The Journal of Veterinary Medical Science

NOTE

Virology

Utilization of meat juice from market-purchased pork for serological surveillance of mosquito-borne arboviruses

Ryusei KUWATA[1)]*, Le Nu Anh THU[2)], Dang Thi Mai PHUONG[3)], Makoto TAKEISHI[4,5)], Yoichi INOUE[1)], Naomi OHTA[1)], Violeta Ivonne FLORES LINO[1)], Hirohito OGAWA[1)], Shumpei WATANABE[1)], Hiroshi SHIMODA[6)], Yasutsugu SUZUKI[7)], Phan Thi HANG[2)], Murasaki AMANO[3)], Haruka ABE[3)], Yukiko HIGA[4)], Haruhiko ISAWA[4)], Ken MAEDA[4)], Le Dinh PHUNG [2)], Futoshi HASEBE[3)], Shinji KASAI[4)]

[1)]Okayama University of Science, Ehime, Japan
[2)]University of Agriculture and Forestry, Hue University, Hue city, Vietnam
[3)]Institute of Tropical Medicine (NEKKEN), Nagasaki University, Nagasaki, Japan
[4)]National Institute of Infectious Diseases, Japan Institute for Health Security, Tokyo, Japan
[5)]The Institute of Medical Science, The University of Tokyo, Tokyo, Japan
[6)]Yamaguchi University, Yamaguchi, Japan
[7)]Center for Marine Environmental Studies, Premier Institute for Advanced Studies, Ehime University, Ehime, Japan

J Vet Med Sci
88(8): 1280–1284, 2026
doi: 10.1292/jvms.26-0242

Received: 11 May 2026
Accepted: 28 May 2026
Advanced Epub:
11 June 2026

ABSTRACT. This study evaluated the utility of meat juice (MJ) from market-purchased pork in Japan and Vietnam for serological surveillance of mosquito-borne viruses, specifically Japanese encephalitis virus (JEV) and Getah virus (GETV). We confirmed the presence of specific antibodies against JEV and GETV in the MJ samples using enzyme-linked immunosorbent assay and virus neutralization test. The lack of correlation between JEV- and GETV-specific optical density values suggests independent infections in Vietnamese pig populations. The high seroprevalence observed in Vietnam highlights the practical utility of MJ as a viable alternative to serum for effective large-scale arboviral surveillance in field settings. These findings underscore the need for comprehensive mosquito-borne virus control in swine health management.

KEYWORDS: antibodies, arboviruses, market-purchased pork, meat juice

Arthropod-borne viral diseases have emerged as a significant global public health threat. This threat has been driven by the expansion of vector habitats, facilitated by global warming and the increasing globalization of logistics. *Orthoflavivirus japonicus*, commonly referred to as Japanese encephalitis virus (JEV, family *Flaviviridae*, order *Amarillovirales*), causes severe neurologic disorders in humans, such as acute encephalitis. According to a recent WHO report [15], an estimated 100,000 JEV infection cases occur annually, primarily in Southeast Asia and the Western Pacific regions. Conversely, *Alphavirus getah*, commonly referred to as Getah virus (GETV, family *Togaviridae*, order *Martellivirales*), is of paramount importance in the field of veterinary medicine, as it causes outbreaks characterized by pyrexia, exanthema, and hind-limb edema in horses [12] and also affects other livestock animals [6]. Both JEV and GETV share a common transmission cycle in which mosquitoes' function as vectors and pigs as the primary amplifying hosts. The infection cycle is maintained and expanded when mosquitoes feed on the blood of amplifying hosts exhibiting viremia and subsequently transmit the virus to other susceptible humans or animal hosts. As diseases caused by JEV and GETV are widely distributed across Asia, establishing robust epidemic prevention systems remains a critical priority.

