

Serological evidence of coronavirus infections in native hamadryas baboons (*Papio hamadryas hamadryas*) of the Kingdom of Saudi Arabia

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SUMMARY

The hamadryas baboon (*Papio hamadryas hamadryas*) is the only indigenous species of non-human primates (NHP) found in the Kingdom of Saudi Arabia (KSA). There are no peer-reviewed publications on viral infections of the baboons of KSA. Apart from camels, other animals are likely sources of the novel Middle East Respiratory Syndrome coronavirus (MERSCoV) for humans. We investigated evidence of highly pathogenic coronavirus infections including MERSCoV in a large group of commensal baboons accompanied by feral dogs, on the outskirts of Ta'if city, KSA, in February 2013. Fifty baboons (16 juveniles and 34 adults) were screened for serum antibodies to human coronaviruses (HCoV-043/-NL63/-229) and canine coronaviruses (CCoV-1-3) using direct Enzyme-linked Immunosorbent Assay (ELISA) technique and for MERSCoV antibodies using Serum Neutralization Test (SNT). Of the 50 sampled baboons, 22% ($n = 11$) were seropositive to HCoVs, 10% ($n = 5$) were seropositive to CCoVs, while none had detectable MERSCoV antibodies. These findings bear potentially significant implications for public health, canine health and baboon conservation efforts, necessitating follow-up investigations and preventive measures at locations where baboons frequent human habitations, or are regarded as tourist attractions, in KSA.

Key words: Commensal, *Papio hamadryas hamadryas*, coronaviruses, infections, Kingdom of Saudi Arabia.

Key Findings

- Across the southwestern region of the kingdom of Saudi Arabia (KSA), large groups of free-living hamadryas baboons exist in a commensal state, side-by-side with human communities.

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- There is no data available on the viral infections of these baboons, or their potential role in the transmission cycle of novel zoonotic viruses such as the Middle East Respiratory Syndrome coronavirus (MERS-CoV).
- We found evidence of seropositivity to coronaviruses of human and canine origin, but not to MERS-CoV, in commensal baboons coexisting with feral dogs around the city of Ta'if, KSA.

INTRODUCTION

The Middle East Respiratory Syndrome (MERS) is the most important emerging infectious disease (EID) of zoonotic and public health importance, in the Middle East and North Africa. The disease is caused by the MERS Coronavirus or MERS-CoV, a positive-sense single-stranded RNA virus, initially referred to as HCoV-EMC/2012 or Human Coronavirus Erasmus Medical Centre/2012. The MERS virus is a lineage C betacoronavirus that causes pneumonia, renal failure and death in 30–40% of human patients [1] and much higher mortalities in people with concurrent diseases such as diabetes, hypertension, chronic cardiac disease and chronic renal disease [2]. More than in any other country, a higher number of MERS-related human morbidities and deaths have occurred in the Kingdom of Saudi Arabia (KSA), with 1582 out of the 1917 (82.52%) laboratory confirmed cases of the disease and 659 out of the 684 (96.35%) associated case fatalities [3, 4]. It is believed that MERS originated as a zoonosis and that dromedaries are the main source of the primary human infection [5]. However, there is growing evidence that other animals apart from camels may have a role to play in human MERS infections. Coronaviruses have the inherent ability to infect more than one host species [6] and MERS-CoV sequences have been detected in faecal pellets of a *Taphosus perforatus* bat in KSA [7]. The receptor dipeptidyl peptidase 4 or DPP4 that confers susceptibility to MERS-CoV has been identified not only in human beings, camels and bats, but also in non-human primates (NHP) such as baboons, rhesus macaques and marmosets [8].

