NARO
Food for Future Generations

SEACFMD Labnet Meeting
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Recent foot-and-mouth disease research at NIAH

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Topic 1

FMD viruses circulating in Myanmar, 2016–2022: phylogeny and pathogenicity in cattle and pigs

Which lineages are there, how they relate to viruses in neighboring countries, and whether existing diagnostic methods detect them

Kawaguchi R, Nishi T, Fukai K, Lwin KO, Morioka K. Transbound Emerg Dis 2025; 1532487 (open access)

Background

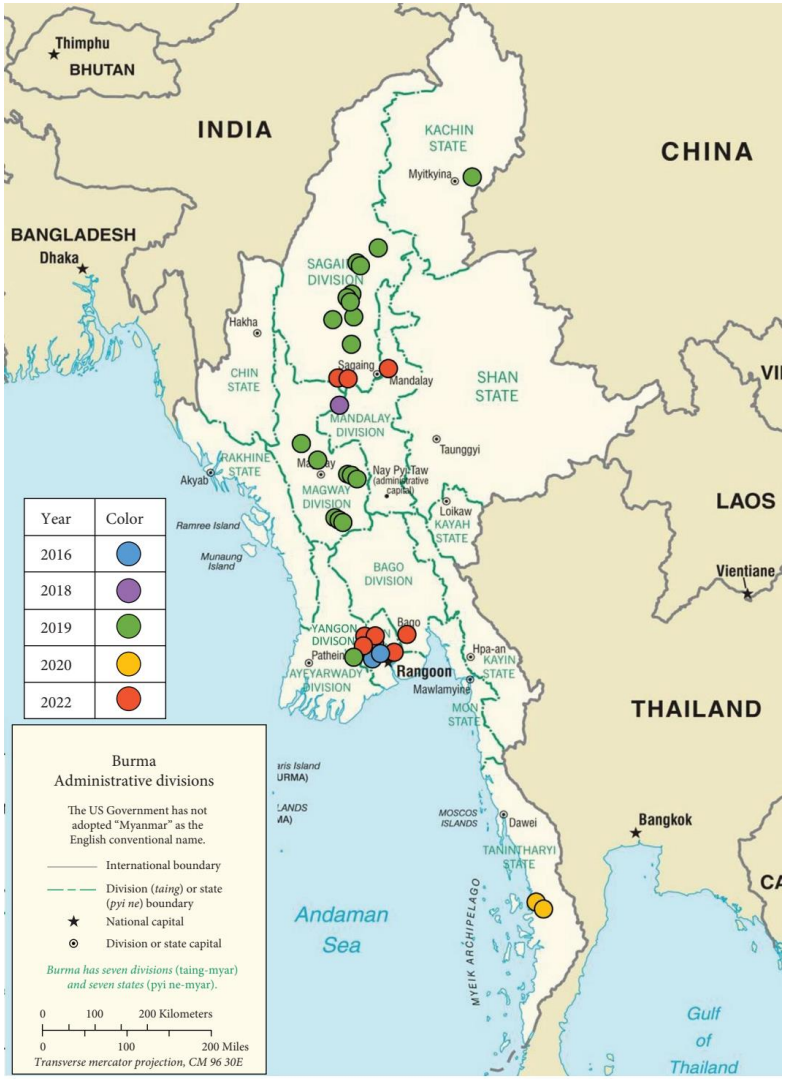


Fig. 1. Sampling sites of FMD cases in Myanmar, 2016–2022 (color = year).

Why Myanmar matters for FMD control in Asia

- Endemic for FMD; borders India and Bangladesh (Pool 2). Viruses of serotypes O and Asia1 have moved from South Asia into Myanmar in the past.
- Largest cattle and buffalo population in Southeast Asia.
- Formal and informal trade of live animals with Thailand, Vietnam, Cambodia, Laos and China: an outbreak in Myanmar can trigger outbreaks in neighboring countries.
- Viruses from past outbreaks in East Asia belonged to the same lineage as viruses circulating in Myanmar at the same time.

Objectives

- Genetic characterization of FMD viruses detected in Myanmar between 2016 and 2022
- Phylogenetic relationship with viruses in neighboring Asian countries
- Pathogenicity in cattle and pigs, and applicability of existing diagnostic methods

Kawaguchi R, Nishi T, Fukai K, Lwin KO, Morioka K. Transbound Emerg Dis 2025; 1532487

Materials and methods

Samples and genetic analysis

- 34 vesicular epithelium samples from cattle and pigs with clinical FMD, 2016–2022 (northern, central and southern Myanmar)
- Samples: FMDV Diagnostic Laboratory, LBVD (Myanmar) → Kodaira Research Station, NIAH (Japan)
- Virus isolation in ZZ-R 127 cells; serotype confirmed by lateral flow antigen detection
- VP1 sequencing of all samples; whole-genome sequencing (Ion PGM) of O/ME-SA/Ind-2001e viruses
- Phylogeny: MEGA 11, maximum likelihood, Kimura 2-parameter model; reference sequences from WRLFMD and GenBank

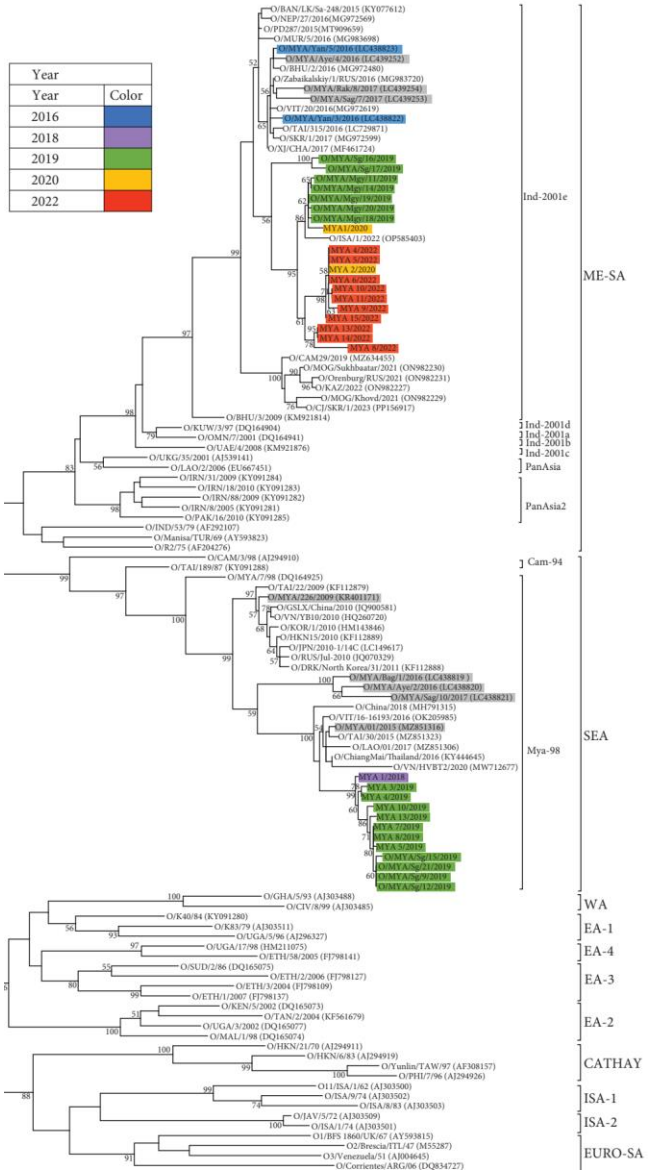
Experimental infection (NIAH)

