalin inactivated vaccine | Other (research); NR; Nursing (n = 2)  | Inconclusive | * The pig with a single injection did not produce antibodies. After the challenge, viremia was “not determined” and HI antibodies were high
* The pig with three injections produced antibodies after vaccination and did not show viremia or abnormal clinical signs after the challenge
 |
| Live attenuated ML-17 strain (adapted from JaOH 0566 strain by authors) | Vaccinate and challenge with JaGAr-01 (GIII) | NR; NR; NR (n = 2)  | Efficacious | * Antibody titers
* Viremia
 | * All pigs showed no viremia
 | Yoshida, I. et al. (1981) |
| Vaccinate, booster, and challenge with JaGAr-01 (GIII) | NR; NR; NR (n = 2)  |
| “Commercial killed JE vaccine for veterinary use” | Vaccinate and challenge with JaGAr-01 (GIII) | NR; NR; NR (n = 2)  | Not efficacious | * Pigs developed viremia similar to non-immunized control pigs
 |
| Vaccinate, booster, and challenge with JaGAr-01 (GIII) | NR; NR; NR (n = 2)  |
| Inactivated JEV vaccine | Vaccinate, booster, and challenge with JEV SA-14 (GIII) | Yorkshire; NR; Weaning (n = 5)  | Efficacious | * Antibody titers
* Mortality
 | * All pigs produced NAb titers after inoculation and survived the lethal challenge
 | Li, P. et al. (2008) |
| Recombinant adenovirus (rAd-TEP) vaccine (author produced) | Yorkshire; NR; Weaning (n = 15)  | Inconclusive | * All pigs produced NAb titers after inoculation
* All pigs (n = 5) vaccinated with a dose of 1×1010.0 TCID50 survived the lethal challenge
* Three of five survived from the group vaccinated with a dose of 3×109.0 TCID50
* Two of five survived from the group vaccinated with a dose of 1×109.0 TCID50
 |
| Commercial inactivated JEV vaccine | Vaccinate and challenge with Beijing P3 (GIII) | NR; NR; Weaning  (n = 3) | Inconclusive | * Antibody titers
* Clinical signs
* Viremia
 | * All vaccinated pigs produced JEV NAb by 14 dpc, although at a lower level compared to those vaccinated with CSF-JE VRP
* Two vaccinated pigs developed viremia at 3 dpc and one pig developed viremia at 7 dpc
 | Yang, Z. et al. (2012) |
| Chimeric classical swine fever-Japanese encephalitis viral replicon (CSF-JE VRP) from a cDNA clone of pA187delE2/JEV-tE (author produced) | NR; NR; Weaning  (n = 3) | Efficacious | * All vaccinated pigs produced JEV NAb by 21 dpc
* One vaccinated pig exhibited viremia (3 dpc)
 |
| **Other**  |
| **Vaccine information** | **Study design and challenge strain (Genotype)** | **Population** **[breed; sex; age] (sample size vaccinated)** | **Author conclusion** | **Efficacy evidence** | **Author evidence** | **Reference(s)** |
| Highly attenuated vaccinia (NYVAC) vectored recombinants (vP908 and vP923) | Vaccinate, booster, & challenge with JEV B-2358/84 | Landrace; Male; Growing (n = 5)  | Efficacious | * Viremia
 | * Two vaccinated swine exhibited viremia > 10 PFU/ml for an average of 2 days
* Nine of ten control swine (negative and positive controls) exhibited viremia for an average of 2.7 days
 | Konishi, E. et al. (1992) |
| JE-DNA vaccine based on pNGVL4a (pNJEME) and a partial dose of a commercial formalin-inactivated vaccine for human use (JEVAX; Takeda Pharmaceutical Osaka, Japan) | Vaccinate, booster, and challenge with Sw/Mie/40/2004 (GI-b) | Other (research); Female; Mature  (n = 2) | Efficacious | * Antibody titers
* Fetal death
 | * Vaccinated sows developed NAb titers (≥ 1:40) by the time of challenge, no control sows (n = 2) had detectable levels of NAb
* None of the fetuses (n = 12) from vaccinated sows were dead/abnormal, whereas six of twelve fetuses from control sows were mummified
 | Imoto, J.-I. et al. (2010)\* |
| TRIP/JEV.prME lentiviral vector vaccine | Vaccinate, booster, & challenge with Nakayama (GIII) | Landrace; NR; Weaning (n = 3) | Inconclusive | * Antibody titers
* Clinical signs
* Tissue tropism
* Viral shedding
* Viremia
 | Vaccinated pigs:* Did not develop viremia or abnormal clinical signs
* Low, but detectable levels of JEV RNA were detected in the following: lymph node, ileum, jejunum, nasal cavity, olfactory bulb, striatum, and brain stem
 | García-Nicolás, O. et al. (2017) |
| GI JEV virus-like particles (VLPs) | Vaccinate, booster, & challenge with YL2009-4 (GI) or CH1392 (GIII) | Other (research); NR; Weaning to growing (n = 9) | Efficacious | * Antibody titers
* Clinical signs
* Tissue tropism
* Viremia
 | * After the first vaccination, three of five pigs displayed NAb and 4 weeks after the second vaccination all pigs had NAb against GI and GIII JEV. The NAb titer was 1.9-fold higher against GI than GIII
* All vaccinated pigs did not display abnormal clinical signs or viremia
* No tissue tested had detectable levels of viral RNA
* No observable lesions in the brain parenchyma or vessels
 | Fan, Y.-C. et al. (2018) |
| Passive immunization with monoclonal antibodies (JEV-31 mAb or JEV-169 mAb) | Passive immunization & challenge with JE-91 (GI) | Other (research); NR; Weaning (n = 10)  | Efficacious | * Antibody titers
* Clinical signs
* Tissue tropism
* Viral shedding
* Viremia
 | * All pigs developed NAb (> 1:10 PRNT50)24 hours after immunization
* Control pigs (n = 5) developed transient viremia between 1 and 2 dpc; viremia was detectable in three pigs at 1 dpc
* None of the immunized pigs had febrile signs or showed detectable viremia at 1 dpc; eight had detectable viremia at 3 dpc
 | Young, C.L. et al. (2020) |

