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Association between seroprevalence of measles virus in monkeys and degree of human-monkey contact in Bangladesh

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ABSTRACT

Measles infections can cause significant morbidity and mortality in human and monkey populations. The endemicity of measles in human populations and viral circulation within populations of free-living monkeys may have important repercussions for potential zoonotic transmission events and for the long-term health of monkey populations. Yet, there has not yet been a rigorous investigation of the dynamics of measles transmission where human and monkey populations coexist. In this study, to determine the difference in seroprevalence of the measles virus across different contexts of human-monkey contact, we analyzed serum samples collected from 56 apparently healthy *Macaca mulatta* monkeys who occupied diverse contexts, with different degrees of human-monkey contact, in Bangladesh. This is the first report of measles virus seroprevalence in monkeys in Bangladesh. We found a clear association between measles virus seropositivity in monkeys and the context in which they interact with humans. Seroprevalence was the lowest in wild areas (0.0%) and increased in shrines (4.8%), urban areas (5.9%), and was highest among monkeys who are used as performance animals (50.0%). This work suggests that a One Health approach informed by local interspecies transmission dynamics is necessary to develop strategies that both improve measles vaccination coverage, achieve long-term surveillance in monkey populations, and prevent measles spillback to monkeys. This approach aims to inform conservation efforts and protect the long-term health of human and monkey populations.

1. Introduction

Measles is a highly contagious febrile disease caused by a virus of the genus Morbillivirus in the family Paramyxoviridae and is easily spread through respiratory droplets [1,2]. The measles virus has the highest basic reproductive number; an infected person can infect between 12 and 18 other individuals [3]. Despite the development of an effective vaccine in the 1960s, measles infection is associated with over 140,000 measles deaths worldwide [2,4]. While humans are natural hosts of the measles virus, viral circulation occurs in populations of free-living monkeys, likely a result of human-monkey transmission followed by within-population transmission [5]. Currently, more than 90% of deaths related to measles occur in Southeast Asia, a geographic area where, coincidentally, the human-monkey interface is diverse and has contexts with different degrees of human-monkey contact [5,6].

The research published to date on the prevalence of measles infections in monkeys has not been sufficient to fully understand the transmission dynamics of the virus between human and monkey populations [5]. The World Health Organization considers humans as the only reservoir [4]. However, studies have shown that measles infection can occur among free-ranging populations of macaques [5,7]. Despite the potential impact that this disease can have on human and animal health until now it has not been possible to establish adequate strategies for its surveillance and control of interspecies transmission [1,8]. For example, Bangladesh, a country that has strengthened its surveillance system, still faces challenges in eliminating continuous small-scale

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outbreaks in humans [1,9], which may spill over to local monkey populations [5,10–13].

Bangladesh is characterized by a high human population density, a high level of poverty, and a tropical climate [9,14]. Additionally, it has many areas with human-wildlife contact, turning this place vulnerable to interspecies pathogens transmission [15]. In addition, Bangladesh is home to one of the most widely distributed monkey species, *Macaca mulatta* [16]. These monkeys are often chosen by locals to perform in circuses, are kept as household pets, and participate in various Bangladesh cultural traditions [16]. Given the importance of these monkeys in local communities, there are many contexts in which they come into contact with humans (i.e. natural areas, urban areas, shrines, and performing environments). These contexts of human-monkey contact are created by the characteristics of the associated human population. Additionally, the degree and extent of human contact with the monkeys varies across contexts regarding time of interactions, proximity, and frequency [5,7,17]. Therefore, it is essential to study the presence of measles virus, a pathogen that poses a significant threat to both humans and monkeys, in areas that allow different degrees of contact between humans and monkeys, and where outbreaks occur regularly [1,6,9].

Laboratory monkeys that get infected with the measles virus show a range of clinical signs from asymptomatic, to mild gastrointestinal signs, secondary bacterial infection, pneumonia, encephalitis, abortion, and to death [8,18]. Wild monkeys, which are known to become infected when they are in contact with infected humans, show temporary immunosuppression similar to or often more acute and severe than it presents in humans [18,19], causing mortality of up to 100% [5]. The study of the seroprevalence of the measles virus in populations of monkeys can allow a better understanding of its transmission dynamic and determine the risk that contact with humans represents for the health of local monkey populations and conservation areas [5].