Serum is typically used for the detection of pathogen-specific antibodies. However, obtaining blood samples for antibody detection remains a significant challenge both domestically and internationally and can result in a lack of sufficient serum samples for analysis, particularly in short-term epidemiological surveys. Meat juice (MJ)—exudate released from tissue cells in response to repeated freeze-thaw cycles—has garnered attention as a viable alternative specimen for overcoming blood sampling constraints. Recent studies have demonstrated the utility of specific antibody detection using MJ for epidemiological surveillance of various infectious

*Correspondence to: Kuwata R: r-kuwata@ous.ac.jp, Faculty of Veterinary Science, Okayama University of Science, 1-3 Ikoinooka, Imabari-shi, Ehime Prefecture, 794-0085, Japan

conditions, such as parasite infection [4, 11], toxoplasmosis [10], tuberculosis [1], and salmonellosis [2], as well as viral diseases, including Aujeszky's disease [3], foot-and-mouth disease [16], and African swine fever [13]. We previously reported the detection of specific antibodies against JEV and hepatitis E virus using MJ derived from the heart and diaphragm of wild boars in Japan [17]. As an application of these findings, the present study utilized MJ obtained from commercially available pork as specimens to investigate and evaluate the prevalence of JEV- and GETV-specific antibodies.

Portions of edible pork (50–100 g of short ribs or loin) were obtained from a meat processing plant in Ehime Prefecture, Japan, and from vendors at local markets in Hanoi and Hue, Vietnam. The survey was conducted between 2024 and 2025. In Japan, processed meat from pigs raised at seven pig farms in Ehime Prefecture was sampled. A total of 306 specimens were collected, comprising 191 in December 2024 and 111 in November 2025. Pork short ribs were obtained from individual carcasses to ensure the distinct identification of each animal source. In Vietnam, samples were purchased in two cities: Hanoi and Hue, in January (Hanoi; n=72, Hue; n=50) and June (Hanoi; n=74, Hue; n=55) 2024 and March (Hanoi; n=72, Hue; n=72) and June (Hanoi; n=80, Hue; n=68) 2025. During each period, meat portions were purchased from multiple vendors in multiple markets within a single day. In the Vietnamese market system, vendors typically purchase a single whole carcass and then cut them up for sale. Therefore, while samples collected from different vendors are likely to have been taken from distinct individual animals, it should be noted that it cannot be strictly guaranteed that all samples are completely independent of one another.

MJ samples were obtained by collecting the liquid that seeped out of meat tissue after freezing and thawing the pork pieces at least three times. In this study, the presence of arbovirus-specific antibodies in MJ was evaluated using the 80% plaque reduction neutralization test ($PRNT_{80}$) and enzyme-linked immunosorbent assay (ELISA). $PRNT_{80}$ and ELISAs were performed in Japan for Japanese samples, and based on the results, ELISA cut-off values were established and subsequently applied to evaluate ELISA results for Vietnamese samples. Viral strains utilized in the study included JEV strain JEV/MQ/Yamaguchi/803/2016 [5] and GETV strain 14-I-605-C1 [12]. $PRNT_{80}$ and ELISAs were conducted according to previously described methods [14]. MJ samples were used as 1:50 dilution, rather than a 1:100 dilution typically used for serum samples. Furthermore, heat-based inactivation was excluded from the final protocol due to significant protein aggregation that interfered with liquid phase recovery; instead, MJ samples were centrifuged at 4°C and 10,000 *g* for 15 min, and the resulting supernatant was examined. All statistical analyzes were performed using EZR statistical software, version 1.54 [7].

First, JEV-specific antibodies in MJ samples from Japan (Ehime) were detected using an ELISA. This analysis identified several MJ samples with high optical density (OD) values. To verify the effectiveness of the ELISA system as a screening tool, we evaluated the correlation between ELISA OD value and virus neutralizing antibody titer (VN titer) using the 30 MJ samples with the highest ELISA OD values (Fig. 1). This analysis revealed an extremely strong positive correlation between the two indicators (coefficient of determination R^2=0.892, P<0.001). As the VN titer increased from 1:10 to 1:80, the mean ELISA OD value increased in a stepwise manner as follows: 0.170, 0.518, 0.864, and 1.567, respectively. The regression equation was determined as: y=0.449x−0.355 (where x represents the logarithmic dilution stage of the VN titer, and y represents the ELISA OD value), suggesting that ELISA absorbance values can be used to estimate neutralizing activity with high precision.

The ELISA cut-off value, sensitivity, and specificity were analyzed using receiver operating characteristic (ROC) analysis. VN titers of less than 1:10 were defined as "negative", and VN titers greater than 1:20 were defined as "positive". The optimal cut-off value at which both sensitivity and specificity are maximal was calculated as OD=0.28. Application of this threshold enabled the determination of antibody status without causing false-positive or false-negative results. These data confirm that the ELISA using MJ samples is a highly effective alternative for the quantitative assessment of JEV-neutralizing antibodies.

