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Original Research

Comparison of Aerosol- and Percutaneous-acquired Venezuelan Equine Encephalitis in Humans and Nonhuman Primates for Suitability in Predicting Clinical Efficacy under the Animal Rule

Janice M Rusnak,[1,*] Lesley C Dupuy,[2] Nancy A Niemuth,[3] Andrew M Glenn,[4] and Lucy A Ward[4]

Licensure of medical countermeasure vaccines to protect against aerosolized Venezuelan equine encephalitis virus (VEEV) requires the use of the Animal Rule to assess vaccine efficacy, because human studies are not feasible or ethical. We therefore performed a retrospective study of VEE cases that occurred in at-risk laboratory workers and support personnel during the United States Biowarfare Program (1943–1969) to better define percutaneous- and aerosol-acquired VEE in humans and to compare these results with those described for the NHP model (in which high-dose aerosol VEEV challenge led to more severe encephalitis than parenteral challenge). Record review and analysis of 17 aerosol- and 23 percutaneous-acquired human cases of VEE included incubation period, symptoms, physical examination findings, and markers of infection. Human VEE disease by both exposure routes presented as acute febrile illness, typically with fever, chills, headache, back pain, malaise, myalgia, anorexia, and nausea. Aerosol exposure more commonly led to upper respiratory tract-associated findings of sore throat (59% compared with 26%), pharyngeal erythema (76% compared with 52%), neck pain (29% compared with 4%), and cervical lymphadenopathy (29% compared with 4%). Other disease manifestations, including encephalitis, were similar between the 2 exposure groups. The increase in upper respiratory tract findings in aerosol-acquired VEE in humans has not previously been reported but is supported by the mouse model, which showed nasal mucosal necrosis, necrotizing rhinitis, and an increase in upper respiratory tract viral burden associated with aerosol VEEV challenge. Fever, viremia, and lymphopenia were common markers of VEE disease in both humans and NHP, regardless of the exposure route. Taken collectively, our findings provide support for use of the nonlethal NHP model for advanced development of medical countermeasures against aerosol- or percutaneous-acquired VEE.

Abbreviations: TrD, Trinidad donkey; VEEV, Venezuelan equine encephalitis virus

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Venezuelan equine encephalitis virus (VEEV) has been designated as a Category B biothreat agent, because aerosol exposure to as few as 10 to 100 organisms results in symptomatic disease in nearly all humans. No FDA-approved vaccine is currently available to prevent VEE in humans. However, the US Department of Defense is supporting vaccine development efforts to protect against aerosol VEEV exposure, including virus-like particles, modified Vaccinia Ankara, and DNA vaccine platforms.[29,49]

Naturally occurring VEEV infection is a mosquito-borne illness endemic to northern South America, Central America, Mexico, Florida, and Trinidad.[1,14,33,48] Mosquito-borne VEEV infection typically manifests as an acute self-limiting febrile illness of 3 to 5 d in duration, with abrupt onset of fever and chills, severe headache, malaise, and myalgia. Other common symptoms include nausea, vomiting, photophobia, and sore throat, as well as residual asthenia that may persist for 1 to 2 wk. Encephalitis and death are uncommon and are reported in fewer than 0.5% of cases (mostly children) and less than 1% of cases, respectively.[1,4,14,36]

In contrast, VEE acquired by aerosol exposure does not occur naturally. According to a limited number of reported VEE cases in laboratory workers accidentally exposed to aerosolized VEEV, aerosol-acquired VEE in humans appears to have a similar clinical presentation as mosquito-borne disease.[5,25,26,40]

Because aerosol-acquired VEEV infection does not occur naturally, FDA licensure of a vaccine to protect against aerosol VEEV exposure will require the use of the Animal Rule (that is, Approval of Biologic Products when Human Efficacy Studies

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[1]Joint Vaccine Acquisition Program, Medical Countermeasure Systems, and Battelle, Fort Detrick, Maryland; [2]United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland; [3]Department of Statistics, Battelle, Columbus, Ohio; [4]Joint Vaccine Acquisition Program, Medical Countermeasure Systems, Fort Detrick, Maryland
[*]Corresponding author. Email: Janice.m.rusnak.ctr@mail.mil

are not Ethical or Feasible) to demonstrate vaccine efficacy. Such approval under the Animal Rule requires demonstration of vaccine protection in an animal model that is predictive of the response expected in humans.[4,16,18,22] Therefore, the animal model selected for vaccine efficacy testing should exhibit similar disease and pathophysiology as observed in humans for a given exposure route (for example, aerosol exposure).

Clinical and pathologic manifestations of VEEV have been studied primarily in lethal mouse and nonlethal NHP models.[42] The NHP model for parenteral-acquired VEEV infection demonstrated remarkable similarities to mosquito-borne VEE in humans. However, somewhat different than reported in humans, VEE infection acquired by aerosol and intranasal exposure routes in mice and NHP was associated with an earlier onset and more severe CNS infection than parenteral challenge. VEEV exposure by these routes allowed direct viral entry into the brain through the olfactory system and resulted in necrosis of the nasal and olfactory mucosa (mice only) as well as early detection of VEEV in lungs.[6-8,17,38,42,44-46]

To characterize aerosol-acquired VEE disease in humans, we reviewed detailed clinical records of 17 aerosol-acquired VEE cases that occurred within the United States Biowarfare Program at Fort Detrick from 1943 to 1969. We then compared the 17 aerosol-acquired VEE cases with 23 percutaneous-acquired VEE infections diagnosed within the Biowarfare Program and with published human VEE cases to assess potential differences in disease presentation as a result of exposure route. We discuss the findings from the human retrospective studies within the context of published animal data to highlight similarities and differences of VEEV disease among species.

Materials and Methods

Permission was obtained from the Human Use Committee at the US Army Medical Research Institute of Infectious Diseases to perform a retrospective analysis of laboratory workers and support personnel with VEE infections that occurred during the United States Biowarfare Program at Fort Detrick from 1943 to 1969. Measures to protect the identity of all persons were maintained throughout the review and analysis. Research records of primary VEE infections (both laboratory-acquired and vaccine-associated infections) were reviewed for demographics, route of exposure, incubation period, clinical signs and symptoms, physical exam findings, duration of fever, clinical laboratory tests, VEEV viremia, and VEEV isolation in pharyngeal secretions.

The analysis excluded secondary VEE infections and VEE infections in personnel who had received a live-attenuated VEE vaccine at least 14 d before the onset of illness, because preexisting immunity might ameliorate VEE symptomatology. The analysis did not exclude prior immunization with early formalin-inactivated vaccines (precursors of the C84 VEE vaccine), because those vaccines were unlikely to have significant protective benefit against VEEV exposure.[13,31,44]

Given that the incubation period of VEEV generally occurs within 7 d of exposure (and never reported as being longer than 14 d), VEE infection was assessed as laboratory-acquired when the patient 1) had been in a laboratory with ongoing VEEV research within the previous 2 wk, 2) had not received a VEE vaccine in the 2 wk before the onset of symptoms, and 3) had laboratory confirmation of VEE infection by either demonstration of VEEV in the blood or pharynx or a 4-fold rise in VEE-specific serology. A laboratory-acquired infection was assessed as percutaneous-acquired when a known VEEV percutaneous exposure had occurred within 14 d of illness onset, and as aerosol-acquired when no percutaneous exposure or possible direct VEEV contact had occurred within 14 d before illness onset.

Vaccine-associated VEE infections were attributed to either incomplete inactivation of a formalin-inactivated VEE vaccine or inadequate attenuation of a live-attenuated VEE vaccine.[41,44] Vaccine-associated VEE infection was defined as a febrile illness with VEE symptomatology that occurred within 14 d after receiving either an investigational live-attenuated or formalin-inactivated VEE vaccine and was not associated with a known VEEV laboratory exposure event within the past 14 d (Table 1).

Charts were reviewed to assess the incubation period, clinical symptoms, physical exam findings, total WBC count, lymphocyte count, neutrophil count, and VEEV isolation from serum and pharyngeal specimens. The day of initial occurrence and the frequencies of these clinical findings and abnormal laboratory values were assessed for both aerosol- and percutaneous-acquired VEE infections. Serologic VEE-specific laboratory confirmation was performed by neutralizing antibody assay, and in a few cases, by complement fixation or hemagglutination inhibition assay. Serum neutralizing antibody tests were performed by using various mixtures of undiluted serum and appropriate dilutions of VEEV in mouse brain suspensions and a standard intraperitoneal test in mice, as described in earlier publications.[34,41,44] Detection and isolation of VEEV was assessed by in vivo testing that involved intraperitoneal injection of mice or guinea pigs with the clinical serum or pharyngeal specimen, followed by observation of the animal for signs of CNS infection or death, as described in earlier publications.[41,44] Throat wash specimens were treated with streptomycin (1000 μg/mL) and penicillin (100 U/mL) before animal injection.

Statistical analysis used *t* tests (a *P* value less than 0.05 was required for statistical significance) for comparison of demographics, incubation period, and frequency of symptomatology of persons with aerosol compared with percutaneous-acquired VEE infection. Statistical analysis to compare laboratory results between the 2 groups was not performed, because laboratory testing was done less frequently in vaccine-associated VEE cases. Selected aerosol-acquired and mosquito-borne VEE cohorts in the literature with detailed clinical information were reviewed and are presented. Descriptive analysis (including upper respiratory tract symptomatology, encephalitis, and markers of disease) was used to compare published clinical presentation with the 2 VEE cohorts from the Biowarfare Program. In addition, results from existing VEE NHP and mouse models were summarized and compared with human aerosol- and percutaneous-acquired VEE disease.

Results

Review of VEE Cases. ***VEE cases.*** A total of 43 patients were diagnosed with primary VEE infection during the Biowarfare Program from 1950 to 1967. Three cases that occurred in persons who had received a live-attenuated VEE TC82 more than 14 d before illness onset were excluded from analysis, due to the likelihood of persistent vaccine-induced immunity resulting in ameliorated disease. In addition, a case of secondary VEE infection that occurred soon after a person received the third dose of a formalin-inactivated VEE vaccine series was excluded from analysis (only the primary VEE infection that occurred after his first vaccine dose was included in the analysis).

Of the remaining 40 VEE cases, 17 were aerosol-acquired and 23 percutaneous-acquired (Table 1). All 17 aerosol-acquired VEE infections were secondary to laboratory exposures. Only 2 of the 23 percutaneous-acquired VEE infections were due to laboratory

Table 1. Demographics and incubation period of primary VEE infection by aerosol compared with percutaneous exposure routes

	Aerosol laboratory exposure	Percutaneous exposure			
		Laboratory	LA vaccine	FI vaccine	All groups
Group	Aerosol	Group 1	Group 2	Group 3	Groups 1, 2, and 3
No. of cases	17	2	5	16	23
Male (no. [%])[a]	16 (94.1%)	1 (50%)	4 (80%)	16 (100%)	21 (91%)
Female (no. [%])	1 (5.9%)	1 (50%)	1 (20%)	0	2 (9%)
Caucasian (no. [%])[a]	17 (100%)	2 (100%)	5 (100%)	15 (94%)	22 (96%)
Black(no. [%])	0	0	0	1 (6%)	1 (4%)
Mean age (y; range)[a]	27.6 (21–41)	31.5 (24–35)	32.2 (23–44)	33.9 (22–55)	33.4 (22–55)
Incubation period (mean [range])[b]	2.71 ± 1.70 d (1–5 d)[c]	3 ± 2.83 d (1–5 d)	3 ± 3.39 d (2 h–8 d)[d]	4.4 ± 1.84 d (2–8 d)[e]	4.09 ± 2.33 (2 h–8 d)[d]

FI, formalin-inactivated vaccine (precursor to the investigational C84 vaccines, derived from VEE IA/B TrD strain); LA, live–attenuated vaccine (precursor to investigational TC83 vaccine, derived from VEE IA/B TrD strain).