During the last two decades, infection with the measles virus has been studied in wild monkeys from different parts of Asia such as Indonesia, Singapore, and Nepal [5,20]. For example, in Nepal, researchers reported 100% seroprevalence in *Macaca mulatta* individuals sampled in a temple [5]. However, none of these studies included multiple contexts of human-monkey interactions which would have facilitated a stratified analysis across contexts that offer a gradient of human-monkey contact such as natural areas, urban areas, shrines, and performing environments. Also, the seroprevalence of the measles virus in monkeys in Bangladesh has not yet been determined. Thus, in this study, our objectives were to estimate of seroprevalence of the measles virus in monkeys of the species *Macaca mulatta* in Bangladesh and identify an association between the level of seropositivity and the degree of human-monkey contact.

2. Methods

2.1. Ethics statement

Ethical approval was obtained from the University of Washington's Institutional Animal Care and Use Committee (approval number: 3143–03).

2.2. Study Site

Bangladesh is one of the most densely populated countries in the world. Its geographical location has positioned it for centuries as an important point of Asian and world trade [14,16]. It also has many urban areas located within or near monkey habitats [15,16]. Data collected from seven geographically distinct areas in Bangladesh were included, chosen because they represent areas with defined human-monkey contact [21] (Fig. 1).

2.3. Geographical areas

The seven different geographical areas from which the samples were obtained were chosen based on the human and monkey density, the transportation routes, and the degree of human-monkey contact [15,17].

2.4. Contexts

This study focuses on 4 different contexts: wild areas, urban areas, shrines, and performing environments:

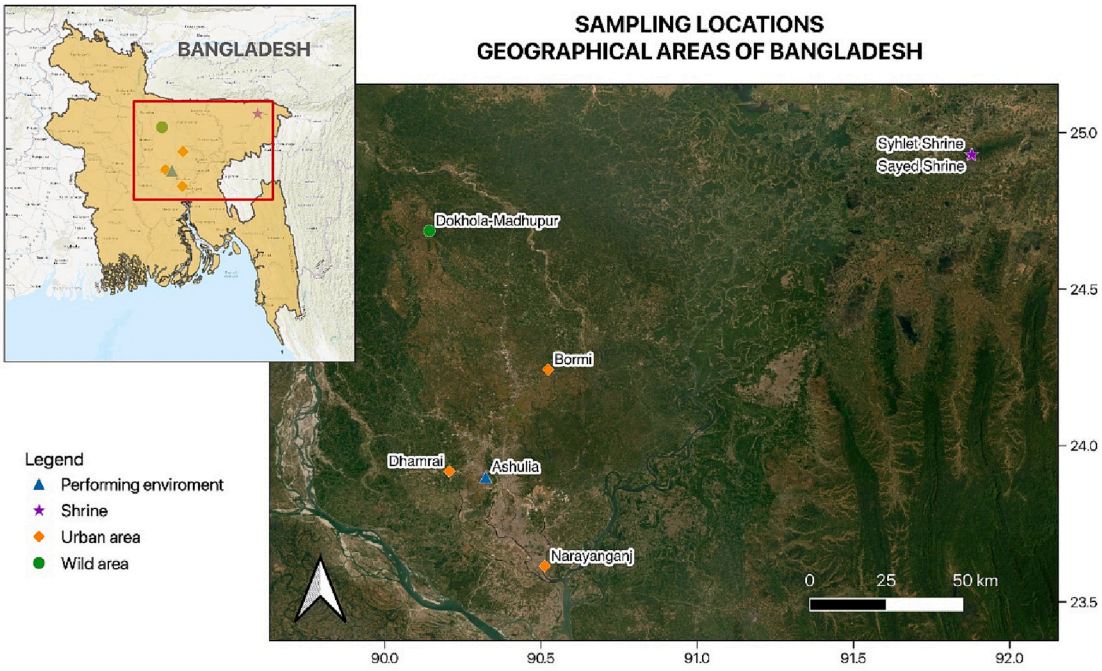

Fig. 1. Geographical areas for monkey sampling according to context in Bangladesh.

