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Species-specific impacts of radiofrequency exposure on the structural and morphological characteristics of *Aedes aegypti* and *Aedes albopictus* eggs

Nazri Che Dom[1,2,3], Nik Muhammad Hanif Nik Abdull Halim[1,4], Nurul Huda Abd Rahman[5], Rahmat Dapari[6,7]✉, Hasber Salim[8] & Ahmad Taufek Abdul Rahman[9,10]

Background Radiofrequency electromagnetic fields (RF-EMFs) are integral to wireless communication systems, and their potential biological impacts on non-target organisms, such as mosquitoes, remain poorly understood. This study aimed to investigate the effects of RF exposure on the structural and morphological characteristics of *Aedes aegypti* and *Aedes albopictus* eggs, two major vectors of dengue and other arboviral diseases, under controlled experimental conditions.
Methods Laboratory and field strain eggs of *Ae. aegypti* and *Ae. albopictus* were exposed to RF at frequencies of 900 MHz and 18 GHz for 24 h, with unexposed eggs serving as controls. Structural changes were analyzed using scanning electron microscopy (SEM), while morphometric parameters (length, width, egg index, and surface area) were measured using ImageJ software. Statistical analysis was conducted using t-tests, with significance set at $p < 0.05$.
Results Significant structural alterations were observed in the outer chorionic cells (OCC) of eggs exposed to RF, particularly at 900 MHz. *Ae. aegypti* eggs exhibited greater damage, including cell disappearance, surface irregularities, and disrupted exochorionic networks (EN), compared to *Ae. albopictus*. At 18 GHz, structural changes were milder and limited to surface disorganization. Morphometric analysis revealed that *Ae. aegypti* eggs were significantly larger than *Ae. albopictus* eggs in the control group ($p < 0.05$). RF exposure at 900 MHz reduced egg size in both species, while 18 GHz exposure resulted in species-specific effects.
Conclusion This study highlights the frequency-dependent and species-specific impacts of RF exposure on *Aedes* egg morphology and structure. The findings suggest that RF exposure, particularly at 900 MHz, may affect mosquito reproduction and population dynamics, with potential implications for vector control strategies.

Keywords Aedes aegypti, Aedes albopictus, Radiofrequency, Mosquito eggs, Scanning electron microscopy, Vector control

Mosquitoes remain a critical public health challenge worldwide, acting as vectors for diseases that disproportionately affect tropical and subtropical regions[1]. Among them, Aedes mosquitoes, particularly *Aedes aegypti* and *Aedes albopictus*, are prominent vectors of arboviruses such as dengue fever (DF), chikungunya, and

[1]Faculty of Health Sciences, Universiti Teknologi MARA (UiTM), UITM Cawangan Selangor, Puncak Alam 42300, Selangor, Malaysia. [2]Integrated Mosquito Research Group (I-MeRGe), Universiti Teknologi MARA (UiTM), Puncak Alam, 42300 Selangor, Malaysia. [3]Institute for Biodiversity and Sustainable Development (IBSD), Universiti Teknologi MARA, Shah Alam, Malaysia. [4]Setiu District Health Office, Bandar Permaisuri, Setiu 22100, Terengganu, Malaysia. [5]Antenna Research Centre, School of Electrical Engineering, College of Engineering, Universiti Teknologi MARA, Shah Alam 40450, Selangor, Malaysia. [6]Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia. [7]Integrated Dengue Research and Development, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang 43400, Selangor, Malaysia. [8]School of Biological, Sciences, Universiti Sains Malaysia, Penang 11800, Malaysia. [9]School of Physics and Material Studies, Faculty of Applied Sciences, Universiti Teknologi MARA,, Shah Alam 40450, Selangor, Malaysia. [10]Centre for Astrophysics and Applied Radiation, Institute of Science, Universiti Teknologi MARA, Shah Alam 40450, Selangor, Malaysia. ✉email: drrahmat@upm.edu.my

Zika virus. *Ae. aegypti*, commonly referred to as the "yellow fever mosquito," thrives in urban areas, breeding in small, transient water containers, and exhibits a strong preference for close human proximity[2]. In contrast, *Ae. albopictus*, or the "Asian tiger mosquito," predominates in rural and suburban environments[3–5]. Both species are widespread across Malaysia, especially in densely populated regions like Selangor and Kuala Lumpur, where environmental conditions, urbanization, and human activity create ideal breeding grounds. The increased frequency and severity of dengue outbreaks in Malaysia underscore the urgent need for effective mosquito control strategies.

Efforts to control Aedes populations have historically relied on chemical insecticides, yet growing insecticide resistance, environmental concerns, and operational challenges necessitate alternative approaches[6]. Biological and environmental management methods, while promising, often lack the scalability or immediate impact required for large-scale vector control[7]. Against this backdrop, understanding how emerging anthropogenic factors, such as exposure to radiofrequency electromagnetic fields (RF-EMFs), influence Aedes mosquitoes could offer new insights into their biology and control. RF-EMFs, integral to global wireless communication networks, have expanded significantly with the advent of fifth-generation (5G) technologies[8]. In the context of integrated vector management (IVM), understanding RF impacts offers a complementary perspective alongside chemical, biological, and environmental control methods, potentially informing multi-pronged intervention strategies in areas with high RF exposure levels.