Dpc = Days post challenge; Nab = Neutralizing antibody; NR = Not reported.

\*Report also includes studies comparing inoculation route of the vaccine of interest and other positive controls.

**Supplemental Table S8.** Description of Japanese encephalitis virus (JEV) infectious disease models (compartments evaluated in pigs are highlighted in bold), control strategies evaluated, and reported basic reproduction number.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Compartments‡ (population)**  | **Basic reproduction number [route of transmission**†**]** | **Control strategy evaluated (if applicable)** | **Outcomes from control strategies** | **Location** | **Reference(s)** |
| SIR (humans); **I (pigs)**; SI (mosquitoes) | -1.00 – 0.50[vector-borne] | Evaluated control strategies (electronic devices, insecticide treated bed nets, mosquito repulsive lotions, pig vaccination, human treatment, vaccination, and insecticides). | Adopting the three optimal control interventions is the best control strategy to minimize the number of infective pigs and to reduce disease transmission rate. | India  | Goswami, N.K. (2022)  |
| SIR (humans); **SI (pigs)**; SI (mosquitoes) | 2.01[vector-borne] | Evaluated optimal control strategies (human vaccination, human treatment, pig vaccination, insecticide application) and their costs.  | Combining all control strategies provided optimal disease control and cost.  | Indonesia | Kharismawati, H., Fatmawati, and Windarto (2019) |
| SVIR (humans); **SI (pigs)**; SI (mosquitoes) | 0.02 – 2.90[vector-borne] | Evaluated impact of vaccination and mosquito reduction strategies on controlling disease. | Presented how R0 varies based on mortality rate of mosquitoes, humans, and pigs.  | India | Baniya, V., and Keval, R. (2020) |
| SVIR (humans); **SVI****(pigs)**; SI (mosquitoes) | NR | Evaluated control strategies (vaccination only, medicine only, insecticide only, vaccination and medicine, vaccination and insecticide, medicine and insecticide, medicine, vaccination and insecticide and no control). | Simultaneous use of all the controls always gives the best result compared to single control or no control. Vaccination control alone (without medicine or insecticide control) cannot reduce the infected population. Pig control plays a significant role in controlling the disease from the whole system. | India | De, A. et al. (2016)  |
| **SEIR (pigs**) | 1.20 [vector-borne] | Evaluated different vaccination programs (vaccination of 25%, 50%, or 75% of pigs) assuming that efficacy is 95% and that 5% of pigs’ infections result from external introductions.  | They found there would be a 61%, 82%, and 89% reduction in annual incidence in pigs when 25%, 50%, or 75% of the pigs are vaccinated, respectively. Other assumptions were evaluated in sensitivity analysis.  | Bangladesh | Khan, S.U. et al. (2014) |
| **SEICR (pigs**); spill-over ratio from reservoirs to humans | 0.0013 (95% CI: 0.00 – 0.31) [pig-to-pig] | Assessed the mechanism underlying the JEV skip-and-resurgence patterns between December 2003 and May 2017. | Pig-to-pig transmission increases the size of JEV epidemics, but it is unlikely to maintain the same level of transmission among pigs compared to vector-borne transmission.  | Hong-Kong | Zhao, S. et al. (2018) |