i) *Wild areas* typically contain ecotourism attractions. These areas maintain sporadic and casual human-monkey contact [15].
ii) *Shrines* have been reported as one of the places where the most diverse human-monkey contact occurs [15]. These areas are refuges for monkeys [21] that come into direct contact with shrine visitors that often offer food to the monkeys [15].
iii) *Urban areas* contain the highest human population density and the presence of synanthropic monkeys is very common; therefore, they represent areas where the presence of the measles virus in the human population is constant with frequent outbreaks, as measles has not been eliminated from Bangladesh [15].
iv) *Performing environments* are circuses run by the "Bedey" community [15,22]. Bedey people are nomadic and marginalized; 98% of them live below the poverty and have scant access to healthcare and education [15,22]. In these circuses, Bedey individuals own and train the performing monkeys resulting in frequent and prolonged human-monkey contact [22].

2.5. Study population

The study population was chosen by convenience. Study subjects were obtained through capture and/or by agreement with the owners of performing monkeys. A total of 56 (28 females and 28 males) rhesus macaques *Macaca mulatta* were evaluated. All animals were apparently healthy and did not show any signs of disease at the time of capture.

2.6. Capture and Sampling

Physical or chemical immobilization methods were used to capture and restrain individuals in all settings. In the case of performing monkeys, physical restraint was achieved with the help of the owners [16,28]. Free-ranging monkeys were restrained using a remote darting system (Telinject -Agua Dolce, CA, USA) and a Tiletamine–Zolazepam protocol (Telazol 3 mg/kg) [16,17,28]. Blood samples (5-10 ml) were obtained from the femoral vein of physically restrained or anesthetized monkeys, following a complete clinical examination and morphometric measurements [16,17,23,28]. Animals were weighed using manual spring scales of 1, 5, and 15 kg. Age category (older juvenile, subadult, and adult) was determined according to the dental formula [24].

To minimize the risk of accidental injuries due to capture, younger animals and pregnant females were excluded, as they are prone to acute stress conditions [5,15,25]. After sampling and examination, animals were placed in portable boxes (the capture boxes of approximately 2.5 m3, and the recovery boxes of 0.75 m3; manufactured at the National Primate Research Center in Washington), until they were fully recovered from anesthesia to later be released into the area where they were initially trapped [5].

2.7. Sample processing and serology

Blood samples were collected in Vacutainer tubes with EDTA and then separated by centrifugation to obtain the plasma [5]. Plasma samples were placed in containers with ice packs and then stored at −80 °C, to be sent to the Laboratory of the University of Washington, United States of America.

Samples were analyzed using the multiplex immunoassay (MIA) technique [26], which have demonstrated its efficiency, validity, and reliability in serological studies of herd immunity against vaccine-preventable infections to measles, mumps, rubella, varicella-zoster virus and hepatitis B [26,27]. Also, this technique is suitable for large-scale surveillance studies because it requires small amounts of serum, and is faster than the enzyme-linked immunosorbent assays (ELISAs) [27–31]. More importantly, they are more sensitive than the ELISAs and have high specificity [26,32]. In addition, this technique has shown low cross-reaction in multiple studies on viral pathogens [33,34].

2.8. Statistical analysis

The database was imported into the statistical program R [35]. A descriptive analysis was performed to determine the absolute and relative frequencies of each of the variables. In the bivariate analysis, associations between context, geographic area, categorical age, and sex with measles virus seropositivity were determined using the Chi-square test in variables with 10 or more observations per group, Fisher's exact test for categorical variables with fewer than 10 observations in any subgroup. The association between seropositivity and the monkeys' weight was evaluated using a student's *t*-test. We built logistic models for the multivariate analysis. All statistical tests were 2-sided, and the significance level was 0.05.

3. Results

3.1. Demographics of Macaca mulatta in the Bangladesh study

From a total of 56 samples, 50% were males, with an average weight of 7.3 kg (SD = 3.1), and 50% were females, with an average weight of 6 kg (SD = 1.6). The area from which the largest number of samples was obtained was Syhlet Shrine (21.4%) and the area with the least number of samples was Narayanganj (5.4%). The age distribution of the sampled individuals was 17.9% older juvenile, 16.1% subadult, and 66.1% adult (Table 1).