Note that 15 of 17 aerosol and the 2 group 1 cases received at least 1 dose of an early, poorly immunogenic FI vaccine.

[a]No significant differences in gender ($P = 9.575$) or race ($P = 0.645$). Mean age in percutaneous-acquired VEE groups was greater ($P = 0.025$) than aerosol-acquired VEE. Too few data points were available to determine race- or gender-associated differences within the 5 VEE groups according to exposure route.

[b]No difference in incubation period between aerosol compared with percutaneous-acquired VEE ($P = 0.162$; absolute P value by t test) or between the 3 groups of percutaneous-acquired VEE ($P = 0.351$).

[c]Calculated for the 7 aerosol-acquired VEE cases with an identified laboratory exposure date.

[d]Onset of myalgia was 2 h after vaccination in 1 case (cannot exclude myalgia due to vaccine-related adverse event), with onset of other VEE symptoms not until 24 h later.

[e]Incubation period could not be determined in 1 case in group 3 vaccinated 7 d before and on day 0 of illness.

exposure, both occurring after accidental injection with a contaminated needle (group 1). The other 21 VEE percutaneous-acquired infections were related to vaccination. Five of the 21 vaccine-related VEE cases (group 2) were associated with use of live-attenuated VEE TC50 or VEE TC80 vaccine candidates that were precursors to the investigational live-attenuated TC83 VEE vaccine. The remaining 16 vaccine-related cases (group 3) were attributed to inadequate inactivation of formalin-inactivated VEEV vaccine candidates; 15 cases were associated with the initial VEE Trinidad donkey (TrD) strain formalin-inactivated vaccine candidate (3-dose primary series on days 0, 7, and 42; given from 1950 to 1952) that occurred after vaccine dose 1 ($n = 10$), dose 2 ($n = 4$), or dose 4 ($n = 1$).[34,41,44]

Of note, 15 of the 17 patients with aerosol-acquired VEE and the 2 percutaneous laboratory-acquired VEE cases had previously received at least one dose of a formalin-inactivated VEE vaccine candidate more than 3 wk before illness onset. These early formalin-inactivated VEE vaccines were precursors to the current investigational C84 VEE vaccine, which was developed by formalin inactivation of the live-attenuated TC83 vaccine. The C84 vaccine was demonstrated to confer short-term protection against parenteral VEEV challenge in animal models but offered inadequate protection against aerosol VEEV challenge.[31]

Demographics. The 40 primary VEE infections occurred predominantly in Caucasians (39 of 40 subjects) and males (37 of 40 subjects), with ages ranging from 21 to 55 y (Table 1). No age difference was observed in the 3 groups of percutaneous-acquired VEE ($P = 0.886$), but the mean age of the 23 percutaneous-acquired VEE cases (33.4 y; range, 21 to 55 y) was significantly ($P = 0.025$) higher than that of the 17 aerosol-acquired VEE cases (27.6 y; range, 21 to 41 y). The slightly higher age in percutaneous-acquired VEE cases was reflective of the policy that included vaccination of laboratory support personnel (that is, facility maintenance and repair personnel), who were generally older in age and at similar risk for vaccine-acquired VEE but at lower risk for aerosol VEEV exposure. Given that nearly all VEE cases occurred in Caucasian men (reflective of the demographics of most workers in the Biowarfare Program), race- and gender-associated differences in VEE infection could not be assessed.

Aerosol-acquired VEE infection. Most of the 17 aerosol-acquired VEE infections occurred in persons who worked directly with VEEV or VEE-infected animals, including 9 laboratory technicians, 5 scientists, and 1 veterinarian. However, 2 infections occurred in maintenance personnel who had been in the laboratory but did not directly perform work with VEEV. Seven cases were associated with a single identified high-risk aerosol exposure event that resulted in VEE infection, whereas the remaining 10 cases (58.8%) were either assessed as due to unrecognized VEEV aerosol exposure or had more than one potential aerosol exposure event in the previous 2-wk period.

Incubation period, symptoms, and physical examination findings. The mean incubation period of the 7 aerosol-acquired VEE infections associated with a single identified laboratory exposure was 2.7 d (range, 1 to 5 d) (Table 1). The majority of the 17 VEE cases presented as acute febrile illness characterized by abrupt onset of high fever (100%), severe headache (100%), chills (86%), back pain (76%), myalgia (59%), malaise (59%), anorexia (59%), nausea (59%), or sore throat (59%; Tables 2 and 3). Common physical exam findings included pharyngeal erythema (76%), pharyngeal edema (12%), cervical or mandibular lymphadenopathy (35%), axillary lymphadenopathy (24%), conjunctival injection (29%), and tender abdomen (24%; Table 4). In one case, mild encephalitis was manifested as a slight arm tremor for 24 h (no somnolence or lethargy).

Fever was often 102 °F or greater, and the maximum elevated temperature ranged from 100.7° to 104.6 °F. Fever onset (at least 100.5 °F) was documented in 13 of the 17 cases on the initial day of illness (day 0) or the following morning (day 1) and in 3 cases on day 2 or 3 (Table 2). One case with a delayed fever onset presented with an atypical 7-d prodromal period before the abrupt onset of a typical VEE febrile illness. The mean time to fever resolution to less than 100.5 °F was 2.3 d (range, 1 to 5 d), and the mean time to fever resolution to less than 99.5 °F was 4.8 d (range, 2 to 9 d). A biphasic pattern of fever was observed in 4

Table 2. Fever onset and duration in aerosol-acquired VEE infection

				Duration of fever	
	Onset fever (≥100.5 °F)	Peak temperature		<100.5 °F for 24 h	<99.5 °F for 24 h
Subject no.	(Day of illness)	(°F)	Day of illness	(No. of days)[a]	No. of days
1	0	104	0	2	7
2	0	102.6	0	2	3
3	0	103	0	3	7
4	0	101.2	0	No data	No data
5	0	104	1	5	5
6	0	104	2	4	6
7	0	101.5	2	3	3
8	1	104.6	1	2	9
9	1	103.4	1	2	5
10	1	103	2	2	4
11	1	102.4	2	3	6
12	1	103	2	2	7
13	1	103	3	3	4
14	2	102.4	2	3	4
15	3	101.6	3	1	2
16	3	100.7	4	2	3
17	7[b]	102	7	2	5

[a]Mean of 2.3 d (range, 1 to 4 d) for fever resolution to a temperature of less than 100.5 °F. A biphasic pattern of fever was observed in 4 cases (temperature <100.5 °F for 1 or 2 d before recurrence).
[b]Subject 17 had a 7-d prodromal period before onset of typical VEE symptoms.

cases, in which the temperature was normal or remained below 100.5 °F for 1 or 2 d before increasing again.

Laboratory findings. Lymphopenia, defined as fewer than 1500 cells/mm^3, was common in the initial 3 d of illness. In the 16 patients evaluated, the nadir mean lymphocyte count was 1322 cells/mm^3 (range, 720 to 1710 cells/mm^3), which occurred on mean day 0 of illness (Table 5). In a subset of 11 persons whose lymphocyte counts were evaluated in the initial 3 d of illness, lymphopenia was observed in all 11 cases and was present on the initial CBC in 9 of the 11 cases (obtained on day 0 [n = 5], day 1 [n = 3], and day 3 [n = 1] of illness; Table 6). For these 11 cases, lymphopenia was initially observed on mean day 1 of illness (range, day 0 to 3). The nadir lymphocyte count occurred on mean day 2 of illness, with initial improvement observed on mean day 3 and resolution on mean day 5 of illness. The mean nadir lymphocyte count in these 11 cases was 957 cells/mm^3 (range, 492 to 1455 cells/mm^3).

Leukopenia, defined as a total WBC count of less than 4500 cells/mm^3, was documented in 9 of the 17 persons with aerosol-acquired VEE infections and occurred on mean Day 4 of illness (range, day 1 to 7; Table 6). The mean nadir leukocyte count of these 9 persons was 3533 cells/mm^3 (range, 2650 to 4300 cells/mm^3), with the nadir count occurring on mean day 5 of illness (range, days 1 to 7). Recovery from the mean leukopenia occurred initially on mean day 8 of illness (range, day 6 to 12). Neutropenia, defined as a neutrophil count of less than 1500 cells/mm^3, was documented in 6 persons (nadir range, 1054 to 1411 cells/mm^3), was initially observed on mean day 5 of illness (range, 3 to 7 d), and lasted a mean of 2 d (range 1 to 3 d) (Table 6). The nadir mean neutrophil count of 2527 cells/mm^3 (range, 1200 to 5845 cells/mm^3) was not observed until mean day 6 of illness. Because CBC were not obtained after day 3 in 3 persons, the incidence of leukopenia and neutropenia may be underestimated.

Analysis of CSF was performed in 2 patients. Results were noteworthy only for a minimally elevated CSF WBC count of 7 cells/mm^3 in 1 case on day 0.

VEEV was present in the blood or pharyngeal secretions in all 16 subjects who were tested (Table 7). VEEV testing was positive in the serum of 11 of 14 persons, in the pharyngeal secretions of 13 of 15 persons, in both serum and pharyngeal specimens of 9 of 14 persons, and in an unspecified source (either serum or pharyngeal specimen) in 1 person. VEEV was documented in serum and pharyngeal secretions as early as day 0 to as late as day 7 of illness.

In one case, VEEV was detected in bronchial washes obtained on day 2 of illness. Because the throat cultures of this person were positive from day 2 to 7, this finding may represent contamination of bronchial washes by pharyngeal secretions. Gastric cultures obtained from this patient on days 2 and 4 of illness were negative. In second case, VEEV was detected in a gastric specimen on day 1 of illness but was negative on Day 2. However, contamination could not be excluded because throat cultures from days 0 to 5 and blood cultures from days 0 to 4 of illness were positive. In this case, testing of CSF for VEE was negative. In a third case in which VEEV was detected in pharyngeal secretions on days 1, 3, and 5 of illness, testing for VEEV in urine and stool on days 3, 5, and 7 of illness was negative.

Percutaneous-acquired VEE cases and comparison with aerosol-acquired VEE. Of the 23 percutaneous-acquired VEE infections, the 2 laboratory-acquired infections associated with contaminated needles occurred in laboratory workers. The majority (n = 16) of the vaccine-associated VEE cases occurred in maintenance or other support personnel (for example, engineers, carpenters) who were at lower risk for VEEV exposure than laboratory workers but still received VEE vaccination (Table 1). Only 5 of the 21 vaccine-associated VEE cases involved laboratory or animal-care personnel who worked directly with VEEV or VEEV-infected animals.