| **MSIR (pigs**); SEI (mosquitoes) | 2.27 – 2.90 [overall]2.00 – 2.48 [vector-borne]0.35 – 0.83 [pig-to-pig] | Evaluated the contribution of direct transmission between pigs to the epidemiological cycle of JE. | The contribution of the pig-to-pig transmission on R0 is 7.5 to 11.9%. | Cambodia | Diallo, A.O. et al. (2018)  |
| **MSEIR (pigs, sows,** ducks, chickens, cattle, humans, dogs); IES (mosquitoes) | .07 (95% CI: 0.99 – 1.20) [District 1]1.25 (95% CI: 1.16 – 1.37) [District 2]1.38 (95% CI: 1.29 – 1.53) [District 3]Districts in an average village of Kandal province. | Evaluated the influence of host community composition on R0 for a multi-host system (7 hosts and *Culex* vectors [vector-borne transmission only]): relative share of competent vs non-competent host body surface area (BSA); relative share of pigs, chickens and ducks among competent hosts BSA; and relative share of cattle among cattle-and-pigs BSA. | When there was a 15% of competent hosts reaching the whole system’s BSA R0 became > 1; as the proportion of pigs BSA increased, so did the R0, regardless of the percent of chickens; as the percentage of cattle increased, R0 decreased and when reaching a 65% of cattle among total cattle and pigs BSA, R0 became < 1. | Cambodia | Ladreyt, H., Chevalier, V., and Durand, B. (2022) |
| **SEI (feral pigs)**; mosquitoes, birds**\*** | NR | Compared the number of infected feral pigs for different bird migration parameters and mosquito vectorial capacity during fall and spring migration, in Miami-Dade, FL, Charleston county, SC, and Carteret County, NC. | Found that the bird migration parameter increases the number of infected feral pigs, especially in spring, but that depends on the mosquito vectorial capacity. | USA (Florida, North Carolina, and South Carolina) | Riad, M.H. et al. (2017)   |
| Mathematical equations¥**Pigs**, mosquitoes | NR | Evaluated epizootics in pigs considering pig (number of susceptible and immune pigs) and mosquito parameters (density, number of transmissible mosquitoes) and considered implications relative to epidemics in humans. | The density of mosquitoes much less sensitively influences the scale of pig epizootics than of human epidemics. The time of initiation of pig infection affects the number of infected pigs and the number of transmissible mosquitoes. An increased mosquito density and daily survival influences the pig epizootics. Pig immunization reduces the number of transmissible mosquitoes, although the level of reduction depends on the mosquito density. | Japan | Wada, Y. (1975) |

**‡**C = Convalescent; E = Exposed; I = Infected/infectious; M = Maternal antibodies; R = Recovered; S = Susceptible; V = Vaccinated.

†Route of transmission described for pigs only.

\*Described as a Generalized Epidemic modeling Framework (GEMF), including an individual-level network model that explicitly considers the feral pig population and implicitly considers mosquitoes and birds.

¥Not described as a compartmental model.

NR = Not reported; 95% CI = 95% Confidence Interval.