3.2. Measles seroprevalence varies by sex and age

We observed a much higher percentage of seropositivity in the sampled females (21.43%) versus males (3.57%) and this difference was statistically significant (x2 = 4.08; $p = 0.043$). The samples of adult individuals resulted in a higher percentage of seropositivity (16.22%) compared to the other categories (older juvenile 0%, subadult 11.11%), however, this difference was not statistically significant (Fisher's exact test, $p = 0.513$). We did not find an association between weight and seropositivity (Table 2).

3.3. Measles seroprevalence across geographical area

Across geographical areas, the highest measure of seroprevalence was observed in monkeys sampled in Ashulia (50%) compared to the other areas (Bormi 0%, Dhamrai 12.5%, Dokhola Modhupur 0%, Narayanganj 0%, Sayed Shrine 11.11%, Syhlet Shrine 0%), and this imbalance distribution was statistically significant (Fisher's exact test, $p = 0.009$) (Table 2).

3.4. Association between seroprevalence and degree of human-monkey contact

The highest percentage of seropositivity was obtained in the performing environments (50%), compared to the urban areas (5.88%), shrines (4.76%), and wild areas (0%). We found a statistical association between the context of human-monkey contact and the seroprevalence of the measles virus in monkeys in the bivariate analysis (Fisher's exact test, $p = 0.002$) (Table 2) and in the regression models adjusted for other variables (Table 3), providing evidence of higher levels of measles seropositivity in monkeys with increasing degree of human-monkey contact.

4. Discussion

We found a highly significant association between the context of human-monkey contact and measles virus seroprevalence in monkeys. There is a clear gradient between the degree of human-monkey contact that each of these contexts offers and the probability of seropositivity in monkeys. Seroprevalence was the lowest in wild areas and increased in

Table 1
Distribution of *Macaca mulatta* monkeys in Bangladesh according to the context of contact with humans, sex, area, and age.

		Context									
		Performing		Shrine		Urban		Wild		Total	
Variable	Levels	N	%	N	%	N	%	N	%	N	%
Sex	Female	9	16.1%	7	12.5%	6	10.7%	6	10.7%	28	50.0%
	Male	1	1.8%	14	25.0%	11	19.6%	2	3.6%	28	50.0%
	Ashulia	10	17.9%	0	0.0%	0	0.0%	0	0.0%	10	17.9%
	Bormi	0	0.0%	0	0.0%	6	10.7%	0	0.0%	6	10.7%
	Dhamrai	0	0.0%	0	0.0%	8	14.3%	0	0.0%	8	14.3%
Geogr. Area	DokholaModhupur	0	0.0%	0	0.0%	0	0.0%	8	14.3%	8	14.3%
	Narayanganj	0	0.0%	0	0.0%	3	5.4%	0	0.0%	3	5.4%
	Sayed Shrine	0	0.0%	9	16.1%	0	0.0%	0	0.0%	9	16.1%
	Syhlet Shrine	0	0.0%	12	21.4%	0	0.0%	0	0.0%	12	21.4%
Weight	Mean in kg (SD)	10	5.7 (1.3)	21	5.4 (2.3)	17	8.5 (2.6)	8	6.6 (1.8)	56	100.0%
	OlderJuv	0	0.0%	8	14.3%	1	1.8%	1	1.8%	10	17.9%
Age	Subadult	2	3.6%	4	7.1%	3	5.4%	0	0.0%	9	16.1%
	Adult	8	14.3%	9	16.1%	13	23.2%	7	12.5%	37	66.1%
Total		10	17.9%	21	37.5%	17	30.4%	8	14.3%	56	100.0%

Table 2
Measles virus seroprevalence in *Macaca mulatta* in Bangladesh by contextual and individual variables.