Based on the calculated cut-off values, we compared the seroprevalence of JEV-specific antibodies in the three study locations: Ehime Prefecture, Hanoi, and Hue (Fig. 2). The antibody positivity rate among all 845 samples tested was 14.0% (118/845). By location, Hanoi had the highest prevalence, at 19.1% (57/298), followed by Hue at 15.5% (38/245), whereas Ehime showed the lowest prevalence, at 7.6% (23/302). A chi-square test of independence was conducted to evaluate the distribution of positive cases across the three locations, which revealed a statistically significant difference [$\chi^2(2)$=18.39, P<0.001]. Post-hoc comparisons revealed that the prevalence in Ehime was significantly lower than that in both Hanoi (P<0.001) and Hue (P<0.05). By contrast, no significant difference in prevalence was observed between the two cities in Vietnam (P=0.27).

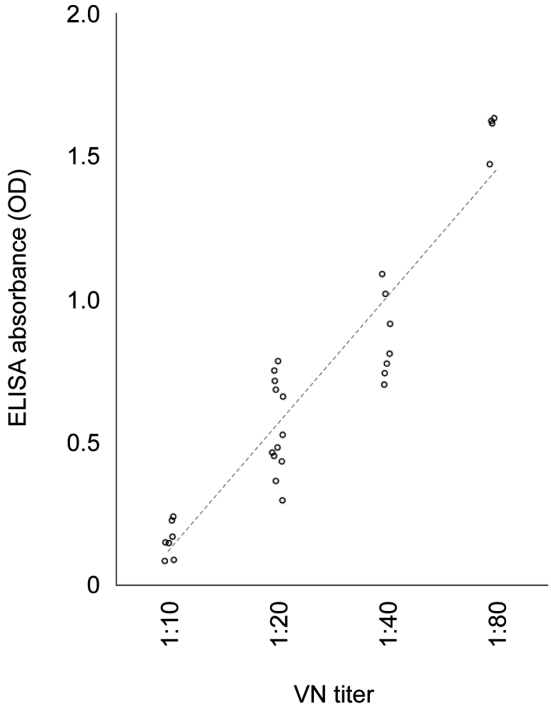

Fig. 1. Comparison of enzyme-linked immunosorbent assay (ELISA) absorbance and virus-neutralizing antibody (VN) titers against Japanese encephalitis virus in pork meat juice in Ehime Prefecture, Japan. The correlation between ELISA and 80% plaque reduction neutralization test results was analyzed using 30 pork meat juice samples. The x- and y-axes represent the VN titer in logarithmic dilutions (ranging from 1:10 to 1:80) and the ELISA absorbance (OD), respectively. Dashed line indicates the linear regression result. Statistical analysis showed a strong correlation between ELISA absorbance and VN titer (R^2=0.892, P<0.001).