Exp.	Virus (lineage)	Animals	Route	Dose
1	O/MYA/Yan/5/2016 (Ind-2001e)	2 pigs	Intraoral	$10^{6.0}$ TCID50
1	O/MYA/Mgy/11/2019 (Ind-2001e)	2 pigs	Intraoral	$10^{6.0}$ TCID50
2	O/MYA/Mgy/11/2019 (Ind-2001e)	2 cattle 4 pigs	Intradermal (tongue / heel bulb)	$10^{6.0}$ TCID50

- Daily clinical score (max 7): vesicles on each limb, snout and lip (1 point each), fever ≥ 40 °C (1 point)
- Blood, oral and nasal swabs to 8–10 dpi: virus titration (LFPK-αvβ6 cells), RT-PCR (3D region, FM8/9 primers), antibody ELISA (PrioCHECK FMDV type O)
- Results compared with O/JPN/2010 infections from our previous study

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Results: VP1 phylogeny of serotype O viruses



- 2016–2019: two lineages of serotype O – ME-SA topotype / Ind-2001e lineage and SEA topotype / Mya-98 lineage. One serotype A virus (ASIA / Sea-97) in 2019; Asia1 reported in 2017 (other report).
- 2020–2022: all 12 samples belonged to O/ME-SA/Ind-2001e.
- Ind-2001e viruses in Myanmar were close to viruses in Pools 1 and 2; Mya-98 viruses were close to Pool 1 viruses (Cambodia, Laos, Thailand, Vietnam, China).

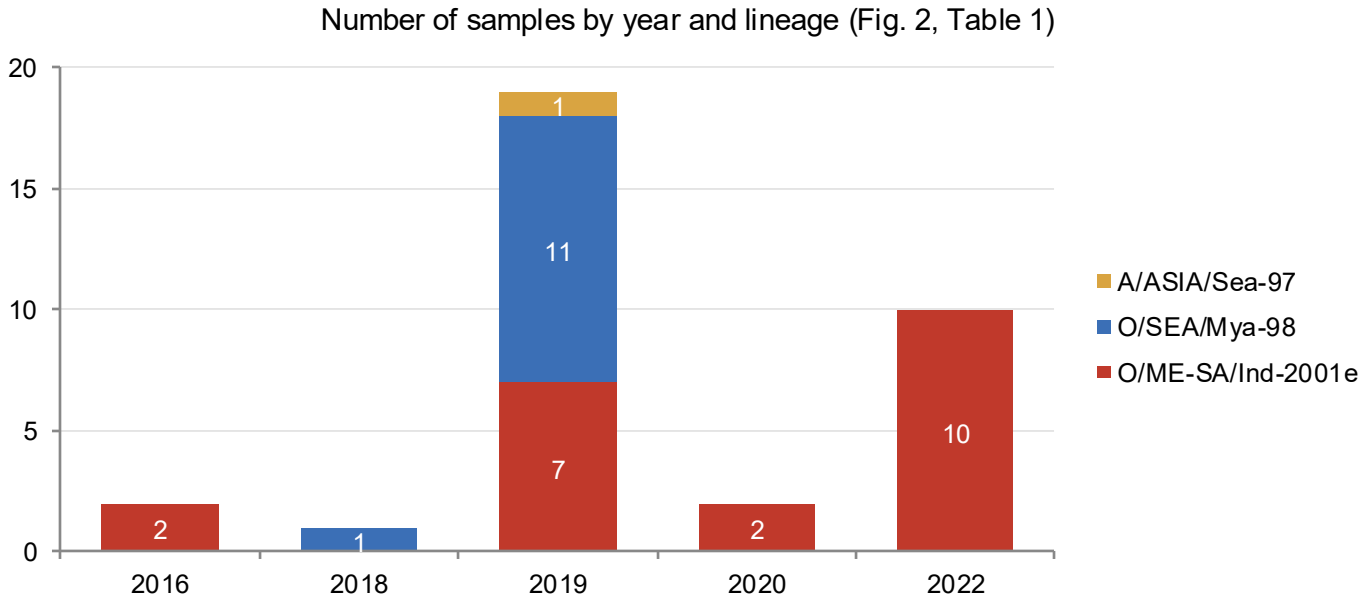


Fig. 2. VP1 tree (ML, 500 bootstraps).

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Results: experimental infection of pigs and cattle

Experiment (route)	Virus	Animals	Vesicles first seen (dpi)	Peak clinical score	Viremia (dpi / peak titer*)	Nasal swab (dpi / peak titer*)	Oral swab (dpi / peak titer*)
1 (intraoral)	O/MYA/Yan/5/2016	pigs	4	6	3–5 / $10^{6.0}$	3–8 / $10^{7.5}$	1–8 / $10^{7.5}$
1 (intraoral)	O/MYA/Mgy/11/2019	pigs	3–5	6	3–5 / $10^{6.8}$	3–5 / $10^{4.8}$	1–9 / $10^{8.5}$
2 (intradermal)	O/MYA/Mgy/11/2019	cattle	2–4	6	1–3 / $10^{6.5}$	1–10 / $10^{7.0}$	1–10 / $10^{7.0}$
2 (intradermal)	O/MYA/Mgy/11/2019	pigs	1–3	6	1–3 / $10^{5.8}$	1–5 / $10^{5.5}$	1–5 / $10^{6.5}$

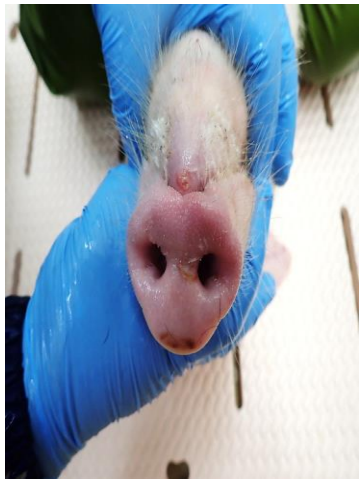
Table 2. Detection period and peak clinical score or viral titer. *TCID50/mL.