Table 3. Frequency and time of onset of symptoms in aerosol- and percutaneous-acquired VEE cohorts

	Aerosol exposure (n = 17)								Percutaneous exposure (n= 23)						
		Day of illness onset		Day of onset of symptom (no. of cases)						Day of onset of symptom (no. of cases)					
Symptom[a]	No. (%)	Mean ± 1 SD	Range	0	1	2	3	≥4	No. (%)	0	1	2	3	≥4	P
Fever or feverish	17 (100%)	0.6 ± 1.73	0–7	13	2	1	0	1	22 (96%)	20	2	0	0	0	0.38
Documented fever	17 (100%)	—	0–7	7	6	1	2	1	21 (92%)[b]	9	8	4	0	0	—
Headache	17 (100%)	0.5 ± 1.5	0–6	12	3	1	0	1	23 (100%)	20	1	1	1	0	1.0
Chills	14 (86%)	0.3 ± 0.61	0–2	11	2	1	0	0	19 (83%)	13	4	0	1	1	0.98
Back pain	13 (76%)	0.9 ± 2.22	0–8	9	2	1	0	1	12 (52%)	7	4	0	0	1	0.12
Malaise	10 (59%)	0.7 ± 0.82	0–2	5	3	2	0	0	16 (70%)	12	3	0	1	0	0.48
Myalgia	10 (59%)	0.3 ± 0.67	0–2	8	1	1	0	0	18 (78%)	16	1	1	0	0	0.18
Anorexia[c]	10 (59%)	0.6 ± 0.7	0–2	5	4	1	0	0	14 (61%)[c]	7	4	0	1	1	0.90
Nausea	10 (59%)	2.1 ± 3.0	0–8	4	3	0	1	3	14 (61%)	9	3	1	1	0	0.90
Sore throat or irritation	10 (59%)	2.1 ± 3.03	0–10	3	3	2	0	2	6 (26%)	1	1	0	3	1	0.04
Fatigue or tired	6 (35%)	2 ± 2.9	0–7	3	1	0	0	2	7 (30%)	7	0	0	0	0	0.75
Weakness[c]	6 (35%)	1 ± 1.73	0–4	3	1	0	0	2	9 (39%)[c]	5	1	1	0	1	0.80
Neck pain	5 (29%)	0.4 ± 0.55	0–1	3	2	0	0	0	1 (4%)	0	1	0	0	0	0.03
Retroorbital pain	5 (29%)	1.6 ± 1.14	0–3	1	1	2	1	0	3 (12%)	2	1	0	0	0	0.20
Photophobia	4 (24%)	1.5 ± 0.58	1–2	0	2	2	0	0	1 (4%)	0	0	1	0	0	0.07
Vomiting	4 (24%)	2 ± 3.37	0–7	2	1	0	0	1	3 (12%)	2	0	0	0	1	0.39
Cough	4 (24%)	0.5 ± 1	0–2	3	0	1	0	0	5 (22%)	3	0	1	1	0	0.89
Insomnia	3 (18%)	1 ± 1	0–2	1	1	1	0	0	0	0	0	0	0	0	0.04
Diaphoresis	3 (18%)	1.7 ± 1.15	1–3	0	2	0	1	0	1 (4%)	0	1	0	0	0	0.17
Sweats	3 (18%)	0.7 ± 0.58	0-1	1	2	0	0	0	5 (22%)	2	1	2	0	0	0.75
Arthralgia	2 (12%)	1 ± 1.41	0-2	1	0	1	0	0	2 (9%)	1	0	0	1	0	0.75
Dizziness	2 (12%)	1.5 ± 2.12	0-3	1	0	0	1	0	3 (12%)	2	1	0	0	0	0.90
Abdominal pain	2 (12%)	0.5 ± 0.71	0-1	1	1	0	0	0	2 (9%)	1	0	1	0	0	0.75
Nasal congestion	2 (12%)	2 ± 0	2	0	0	2	0	0	3 (12%)	2	0	1	0	0	0.90
Lethargy	1 (6%)	1 ± 0	1	0	0	0	0	1	3 (12%)	3	0	0	0	0	0.46
Vertigo	1 (6%)	1 ± 0	1	1	0	0	0	0	3 (12%)	3	0	0	0	0	0.46
Blurred vision	1 (6%)	1 ± 0	1	1	0	0	0	0	1 (4%)	0	1	0	0	0	0.83
Drowsiness	0	0	0	0	0	0	0	0	4 (17%)	4	0	0	0	0	0.07
Urine frequency	0	0	0	0	0	0	0	0	3 (12%)	3	0	0	0	0	0.12
Giddiness	0	0	0	0	0	0	0	0	2 (9%)	2	0	0	0	0	0.21
Diarrhea	0	0	0	0	0	0	0	0	2 (9%)	2	0	0	0	0	0.21

[a]One occurrence each of: irritability, malaise/weak/tiredness, arm tremor, burning eyes, rhinorrhea, ear pain, neck stiffness, back stiffness, and chest in aerosol-acquired VEE and 1 occurrence of axillary pain in percutaneous-acquired VEE not included in above table.
[b]Fever not documented in outpatient management of retrospectively diagnosed VEE case associated with formalin-inactivated vaccine.
[c]Day of onset unknown in one case each of anorexia and weakness in VEE associated with FI vaccine.

Incubation period. The incubation period for the 2 percutaneous laboratory-acquired VEE cases (mean, 3 d; range, 1 to 5 d) was not significantly different from VEE due to live-attenuated VEE vaccines (mean, 3 d; range, 2 h to 8 d) or formalin-inactivated VEE vaccines (mean, 4.4 d; range, 2 to 8 d; $P = 0.886$; Table 1). The case with a 2-h incubation period involved a person with onset of myalgia 2 h after receiving a live-attenuated vaccine; the febrile illness typical of VEE did not occur until 24 h later. Thereby, myalgia due to a nonvirus-related vaccine reaction could not be excluded. The mean incubation period for the 23 percutaneous-acquired VEE cases was 4.09 d (range, 2 h to 8 d) which was not significantly different from aerosol-acquired VEE, which had a mean incubation period of 2.71 d (Table 1).

Symptoms. Symptomatology of the 23 percutaneous-acquired VEE cases was similar to the acute febrile illness observed in aerosol-acquired VEE (Table 3). Feverishness or fever (often 102

Table 4. Frequency and time of onset of physical examination findings in aerosol- and percutaneous-acquired VEE cohorts

	Aerosol exposure (n = 17)							Percutaneous exposure (n = 23)						
		Day of onset of symptom (no. of cases)							Day of onset of symptom (no. of cases)					
	No. (%)	0	1	2	3	4	≥5	No. (%)	0	1	2	3	4	≥5
Pharyngeal exam														
Erythema	13 (76%)	4	6	0	2	0	1[a]	12 (52%)	4	6	1	0	0	1
Edema	2 (12%)	0	1	0	0	0	1[a]	3 (12%)	1	2	0	0	0	0
Ulcer	1 (6%)	0	0	0	0	1	0	0	0	0	0	0	0	0
Exudate	0	0	0	0	0	0	0	1 (4%)	0	1	0	0	0	0
Inflamed tonsils	1 (6%)	0	0	0	1	0	0	0	0	0	0	0	0	0
Lymphoplasia	1 (6%)	0	0	0	1	0	0	1 (4%)	0	1	0	0	0	0
Cervical lymphadenopathy	5 (29%)	1	3	0	1	0	0	1 (4%)	0	1	0	0	0	0
Mandibular lymphadenopathy	1 (6%)	1	0	0	0	0	0	0	0	0	0	0	0	0
Axillary lymph-adenopathy	3 (18%)	1	1	1	0	0	0	4 (17%)	1	1	0	0	1	1
General lymph-adenopathy	1 (6%)	1	0	0	0	0	0	0	0	0	0	0	0	0
Conjunctival injection	5 (29%)	1	3	1	0	0	0	6 (26%)	3	2	1	0	0	0
Congested nasal mucosa, postnasal drip, or rhinorrhea[b]	3 (18%)	0	3	0	0	0	0	1 (4%)	1	0	0	0	0	0
Facial flushing	1 (6%)	0	1	0	0	0	0	0	0	0	0	0	0	0
Chest[c]	0	0	0	0	0	0	0	2 (8%)[c]	1	1	0	0	0	0
Tender abdomen[d]	4 (24%)	0	2	0	0	0	0	3 (12%)	0	1	2	0	0	0
Tremor	1 (6%)	0	1	0	0	0	0	0	0	0	0	0	0	0
Lethargy	1 (6%)	0	0	0	0	0	1[a]	0	0	0	0	0	0	0

[a] Case with 1 wk prodrome before fever onset.
[b] One case each of congested nasal mucosa, postnasal drip, or rhinorrhea in aerosol-acquired VEE, and 1 case of postnasal drip in percutaneous-acquired VEE.
[c] One case each of rales or crackles in percutaneous-acquired VEE.
[d]Unknown-onset tender abdomen in 2 cases.

°F or greater) was observed in 96% (22 of 23) of cases and was initially observed on day 0 (n = 20) or day 1 (n = 2) of illness. The mean time to fever resolution to a temperature less than 100.5 °F for groups 1, 2, and 3 was 2.7, 1.9, and 2 d, respectively, which was similar to the time for fever resolution in aerosol-acquired VEE cases (mean, 2.3 d). However, sore throat, (with or without pharyngeal erythema), neck pain, cervical lymphadenopathy, and insomnia were significantly ($P < 0.05$) more common with aerosol-acquired VEE than percutaneous-acquired infection (Tables 3 and 4). In addition, photophobia tended ($P = 0.07$) to occur more commonly and drowsiness less commonly in aerosol-acquired infection. Similar occurrences between percutaneous- and aerosol-acquired VEE were observed for other upper or lower respiratory symptoms, such as cough (5 of 23 cases [22%] compared with 4 of 17 cases [24%]; $P = 0.8934$) and nasal congestion (3 of 23 cases [12%] compared with 2 of 17 [12%]; $P = 0.9038$). Severe encephalitis was not observed, and severe CNS symptoms were infrequent in both aerosol- and percutaneous-acquired VEE cohorts.

Laboratory findings. Laboratory tests were performed less frequently in cases of vaccine-associated VEE infections than aerosol-induced cases. Regardless, lymphopenia was observed early in the illness associated with percutaneous-acquired VEE (on mean day 1 of illness) and was documented in 16 of the 21 persons who had at least one CBC performed (Table 6). Leukopenia was observed in 15 and neutropenia in 6 of the 21 persons tested. CSF analysis, performed in 2 persons, showed normal protein levels and an absence of WBC. VEEV was detected in the serum of 8 of the 9 persons tested and in the pharyngeal secretions of all 9 persons. Similar to the findings for aerosol-acquired infection, tests for VEEV detection were positive as early as day 0 and as late as day 6 of illness (Table 7). VEEV testing was performed on blood or pharyngeal secretions in only 8 of the 16 VEE cases associated with a formalin-inactivated vaccine and in 2 of the 5 cases associated with a live-attenuated vaccine, but VEEV was detected in at least one specimen in all 10 of these cases in which testing was performed. In addition, VEEV testing was positive in both laboratory-acquired VEE cases that occurred after exposure to contaminated needles.

Reported VEE cases compared with Biowarfare Program VEE cohorts. The symptomatology of our aerosol-acquired VEE cohort (VEEV subtype IAB) and of aerosol-acquired and mosquito-borne VEE cohorts selected from published reports containing substantial clinical information is summarized in Table 8. Comparisons of our aerosol-acquired VEE cohort with aerosol- and mosquito-borne VEE cohorts from the literature were limited primarily to descriptive analysis of upper respiratory tract symptomatology (for example, sore throat, pharyngeal erythema, cough), photophobia, neck pain, cervical lymphadenopathy, encephalitis, and markers of disease.