Operating at frequencies up to 300 GHz, the higher ranges of these networks overlap with the size of insect bodies, raising questions about their potential biological impact. Although prior studies have explored RF effects on various organisms, including reduced reproductive capacity in fruit flies, impaired navigation in honeybees, and disrupted microbial communities, the specific consequences for mosquito populations remain unclear[9–15]. Limited research on *Ae. aegypti* has revealed inconsistent impacts on flight ability and morphology under RF exposure, but these studies are primarily confined to adult mosquitoes[16]. The effects of RF on earlier developmental stages, particularly eggs, which are critical to the population dynamics of mosquitoes, are poorly understood[17].

In Malaysia, where dengue cases place a significant burden on the healthcare system, understanding the potential role of RF exposure on *Aedes* populations is particularly relevant. Urban areas with high population densities and extensive telecommunications infrastructure may inadvertently influence mosquito behavior and life cycles through RF emissions. This raises critical questions: Could RF exposure alter the development, viability, or structure of mosquito eggs? Might these changes affect the survival and proliferation of Aedes populations, thereby impacting disease transmission dynamics? This study seeks to address these gaps by investigating the effects of RF exposure at 900 MHz and 18 GHz on the structural and morphological characteristics of *Ae. aegypti* and *Ae. albopictus* eggs. By employing high-resolution scanning electron microscopy (SEM), this research aims to elucidate species-specific responses to RF exposure during early developmental stages. The findings will provide foundational insights into how anthropogenic RF exposure influences mosquito biology, with potential implications for innovative dengue vector control strategies.

From a field application perspective, the potential influence of RF exposure on early mosquito developmental stages could have significant implications in urban and peri-urban environments where wireless communication infrastructure is dense. In such RF-rich settings, any structural compromise to eggs may reduce hatching success or weaken subsequent larval development, thereby complementing existing vector control interventions. Unlike conventional insecticide-based methods, which often face operational resistance and environmental constraints, RF-related impacts represent an indirect, passive environmental pressure that could modulate population dynamics without direct chemical intervention. By exploring these interactions, this study not only advances fundamental understanding of mosquito egg biology under RF influence but also offers preliminary insights into a novel, non-chemical dimension of integrated vector management (IVM). This study hypothesizes that the structural and morphological changes in eggs differ significantly between the two species upon RF exposure, reflecting their distinct biological adaptations and susceptibilities.

METHODS

Mosquito strain collection

Laboratory strains of *Ae. aegypti* and *Ae. albopictus* eggs were sourced from the Department of Medical Entomology, Infectious Diseases Research Centre, Institute of Medical Research, Kuala Lumpur, Malaysia. Field strains were collected over a 16-week period (March–June 2022) using ovitrap surveillance in strategic sites with established Aedes populations in Puncak Alam, Selangor. The ovitraps were black plastic containers (8 cm diameter × 11 cm height) with a 4 cm central opening and a 14 cm × 3 cm paddle as oviposition sites. Approximately two-thirds of the containers were filled with water, following established collection protocols[18–20].

Mosquito rearing and cohort establishment

Both field and laboratory strains were maintained under controlled conditions (29 ± 3 °C, 75 ± 10% RH, 12:12 light-dark cycle) in the vector insectary at Universiti Teknologi MARA, Puncak Alam. Field strain eggs were submerged in deionized water to induce hatching, and larvae were identified taxonomically using stereomicroscopy based on key morphological features[21]– [22]. For both species, larval development was monitored daily with nutritional supplementation using fine fish food (TetraMin Plus Tropical Fish Flakes). To ensure virgin adult mosquitoes, sex separation was performed at the pupal stage. Mating pairs (15 males and 15 females) were introduced into rearing cages (30 × 30 × 30 cm) with a 10% sucrose solution. A blood meal was administered using a confined white mouse to facilitate egg production. Eggs were collected daily on filter paper substrates over a week, and the F2 generation was used for experiments to ensure consistent traits[23].

RF exposure setup
A custom-built polystyrene exposure chamber (49.5 cm × 62.0 cm × 37.0 cm) was utilized, equipped with a wideband antenna (A-Info LB8180-NF; 0.8–18 GHz, 12 dB gain). The RF source was a wideband signal generator (Keysight N5173B EXG X-Series; 9 kHz–40 GHz) connected to a super ultra-wideband amplifier. RF signals were transmitted via coaxial cables to the antenna for precise exposure (Fig. 1).

RF exposure protocol
Eggs of the F2 generation were visually inspected for defects using a stereomicroscope before placement on filter paper within plastic containers (16.5 cm × 7.5 cm × 10.0 cm). Eggs were used within 24–36 h post-oviposition to minimize age-related structural changes. Prior to exposure, eggs were partially air-dried for approximately 2 h to standardize surface moisture content. Eggs were exposed to RF frequencies of 900 MHz and 18 GHz for 24 h. The 24-hour exposure duration was selected based on preliminary trials and published studies demonstrating observable ultrastructural changes in insect eggs within this time frame, while avoiding complete desiccation. The 900 MHz frequency corresponds to widely used GSM cellular communication, whereas 18 GHz lies within the millimeter-wave band of emerging 5G networks. Both frequencies were chosen for their environmental relevance in urban areas where Aedes mosquitoes are prevalent. A control group was maintained under identical conditions without RF exposure.