Cow 233, lip, 3 dpi



Cow 233, hindlimb, 5 dpi



Pig 213, lip, 3 dpi



Pig 213, hindlimbs, 5 dpi

Fig. S2. Lesions after intradermal inoculation with O/MYA/Mgy/11/2019.

- The 2016 and 2019 strains caused similar disease in pigs.
- The 2019 strain caused typical FMD in cattle and pigs: vesicles on the mouth, snout and feet, viremia and virus excretion.
- Vesicle formation and virus excretion were comparable to O/JPN/2010.

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Results: detection by existing diagnostic methods

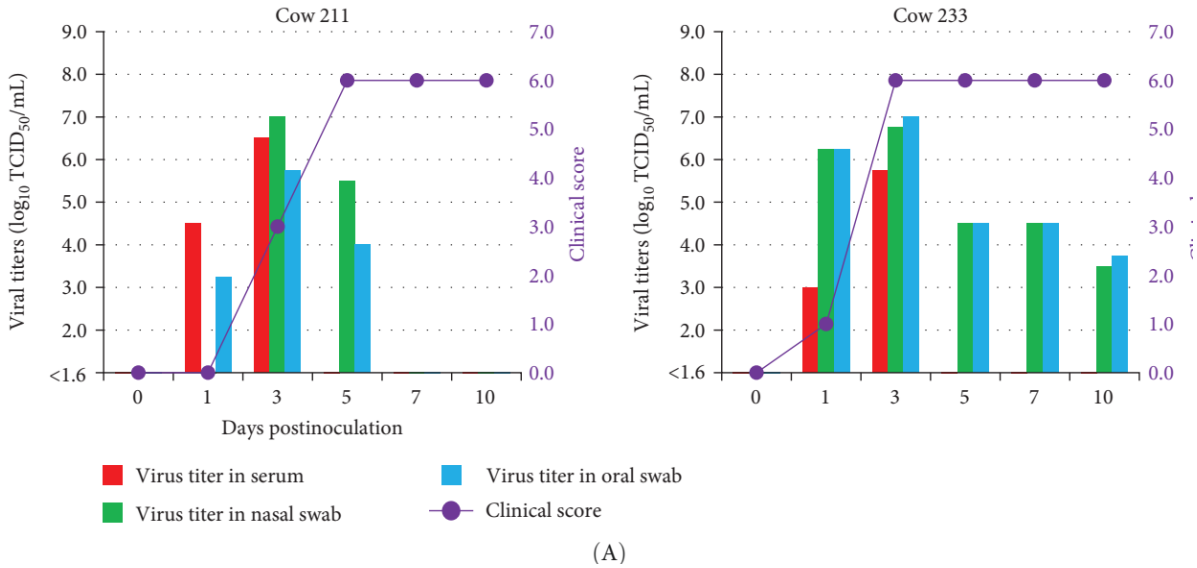


Fig. 5A. Virus titers (bars) and clinical scores (line) in cattle inoculated intradermally with O/MYA/Mgy/11/2019.

- Cattle: infectious virus in serum (1–3 dpi) and in nasal and oral swabs (1–10 dpi); clinical score 6 by 5 dpi.
- Pigs (intradermal): virus excretion 1–5 dpi; vesicles from 1–3 dpi.

Detection period, dpi (Table 3)

Animals (virus, route)	Serum RT-PCR	Oral swab RT-PCR	Nasal swab RT-PCR	Antibody ELISA
Pigs, 2016 strain, intraoral	3–5	3–8	3–8	7–8
Pigs, 2019 strain, intraoral	3–5	1–9	3–7	9
Cattle, 2019 strain, intradermal	1–5 (–10)	1–10	1–10	7–10
Pigs, 2019 strain, intradermal	1–5	1–10	1–10	5–10

Ranges cover all animals in the group.

- Lateral flow antigen test confirmed serotype O and A in the field isolates.
- Viral genes, infectious virus and antibodies were all detected with the existing WOAH-standard methods.
- These methods are applicable to the viruses now circulating in Myanmar and the surrounding Asian region.

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Summary

1. From 2016 to 2019, serotype O (ME-SA/Ind-2001e and SEA/Mya-98) and serotype A (ASIA/Sea-97) co-circulated in Myanmar. From 2020 to 2022, all samples belonged to O/ME-SA/Ind-2001e.

2. Whole-genome analysis showed that viruses detected after 2019 form a genetic group separate from the 2016 viruses, together with the Indonesian isolate of 2022.

3. The 2019 strain caused typical FMD in cattle and pigs, with virus excretion comparable to O/JPN/2010. Such a virus is a risk for FMD-free areas.

4. Existing WOAH-standard methods detected infectious virus, viral genes and antibodies. Continued surveillance and rapid diagnosis in Myanmar are important for Southeast and East Asia.

Joint study with the Livestock Breeding and Veterinary Department, Myanmar. Funded by MAFF, Japan. Open access: doi.org/10.1155/tbed/1532487

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Topic 2

Infectious FMD virus isolated from a meat product illegally brought into Japan and confiscated at quarantine inspection

The first isolation of infectious FMD virus from an animal product confiscated by the Animal Quarantine Service of Japan

Kawaguchi R, Odawara N, Nishi T, Fukai K, Kusakari M, Asaoka M, Morioka K. J Vet Med Sci 2026 (advance publication)

Background

Incursion of FMD virus into previously unaffected regions

- 2022: outbreaks caused by O/ME-SA/Ind-2001e in Indonesia, which had been free of FMD for 40 years.
- 2025: outbreaks caused by strains originating from South and Central Asia in Germany, Hungary and Slovakia.
- 2026: serotype SAT1 reported for the first time in China, and subsequently in Mongolia.
- Routes of incursion often remain unclear. Contaminated animal products are regarded as a major risk factor, because the virus is transmitted not only by direct contact but also through products and the environment.