Aerosol-acquired VEE cases. Detailed clinical information was available for the 14 initial aerosol-acquired VEE cases in unvaccinated laboratory workers reported in the literature.[5,25,26] The incubation period ranged from 36 h to 4 d in 11 cases, was 7 d in one case, and was undetermined in 2 cases. Similar to our aerosol-acquired VEE cohort, VEE symptoms manifested as abrupt onset of an acute febrile illness with severe headache (93%), chills or chilly sensation (78%), myalgia (78%), weakness (78%), malaise (43%), fatigue (43%), lower back pain (43%), photophobia (43%), anorexia (71%), and nausea or vomiting (43%); Table 8). Onset of most symptoms occurred on the initial day of illness or by the following morning. Sore throat or hyperemia

Table 5. Aerosol-acquired VEE: mean WBC, lymphocyte, and neutrophil counts by day of illness

Parameter[a]	Day of illness	No. of subjects with at least 1 CBC test	Total no. of CBC tests	Mean count (no. of cells/mm^3 [1 SD])	Range of count (no. of cells/mm^3)
Total WBC count	0	7	7	8186 (2346)	4850–11,400
	1	10	10	6606 (2119)	3000–9800
	2	11	11	6055 (1264)	4050–7600
	3	10	10	4980 (1592)	3000–8250
	4	11	11	4805 (1634)	2650–7850
	5	8	8	4963 (2136)	2800–8500
	6	10	10	5290 (1814)	3500–8600
	7	10	10	5920 (2622)	3850–10,950
	8–11	7	12	6538 (1924)	4450–9300
	≥12	4	8	7644 (1156)	6100–10,000
Lymphocyte count	0	7	7	1322 (384)	720–1710
	1	9	9	1417 (1045)	492–3450
	2	11	11	1765 (845)	621–3799
	3	10	10	1924 (596)	1219–3332
	4	11	11	1746 (833)	742–3297
	5	8	8	1964 (538)	1134–2868
	6	10	10	2222 (769)	1218–4042
	7	10	10	2339 (695)	1694–4161
	8–11	7	12	2676 (704)	2046–4070
	≥12	4	8	3515 (911)	2757–5600
Neutrophil count	0	7	7	6308 (1917)	3298–8550
	1	9	9	4430 (1924)	1560–7636
	2	11	11	3812 (1294)	2147–5658
	3	10	10	2672 (1210)	1230–5363
	4	11	11	2797 (1121)	1120–4469
	5	8	8	2543 (1397)	1054–4760
	6	10	10	2527 (1465)	1200–5845
	7	10	10	3179 (2304)	1411–8414
	8–11	7	12	3367 (1573)	1776–6789
	≥12	4	8	3558 (625)	2800–4495

[a]Normal ranges of these manual WBC counts performed from 1950 to 1967 were not available. Reference ranges of WBC counts currently performed on commercial laboratory equipment are generally as follows: total WBC, 4500–11,000 cells/mm^3; absolute lymphocyte count, 1000–4800 cells/mm^3; and absolute neutrophil count, 1500–7700 cells/mm^3).

of the pharynx was observed in 35% of cases, as compared with 59% of cases in our aerosol-acquired VEE cohort and 26% of cases in our percutaneous-acquired VEE cohort (26% of cases). Severe encephalitis was not observed, but 2 cases that exhibited prolonged drowsiness (no disorientation or confusion) had residual insomnia and slight intention tremors. VEEV was detected in the serum in 10 of 12 cases, and pharyngeal colonization was present in both of the 2 cases in which this testing was performed.

The largest cohort of VEEV aerosol exposure reported in the literature occurred in 24 laboratory workers of unknown vaccination status in Russia after the breakage of 9 ampules containing suspensions of VEE-infected freeze-dried mouse brain (Table 8).[39,40] The incubation period ranged from 1.5 to 4 d, with 15 persons developing symptoms the following day and 7 others within the next 24 h. All 24 workers developed a self-limited febrile illness consisting of a mild to severe form of the disease, comprised of severe headache, malaise, and shivering but no signs of encephalitis. VEEV was isolated from serum or nasopharyngeal washes between days 2 to 6 of illness in a subset of workers. Although VEEV was isolated from the nasopharynx, most patients had no evidence or only slight mucosal membrane effects of infection. In a related publication of this Russian VEE laboratory outbreak, baseline neutralization serology was obtained from 13 cases between days 3 and 7 of illness.[39] This testing revealed negative titers in 9 workers and low-level titers (46, 79, 148, and 218; units not specified) in the remaining 4 workers; these initial titers were followed by neutralizing titers of 10,000 (units not specified) or greater in all 13 persons between days 25 and 40 of illness. These findings suggest that most patients had no preexisting immunity to VEEV.

Percutaneous-acquired VEE cases. An epizootic VEE subtype IC outbreak in Texas consisting of 79 cases (both adults and children) with detailed clinical and laboratory findings reported an incubation period ranging from 27.5 h to 4 d in the 11 cases with a defined exposure day (Table 8).[3] Patients presented with illness characterized by an abrupt onset of fever (100%), headache (89%), myalgia (66%), lethargy (43%), vomiting (39%), chills (33%), somnolence or drowsiness (29%), sore throat (20%), pharyngitis (22%), diarrhea (22%), and arthralgia (11%). Cough was not reported. The neck pain present in 19% cases was likely related (at least in part) to the nuchal rigidity that was observed

Table 6. Frequency and time of onset of lymphopenia, leukopenia, and neutropenia in aerosol- and percutaneous-acquired VEE

	Aerosol exposure		Percutaneous exposure		
	No. of subjects with parameter /total no. tested	Day of onset of parameter (mean ± 1 SD [range])	No. of subjects with parameter / total no. tested	Group (no. of subjects)	Day of onset of parameter (mean ± 1 SD [range])
Lymphocytes <1500 cells/mm^3	11/16[a]	Day 1 ± 0.9 (0-3)	16/21[b]	1 (n = 2)	Day 1 ± 0.7 (0–1)
				2 (n = 3)	Day 1 ± 0.6 (0–1)
				3 (n = 11)	Day 1 ± 1.1 (0–4)
Total WBC count <4500 cells/mm^3	9/17	Day 4 ± 2.4 (1-7)	15/21[b]	1 (n = 2)	Day 3 ± 2.2 (1–4)
				2 (n = 2)	Day 4 ± 3.5 (1–6)
				3 (n = 11)	Day 2 ± 1.4 (0–4)
Neutrophils <1500 cells/mm^3	6/16[a]	Day 5 ± 1.5 (3-7)	6/21[b]	1 (n = 1)	Day 2 ± 0 (2)
				2 (n = 1)	Day 6 ± 0 (6)
				3 (n = 4)	Day 3 ± 1.8 (1–5)

Normal ranges of the WBC counts are not available from 1950 to 1967. Criteria for lower range of lymphocyte, total WBC, and neutrophil counts were based on the ranges of traditional definitions of leukopenia, neutropenia, and lymphopenia in past years.
[a]No WBC differential performed in 1 subject with aerosol-acquired VEEV.
[b]No CBC performed in 2 subjects with percutaneous-acquired VEEV.

in 10% of cases and not likely due to cervical lymphadenopathy, given that lymphadenopathy (site unspecified) was only reported in one case. Young age was the major risk factor for severe encephalitis that included seizure, paralysis, or coma, with all 6 cases of severe encephalitis occurring in children. In addition, 4 adults and 6 children with excessive drowsiness reported confusion, hallucinations, or gait abnormalities, consistent with mild encephalitis. None of the 79 patients died. Hematology findings—available in 89% of the cases—were similar to those of our cohorts. Lymphopenia was observed early in the disease course, generally from day 1 through day 4 of illness (more than 80% of lymphocyte counts ranged from 150 to 1490 cells/mm^3 and 9% from 150 to 500 cells/mm^3 on days 1 through 4 of illness). Increases in the lymphocyte count were observed initially on day 4, but counts remained less than 1500 cells/mm^3 in most patients, and lymphocyte counts greater than 1500 cells/mm^3 were noted on days 5 through 9 of illness. Leukopenia (fewer than 4500 cells/mm^3) occurred in at least 75% of cases. Decreases in neutrophils were observed initially on day 3 of illness, with recovery of leukopenia and neutropenia on days 6 to 8. Of the 9 cases in which CSF analysis was performed, CSF analysis revealed no WBC in 7 cases but increased lymphocyte counts in the remaining 2 cases (212 and 333 WBC/μL, 100% lymphocytes).

Disease resulting from an enzootic VEE (subtype ID) mosquito-borne outbreak (7 cases) presented with acute febrile illness, similar to that in our cohorts (Table 8).[15] One adult had neurologic symptoms consistent with a mild encephalitis, as manifested by severe somnolence, delirium, dizziness, tremor, and inability to form words. Sore throat or cough was not reported in any of the 7 cases. Marked lymphopenia (range, 4% to 10% of total WBC) with associated leukopenia (range, 2100 to 3800 WBC/mm^3) was present on days 2 and 3 of illness in 5 of the 7 cases. VEE viremia was documented in all 7 cases, and VEEV was cultured from the pharynx in 2 of 6 cases. Another VEE (subtype ID) cohort from a retrospective review of 33 laboratory-confirmed cases involving both adults and children in Panama demonstrated encephalitis in 9% of cases and a 6% mortality rate. Sore throat (6%) and cough (3%) were infrequent.[32]

VEE-like symptoms occurred in 15 of 40 persons within 12 to 64 h after receiving the live-attenuated TC83 vaccine (Table 8).[2] All 15 subjects presented with a febrile illness, associated with headache, malaise, myalgia, anorexia, nausea, and sore throat. The duration of the febrile illness was approximately 36 h, with a secondary biphasic illness occurring at days 7 to 8 after vaccination in 5 cases. Leukopenia (fewer than 4000 cells/mm^3) was noted in 40% of the 40 vaccinees on days 3 through 5 after vaccination and was documented in 27% (4 of 15) of the ill patients. VEEV was isolated from whole-blood samples between days 3 and 12 after vaccination in 13 of 40 cases, including 8 of the 15 (53%) ill subjects.

Autopsy reports of VEE cases are limited, because VEE in humans is generally nonlethal. In 21 lethal VEE cases in the 1962 to 1963 Venezuelan subtype IC VEE epidemic, the major histopathologic lesions observed were in the CNS (all 20 cases [100%]), lymph nodes (10 of 13 patients tested [77%]), spleen (all 16 samples tested [100%]), liver (13 of 18 samples [72%]), lungs (all 21 samples [100%]), and gastrointestinal tract (all 10 cases [100%]). Histopathology findings revealed inflammatory infiltrates consisting mainly of lymphoid and mononuclear cells in numerous tissues, and evidence of vascular injury manifested as

Table 7. Detection of viremia and VEEV in pharynx by day of illness in aerosol- and percutaneous-acquired VEE

		No. of subjects with positive test / no. of subjects tested				
		Aerosol exposure	Percutaneous Exposure			
	Day of illness	Aerosol (LAI; $n = 16$)[a]	Group 1 (LAI; $n = 2$)	Group 2 (LA vaccine; $n = 5$)[b]	Group 3 (FI vaccine; $n = 16$)[c]	Groups 1, 2, and 3 ($n = 23$)
Viremia	0	2/4	0/1	0/1	1/1	1/3
	1	7/8	1/2	ND	3/3	4/5
	2	4/5	1/2	ND	2/3	3/5
	3	4/8	1/2	1/1	0/1	2/4
	4	3/4	2/2	ND	ND	2/2
	5	2/4	1/2	ND	ND	1/2
	6	1/2	0/2	ND	ND	0/2
	7	1/3	0/2	ND	ND	0/2
	8	ND	0/2	ND	ND	0/2
	≥9	ND	0/2	ND	ND	0/2
Throat	0	3/3	0/1	1/1	0/1	1/3
	1	8/9	2/2	1/1	3/3	6/6
	2	5/6	1/1	0/1	3/3	4/5
	3	8/9	2/2	ND	1/1	3/3
	4	5/6	1/2	ND	ND	1/2
	5	5/6	1/2	ND	ND	1/2
	6	1/3	1/2	ND	ND	1/2
	7	2/4	0/2	ND	ND	0/2
	8	0/1	0/2	ND	ND	0/2
	≥9	0/1	0/4	ND	ND	0/4

FI, formalin-inactivated; LA, live attenuated; LAI, laboratory-acquired infection
Data are given as no. affected / total no. tested.
[a]One subject excluded from analysis as laboratory report specified VEEV isolation source as either "serum or pharynx".
[b]Test for VEEV in blood or pharyngeal secretions were performed in only 2 of the 5 VEE cases associated with live-attenuated vaccines (both cases had at least one positive specimen)
[c]Test for VEEV in blood or pharyngeal secretions performed in only 8 of the 16 VEE cases associated with formalin-inactivated vaccine (all 8 cases had at least one positive specimen)

enlarged reactive endothelial cells and vasculitis. The vasculitis was characterized by inflammatory cells infiltrating blood vessel walls, fibrin thrombi, perivascular hemorrhage and edema, and occasional necrosis of the vessel walls.[10,48] CNS histopathology performed in 20 cases revealed cerebrovascular congestion in 14 cases, edema with inflammatory cell infiltrates in various areas of the brain and spinal cord in 17 cases, intracerebral hemorrhage in 7 cases, vasculitis in 4 cases (2 cases with necrotizing vasculitis), meningitis in 13 cases, encephalitis (5 mild cases and 2 moderate–severe cases), and cerebritis characterized by degeneration and neuronal loss with mixed inflammatory cell infiltrate within the brain in 5 cases. Spleen, lymph nodes, and lymphoid tissue of the gastrointestinal tract commonly had marked lymphoid depletion with extensive follicular necrosis. Hepatic findings most commonly involved diffuse hepatocellular degeneration, congestion, and mild to severe inflammatory infiltrates. Interstitial pneumonia was observed in 19 of 21 cases and was accompanied by marked interstitial and intraalveolar congestion and edema in 11 cases. Except for interstitial pneumonia and severe hepatocellular degeneration, the histopathology of these 21 cases was similar to that observed in NHP animal models.