Experimental design
A factorial $3 \times 3 \times 2 \times 2$ randomized design was used. The four independent variables were: (1) RF frequency (900 MHz, 18 GHz, control), (2) exposure duration (24 h, control), (3) mosquito strain (laboratory, field), and (4) species (*Aedes aegypti*, *Aedes albopictus*).

Egg structure analysis
Structural changes in eggs were analyzed using field emission scanning electron microscopy (FESEM; Leo Supra 50 VP, Carl-Ziess SMT, Germany) with a primary beam energy of 3 kV.

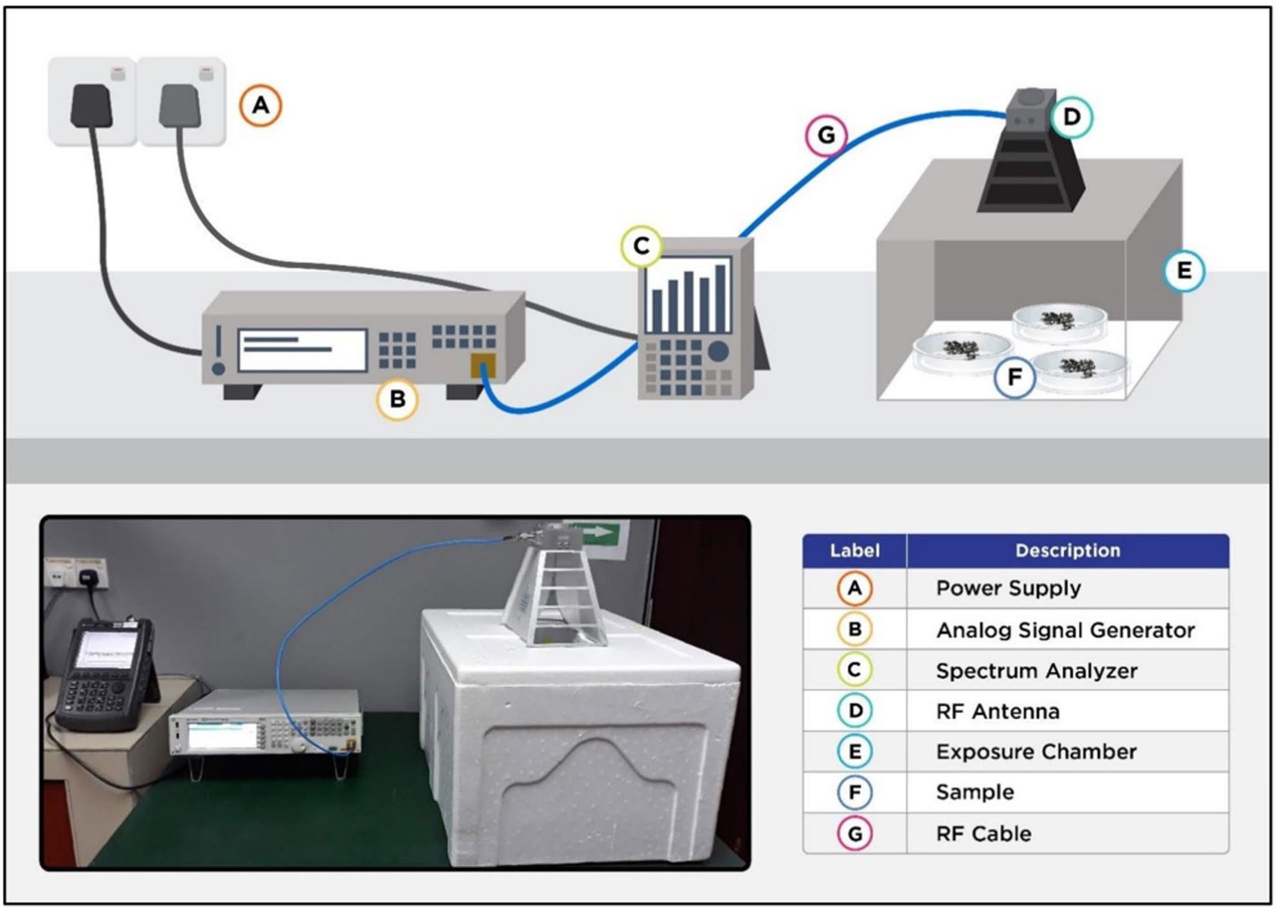

Fig. 1. Experimental setup for RF exposure. The system comprises (**A**) power supply, (**B**) analog signal generator, (**C**) spectrum analyzer, (**D**) RF antenna, (**E**) exposure chamber, and (**F**) sample plates containing mosquito eggs. The RF signal is transmitted from the generator to the antenna via an RF cable (**G**), enabling controlled exposure to specified frequencies (900 MHz and 18 GHz) within the chamber.

Eggs were fixed in 2.5% glutaraldehyde (2 h at 4 °C), rinsed thrice in 0.1 M phosphate buffer (10 min each), post-fixed in 1% osmium tetroxide (1 h at 4 °C), dehydrated through graded ethanol series (30%, 50%, 70%, 90%, 95%, and 100% for 10 min each), and sputter-coated with a 20 nm gold layer before imaging. For each treatment, nine eggs were analyzed, representing three biological replicates (three eggs per replicate) from independent oviposition events. High-resolution images (6,000× magnification) were captured for each treatment group[24–26]. Morphological parameters (length, width, egg index, and surface area) were assessed using ImageJ software based on SEM and light microscope images (Olympus IX73)[27–30]. Measurements were conducted on nine eggs per treatment, with egg index calculated as the length-to-width ratio[31].