Animal quarantine in Japan

- The last FMD outbreak in Japan was in 2010.
- Import of animal products from many countries is prohibited under the Act on Domestic Animal Infectious Diseases Control.
- The Animal Quarantine Service (AQS) inspects and confiscates illegally brought animal products, with passenger screening, information for travelers and detector dogs.
- Infectious highly pathogenic avian influenza virus and African swine fever virus have been isolated from confiscated products; FMD virus had not.

Objective

- To test meat products confiscated at quarantine inspection for FMD virus genes
- To isolate infectious virus from positive products
- To determine the topotype and lineage of the detected viruses and their relationship with viruses circulating in Asia

Kawaguchi R, Odawara N, Nishi T, Fukai K, Kusakari M, Asaoka M, Morioka K. J Vet Med Sci 2026 (advance publication)

Materials and methods: confiscated meat products

Sample ID	Confiscated item (weight)	Month	Departure	Destination	Transport
R7-03	Sausages (3,990 g)	January 2025	China	Chubu	International parcel
R8-05-1	Rib meat (1,376 g)	January 2026	China	New Chitose	Hand-carried (luggage)
R8-05-2	Leg with hoof (93 g)	January 2026	China	New Chitose	Hand-carried (luggage)

Table 1. Sample information for the confiscated meat products. All were judged not heat-treated by the catalase test; R8-05-1 and R8-05-2 were identified as goat by PCR of the mitochondrial cytochrome b gene.



Fig. 1A. Sausages (R7-03).



Fig. 1B. Rib meat (R8-05-1).



Fig. 1C. Leg with hoof (R8-05-2); no visible vesicular lesion.

Laboratory tests

- Tissue sampling: 31 sites (R7-03), muscle (R8-05-1), epithelium of the main and accessory hooves (R8-05-2); 10% homogenates
- FMDV gene: real-time RT-PCR (3D region). Virus isolation: LFPK-αvβ6 and ZZ-R 127 cells, up to three passages
- Antigen detection kit (seven serotypes) and lateral flow serotyping; VP1 and whole-genome sequencing (nanopore); phylogeny with MEGA 12

Kawaguchi R, Odawara N, Nishi T, Fukai K, Kusakari M, Asaoka M, Morioka K. J Vet Med Sci 2026 (advance publication)

Results: virus gene detection and isolation

Sample (year)	Site	Tissue	rRT-PCR (Ct)	Virus isolation / dpi* LFPK-αvβ6	Virus isolation / dpi* ZZ-R 127
R8-05-2 (2026)	Hoof	Interdigital epithelium	23.9	+ / 1	+ / 2
R8-05-2 (2026)	Hoof	Coronary band epithelium 1–3	27.0–27.8	+ / 2	+ / 2–3
R8-05-2 (2026)	Hoof	Accessory digit epithelium	30.0	+ / 3	−
R8-05-1 (2026)	Rib	Muscle tissue	− (not detected)	not tested	not tested
R7-03 (2025)	3 of 31 sites	Lean meat	36.7–37.0	−	−

Table 2. *Days post-inoculation when cytopathic effect (CPE) was confirmed. +: CPE-positive; −: CPE-negative after three passages.

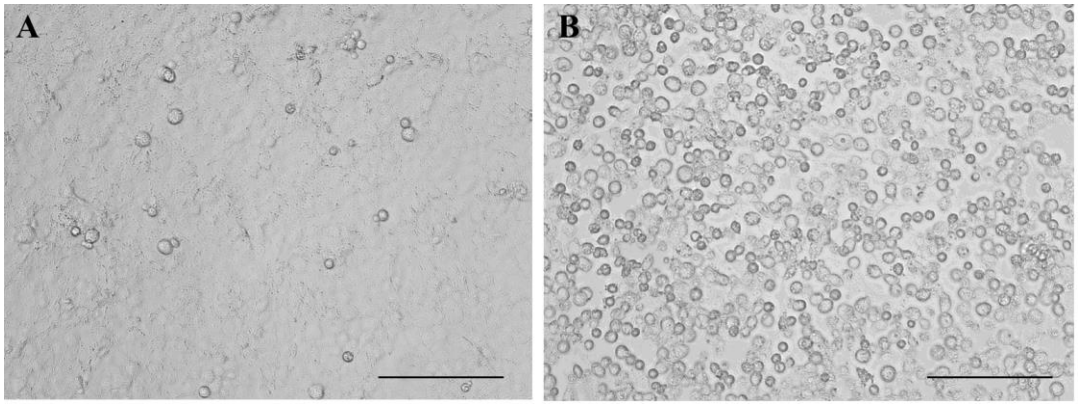


Fig. 2A, B. LFPK-αvβ6 cells: mock-infected (A) and inoculated with interdigital epithelium (B), 1 dpi. Scale bar 150 μm.

Key observations

- Infectious virus was isolated only from the goat hoof epithelium, the site where FMD virus replicates and vesicles form.
- The hoof showed no visible vesicular lesion. In goats and sheep, hoof lesions are often mild or unapparent, so infection may pass unnoticed.
- Muscle tissue was negative: post-mortem acidification of muscle inactivates FMD virus, whereas epithelium, bone marrow and lymph nodes keep a near-neutral pH and the virus can survive.
- Virus was detected in cells inoculated with material equivalent to about 0.1 mg of the original product.

Kawaguchi R, Odawara N, Nishi T, Fukai K, Kusakari M, Asaoka M, Morioka K. J Vet Med Sci 2026 (advance publication)