Autopsy of a 14-y-old boy with enzootic VEE subtype IC infection showed similar autopsy findings as reported in these 21 lethal subtype IC cases, including congested edematous lungs and myocardial involvement. However, only minimal perivascular mononuclear cell infiltrates were observed in the brain. The cause of death was attributed to severe metabolic abnormalities that resulted in irreversible shock and not to encephalitis.[24]

Overview of literature regarding currently available VEE animal models *Mice*. Studies in mice demonstrated that, regardless of the exposure route, VEEV entered the CNS mainly through the olfactory system, due to the increased susceptibility of olfactory neurons to VEEV infection.[6,31,43,46] However, unlike aerosol-acquired infection, subcutaneous challenge of VEEV required a viremia before infection of the olfactory system.

Percutaneous challenge. Viremia in CD1 and BALB/c mice was detected initially as early as 12 h after subcutaneous challenge with 1000 pfu and 8200 pfu, respectively, of VEEV strain V3000, which was derived from a cDNA clone of the TrD strain of VEEV. The viremia was followed by subsequent infection initially in mononuclear phagocytes within various lymphoid tissues. The nasal mucosa exhibited limited histopathology changes and only a few virus-positive cells.[6,43,46] VEEV was not detected in the olfactory neuroepithelium until 18 h to 24 h after challenge. VEEV was noted in the olfactory nerves as early as 30 h after challenge; in the olfactory bulbs, lateral olfactory tracts, and pyriform cortex by 36 h to day 3 after challenge; and subsequently in other areas of the brain.[6,46] Given that VEEV initially was detected in the capillaries underlying the olfactory mucosa at the time of maximal viremia, viral entry into the brain was postulated to be through infection of the olfactory neurons within the olfactory epithelium, which are unprotected by the blood–brain barrier. An alternate route of VEEV entry into the

Table 8. Comparison of aerosol-acquired VEE cohort with VEE cohorts in literature (VEEV subtypes IAB, IC, and ID)

	Aerosol cohort (n = 17)	Aerosol[5,25,26] (IAB; n = 14)	Aerosol[40] (unknown; n = 24)	Natural disease[3] (IC; n = 79)	Natural disease[15] (ID; (n = 7)	Natural disease[32] (ID; n = 33)	TC83 vaccine[2] (IAB; n = 15)
Incubation period	1-5 d; mean, 2.71 d	36–96 h; except 7 d in 1 case	28 h-4 d; 28-48 h in 22 cases	27.5 h-4 d in 11 cases	3-5 d (estimate)	No data	12-64 h
General symptoms							
Fever/feverish	100%	86%	common	100%	100%	94%	100%
Headache	100%	93%	common	89%	100%	55%	common
Chills or chilly sensation	86%	78%	common	33%	common		
Sweats/ diaphoresis	18%	36%					
Malaise	59%	42%	common				common
Fatigue	35%	42%					
Weakness	35%	78%		20%	100%		
Lethargy	6%			43%			
Somnolence or drowsiness	0	36%[a]		29%	14%		
Insomnia	18%	43%					
Agitation or irritability	6%			6%			
Myalgias	59%	78%		66%	100%	21%	common
Arthralgia	12%	43%		11%	100%	21%	
Low back pain	76%	43%			100%	6%	
Neck pain	29%	7%		19% (10% nuchal rigidity)			
Neck or back stiffness	12%						
Cervical adenopathy	35%						
General adenopathy	24%			1%			
Gastrointestinal symptoms							
Anorexia	59%	71%			100%		common
Nausea or vomiting	59%	43%		39% vomiting	occasional	21%	common
Diarrhea	0			22%		9%	
Abdominal pain	12%						
Ocular symptoms							
Periocular pain	29%	14%		15%		27%	
Photophobia	24%	43%					
Visual blurring	6%						
Conjunctival injection	29%			3%			
Upper respiratory symptoms, vertigo, and pulmonary symptoms							
Sore throat	59%	14%		20%		6%	sporadic
Hyperemia pharynx	76%	21%		22% (3% tonsillitis)			
Palatal ulcers or petechiae	6%			9%			
Nasal congestion	12%						
Rhinorrhea or ear pain	1 each (6%)						
Cough	24%	7%	0			3%	
Respiratory or chest	6%[b]	21%[c]					
Thirst	0	7%					
Vertigo	6%	29%					
Dizziness	12%	7%			100%		
Neurologic symptoms, death, and duration of illness							
Tremor	6%[d]	14%[a]	0	0	14%	27%	0
Seizures	0	0	0	6%	0	3%	0
Delirious	0	7%	0	6%	14%		0
Encephalitis	Mild (6%)[d]	Mild (14%)[a]	0	Severe 8% Mild 11%[e]	Mild 14%[f]	9%	0

Table 8. Continued.

	Aerosol cohort (n = 17)	Aerosol[5,25,26] (IAB; n = 14)	Aerosol[40] (unknown; n = 24)	Natural disease[3] (IC; n = 79)	Natural disease[15] (ID; (n = 7)	Natural disease[32] (ID; n = 33)	TC83 vaccine[2] (IAB; n = 15)
Death	0/17	0/14	0/24	0/79	0/7	2/33	0/40
Duration of illness	Recovery by 1 wk; asthenia for 2–3 wk	6 to 15 d (mean, 8.5 d)		Most recovered within 1 wk	2 to 5 d; asthenia for 2–3 wk		36-h duration in most cases
Laboratory tests							
Viremia or VEEV in nasopharynx	Blood 11/14	Blood 10/12	Isolated from blood and nasopharynx[g]	Viremia or serology- confirmed cases; Viremia documented in 40 cases[h]	Blood 7/7 Pharynx 2/2	33/33[i]	Blood 8/15
Serology	17/17	14/14	Nearly all		7/7	NA[i]	15/15
CSF	Mild increase in WBC in 1 of 2 cases	Normal in 1 case	ND	Mild increase in lymphocytes in 2 of 9 cases; increased protein	ND	No data	ND
WBC	Lymphopenia, leukopenia, neutropenia			Lymphopenia, leukopenia, neutropenia	Lymphopenia, leukopenia		Leukopenia in 4/15 cases

NA, not applicable; ND, not done
Where applicable, data are given as no. affected / total no. tested.
[a]2 cases with significant and prolonged drowsiness had persistent insomnia with mild intention tremor.
[b]1 case with chest pain.
[c]1 case each of chest tightness, mild dyspnea, or irregular labored breathing associated with somnolence.
[d]1 case with "slight" tremor of right arm for 24 h; irritable but no drowsiness.
[e]9% ataxia, 6% seizures, 6% hallucinations, 2% paralysis, 2% coma, 4% paresthesias; severe encephalitis in 6 children (8%); mild encephalitis (confusion, hallucination, abnormal gait, or drowsiness) in 4 adults and 6 children (13%); encephalopathy in 1 case (1%).[3]
[f]One patient delirious and "unable to form words."
[g]Isolated on day 3 (8 cases), day 4 (2 cases), day 5 (3 cases), and day 6 (1 case).
[h]Viremia in a subset of 40 cases observed days 0 to 8 illness; most common on initial 3 d of illness (peak viremia day after illness onset).[2]
[i]Retrospective review of VEE subtype ID cases in Panama (1961–2004) with viremia or VEEV isolation.[32]

CNS identified in these studies was by means of the trigeminal nerve, which was associated with VEEV replication in the tooth pulp.[6]

Recent bioluminescence studies involving subcutaneous challenge of adult CD1 mice with 1×10^4 pfu of VEEV subtype IC (strain 3908) that expresses firefly luciferase suggested VEEV may initially enter the brain directly through a hematogenous route at specific sites in the brain where the blood–brain barrier is naturally absent, as was reported after subcutaneous challenge of mice with Eastern and Western equine encephalitis viruses.[19-21,30] An earlier study of VEE TrD strain IP challenge (1.0×10^4 pfu) of C57BL/6 mice suggested that infection of the brain might also occur through a hematogenous route, in light of detection of VEEV antigen initially in the cerebral cortex at 3 d after challenge but not until day 4 in the hippocampus, thalamus, and brainstem.[23]

Aerosol and intranasal challenge. Results of aerosol and intranasal challenge studies in mice yielded a lethal model, with death mainly due to encephalitis. Mouse lines studied included CD1 (intranasal challenge with 1000 pfu of V3000 strain), BALB/c (aerosol challenge with 10,000 pfu of V3000 strain or 7400 pfu of TrD strain), outbred ICR (aerosol challenge with 1000 pfu and intranasal challenge with $1 \times 10^{2.5}$ pfu of TrD strain), and C3H/HeN (aerosol challenge 9600 pfu TrD strain).[6,38,43,46]

In contrast to percutaneous-acquired VEE, aerosol and intranasal VEEV challenge of mice was associated with significantly increased histopathologic lesions and viral burden in the upper respiratory tract, nasal mucosa (mucosal necrosis and more virus-positive cells in the nasal mucosa), and CNS.[6,38,43,46] Direct infection of the nasal mucosa with subsequent spread to the nearby olfactory system resulted in an early neuroinvasion that occurred before the onset of viremia. CNS infection was noted as early as 16 h (and in all mice by 48 h) after challenge and was generally associated with necrotizing rhinitis; massive infection of the olfactory epithelium; and bilateral infection of the olfactory nerves, bulbs, and tracts.[6,46] Virus levels were 3 times higher in the olfactory bulb than in the brain at 16 to 24 h after aerosol challenge but were similar to those in the brain by 60 h afterward, thus supporting the hypothesis that VEEV entry into the brain was through the olfactory system.[38] Findings from electronmicrography and histopathology after intranasal VEEV challenge supported axonal transport as the mechanism of viral spread from the olfactory bulb neurons into the CNS. Within 48 h after intranasal challenge and before viral detection in the olfactory bulbs and cortex, evidence of viral replication in the nasal mucosa and subsequent necrosis in the olfactory epithelial and secretory cells of Bowman glands were observed. Spread of VEEV to the CNS through the trigeminal nerve, resulting from dental tissue infection was a less frequent route of CNS entry. In addition, aerosol challenge resulted in virus detection in the lungs within 12 h after challenge, with subsequent viremia and viral spread to lymphoid tissues.