Variables	Levels (N)	Seropositivity	95% CI	*p*-value
Context	Performing environment (10)	50.00%	(0.24–0.76)	0.002[a]
	Urban area (17)	5.88%	(0.01–0.27)	
	Shrine (21)	4.76%	(0.01–0.23)	
	Wild area (8)	0.00%	(0–0.32)	
Geographical areas	Ashulia (10)	50.00%	(0.24–0.76)	0.009[a]
	Bormi (6)	0.00%	(0–0.39)	
	Dhamrai (8)	12.50%	(0.02–0.47)	
	DokholaModhupur (8)	0.00%	(0–0.32)	
	Narayanganj (3)	0.00%	(0–0.56)	
	Sayed Shrine (9)	11.11%	(0.02–0.43)	
	Syhlet Shrine (12)	0.00%	(0–0.24)	
Sex	Female (28)	21.43%	(0.10–0.4)	0.043[b]
	Male (28)	3.57%	(0–0.18)	
Weight median (6.3 kg)	Below median (28)	7.1%	(0.04–0.27)	0.760[c]
	Above median (28)	5.4%	(0.06–0.31)	
Age	Older Juvenile (10)	0.00%	(0–0.28)	0.513[a]
	Subadult (9)	11.11%	(0.02–0.43)	
	Adult (37)	16.22%	(0.08–0.31)	

CI = Confidence interval; a = Fisher's exact test; b = Chi-square test; c = t-student test.

Table 3
Association between context and degree of human-monkey contact and measles seroprevalence in *Macaca mulatta* in Bangladesh.

Variables	OR	95% CI	p-value
Context			
Shrine	Ref.	Ref.	Ref.
Urban	0.74	(0.04–13.33)	0.840
Performing	1.16	(1.05–127.35)	0.046
Wild	0.0000003	(0 - Inf)	0.996
Age			
Adult	Ref.	Ref.	Ref.
Subadult	0.48	(0.04–5.99)	0.569
OldJuv	0.000003	(0- Inf)	0.996

OR = Odds ratios estimated with multiple logistic regression; CI = Confidence interval.

shrines, urban areas, and was highest in performing environments. These data suggest that seroprevalence (and exposure) is associated with the degree of human-monkey contact permitted by each context.

Wild areas maintain sporadic and casual human-monkey contact due to ecotourism attractions [15], likely limiting the transmission of the measles virus from humans to monkeys and explaining the observed nil seroprevalence. *Shrines* are visited by many people, who frequently come into direct contact with the monkeys [15]. However, the measles vaccination rates among these visitors are expected to be higher than those in the Bangladeshi population. *Urban* areas contain the highest human population density and measles outbreaks are common since measles has not been eliminated in Bangladesh and measles vaccination coverage is suboptimal in multiple areas [36]. These urban areas offer a greater possibility for monkeys to acquire the virus [15]. *Performing environments* are run by the "Bedey" community. This human community is largely marginalized in Bangladesh and has limited access to healthcare, so measles vaccination coverage is very low among them [37]. In addition, performing monkeys have a different ecology than monkeys from wild, urban, or shrines [38–40]. The performing monkeys are in constant proximity to their owners and family members [39] creating optimal conditions for monkeys to acquire human pathogens [38]. The nomadic performing practices of this group also increase the likelihood that the monkeys will come into contact with other villagers in various parts of the country and the world (tourists), and with other domestic or even wild animals [39]. Our performing monkeys were sampled in Ashulia village, a resting point for performing groups, which explains that high seroprevalence reported for that area [39].

.We observed greater measles seropositivity in female monkeys than in males and this difference was statistically significant. Similarly, although higher seropositivity was observed in older individuals, the present study could not determine that there is a statistical association between the seroprevalence of the measles virus and age in monkeys from Bangladesh. We observed effect sizes and trends that suggest an association, but we failed to detect statistical associations likely because of our sample size. It will be necessary to carry out a larger study to clarify these associations.

Although a few authors consider the monkey as a possible reservoir of the measles virus [40], many others state that the human being is the only reservoir known to date [5,9,11,41]. Our definition of reservoir, shared by others, is a species or a community of species that can maintain transmission of a pathogen indefinitely [42]. For measles persistence, the extremely high transmissibility of the measles virus among humans requires populations greater than 250,000 individuals to maintain transmission, otherwise, the susceptible host population would be depleted. If the same transmission parameters operate in the monkey population, which cannot reach 250,000 individuals, the endemic or persisting transmission of the measles virus would not be possible [41]. However, isolated measles outbreaks in monkey populations can occur following interspecies viral transmission from humans to monkeys [43].