Results: antigen detection and serotyping of the isolate

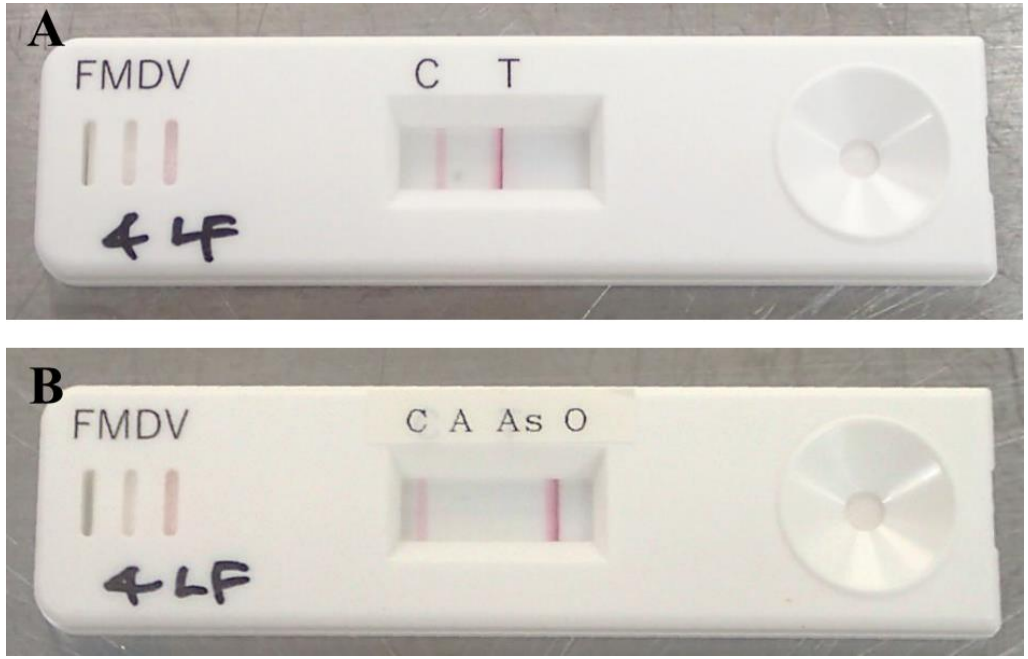


Fig. 3. (A) Antigen detection kit for all seven serotypes: test line (T) and control line (C) positive. (B) Lateral flow serotyping: positive line at O. A, As and O indicate serotypes A, Asia1 and O.

Identification of the isolate

- Supernatants of CPE-positive LFPK-αvβ6 and ZZ-R 127 cells inoculated with the interdigital epithelium of R8-05-2 were positive in the antigen detection kit (all seven serotypes).
- The lateral flow serotyping assay gave a positive line for serotype O.
- **The isolate was designated O/JPN/AQS/2026.**

First isolation of infectious FMD virus from an animal product confiscated during animal quarantine inspection in Japan.

Kawaguchi R, Odawara N, Nishi T, Fukai K, Kusakari M, Asaoka M, Morioka K. J Vet Med Sci 2026 (advance publication)

Results: genetic characterization of the detected viruses

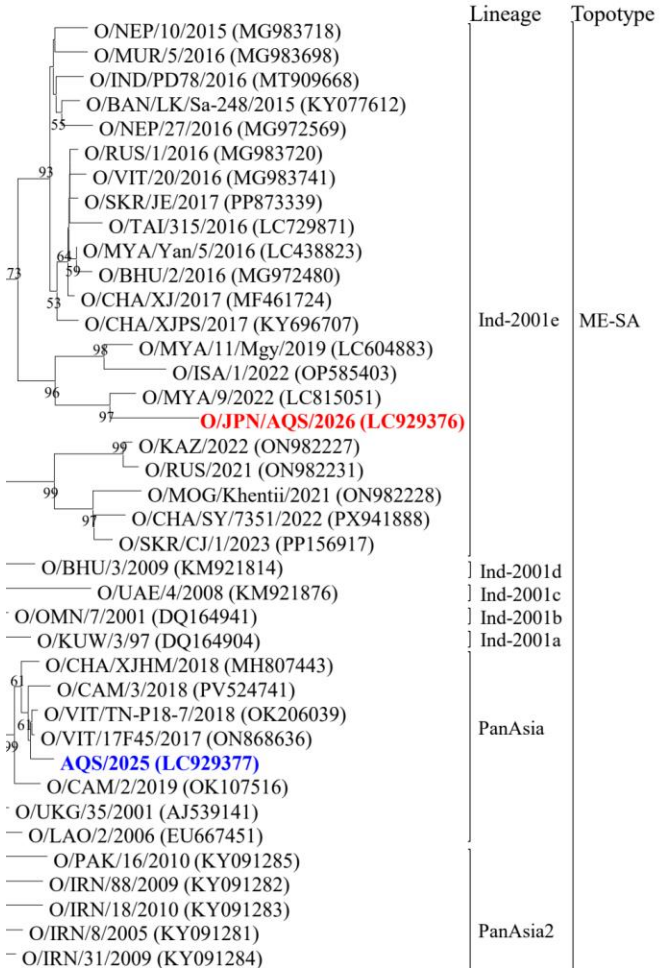


Fig. 4. VP1 tree (ME-SA part; neighbor-joining, 500 bootstraps).