RESULTS

Effect of RF exposure on egg structure

The control group revealed well-organized outer chorionic cell (OCC) structures in both species, characterized by polygonal cells (predominantly pentagonal and quadrilateral) and smooth, uniform surfaces (Fig. 2). Notable differences were observed in the exochorionic network (EN), with *Ae. aegypti* displaying a larger, reticulated EN pattern, while *Ae. albopictus* exhibited a solid, perforated EN structure. These findings indicate species-specific morphological markers under natural conditions.

Exposure to 900 MHz RF induced significant structural damage in both species, with distinct degrees of alteration (Fig. 3). In *Ae. aegypti*, the OCC cells became highly distorted or disappeared entirely, and surface abnormalities such as ruptures and debris were prominent. Key OCC components, including the central tubercle (CT), peripheral tubercle (TP), and EN, were poorly defined, and the overall structure appeared disorganized. Similarly, *Ae. albopictus* eggs displayed fragmented OCC structures and surface damage, but the alterations were comparatively less severe. While both species showed signs of RF-induced damage, *Ae. aegypti* exhibited more pronounced effects, including greater loss of OCC cells and disrupted EN patterns.

The effects of 18 GHz RF exposure were relatively mild compared to 900 MHz (Fig. 4). In *Ae. aegypti*, minor inconsistencies in OCC organization and surface texture were observed, while *Ae. albopictus* showed slight disorganization of OCC components. In both species, the CT and TP remained discernible but displayed subtle signs of degradation, and the EN was less cohesive than in the control group. These findings suggest that while 18 GHz RF exposure induces structural changes, its impact on egg morphology is less significant than that of 900 MHz RF.

Effect of RF exposure on egg morphology

Morphometric analysis of egg parameters, including length, width, surface area, and egg index, revealed significant differences between *Ae. aegypti* and *Ae. albopictus* across control and RF exposure groups (Tables 1 and 2).

In the control group, *Ae. aegypti* eggs were significantly longer than those of *Ae. albopictus* in the laboratory strain ($p = 0.04$), while no significant difference was observed in the field strain ($p = 0.74$). Under 900 MHz RF exposure, *Ae. aegypti* eggs consistently exhibited greater lengths than *Ae. albopictus* in both laboratory ($p = 0.04$) and field strains ($p = 0.04$). However, at 18 GHz RF, no significant differences in egg length were observed between the two species ($p > 0.05$). Egg width analysis revealed no significant differences between species in the laboratory strain control group ($p = 0.85$), but in the field strain, *Ae. albopictus* eggs were significantly wider than those of *Ae. aegypti* ($p = 0.03$). RF exposure at 900 MHz and 18 GHz showed no significant effects on egg width for either species in both strains ($p > 0.05$). Similarly, egg surface area differed significantly in the control laboratory strain, where *Ae. aegypti* eggs had a larger surface area than *Ae. albopictus* ($p = 0.04$). However, no significant differences were observed in the field strain under control conditions ($p = 0.30$). RF exposure at 900 MHz did not significantly alter egg surface area in either species, but at 18 GHz, *Ae. albopictus* eggs in the laboratory strain exhibited a significantly larger surface area than *Ae. aegypti* eggs ($p = 0.03$). Egg index values in the control group showed no significant differences between species in the laboratory strain ($p = 0.55$). However, in the field strain, *Ae. aegypti* eggs had a significantly higher egg index than *Ae. albopictus* ($p = 0.01$). Under 900 MHz RF exposure, egg index values were comparable between species for both strains ($p > 0.05$). At 18 GHz, no significant differences were observed in the laboratory strain ($p = 0.48$), but in the field strain, *Ae. aegypti* eggs had a significantly higher egg index than *Ae. albopictus* eggs ($p = 0.03$).

DISCUSSION

This study provides novel insights into the effects of radiofrequency (RF) exposure on the structural and morphological properties of *Aedes aegypti* and *Aedes albopictus* eggs, focusing on two RF frequencies, 900 MHz and 18 GHz. The findings align with the growing concern about the biological impacts of RF exposure, especially in light of rapid urbanization and increasing reliance on wireless technologies. Both species, as significant vectors of dengue fever and other arboviral diseases, are known for their adaptability to urban ecosystems, which makes understanding their susceptibility to environmental factors, such as RF exposure, critical[32].