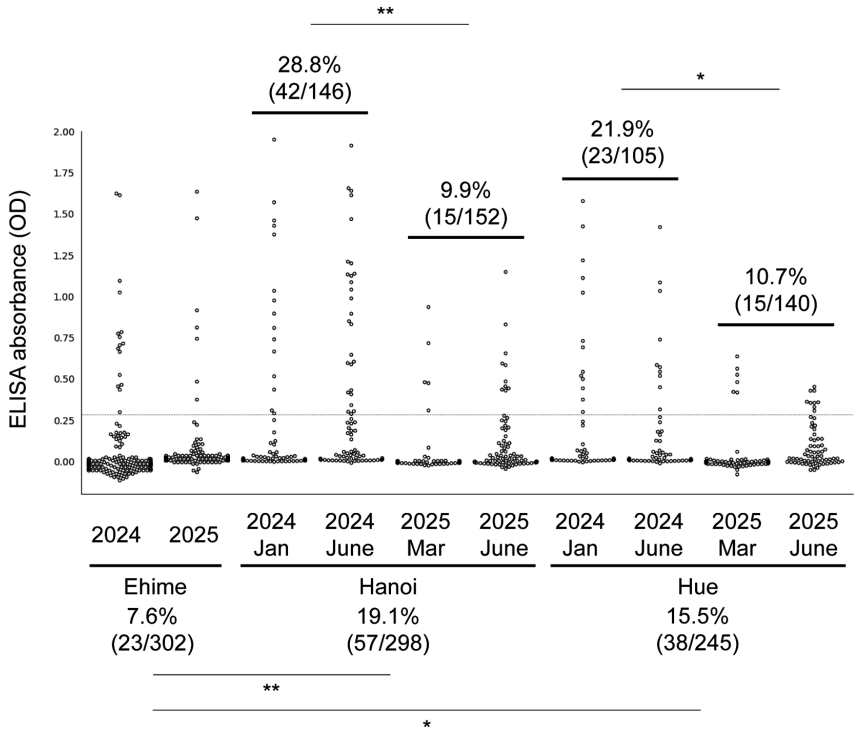

Fig. 2. Chronological comparison of anti-Japanese encephalitis virus antibody levels and seroprevalence between Ehime, Hanoi, and Hue. Individual ELISA absorbance (OD) values are shown for pork meat juice samples collected in 2024 and 2025 in Ehime Prefecture, Japan, and the cities of Hanoi and Hue in Vietnam. Horizontal dashed line represents the positive cut-off value. Percentages and parentheses above the brackets indicate seroprevalence and the number of positive samples relative to the total number of samples. Significant differences are indicated by asterisks: *P<0.001 and **P<0.05.

These results indicate that the prevalence of JEV-specific antibodies in Ehime is significantly lower than in the two cities in Vietnam.

A comparison of the seroprevalence in 2024 and 2025 revealed no statistically significant difference in Ehime, with a rate of 8.4% (16/191) in 2024 and 6.3% (7/111) in 2025 [$\chi^2(2)=0.44$, $P=0.506$]. By contrast, the two cities in Vietnam exhibited significant year-over-year fluctuations. In Hanoi, the prevalence decreased from 28.8% in 2024 (42/146) to 9.9% in 2025 (15/152), a statistically highly significant difference [$\chi^2(2)=17.55$, $P<0.001$]. A similar trend was observed in Hue, where the prevalence decreased from 21.9% in 2024 (23/105) to 10.7% in 2025 (15/140); this change was also statistically significant [$\chi^2(2)=5.76$, $P=0.016$]. The stability of the seroprevalence in Ehime during this period suggests that there were no significant changes in the external environment, such as a change in the population dynamics of vector mosquitoes. However, the significant decline in prevalence observed in Hanoi and Hue in 2025 may reflect either a drastic change in environmental factors within Vietnam or periodic fluctuations in the infection cycle. In the case of arbovirus infections in particular, annual precipitation and temperature significantly impact the density of mosquito vectors. Therefore, it is highly probable that changes in climatic conditions in 2025 contributed to a suppression of viral transmission efficiency.

In the GETV ELISA, none of the samples from Japan (Ehime) exhibited high OD values. Furthermore, the 15 samples yielding the highest OD values (ranging from 0.06 to 0.11) were confirmed to be negative by the $PRNT_{80}$ assay. Therefore, all samples from Japan (n=302) were designated a provisional negative control group, and the cut-off value was determined using statistical methods. Specifically, the threshold was established as the mean OD of the negative group plus three times the standard deviation (*Cut-off*=Mean + 3*SD*). For the samples from Ehime, the mean OD was 0.0096, and the standard deviation was 0.03548; the calculated cut-off value for a positive result was thus OD = 0.116. Based on the calculated cut-off values, we compared the GETV-specific antibody prevalence across the three study locations (Fig. 3). Statistically significant differences between locations were observed, and multiple comparisons using the Bonferroni correction showed that the prevalence in Ehime was significantly lower than that in both Hanoi and Hue ($P<0.001$). By contrast, no significant differences were observed between the two cities in Vietnam. Comparing the prevalence between 2024 and 2025, Hanoi showed a rate of 8.2% in 2024 (12/146) and 5.3% in 2025 (8/152). Although a downward trend was observed, the difference was not statistically significant ($P=0.312$). Similarly, in Hue, the prevalence was 6.7% in 2024 (7/105) and 2.1% in 2025 (3/140). Although there was a numerical decrease in the positive rate in both cities, an analysis using Fisher's exact test revealed that the difference between the two years was not statistically significant ($P=0.096$).