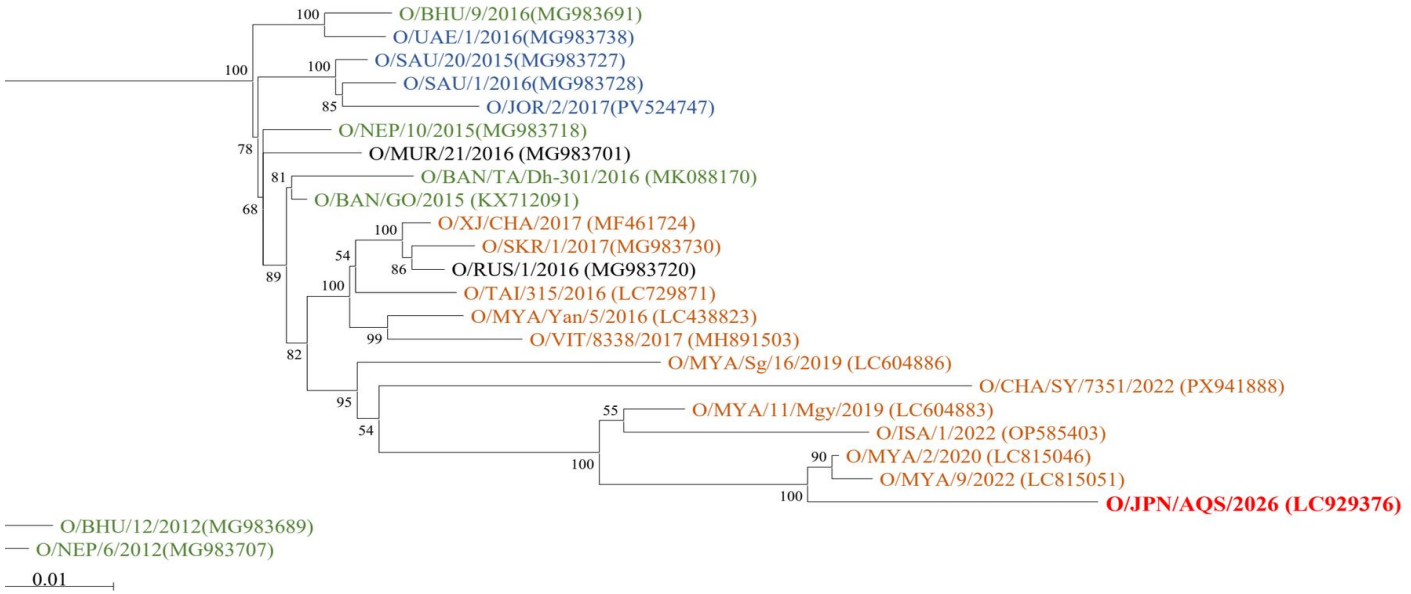


Fig. 5. Whole-genome tree of O/ME-SA/Ind-2001e (maximum likelihood, Tamura-Nei). Colors: Pool 1 orange, Pool 2 green, Pool 3 blue.

Sample	Virus	Topotype / lineage	Closest relative (identity)
R8-05-2 (goat hoof, 2026)	O/JPN/AQS/2026	O / ME-SA / Ind-2001e	O/MYA/9/2022, Myanmar (97.3% VP1)
R7-03 (sausages, 2025)	AQS/2025 (gene only)	O / ME-SA / PanAsia	O/VIT/17F45/2017, Vietnam (99.5% VP1)

- Whole genome of O/JPN/AQS/2026 (7,575 nt) was closely related to isolates from Myanmar in 2019–2022 (96.2–98.0%) and Indonesia in 2022 (95.9%).
- The PanAsia lineage detected in the sausages has not been officially reported since 2023, which suggests that more viruses may circulate in the field than are captured by surveillance.

Kawaguchi R, Odawara N, Nishi T, Fukai K, Kusakari M, Asaoka M, Morioka K. J Vet Med Sci 2026 (advance publication)

Summary

1. Infectious FMD virus (O/JPN/AQS/2026) was isolated for the first time from an animal product confiscated at animal quarantine inspection in Japan: the hoof epithelium of goat meat brought from an endemic region.

2. The hoof had no visible vesicular lesion. Products without gross lesions can still carry infectious virus and act as fomites, while muscle tissue was negative.

3. The isolate belongs to O/ME-SA/Ind-2001e and is closely related to viruses from Myanmar (2019–2022) and Indonesia (2022). A PanAsia lineage gene, not officially reported since 2023, was detected in sausages.

4. Animal products are a cryptic but critical route of transboundary FMD virus movement. Quarantine inspection and border control are essential to prevent incursion.

Joint study with the Animal Quarantine Service, Ministry of Agriculture, Forestry and Fisheries, Japan.

Kawaguchi R, Odawara N, Nishi T, Fukai K, Kusakari M, Asaoka M, Morioka K. J Vet Med Sci 2026 (advance publication)

Reference

Effect of doubled-dose FMD vaccination against heterologous virus infection in cattle

Can a doubled dose of a pre-stocked vaccine protect cattle against field viruses that do not match the vaccine strains?

Kawaguchi R, Nishi T, Fukai K, Ikezawa M, Kokuho T, Morioka K. J Vet Med Sci 2024; 86: 777–786 (open access)

Background

Emergency vaccination in FMD-free countries

- FMD-free countries stock ready-to-use vaccines or antigens for emergency use. Japan had outbreaks in 2000 and 2010.
- A single dose is effective against homologous strains, but a newly emerging virus may be antigenically distant from the stored strains (heterologous).
- Example: serotype A, ASIA topotype, G-VII lineage in the Middle East (2015) was poorly controlled by the vaccines used in that region.
- Trans-pool movement of antigenically distant viruses makes vaccine selection difficult in advance.

Objective

- To test whether a doubled dose of a pre-stocked trivalent vaccine improves protection of cattle against heterologous field viruses of the same serotype.

Stored vaccine and challenge viruses

Stored trivalent vaccine (Aftopor)	Challenge viruses (field strains)
O Manisa (ME-SA topotype) O 3039 or O Campos A22 Iraq (ASIA topotype, A22 lineage) > 6 PD50 of each antigen per 2 mL dose	O/JPN/2010 SEA topotype, Mya-98 lineage (Japan, 2010 outbreak) A/IRN/2016 ASIA topotype, G-VII lineage (Middle East, 2015–16)

- O/JPN/2010: r1 value below 0.3 against O Manisa (above 0.3 against O 3039).
- A/IRN/2016: poorly matched to eleven common serotype A vaccine strains, including A22 Iraq.
- Both challenge viruses are therefore heterologous to the stored vaccine.

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Materials and methods

Vaccination and challenge in cattle

Experiment	Group	Vaccine (intramuscular)	Dose	Cattle	Challenge virus (intradermal, tongue)	Day of challenge
1	1–1	O Manisa + O 3039 + A22 Iraq	Single (2 mL)	4	O/JPN/2010 (SEA/Mya-98)	29–30 dpv
1	1–2	O Manisa + O 3039 + A22 Iraq	Doubled (4 mL)	2	O/JPN/2010 (SEA/Mya-98)	29–30 dpv
1	1-Uv	None	—	6*	O/JPN/2010 (SEA/Mya-98)	—
2	2–1	O Manisa + O Campos + A22 Iraq	Single (2 mL)	2	A/IRN/2016 (ASIA/G-VII)	28 dpv
2	2–2	O Manisa + O Campos + A22 Iraq	Doubled (4 mL)	2	A/IRN/2016 (ASIA/G-VII)	28 dpv
2	2-Uv	None	—	2	A/IRN/2016 (ASIA/G-VII)	—

Table 1. Cattle were 6 months old; vaccine Aftopor, > 6 PD50 per dose; challenge dose 10^6 TCID50 in 1 mL. *Data of unvaccinated cattle from our previous studies. dpv: days post-vaccination.