The results demonstrate significant species-specific responses to RF exposure. The structural integrity of the eggs, particularly the outer chorionic cells (OCC), was compromised at 900 MHz, as evidenced by the disappearance and distortion of OCC cells. This level of RF exposure resulted in visible surface damage, including cracks and debris, particularly in *Ae. aegypti* eggs, which appeared more vulnerable compared to *Ae. albopictus*. These findings corroborate earlier research indicating that RF exposure can induce cellular damage and morphological alterations in insects, suggesting potential implications for mosquito reproduction and population dynamics[33–36]. The differences in susceptibility between the two species may be attributed to physiological variations, such as differences in eggshell structure and exochorionic network (EN) configurations, which appeared more robust in *Ae. albopictus*[37–39]. From an applied entomology standpoint, these findings

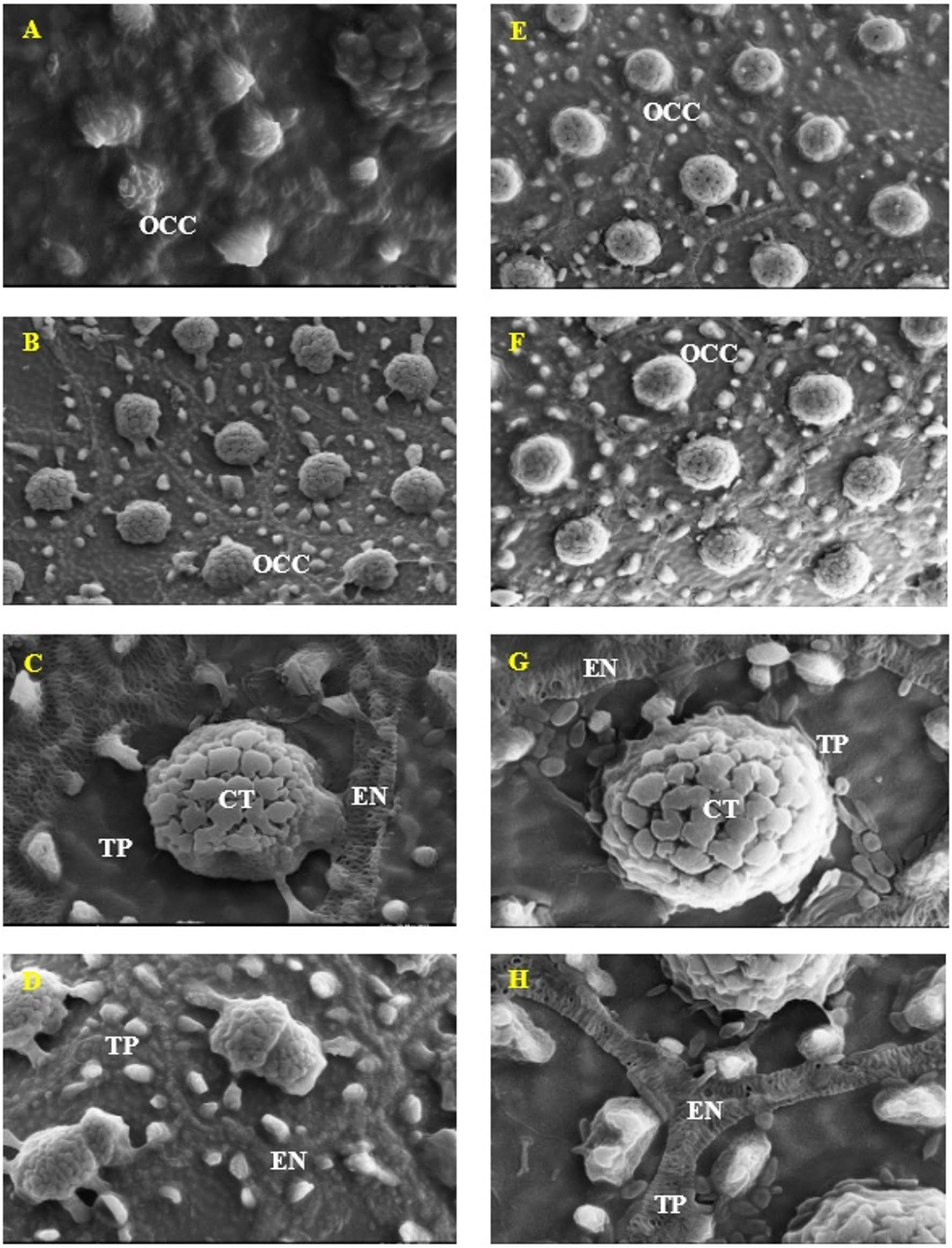

Fig. 2. Scanning electron microscopy (SEM) of eggs of *Ae. aegypti* (**A**–**D**) and *Ae. albopictus* (**E**–**H**) at control RF exposure. The figures represent details of outer chorionic cell (OCC) with central tubercle (CT), peripheral tubercle (TP), and exochorionic network (EN), respectively.

provide a conceptual framework for integrating RF exposure effects into mosquito management considerations, particularly in high-density urban zones where both vector abundance and RF emission levels are elevated. While direct RF-based control is not currently feasible, understanding the species-specific vulnerabilities of eggs can help refine predictive population models and inform risk mapping in urban epidemiology. Such knowledge may be particularly relevant for anticipating how environmental RF 'background noise' interacts