NHP The NHP model has been the primary animal model for both mosquito-borne and aerosol-acquired VEE in humans. Both rhesus (*Macaca mulatta*) and cynomolgus (*Macaca fascicularis*) macaques (country of origin unknown) have been used to

develop a nonlethal model of VEEV infection that shows similar disease as in humans. NHP generally had onset of fever, viremia, and lymphopenia within 1 to 3 d after VEEV aerosol or parenteral challenge. Although some NHP exhibited signs of encephalitis a few days later, nearly all NHP survived infection, as do humans.[42] Also similar to VEE disease in humans, the primary markers of infection in NHP were fever, viremia, and lymphopenia. CNS histopathology findings in NHP included gliosis, satellitosis, multifocal perivascular cuffs composed mainly of lymphocytes, neuronal death, and a few microhemorrhages.[37,42]

Reports in the literature of earlier NHP studies comparing aerosol or intranasal with parenteral subcutaneous or intraperitoneal challenge are often limited, due to the absence of immunohistochemistry staining, electronmicrography, and characterization of VEEV. Nevertheless, as observed in mice, NHP in these studies demonstrated earlier onset and more severe CNS disease after aerosol and intranasal challenge as compared with parenteral challenge. Unlike in the mouse model, VEEV neuroinvasion and neurovirulence in NHP were more limited, resulting in a nonlethal VEEV infection more similar to human disease.

Subcutaneous challenge. Subcutaneous challenge of rhesus macaques with VEEV serotype IA Trinidad, VEEV IB PTF39, or VEEV IC P676 epizootic strains with inoculum doses of 16,000, 20,000, and 160,000 intracerebral LD_{50} doses for suckling mice, respectively, resulted in signs of disease (for example, fever, anorexia, loose stools, irritability, depression, diminished muscular strength) initially observed from 2 to 7 d after challenge.[28] Viremia was observed the day after challenge, peaked at days 1 through 3, and resolved by days 4 through 6 after challenge. In addition, VEEV was isolated from the pharynx during this time.[28] Only one NHP developed overt neurologic signs, including tremors, myoclonus, and choreiform movements on day 2 after challenge that was accompanied by VEEV detection in the CSF.

Intraperitoneal challenge. Similarly, intraperitoneal challenge of 67 rhesus macaques with an unspecified range of doses of VEEV Trinidad strain was associated with a febrile illness, with fever onset between 12 to 72 h and viremia on days 1 to 2 after challenge.[17,42] Histopathology showed initial evidence of infection in the lymphoid tissues, with later changes in the olfactory cortex and thalamus by day 6, and hypothalamus and throughout the brain by day 8. Brain lesions, characterized by gliosis and multifocal perivascular cuffing composed mainly of lymphocytes, varied in severity and location, and were most severe between 14 to 21 d after infection and present in 18 of 20 NHP euthanized during this time.

Aerosol and intranasal challenge. In contrast, intranasal challenge of rhesus macaques with a 10^6 median culture infective dose of VEEV as titrated in chick embryo cells initially resulted in the presence of VEEV in the nasal mucosa, lungs, and cervical and hilar lymph nodes within 18 h after challenge; earlier infection of the olfactory bulb by 48 h (compared with 6 d after intraperitoneal challenge); and more severe CNS infection as determined by histopathology, with higher viral titers and increased neuronal damage, neuronophagia, and neutrophil infiltration as compared with intraperitoneal challenge.[7,8,42] Detection of virus in the olfactory bulb before the onset of viremia supported the olfactory pathway as the route of early CNS infection. As observed with parenteral challenge, VEEV challenge by the intratracheal route that bypassed the upper respiratory tract and VEEV aerosol challenge after surgical interruption of the olfactory tracts resulted in a delayed and less severe CNS infection that did not occur until after the onset of viremia (similar to parenteral VEEV challenge). With intratracheal challenge of NHP, CNS infection was not observed before infection of the nasal mucosa.

Although clinical and histopathologic findings support both the rhesus and cynomolgus macaques as potential nonlethal animal model candidates for the development of medical countermeasures for VEEV, most vaccine candidates in recent years have used a cynomolgus macaque model to demonstrate vaccine efficacy against aerosol challenge in NHP. For example, a VEEV DNA vaccine given to adult cynomolgus macaques by gene gun demonstrated partial protection against aerosol challenge with 1×10^8 pfu of VEEV IAB (TrD strain).[12] All 3 control NHP had viremia that was initially detected on day 1 after challenge, peaked at day 2, and persisted until day 3 or 4 after challenge. In comparison, only 1 of 3 vaccinated NHP had a low-level viremia (10 pfu/mL) detected only on day 3 after challenge. Fever was observed within 24 h of exposure in all 3 control NHP, which peaked at day 5 after challenge and persisted for an average of 7.3 d (145.4 h). In contrast, the duration of fever in vaccinated NHP was reduced to 3.7 d (87.3 h), with the peak fever occurring at day 3 after challenge and a mean maximal temperature elevation (3.8 °C) that was similar to that in control NHP (4.0 °C). An average 30.9% decrease in lymphocyte count was observed in control NHP, compared with only a 2.6% average increase in vaccinated NHP.

The cynomolgus macaque model has also been used to evaluate vaccine efficacy against aerosol challenge with enzootic VEEV strains. Cynomolgus macaques were used to evaluate the efficacy of an internal ribosome entry site-based VEEV vaccine against aerosol challenge with 4×10^4 pfu of the enzootic VEEV IE strain 68U201.[37] VEEV viremia was detected in control NHP ($n = 2$) on the 2 d after challenge but was not detected in the 5 vaccinated NHP. The maximal elevation of fever was significantly higher in control NHP compared with vaccinated NHP, which did not demonstrate a fever during initial 88 h after challenge (temperatures not monitored from 88 h to 142 h due to equipment failure). Lymphopenia was not assessed in this study.[37]

Another vaccine efficacy study against aerosol challenge (1×10^8 pfu) with VEEV subtype IE enzootic strain 68U201 in 8 unvaccinated cynomolgus macaques demonstrated fever, viremia, and lymphopenia as reliable markers of disease.[35] All 8 control NHP developed viremia with viral titers peaking at 2×10^5 pfu on the day after challenge and persisting for 3 d, compared with no viremia detected in V3526- and 1E1009-vaccinated animals. Although throat swabs revealed VEEV in both control and vaccinated NHP, control NHP had higher VEEV titers, which persisted until day 6, compared with vaccinated NHP, which had 4-log lower viral titers in throats on day 1 and undetectable titers by day 2 after challenge. Fever onset, duration, and maximal temperature elevation were greater in unvaccinated control NHP (150 fever h for 88 h duration compared with 54 and 40 fever h for 32 and 35 h duration, respectively, for the 2 vaccinated NHP groups). Lastly, lymphopenia was greater in unvaccinated control NHP (mean 48% decrease compared with a mean 15% and 7% decrease, respectively, in the 2 vaccinated NHP groups).

Discussion

The major pathophysiologic effects of encephalitis resulting from VEEV infection occur after viral entry into the CNS.[27] However, regardless of the exposure route, most adult humans infected with VEEV do not manifest signs or symptoms of severe encephalitis such as seizures, coma, cranial nerve

dysfunction, or paralysis.[14,48] Likewise, severe encephalitis was not observed in our VEE cohort; and a slight arm tremor in one case of aerosol-acquired VEE was the only observed major neurologic manifestation. The infrequent CNS symptomatology observed in our aerosol-acquired VEE cases and in those from the published literature support that, unlike that observed after high-dose VEEV aerosol challenge in animal models, aerosol-acquired VEE in humans at exposure doses encountered in research laboratories is not associated with an increased frequency or severity of CNS disease. The higher incidence of insomnia in our aerosol-acquired VEE cohort and in the initially reported 14 aerosol-acquired VEE cases in laboratory workers was possibly due to the variability in the assessment of symptoms by different clinicians,[5,25,26] given that insomnia was rarely reported in subsequent literature regardless of the exposure route.[2,3,15,32,40] In addition, the trend for an increased frequency of drowsiness in our percutaneous-acquired VEE cohort was not supported by the published literature.

The statistically significant increases in the frequencies of sore throat, (with or without pharyngeal erythema), neck pain without nuchal rigidity, and cervical lymphadenopathy associated with the aerosol-acquired VEE cases suggest an increase in upper respiratory tract symptomatology with aerosol exposure. VEEV was commonly detected in the pharyngeal secretions of both aerosol- and percutaneous-acquired VEE cases. However, animal studies suggest that the increase in upper respiratory tract findings in aerosol-acquired VEE may be due to higher pharyngeal viral burdens associated with aerosol exposure. Higher viral burdens in the nasal mucosa with associated nasal mucosal necrosis and necrotizing rhinitis were observed in mice after both intranasal and aerosol challenge but not after subcutaneous exposure.[31,42,46] Intranasal challenge of NHP was associated with early viral detection in the nasal mucosa but was not associated with significant nasal mucosal changes on histopathology, supporting the hypothesis that VEE disease in NHP is more similar to humans.[7,8] Whereas cough was reported in both aerosol (24%) and percutaneous (22%) acquired VEE, lower respiratory tract symptoms were uncommon.

Cervical or mandibular lymphadenopathy was observed in 35% of aerosol-acquired VEE cases, but only in one case (4%) of percutaneous-acquired VEE. Potentially higher pharyngeal viral burdens observed with aerosol-acquired infection may have contributed to the localized lymphadenopathy. Cervical lymphadenopathy was present in 4 of the 5 cases with neck pain and was likely associated with the increased frequency of neck pain reported in aerosol-acquired VEE cases. Axillary lymphadenopathy was common in both our percutaneous (17%) and aerosol-acquired (24%) VEE cohorts. However, cervical or generalized lymphadenopathy was infrequently reported in the literature, regardless of the exposure route.[3,5,11,15,25,26,32,40] Other than the earlier evaluation during the Biowarfare Program (often performed within 24 h of illness onset) and more detailed records of daily clinical evaluations, the reason for the discrepancy in lymphadenopathy is unclear.

For unclear reasons, conjunctival injection was commonly observed in both aerosol- and percutaneous-acquired VEE cohorts (29% and 22%, respectively) but was reported less frequently in the majority of cohorts in the literature.[3,5,25,26,40] Although conjunctival injection in our cohorts often occurred within 24 h of illness onset and was short-lived (1 to 3 d in duration), delayed clinical presentation alone may not fully explain the reporting discrepancy of conjunctival injection in the literature.[1]

Photophobia was reported in 24% of our aerosol-acquired VEE cases and in 43% of the initial 14 aerosol-acquired VEE cases in laboratory workers reported in the literature,[5,25,26] but this symptom was reported in only 4% of our percutaneous-acquired VEE cases. Why photophobia was uncommonly reported in many mosquito-borne VEE cohorts in the literature, even in the presence of severe encephalitis, but commonly reported in other mosquito-borne VEE cohorts is unclear.[3,9,11,15,32] The occurrence of conjunctival injection in 3 of the 5 photophobia cases raises the possibility that the photophobia may due to viral conjunctivitis, possibly related to higher pharyngeal viral burdens or direct ocular infection from aerosol VEEV exposure.