Finally, a detailed comparison of Vietnamese samples with the positive for JEV- and/or GETV-specific ELISA OD values revealed three distinct reaction patterns (Fig. 4). The majority of samples (n=79) exhibited a high OD value for JEV while remaining negative for GETV. By contrast, a smaller subset (n=14) of samples were negative for JEV-specific antibodies but exhibited a marked increase in OD values indicative of GETV-specific antibodies. Furthermore, the final cohort (n=16) showed moderate to high OD values for

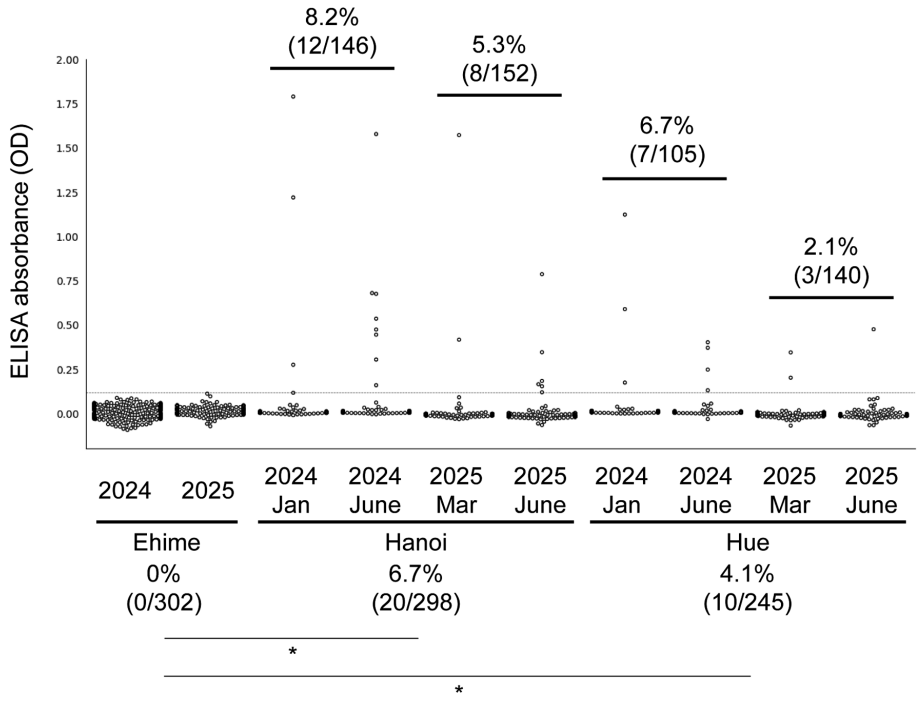

Fig. 3. Chronological comparison of anti-Getah virus antibody levels and seroprevalence between Ehime, Hanoi, and Hue. Individual ELISA absorbance (OD) values are shown for pork meat juice samples collected in 2024 and 2025 in Ehime Prefecture, Japan, and the cities of Hanoi and Hue in Vietnam. Horizontal dashed line represents the positive cut-off value. Percentages and parentheses above the brackets indicate seroprevalence and the number of positive samples relative to the total number of samples. Significant differences are indicated by asterisks: *P<0.001.