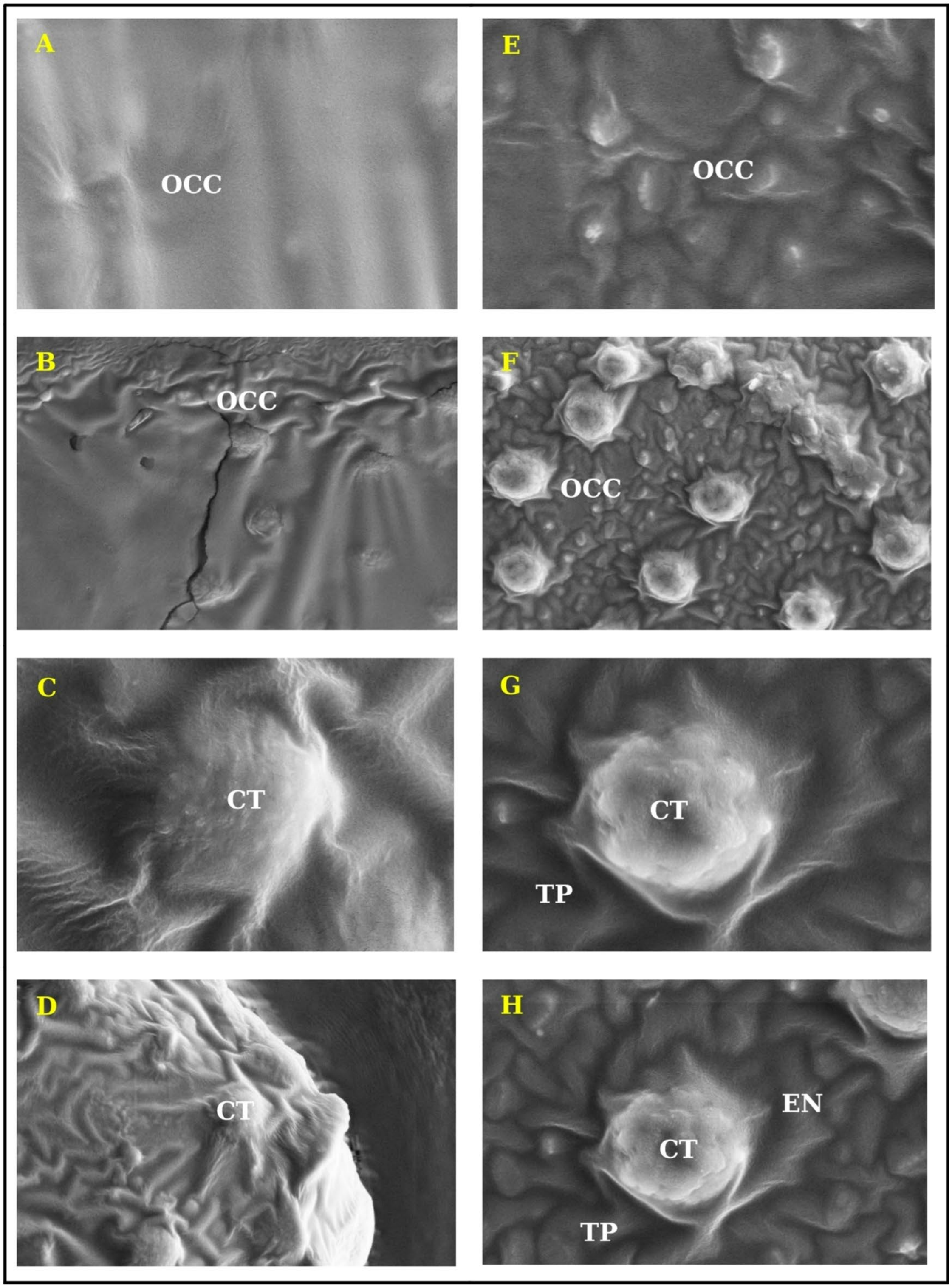

Fig. 3. Scanning electron microscopy (SEM) of eggs of *Ae. aegypti* (**A**–**D**) and *Ae. albopictus* (**E**–**H**) at 900 MHz RF exposure. The figures represent details of outer chorionic cell (OCC) with central tubercle (CT), peripheral tubercle (TP), and exochorionic network (EN), respectively.

with other ecological factors, potentially shaping Aedes egg survival rates and influencing vector-borne disease transmission patterns. This applied relevance directly addresses the importance of the study beyond laboratory settings, despite its current limitations in demonstrating direct field efficacy.

The effects of 18 GHz RF exposure were relatively mild compared to 900 MHz. In *Ae. aegypti*, minor inconsistencies in OCC organization and surface texture were observed, while *Ae. albopictus* showed slight