Observations after challenge (8 days)

- Daily clinical score (max 7): vesicles on each limb, snout and lip, and fever ≥ 40 °C
- Virus titration of serum, nasal and oral swabs (LFPK-αvβ6 cells); FMDV gene detection by RT-PCR
- Total clinical score and total viral load (area under the curve) per animal

Antibody

- Virus neutralization test (BHK-21 cells) on the day of challenge
- Titers measured against both the challenge virus (O/JPN/2010, A/IRN/2016) and the vaccine strain (O Manisa, A22 Iraq)

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Results: clinical signs after challenge

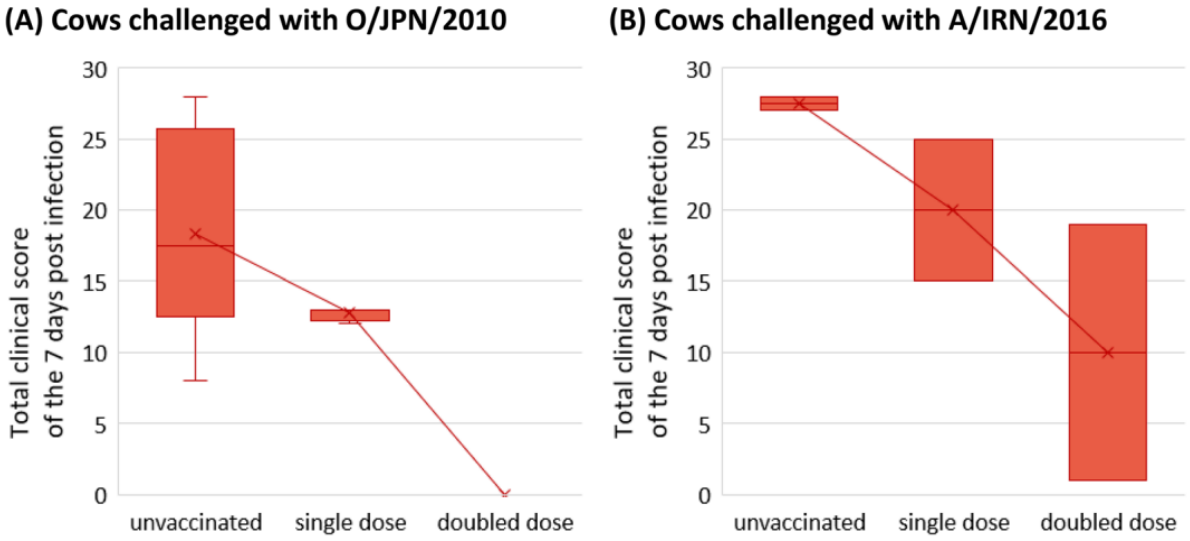


Fig. 3. Total clinical score over 7 days post-infection. × = group average.

- Preventing vesicle formation is the main goal of emergency vaccination: vesicular epithelium and fluid contain large amounts of infectious virus.

Total clinical score (sum over 7 days)

Group	Exp. 1 O/JPN/2010	Exp. 2 A/IRN/2016
Unvaccinated	8–28	27, 28
Single dose	12–13	15, 25
Doubled dose	0, 0	1, 19

Values of individual cattle, or the range within the group.

- All single-dose cattle developed vesicles, but scores were lower than in unvaccinated cattle.
- Experiment 1: both doubled-dose cattle showed no fever, no vesicles, no viremia and no virus excretion (complete protection).
- Experiment 2: one doubled-dose cow had fever and virus in oral swabs but no vesicles; the other developed vesicles as in the single-dose group.

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Results: virus shedding after challenge

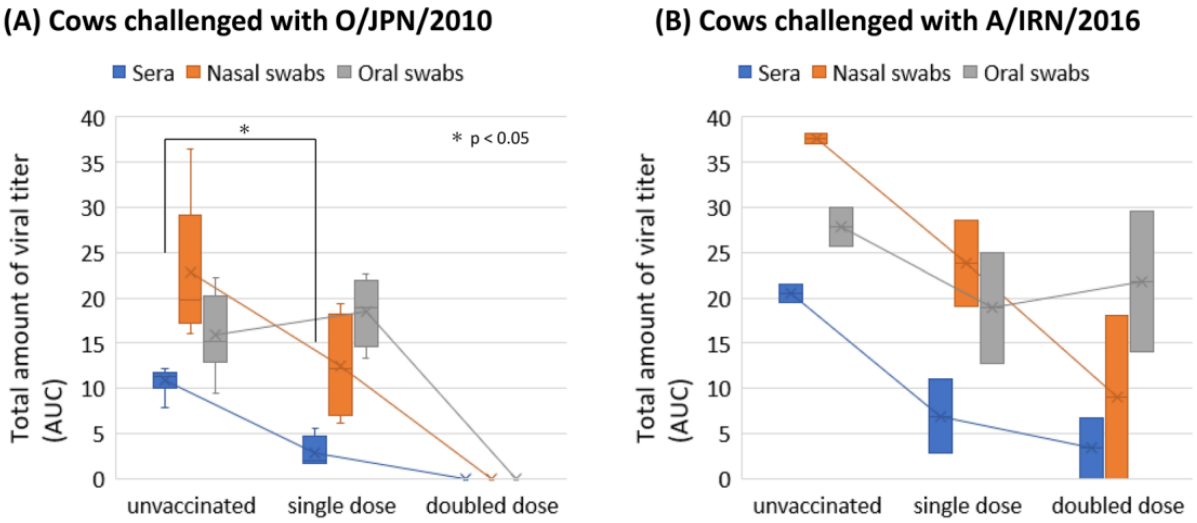


Fig. 4. Total viral titer (area under the curve) in serum, nasal swabs and oral swabs. * P < 0.05.

Effect of the vaccination dose

- Serum: single dose significantly lower than unvaccinated in Exp. 1 (P < 0.05); no virus at all in the doubled-dose group.
- Nasal swabs: viral load decreased as the dose increased, in both experiments.
- Oral swabs: no clear reduction, except the doubled-dose group of Exp. 1. Virus probably leaks from the inoculation site in the tongue.

- Vesicular lesions and nasal discharge contain large amounts of infectious virus. Suppressing them reduces transmission within a herd and between farms.
- A doubled dose reduced both clinical signs and virus shedding against viruses that a single dose did not control.