To better understand transmission within monkey populations and

between monkeys and humans, greater sampling efforts of monkey populations will be required, where longitudinal observational health is integrated with non-invasive sampling methodologies (e.g., urine samples or saliva found in food waste). In addition, it will be necessary to develop new strategies to improve the measles vaccination coverage in endemic areas to prevent transmission from human to monkey populations [9]. This approach would aim to protect the long-term health of both human and monkey populations in Bangladesh and other regions where interspecies measles transmission occurs. Additionally, this One Health approach also emphasizes the importance of establishing a measles prevention and control plan that considers both humans and monkeys, understanding that measles virus infection is a serious threat to both groups [2,11,12,43].

We faced some challenges during the execution of this study. Due to the limited access to these animals, part of the sample was self-selected; for performing monkeys the owners had to agree to participate. Also, in the case of free-living monkeys, samples were obtained from individuals captured by traps; both sample schemes can introduce selection bias. It is possible that there are temporal trends in measles virus exposure at the population and individual level, but our cross-sectional study with limited variation in age was not set up to detect those patterns. The sample size was too small for stratification and identifying other associated variables, however this does not alter our conclusions. This is an initial study, which will inform future research in measles virus in monkeys.

The use of MIA to detect measles antibodies in monkeys was a strength of our study. This technique has proven to be particularly valuable for conducting extensive surveillance studies [30] due to its fast, sensitivity and reproducible characteristics [34]. Also, studies where MIA was used to detect Human Papillomavirus, and Arboviruses show no evidence of cross-reaction with other pathogens [33,34]. However, due to the little attention received by the measles virus in monkeys, no studies have reported evaluation of cross-reactivity using MIA. Although, based on the findings obtained in analogous studies, we expected no or minimal cross reactivity—if any—using MIA to detect measles antibodies in monkeys.

The ability of the measles virus to infect macaques has been proven by others using molecular techniques. In 1999, during an outbreak at a primate facility, the measles virus was detected in *Macaca mulatta* using reverse transcriptase-polymerase chain reaction (RT-PCR) [45]. In another measles outbreak in a different primate facility, the measles virus was detected in urine specimens using virus isolation or RT-PCR [11], and viral detection correlated with measles virus-specific IgG and IgM antibodies [11]. Also, multiple strains of measles virus have been identified and compared in *Macaca fascicularis* using RNA analysis from Vero cells culture [46]. Our serological findings are supported by these molecular studies and most likely represent direct exposure of *Macaca mulatta* to the measles virus and evidence of measles spillback to monkeys.

5. Conclusions

We found a clear association between measles virus seropositivity in monkeys and their degree of contact with humans in Bangladesh, which is linked to the context in which these two species interact. Monkeys from wild areas, where interaction between humans and wildlife is minimal, had the lowest measles seroprevalence. Monkeys from urban areas and shrines had relatively similar and intermediate levels of seroprevalence. Performing monkeys had the highest seroprevalence; it is possible that the health status of the Bedey nomad community, which own and train these animals, may be playing a determining role or may help us, at least partially, understand the dynamic of the virus measles in this context. It is recommended to assess the vaccination coverage in the human populations corresponding to the various contexts of human-monkey contact to determine how vaccination coverage influences interspecies transmission. Also, it is recommended to continue evaluating the measles virus in *Macaca mulatta* monkeys through larger longitudinal studies that include non-invasive methodologies and direct observation of the human-monkey contact rates. This may inform more evidence-based prevention strategies for outbreaks of measles in monkeys, and the greater plan for conservation plans of free-living monkey populations.

CRediT authorship contribution statement

Lizzie Ortiz-Cam: Conceptualization, Methodology, Formal analysis, Writing – original draft. **Lisa Jones-Engel:** Investigation, Resources, Data curation, Validation, Writing – review & editing. **Patricia Mendoza:** Visualization, Writing – review & editing. **Ricardo Castillo-Neyra:** Visualization, Validation, Supervision, Writing – review & editing.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

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