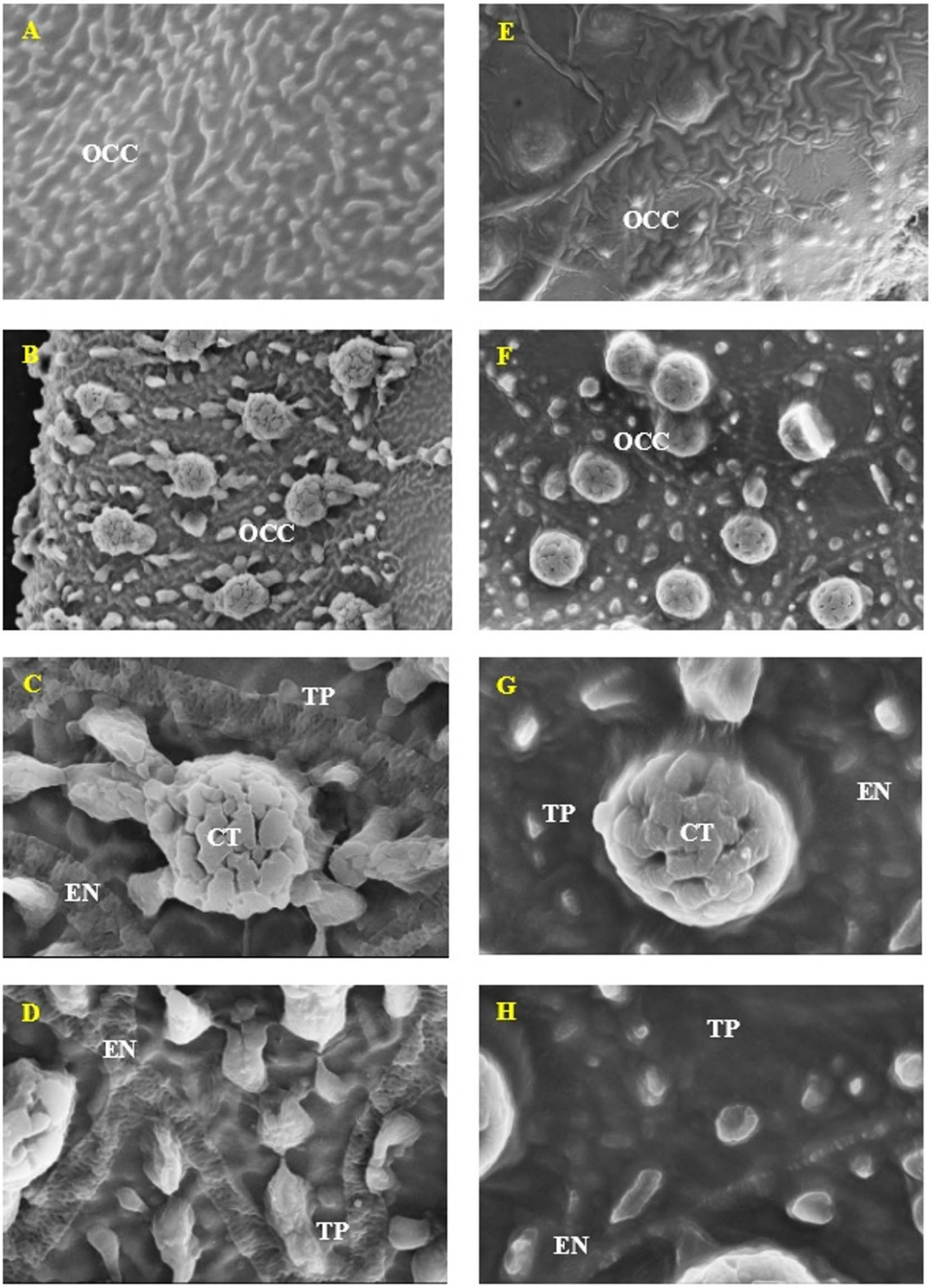

Fig. 4. Scanning electron microscopy (SEM) of eggs of *Ae. aegypti* (**A**–**D**) and *Ae. albopictus* (**E**–**H**) at 18 GHz RF exposure. The figures represent details of outer chorionic cell (OCC) with central tubercle (CT), peripheral tubercle (TP), and exochorionic network (EN), respectively.

disorganization of OCC components. In both species, the CT and TP remained discernible but displayed subtle signs of degradation, and the EN was less cohesive than in the control group. These findings suggest that while 18 GHz RF exposure induces structural changes, its impact on egg morphology is less significant than that of 900 MHz RF. These findings suggest that while 18 GHz RF exposure induces structural changes. It is important to note that EN structures in control eggs appeared less intact than those reported in high-resolution SEM studies

Morphometric characteristics	Control			900 MHz			18 GHz		
	Ae. aegypti	Ae. albopictus	*p* - value	Ae. aegypti	Ae. albopictus	*p* - value	Ae. aegypti	Ae. albopictus	*p* - value
Length (μm)	745.63 ± 19.82	661.24 ± 8.37	0.04**	726.87 ± 23.34	685.76 ± 12.11	0.04**	726.89 ± 34.52	726.20 ± 15.53	0.12
Width (μm)	202.42 ± 16.65	201.20 ± 11.77	0.85	194.32 ± 19.48	176.44 ± 10.24	0.76	198.88 ± 27.49	190.40 ± 8.77	0.66
Egg index (μm)	3.70 ± 0.24	3.49 ± 1.00	0.55	3.76 ± 0.40	4.06 ± 0.88	0.37	3.71 ± 0.50	3.90 ± 0.62	0.48
Surface area (μm²)	751.80 ± 23.75	703.52 ± 2.23	0.04**	726.87 ± 23.34	738.08 ± 3.28	0.08	727.92 ± 36.17	790.24 ± 4.22	0.03**

Table 1. Egg morphology characteristic measurements for the laboratory strain of *Ae. aegypti* and *Ae. albopictus* for each RF exposure. All values are expressed in mean and ± standard deviation (SD). **Significant difference at $p < 0.05$.