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Results: virus-neutralizing antibody titers

VN titers on the day of challenge (Table 4)

Exp. 1	Cow	vs. O/JPN/2010 (challenge)	vs. O Manisa (vaccine)	Vesicles
Single dose	171	22.6	< 16	yes
Single dose	172	64.0	64.0	yes
Single dose	173	45.3	181.0	yes
Single dose	174	45.3	45.3	yes
Doubled dose	181	90.5	90.5	no
Doubled dose	182	90.5	128.0	no

Exp. 2	Cow	vs. A/IRN/2016 (challenge)	vs. A22 Iraq (vaccine)	Vesicles
Single dose	221	< 16	512.0	yes
Single dose	222	22.6	724.1	yes
Doubled dose	223	45.3	256.0	no
Doubled dose	224	16.0	362.0	yes

- Cattle that developed no vesicles had the higher VN titers against the challenge virus (90.5 in Exp. 1; 45.3 in Exp. 2).
- Titers against the vaccine strains themselves did not relate to protection: cow 173 had the highest titer against O Manisa but developed vesicles, and the single-dose cattle of Exp. 2 had the highest titers against A22 Iraq but were not protected.
- A doubled dose raised the titer against the heterologous field virus, which is consistent with reports that serum VN titer against the field virus is a better indicator of vaccine efficacy than the r1 value alone.

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Discussion: use of pre-stocked vaccines in an emergency

Implications

- **An option when the stock does not match the emerging virus.**
  - A doubled dose of a serotype-matched vaccine gave complete protection against O/JPN/2010 and reduced clinical signs and virus shedding against A/IRN/2016.
- **A trade-off in the number of animals vaccinated.**
  - With the same stockpile, a doubled dose halves the number of animals that can be vaccinated. In zone vaccination, the balance between dose and coverage needs to be considered in advance.
- **Criteria for choosing the dose are needed.**
  - Rapid measurement of VN titers against the newly emerging field virus can support the decision; the r1 value alone may not be sufficient.

The goal of emergency vaccination is to prevent vesicle formation, which is highly contagious, and to suppress virus spread within herds, farms and regions.

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Summary

1. A single dose of the pre-stocked trivalent vaccine gave only partial protection against heterologous viruses: O/JPN/2010 (SEA/Mya-98) and A/IRN/2016 (ASIA/G-VII).

2. A doubled dose gave complete protection against O/JPN/2010, and reduced clinical signs and virus shedding against A/IRN/2016.

3. A doubled dose raised VN titers against the heterologous field viruses; protected cattle had the higher titers, whereas titers against the vaccine strains did not predict protection.

4. Raising the dose is a practical way to use pre-stocked vaccines against unexpected strains, but fewer animals can be vaccinated; criteria for determining the effective dose are needed.

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Results: whole-genome phylogeny of Ind-2001e viruses

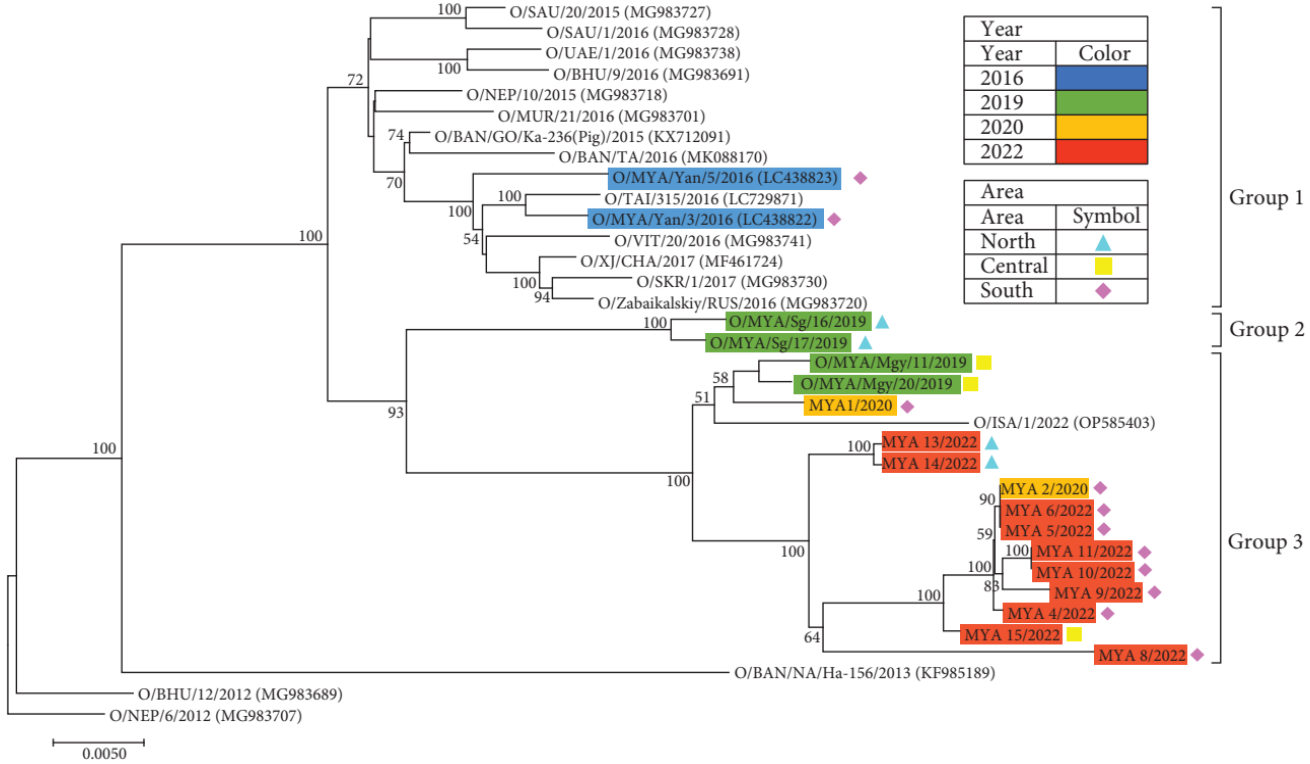


Fig. 3. Whole-genome (ORF) tree of O/ME-SA/Ind-2001e (ML, Kimura 2-parameter). Colored boxes = Myanmar viruses by year; symbols = area.

- 18 Myanmar viruses formed three genetic groups.
- Group 1: 2016 isolates (Yangon). Close to viruses from Thailand, Vietnam, China, Korea and Russia in 2016–2017.
- Group 2: two 2019 isolates from Sagaing.
- Group 3: 2019, 2020 and 2022 viruses from the south, center and north of Myanmar. Includes O/ISA/1/2022 (Indonesia).

- Viruses detected after 2019 belong to a different genetic group from the 2016 viruses.
- Myanmar viruses are linked to viruses that have emerged recently in Southeast Asian countries. Surveillance in Myanmar is important for the region.

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