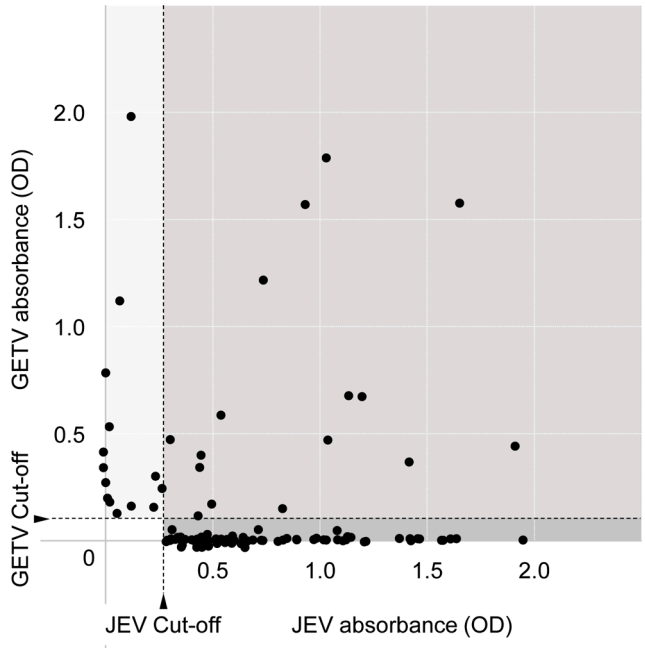

Fig. 4. Comparison of ELISA absorbance (OD) values for Japanese encephalitis virus (JEV)- and Getah virus (GETV)-specific antibodies in the Vietnamese meat juice samples. Individual absorbance values for JEV-specific (x-axis) and GETV-specific (y-axis) antibodies are plotted exclusively for positive pork meat juice samples. Vertical and horizontal dashed lines indicate the positive cut-off values for JEV and GETV, respectively (indicated by arrowheads). Quadrants represent samples that were single-positive or double-positive for the two viruses.

antibodies specific to both viruses, suggesting a different pattern of seroreactivity. Pearson's correlation coefficient was calculated to evaluate the relationship between JEV- and GETV-specific OD values but revealed no significant correlation (r= −0.083, P=0.339). This result indicates that infections caused by these two viruses occur independently of one another in fattening pig populations in Vietnam. Specifically, these data strongly suggest that, under field conditions, prior exposure to one of the viruses does not lead to direct interference or cross-reactivity with respect to either susceptibility to or antibody responses against the other virus [5]. The results of this study suggest that JEV and GETV can circulate either simultaneously or sequentially within populations of fattening pigs in Vietnam. Vietnam is widely recognized as an endemic area for JEV, with previous studies reporting high seroprevalence of JEV in fattening pigs [9]. The fact that the overwhelming majority of samples tested in this study were strongly positive for JEV alone suggests that routine exposure to mosquito-borne JEV and establishment of infection occur in this region. Furthermore, the results of this study indicate that GETV, which has been reported with increasing frequency across Asia in recent years [6], also frequently infects pigs on Vietnamese farms. Pigs serve as potent amplifying hosts for both JEV and GETV. These findings indicated that swine health management practices in Vietnam should include not only conventional JEV vaccination but also a comprehensive control strategy targeting multiple mosquito-borne viruses.

This study demonstrated that MJ obtained from commercially available pork can serve as an effective alternative sample for epidemiological surveys of arboviruses, particularly in field study situations, in which serum samples are difficult to obtain. As the

distribution of arboviruses is expected to undergo further shifts due to climate change and the expansion of logistic networks. The MJ-based monitoring approach developed in this study will facilitate the rapid collection of large-scale epidemiological data, serving as a viable alternative to blood sampling, which can be challenging to procure. This approach should contribute significantly to the development of comprehensive strategies for combating mosquito-borne viral diseases across the world.

CONFLICT OF INTEREST. The authors declare no conflicts of interest.

ACKNOWLEDGMENTS. The authors highly appreciate Dr. Kenjiro Inui and Dr. Yasuhiro Yoshikawa for their valuable advice and contributions to the study design. We would like to express gratitude to Dr. Manabu Nemoto and Dr. Hiroshi Bannai at Equine Research Institute, Japan Racing Association for providing the Getah virus isolate. This work was supported by Grants-in-Aid for the Research Program on Emerging and Re-emerging Infectious Diseases Grant Numbers 21fk0108613, JP23wm0225030, JP23wm0225035, 24fk0108693, JP25wm0125006 from the Japan Agency for Medical Research and development (AMED).