Morphometric characteristics	Control			900 MHz			18 GHz		
	Ae. aegypti	Ae. albopictus	*p* - value	Ae. aegypti	Ae. albopictus	*p* - value	Ae. aegypti	Ae. albopictus	*p* - value
Length (μm)	672.71 ± 36.13	666.84 ± 6.34	0.74	702.21 ± 61.59	658.44 ± 11.89	0.04**	689.49 ± 18.98	659.40 ± 7.16	0.11
Width (μm)	200.14 ± 35.26	239.64 ± 5.52	0.03**	211.02 ± 52.44	198.76 ± 14.42	0.12	209.29 ± 26.21	228.24 ± 7.02	0.09
Egg index (μm)	3.47 ± 0.70	2.81 ± 0.30	0.01**	3.56 ± 1.09	3.66 ± 1.43	0.86	3.34 ± 0.40	2.93 ± 0.37	**0.03****
Surface area (μm²)	703.20 ± 62.13	698.24 ± 1.72	0.30	678.35 ± 38.58	674.56 ± 3.00	0.98	685.14 ± 20.55	681.28 ± 1.86	0.10

Table 2. Egg morphology characteristic measurements for the field strain of *Ae. aegypti* and *Ae. albopictus* for each RF exposure. All values are expressed in mean and ± standard deviation (SD). **Significant difference at $p < 0.05$.

of *Aedes aegypti* eggshells, possibly due to fixation artifacts inherent to our preparation method, highlighting the need for methodological optimization[40]– [41]. This may be attributable to fixation-induced artifacts during sample preparation, which we acknowledge as a methodological limitation. Future work should optimize fixation protocols to preserve ultrastructural integrity. Its impact on egg morphology is less significant than that of 900 MHz RF.

Control group analysis revealed significant differences in egg morphology between the two species. *Ae. aegypti* eggs were larger, with a broader and more reticulated EN, compared to the smaller eggs of *Ae. albopictus*, which exhibited a narrower and more solid EN. These differences reflect species-specific adaptations to their respective habitats. The larger size and reticulated EN in *Ae. aegypti* may enhance nutrient and water retention, supporting embryonic development in urban environments. Conversely, the solid EN in *Ae. albopictus* likely provides additional protection against desiccation and environmental stress, consistent with its ability to thrive in diverse habitats, including rural and suburban areas[42–44].

The observed morphological and structural changes in mosquito eggs exposed to RF can be explained by several mechanisms. At 900 MHz, the longer wavelength facilitates deeper energy penetration, leading to localized heating effects, as reported in prior studies. This thermal effect may disrupt cellular structures, alter membrane properties, and increase evaporation rates, resulting in reduced egg size and surface irregularities. On the other hand, the effects of 18 GHz RF were primarily confined to the surface layers, which could explain the milder changes observed in this study. The findings also suggest that the physiological and genetic makeup of the two species may influence their responses to RF exposure[45]. For instance, the thicker eggshells in *Ae. aegypti* may enhance nutrient retention, while the compact EN in *Ae. albopictus* may confer greater resistance to RF-induced damage. These variations align with previous research on the role of eggshell structure in providing mechanical protection and supporting embryonic development in mosquitoes and other insects[46–48].

While our results indicate potential structural and morphometric changes that may influence reproductive success, we emphasize that this study did not directly measure hatching rates or egg viability. Therefore, any inference about reproductive impact should be treated as preliminary and interpreted with caution. Our observations are consistent with reports from other insect taxa where RF exposure has been linked to altered egg morphology and developmental abnormalities. Similar to findings in honeybees and fruit flies, frequency-dependent structural impacts may influence early-stage survival, though the magnitude of effect is species-specific[48–54]. By extending this research to mosquito eggs, this study highlights the potential for RF exposure to influence early developmental stages, which could have downstream effects on mosquito populations and disease transmission.

While this study provides valuable insights, several limitations warrant further investigation. The study focused on two RF frequencies, 900 MHz and 18 GHz, and future research should explore a broader range of frequencies to better understand the spectrum of RF impacts. Additionally, the long-term effects of RF exposure on mosquito life cycles and reproductive success remain unclear. Expanding this research to include other mosquito species and environmental conditions could provide a more comprehensive understanding of the ecological implications of RF exposure.

CONCLUSION

This study underscores the importance of examining the biological effects of RF exposure on mosquito vectors in the context of global urbanization and increasing wireless technology use. The findings highlight significant species-specific and frequency-dependent impacts on the structural and morphological properties of *Ae. aegypti* and *Ae. albopictus* eggs. By bridging gaps in existing knowledge, this research provides a foundation for future studies on the potential role of RF exposure in vector control strategies and its broader implications for public health.

Data availability

All data generated or analysed during this study are included in this published article.

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Author contributions
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Declarations

Competing interests
The authors affirm that they have no known financial or interpersonal conflict that would have an impact on the research work presented in this study.

Data analysis
Statistical analyses were performed using IBM SPSS Statistics (v25). Differences in morphological parameters between RF-exposed and control groups were assessed using t-tests, with significance set at $p < 0.05$.

Ethics statement
This study was conducted in compliance with ethical standards and guidelines for animal research. The research protocol was reviewed and approved by the Committee on Animal Research and Ethics (UiTM CARE) under the reference number UiTM CARE: 377/2022, dated 13th May 2022. The approved duration for the study was from July 2022 to June 2023. All experimental procedures involving animals were carried out with strict adherence to institutional and national ethical guidelines to ensure the humane treatment of the animals involved.

Author contributions statement
N.M.H.N.A.H., N.C.D., N.H.A.R., R.D., H.S., A.T.A.R. wrote the main manuscript text, tables and figures. All

authors reviewed the manuscript.

Additional information

Correspondence and requests for materials should be addressed to R.D.