After retrospective analysis, 2 cases originally classified as percutaneous-acquired VEE due to recent VEE vaccination may actually have been unrecognized aerosol-acquired VEE. Both subjects had worked in a laboratory with ongoing VEEV studies within the past 2 d, but they had no known exposure or breach in personal protective measures. One subject (an X-ray technician) had VEE onset 8 d after receiving a formalin-inactivated vaccine in 1959 and was the only one of the 17 formalin-inactivated vaccine-associated cases not attributed to the initial TrD strain-based formalin-inactivated vaccine administered from 1950 to 1953. Therefore, there is a strong possibility that this case was likely due to unrecognized VEEV aerosol exposure. The other subject (a veterinarian) developed VEE 8 d after receiving a live-attenuated TC50 VEE vaccine, which is a longer incubation period than expected for VEE due to a live-attenuated vaccine, because 3 of the other cases occurred within 1 d after vaccination. In this case, VEE infection was likely the result of unrecognized exposure from aerosol VEEV experiments performed across the hall 2 d before the onset of his illness. Of note, both cases had multiple upper respiratory tract and ocular symptoms as observed in many aerosol-acquired VEE cases. In addition, one of these patients was the sole patient with cervical adenopathy in the percutaneous-acquired VEE cohort. Eliminating these 2 cases from the percutaneous-acquired VEE analysis would further delineate that the increased upper respiratory tract findings and cervical lymphadenopathy were associated with aerosol-acquired VEE and possibly due to increased viral burden in the upper respiratory tract.

Due to uncontrolled variables, there are known limitations in comparing the aerosol and percutaneous-acquired VEE cohorts. However, even with variables in exposure route, exposure dose, VEEV subtype or strain, history of receiving a formalin-inactivated vaccine, and subject age, the initial febrile illness of VEE was similar between the 2 VEE cohorts in this population of young to middle-aged adults ranging from 27 to 55 y of age. Disease in both VEE cohorts generally manifested as an acute febrile illness, presenting with an abrupt onset of fever, chills, severe headache, malaise, fatigue, weakness, back pain, myalgia (often in the lower back, thigh, or calf muscles), sore throat, anorexia, and nausea. Common physical examination findings were fever, pharyngeal erythema, conjunctival injection, and lymphadenopathy. The increases in upper respiratory tract-related findings of sore throat, pharyngeal erythema, cervical lymphadenopathy, and neck pain observed with aerosol-acquired VEE in humans were the only identifiable differences observed between the 2 cohorts.

Although partial immunity from prior receipt of VEE formalin-inactivated vaccines in some laboratory workers with aerosol-acquired VEE cannot be excluded, several reasons suggest these vaccines were unlikely to have had a significant protective benefit against aerosol-acquired VEE. Antibody responses associated with earlier VEE formalin-inactivated vaccines (1953 to 1969) were short-lived. Neutralizing antibody titers at the time of onset of VEE infection available in 12 of the

15 aerosol-acquired VEE cases with a prior history of formalin-inactivated VEE vaccination were negative in 6 cases and present at only minimal levels in the remaining 6 cases, with the last vaccine dose in most subjects administered between 2 to 6 mo before exposure. Disease in these 15 cases was as severe as that observed in vaccine-naïve, aerosol-acquired VEE cohorts in the literature and in the 2 vaccine-naïve aerosol-acquired VEE cases with VEE-negative baseline serology in this cohort.[5,25,26] The C84 VEE vaccine, a later version of the formalin-inactivated vaccine based on the TC83 strain, elicited poor protection against aerosol VEEV challenge in animal studies when given as a 2-dose primary series (disease in all 3 NHP; death in 13 of 14 hamsters).[13,31] A 3-dose primary series of the C84 vaccine resulted in 10-fold higher serum neutralizing antibody titers in NHP compared with a 2-dose primary series, but disease after aerosol challenge still occurred in 3 of 5 NHP.[31] Therefore, preexisting immunity against VEE was unlikely to have had a significant influence on disease severity in most of these aerosol-acquired VEE cases that had no detectable or minimal VEEV neutralizing antibody levels present at the onset of illness but had previously received a formalin-inactivated VEEV vaccine.

An effect due to preexisting immunity was also unlikely in the 15 VEE cases associated with the formalin-inactivated vaccine given from 1950 to 1952 (3-dose primary series given on days 0, 7, and 42, with subsequent boosters every 6 mo).[44] Most infections (n = 10) occurred within 2 to 6 d after receipt of the initial vaccine dose, with 4 cases occurring within 3 to 8 d after the second vaccine dose. The single VEE case that occurred 3 d after dose 4 was attributed to lower antibody titers due to a delay in the booster dose that was given at 10 mo instead of 6 mo after dose 3. None of the 15 patients had known prior VEE vaccination or VEE infection. In the 2 cases with available post-vaccination serum neutralizing antibody titers, titers were negative as late as 11 d after dose 1 and 13 d after dose 2. With the VEE C84 vaccine (given as a primary series on days 0, 28, and 200), neutralizing antibodies as measured by a VEEV plaque reduction neutralization assay ($PRNT_{80}$) did not initially appear until 8 to 14 d after the initial vaccine dose.[13] The VEE antibody $PRNT_{80}$ titer response (expressed as the reciprocal of the highest dilution that neutralized 80% of the plaques) after dose 1 was low and short-lived (mean titer of 36 at day 14). Higher titers (mean, 226) were not observed until 4 wk after the second vaccine dose. Titers before dose 3 on day 200 were low (mean, 50), but titers 40 d after dose 3 were 6 to 7 times higher than observed after dose 2 (mean, 1613).[13] Therefore, neutralizing antibodies were unlikely present in the 12 VEE vaccine-associated cases occurring within 10 d of vaccine dose 1 and were likely absent (or present at only low levels) in the other 3 cases; and this conclusion is supported by similar disease severity in these 15 cases to that of our aerosol-acquired cohort and other VEE cohorts reported in the literature.

Humans are similar to NHP in that both have a low infective dose by aerosol exposure (as few as 10 organisms) and generally survive VEE infection. However, unlike in NHP, aerosol VEE exposure in our human cohort was not associated with an increase in the severity of CNS disease compared with percutaneous exposure.[41] One possible explanation for this lack of an observed increase in CNS disease in our aerosol-acquired VEE cases is that the laboratory workers likely were exposed to lower doses than those used in NHP aerosol challenge studies, which typically have been 1×10^6 pfu or greater. Therefore, that exposure of humans to increased VEEV doses by the aerosol route could result in increased CNS disease remains a possibility. Mouse studies with Eastern equine encephalitis virus showed an increase in disease severity and death from encephalitis with higher aerosol exposure doses, but this result differs from that seen with VEEV, which is lethal to mice even at low aerosol exposure doses of 10 organisms.[19-21,47] Therefore, a dose-increasing effect may not necessarily observed in VEE aerosol challenge of NHP, due to the low infective dose of 10 organisms and nonlethality of disease in NHP.

An alternative reason for the infrequency of severe CNS disease in adult humans from our aerosol-acquired VEE cohort is that this effect may be species-related. The major risk groups for severe encephalitis in humans with mosquito-borne VEE are children younger than 15 y (particularly infants younger than 12 mo) and elderly people.[1,4] The reported infective doses and lethal doses in early aerosol challenge studies in animals were species-dependent.[45] Whereas mice, guinea pigs, and rabbits succumbed to infection at a lower lethal dose, NHP generally survived VEE infection. Death in hamsters, guinea pigs, and rabbits was related to fulminant necrosis of lymphoid tissue, mainly due to bacteremia and shock associated with gastrointestinal lymphoid tissue that occurred before the onset of CNS infection, whereas the main cause of death in NHP and mice was encephalitis, which is similar to humans.[23,42] Disease severity observed in alphavirus animal models has varied with virus strain, mouse strain or age, and a combination of these factors.

The increased upper respiratory tract findings in our human cases associated with aerosol VEEV exposure is supported by findings from intranasal and aerosol VEEV mouse challenge models. Intranasal and aerosol challenge of mice resulted in increased VEE viral burden in the nasal mucosa, with associated inflammation and necrosis of the nasal mucosa. Although significant VEEV viral burdens were observed in the nasal mucosa in NHP after intranasal challenge, the absence of noteworthy nasal mucosa histopathology (for example, no necrosis of the nasal mucosa) from one study in which this was assessed indicate this may reflect a species difference between NHP and mice, possibly due to viral infectivity or anatomic differences of the nasal turbinates.[7,8] Whether the decreased severity of encephalitis and lethality in the NHP model as compared with mice is related to the local nasopharyngeal viral burden is unclear. Future NHP natural history studies assessing the variability of aerosol challenge doses, nasal mucosal viral burdens, and nasal mucosal histopathology and their effects on encephalitis severity may provide clarification.

In summary, according to the results of our review of human cases, aerosol-acquired VEE is similar to percutaneous-acquired VEE in humans, with the exception of an increase in upper respiratory tract-related findings (sore throat, with or without pharyngeal erythema, neck pain, cervical lymphadenopathy) and possibly photophobia associated with aerosol-acquired disease. The data from cynomolgus and rhesus macaques studies demonstrate the suitability of either NHP model for evaluating medical countermeasures against percutaneous- and aerosol-acquired VEEV under the Animal Rule in light of: (1) the similar disease and pathophysiology in NHP models as in humans and (2) the predictability of the NHP model to recreate the expected response in humans after percutaneous or aerosol VEEV exposure. Both humans and NHP have low mortality after aerosol-acquired VEE, with the major cause of death being encephalitis. Similar to humans, the nonlethal VEE NHP models demonstrated fever, viremia, and lymphopenia as reliable markers of disease. Although high-dose VEEV aerosol challenge of NHP was associated with a higher frequency and greater severity of CNS histopathology findings than in humans, this outcome does not preclude use of the NHP model

to assess medical countermeasures intended to protect humans from aerosol VEEV exposures.