REFERENCES

1. Abrantes AC, Risco D, Carvalho M, Cerrato R, Fernández-Llario P, Vieira-Pinto M. 2024. Meat juice as a feasible alternative sample for tuberculosis surveillance in large game. *Vet Ital* **60**: 1–6. [Medline]
2. Arnold M, Smith RP, Martelli F, Davies R. 2024. Bayesian evaluation of meat juice ELISA for detecting Salmonella in slaughtered pigs without specifying a cut-off. *Zoonoses Public Health* **71**: 369–380. [Medline] [CrossRef]
3. Carella E, Caruso C, Moreno A, Di Blasio A, Oberto F, Vitale N, Masoero L. 2023. Meat juice and oral fluid as alternatives to serum for Aujeszky disease monitoring in pigs. *Microorganisms* **11**: 2418. [Medline] [CrossRef]
4. Cybulska A. 2022. Immunoproteomic analysis of *Trichinella britovi* proteins recognized by IgG antibodies from meat juice of carnivores naturally infected with *T. britovi*. *Pathogens* **11**: 1155. [Medline] [CrossRef]
5. Garciá García J, Takashima I, Kariwa H, Hashimoto N. 1994. Kinetics and cross-reactivity of the virus-specific antibody-forming cells in mice during primary and secondary infection with Japanese encephalitis virus and related flaviviruses. *J Virol Methods* **48**: 31–41.[Medline] [CrossRef]
6. Irekeola AAD, Shueb RH. 2025. Global seroprevalence and distribution of Getah virus in domestic and wild animals: A systematic review and meta-analysis. *Vet World* **18**: 3464–3475. [Medline] [CrossRef]
7. Kanda Y. 2013. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant* **48**: 452–458. [Medline] [CrossRef]
8. Kuwata R, Torii S, Shimoda H, Supriyono S, Phichitraslip T, Prasertsincharoen N, Takemae H, Bautista RCJT, Ebora VDBM, Abella JAC, Dargantes AP, Hadi UK, Setiyono A, Baltazar ET, Simborio LT, Agungpriyono S, Jittapalapong S, Rerkamnuaychoke W, Hondo E, Maeda K. 2020. Distribution of Japanese encephalitis virus, Japan and Southeast Asia, 2016-2018. *Emerg Infect Dis* **26**: 125–128. [Medline] [CrossRef]
9. Lindahl J, Boqvist S, Ståhl K, Thu HT, Magnusson U. 2012. Reproductive performance in sows in relation to Japanese Encephalitis Virus seropositivity in an endemic area. *Trop Anim Health Prod* **44**: 239–245. [Medline] [CrossRef]
10. Liyanage KLDTD, Wiethoelter A, Hufschmid J, Jabbar A. 2021. Descriptive comparison of ELISAs for the detection of *Toxoplasma gondii* antibodies in animals: A systematic review. *Pathogens* **10**: 605. [Medline] [CrossRef]
11. Maganira JD, Kidima W, Mwita CJ, Höglund J. 2021. Detection of porcine cysticercosis in meat juice samples from infected pigs. *Acta Parasitol* **66**: 851–856. [Medline] [CrossRef]
12. Nemoto M, Bannai H, Tsujimura K, Kobayashi M, Kikuchi T, Yamanaka T, Kondo T. 2015. Getah virus infection among racehorses, Japan, 2014. *Emerg Infect Dis* **21**: 883–885. [Medline] [CrossRef]
13. Onyilagha C, Nash M, Perez O, Goolia M, Clavijo A, Richt JA, Ambagala A. 2021. Meat exudate for detection of African swine fever virus genomic material and anti-ASFV antibodies. *Viruses* **13**: 1744. [Medline] [CrossRef]
14. Takeishi M, Kuwata R, Ono T, Sasaki A, Ogata M, Iwata E, Taji S, Koike M, Nemoto M, Bannai H, Isawa H, Maeda K, Morikawa S, Kitagawa H, Yoshikawa Y. 2022. Seroconversion of anti-Getah virus antibody among Japanese native Noma horses around 2012. *J Vet Med Sci* **84**: 1605–1609. [Medline] [CrossRef]
15. World Health Organization. "Japanese encephalitis." (WHO News Room / Fact Sheets). https://www.who.int/news-room/fact-sheets/detail/japanese-encephalitis [accessed on May 5, 2026].
16. Yeo S, Yang M, Nyachoti M, Nfon C. 2022. Detection of antibodies to structural proteins of foot-and-mouth disease virus in swine meat juice. *Can J Vet Res* **86**: 147–152. [Medline]
17. Yonemitsu K, Minami S, Noguchi K, Kuwata R, Shimoda H, Maeda K. 2019. Detection of anti-viral antibodies from meat juice of wild boars. *J Vet Med Sci* **81**: 155–159. [Medline] [CrossRef]