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References

1. **Aguilar PV, Estrada-France JG, Navarro-Lopez R, Ferro C, Haddow AD, Weaver SC.** 2011. Endemic Venezuelan equine encephalitis in the Americas: hidden under the dengue umbrella. Future Virol **6:**721–740. https://doi.org/10.2217/fvl.11.50.
2. **Alevizatos AC, McKinney RW, Feigin RD.** 1967. Live, attenuated Venezuelan equine encephalomyelitis virus vaccine. I. Clinical effects in man. Am J Trop Med Hyg **16:**762–768. https://doi.org/10.4269/ajtmh.1967.16.762.
3. **Bowen GS, Calisher CH.** 1976. Virological and serological studies of Venezuelan equine encephalomyelitis in humans. J Clin Microbiol **4:**22–27.
4. **Bowen GS, Fashinell TR, Dean PB, Gregg MB.** 1976. Clinical aspects of human Venezuelan equine encephalitis in Texas. Bull Pan Am Health Organ **10:**46–57.
5. **Casals J, Curnen EC, Thomas L.** 1943. Venezuelan equine encephalomyelitis in man. J Exp Med **77:**521–530. https://doi.org/10.1084/jem.77.6.521.
6. **Charles PC, Walters E, Margolis F, Johnston RE.** 1995. Mechanism of neuroinvasion of Venezuelan equine encephalitis virus in the mouse. Virology **208:**662–671. https://doi.org/10.1006/viro.1995.1197.
7. **Danes L, Kufner J, Hruskova J, Rychterova V.** 1973. The role of the olfactory route on infection of the respiratory tract with Venezuelan equine encephalomyelitis virus in normal and operated *Macaca* rhesus monkeys. I. Results of virological examination. Acta Virol **17:**50–56.
8. **Danes L, Rychterova V, Kufner J, Hruskova J.** 1973. The role of the olfactory route of infection of the respiratory tract with Venezuelan equine encephalomyelitis virus in normal and operated *Macaca* rhesus monkeys. II. Results of histological examination. Acta Virol **17:**57–60.
9. **Daza E, Frias V, Alcola A, Lopez I, Bruzon I, Montero JT, Alvarez G, Garcia MA, Rodriguez R, Boschell J, de la Hoz F, Riva F, Olano V, Diaz LA, Caceras FM, Aristizabal G, Cardenas V, Cuellar J, Gonzalez E.** 1995. Venezuelan equine encephalitis—Columbia, 1995. MMWR Morb Mortal Wkly Rep **44:**721–724.
10. **de la Monte SM, Castro F, Bonilla NJ, Gaskin de Urdaneta A, Hutchins GM.** 1985. The systemic pathology of Venezuelan equine encephalitis virus infection in humans. Am J Trop Med Hyg **34:**194–202. https://doi.org/10.4269/ajtmh.1985.34.194.
11. **Dietz WH Jr, Peralta PH, Johnson KM.** 1979. Ten clinical cases of human infection with Venezuelan equine encephalomyelitis virus, subtype ID. Am J Trop Med Hyg **28:**329–334. https://doi.org/10.4269/ajtmh.1979.28.329.
12. **Dupuy LC, Richards MJ, Reed DS, Schmaljohn CS.** 2010. Immunogenicity and protective efficacy of a DNA vaccine against Venezuelan equine encephalitis virus aerosol challenge in nonhuman primates. Vaccine **28:**7345–7350. https://doi.org/10.1016/j.vaccine.2010.09.005.
13. **Edelman R, Ascher MS, Oster CN, Ramsburg HH, Cole FE, Eddy GA.** 1979. Evaluation in humans of a new, inactivated vaccine for Venezuelan equine encephalitis virus (C84). J Infect Dis **140:**708–715. https://doi.org/10.1093/infdis/140.5.708.
14. **Ehrenkranz NJ, Ventura AD.** 1974. Venezuelan equine encephalitis virus infection in man. Annu Rev Med **25:**9–14. https://doi.org/10.1146/annurev.me.25.020174.000301.
15. **Franck PT, Johnson KM.** 1970. An outbreak of Venezuelan equine encephalomyelitis in the Panama Canal Zone. Am J Trop Med Hyg **19:**860–865. https://doi.org/10.4269/ajtmh.1970.19.860.
16. **Glaze ER, Roy MJ, Dalrymple LW, Lanning LL.** 2017. A comparison of the pathogenesis of Marburg virus disease in humans and nonhuman primates and evaluation of the suitability of these animal models for predicting clinical efficacy under the 'Animal Rule'. Comp Med **65:**241–259.
17. **Gleiser CA, Gochenour WS Jr, Berge TO, Tigertt WD.** 1962. The comparative pathology of experimental Venezuelan equine encephalitis virus in different animal hosts. J Infect Dis **110:**80–97. https://doi.org/10.1093/infdis/110.1.80.
18. **Golding H, Khurana S, Zaitseva M.** 2017. What is the predictive value of animal models for vaccine efficacy in humans? The importance of bridging studies and species-independent correlates of protection. Cold Spring Harb Perspect Biol. http://doi.org/10.1101/cshperspect.a028902.
19. **Honnold SP, Mossel EC, Bakken RR, Fisher D, Lind CM, Cohen JW, Eccleston LT, Spurgers KB, Erwin-Cohen R, Bradfute SB, Maheshwari RK, Glass PJ.** 2015. Eastern equine encephalitis virus in mice I. clinical course and outcome are dependent on route of exposure. Virol J **12:**1–14. https://doi.org/10.1186/s12985-015-0386-1.
20. **Honnold SP, Mossel EC, Bakken RR, Lind CM, Cohen JW, Eccleston LT, Spurgers KB, Erwin-Cohen R, Maheshwari RK, Glass PJ.** 2015. Eastern equine encephalitis virus in mice II: pathogenesis is dependent on route of exposure. Virol J **12:**1–23. https://doi.org/10.1186/s12985-015-0385-2.
21. **Honnold SP.** [Internet]. 2012. Pathogenesis of Eastern equine encephalitis in mice and development of a 2nd generation vaccine. Uniformed Services University of the Health Sciences, Bethesda, MD. [Cited 20 November 2017]. Available at: http://cdm16005.contentdm.oclc.org/cdm/search/collection/p15459coll1/searchterm/shelley%20honnold.
22. **Ioannidis JPA.** 2012. Extrapolating from animals to humans. Sci Transl Med **4:**151ps15. https://doi.org/10.1126/scitranslmed.3004631. PubMed
23. **Jackson AC, SenGupta SK, Smith JF.** 1991. Pathogenesis of Venezuelan equine encephalitis virus infection in mice and hamsters. Vet Pathol **28:**410–418. https://doi.org/10.1177/030098589102800509.
24. **Johnson KM, Shelokov A, Peralta PH, Dammin GJ, Young NA.** 1968. Recovery of Venequelan equine encephalomyelitis virus in Panama. A fatal case in man. Am J Trop Med Hyg **17:**432–440. https://doi.org/10.4269/ajtmh.1968.17.432.
25. **Koprowski H, Cox HR.** 1947. Human laboratory infection with Venezuelan equine encephalomyelitis virus; report of 4 cases. N Engl J Med **236:**647–654. https://doi.org/10.1056/NEJM194705012361801.
26. **Lennette EH, Koprowski H.** 1943. Human infection with Venezuelan equine encephalitis virus. A report on 8 cases of infection acquired in the laboratory. JAMA **123:**1088–1095. https://doi.org/10.1001/jama.1943.02840520004002.
27. **Ludlow M, Kortekaas J, Herden C, Hoffmann B, Tappe D, Trebst C, Griffin DE, Brindle HE, Solomon T, Brown AS, van Riel D, Wolthers KC, Pajkrt D, Wohlsein P, Martina BEE, Baumgartner W, Verjans GM, Osterhaus ADME.** 2016. Neurotropic virus infections as the cause of immediate and delayed neuropathology. Acta Neuropathol **131:**159–184. https://doi.org/10.1007/s00401-015-1511-3.
28. **Monath TP, Calisher CH, Davis M, Bowen GS, White J.** 1974. Experimental studies of rhesus monkeys infected with epizootic and enzootic subtypes of Venezuelan equine encephalitis virus. J Infect Dis **129:**194–200. https://doi.org/10.1093/infdis/129.2.194.
29. **Paessler S, Weaver SC.** 2009. Vaccines for Venezuelan equine encephalitis. Vaccine **27 Suppl 4:** D80–D85. https://doi.org/10.1016/j.vaccine.2009.07.095

30. **Phillips AT, Rico AB, Stauft CB, Hammond SL, Aboellail TA, Tjalkens RB, Olson KE.** 2016. Entry site of Venezuelan and western equine encephalitis viruses in the mouse central nervous system following peripheral infection. J Virol **90:**5785–5796. https://doi.org/10.1128/JVI.03219-15.
31. **Pratt WD, Gibbs P, Pitt ML, Schmaljohn AL.** 1998. Use of telemetry to assess vaccine-induced protection against parenteral and aerosol infections of Venezuelan equine encephalitis virus in nonhuman primates. Vaccine **16:**1056–1064. https://doi.org/10.1016/S0264-410X(97)00192-8.
32. **Quiroz E, Aguilar PV, Cisneros J, Tesh RB, Weaver SC.** 2009. Venezuelan equine encephalitis in Panama: fatal endemic disease and genetic diversity of etiologic viral strains. PLoS Negl Trop Dis **3:**1–7 https://doi.org/10.1371/journal.pntd.0000472.
33. **Randall R, Mills JW.** 1944. Fatal encephalitis in man due to the Venezuelan virus of equine encephalomyelitis in Trinidad. Science **99:**225–226. https://doi.org/10.1126/science.99.2568.225.
34. **Randall R, Maurer FD, Smadel FE.** 1949. Immunization of laboratory workers with purified Venezuelan equine encephalomyelitis vaccine. J Immunol **63:**313–318.
35. **Reed DS, Lind CM, Lackemeyer MG, Sullivan LJ, Pratt WD, Parker MD.** 2005. Genetically engineered, live, attenuated vaccines protect nonhuman primates against aerosol challenge with a virulent IE strain of Venezuelan equine encephalitis virus. Vaccine **23:**3139–3147. https://doi.org/10.1016/j.vaccine.2004.12.023.
36. **Rivas F, Diaz LA, Cardenas VM, Daza E, Bruzon I, Alcala A, De la Hoz O, Caceres FM, Aristizabul G, Martinez JM, Revelo D, De la Hoz F, Boshell J, Camacho T, Calderon L, Olano VA, Villarreal LI, Roselli D, Alvarez G, Ludwig G, Tsai T.** 1997. Epidemic Venezuelan equine encephalitis in La Guajira, Colombia, 1995–1997. J Infect Dis **175:**828–832. https://doi.org/10.1086/513978.
37. **Rossi SL, Russell-Lodrigue KE, Killeen SZ, Wang E, Leal G, Bergren NA, Vinet-Oliphant H, Weaver SC, Roy CJ.** 2015. IRES-containing VEEV vaccine protects cynomolgus macaques from IE Venezuelan equine encephalitis virus aerosol challenge. PLoS Negl Trop Dis **9:**1–10. https://doi.org/10.1371/journal.pntd.0003797.
38. **Ryzhikov AB, Tkacheva NV, Sergeev AN, Ryabchikova EI.** 1991. Venezuelan equine encephalitis virus propagation in the olfactory tract of normal and immunized mice. Biomed Sci **2:**607–614.
39. **Shubladze AK, Gaidmovich Sla, Gavrilov VI.** 1959. [Virological studies of laboratory cases of Venezuelan equine encephalitis.] Vopr Virusol **4:**305–310. [Article in Russian].
40. **Slepushkin AN.** 1949. An epidemiological study of laboratory infection with Venezuela equine encephalomyelitis virus. Vopr Virusol **3:**311–314.
41. **Smith DG, MAmay HK, Marshall RG, Wagner JC.** 1956. Venezuelan equine encephalomyelitis. Laboratory aspects of fourteen human cases following vaccination and attempts to isolate the virus from the vaccine. Am J Hyg **63:**150–164.
42. **Steele KE, Twenhafel NA.** 2010. Pathology of animal models of alphavirus encephalitis. Vet Pathol **47:**790–805. https://doi.org/10.1177/0300985810372508.
43. **Steele KE, Davis KJ, Stephan K, Kell W, Vogel P, Hart MK.** 1998. Comparative neurovirulence and tissue tropism of wildtype and attenuated strains of Venezuelan equine encephalitis virus administered by aerosol and C3H/HeN and BALB/c mice. Vet Pathol **35:**386–397. https://doi.org/10.1177/030098589803500508.
44. **Sutton LS, Brooke CC.** 1954. Venezuelan equine encephalomyelitis due to vaccination in man. JAMA **155:**1473–1476. https://doi.org/10.1001/jama.1954.03690350015005.
45. **Victor J, Smith DG, Pollack AD.** 1956. The comparative pathology of Venezuelan equine encephalomyelitis. J Infect Dis **98:**55–66. https://doi.org/10.1093/infdis/98.1.55.
46. **Vogel P, Abplanlp D, Kell W, Ibrahim MS, Downs MB, Pratt WD, Davis KJ.** 1996. Venezuelan equine encephalitis in BALB/c mice. Kinetic analysis of central nervous system infection following aerosol or subcutaneous inoculation. Arch Pathol Lab Med **120:**164–172.
47. **Vogel P, Kell WM, Fritz DL, Parker MD, Shoepp RJ.** 2005. Early events in the pathogenesis of Eastern equine encephalitis virus in mice. Am J Pathol **166:**159–171. https://doi.org/10.1016/S0002-9440(10)62241-9.
48. **Weaver SC, Ferro C, Barrera R, Boshell J, Navarro JC.** 2004. Venezuelan equine encephalitis. Annu Rev Entomol **49:**141–174. https://doi.org/10.1146/annurev.ento.49.061802.123422.
49. **Wolfe DN, Heppner DG, Gardner SN, Jaing C, Dupuy LC, Schmaljohn CS, Carlton K.** 2014. Current strategic thinking for the development of a trivalent alphavirus vaccine for human use. Am J Trop Med Hyg **91:**442–450. https://doi.org/10.4269/ajtmh.14-0055.