The hamadryas baboon (*Papio hamadryas hamadryas*) is the only species of NHP that is indigenous and endemic to KSA and the rest of the Arabian Peninsula [9]. In the southwest region of the country, these animals exist in the wild state and as commensals along major highways and around big cities such as Abha and Ta'if, locations where primary

outbreaks of human MERS have occurred. Unlike wild baboons, commensal baboons frequently mingle with people, aggregate in large groups and survive mainly on provisioned food and edible wastes scavenged from household refuse bins and communal refuse dumps [10]. As previously observed, frequent exposure or contact between human beings, NHP and other animals may create avenues for cross-species transmissions of pathogens and introduction of new epidemics [11]. Non-human primates are susceptible to autologous as well as human coronaviruses (HCoVs) and such infections may be inapparent, or manifest as enterocolitis and diarrhoea, or pneumonia [12, 13]. The spectrum of clinical signs observed in human MERS victims [14], is similar to those observed in NHPs that are infected with coronaviruses [12, 13]. Whereas, similar genera of intestinal parasites have been identified in stool samples of baboons and people in locations where commensal baboons thrive in the southwestern region of KSA [15], no peer-reviewed data are available on the viral pathogens of the baboons of KSA, or the veterinary and public health implications of such infections.

The commensal baboons at Ta'if share feeding, watering and sleeping sites with feral dogs. We hypothesized that these commensal baboons harbour infections caused by pathogenic coronaviruses originating from human beings (anthroponoses) and dogs, and that the baboons are reservoirs of MERS zoonosis. To obtain baseline information about the exposure of the baboons to pathogenic human and animal coronaviruses, we screened them for serum antibodies to the human coronaviruses HCoV-043, HCoV-NL063 and HCoV-229E, canine coronavirus genotypes 1–3 (CCoV 1-3) and to MERS-CoV.

METHODS

Ethical approval

The research protocol was approved by the Research Ethics Review Committee of the Engineer Abdullah Bugshan Research Chair for Growth Factors and Bone Regeneration (GFBR), King Saud University, Riyadh, KSA. The protocol conformed to national and institutional methods and safety guidelines for the use of wildlife mammals in research. The fieldwork was conducted with the support of the Saudi Wildlife Authority (SWA), Riyadh, KSA and the Prince Saud al-Faisal Wildlife Research Centre (formerly NWRC: National Wildlife Research Centre) in Ta'if.

Study location

The study site was an abandoned dam located on Latitude 21°13'46.6" North and Longitude 40°26'40.9" East of the Greenwich Meridian, 1672 m above sea level and approximately 7 kms southeast of the city of Ta'if. The mean daily temperature, annual precipitation and average humidity at Ta'if are 22.38 °C, 119 mm and 41.1%, respectively [16]. The location is surrounded by hills, the slopes of which served as sleeping sites for baboons and feral dogs. At the time of this study in mid-February 2013, the vegetation of the area was dominated by thorny *Acacia* trees, perennial shrubs (mainly *Solanum incanum* and *Ochradenus baccatus*), and herbs such as *Aerva javanica*, *Argemone ochroleuca* and *Pennisetum setaceum*. The year-round presence of large numbers of hamadryas baboons at the study site can be attributed to the availability in the area of many shady *Acacia* trees, several watering holes and an abundant, year-round supply of provisioned food and edible wastes from open rubbish heaps.

Collection of diagnostic samples

Sample collection was based on opportunistic capture and release of experimental animals. This method was appropriate because the study was not set up for exhaustive statistical analysis. It was rather exploratory and aimed at getting a first glance at what coronavirus infections exist in native baboons of KSA that coexist with feral dogs, at a location where several primary human MERS cases have been recorded. Infant baboons were exempted from the study because the aim was to detect immunity that was active or based on exposure rather than passive (i.e. transplacental or transcolostral). Non-stratified power analysis indicated that a sample of 50 baboons would provide >95% confidence of detecting infections if they were present at a prevalence of $\geq 1\%$.

The animals were lured with fruits and nuts into a purpose-built steel wire mesh cage and were anesthetized with ketamine hydrochloride intramuscular injections. The anaesthetic was administered at 10 mg/kg body weight using gas-propelled darts (Telinject™ Veterinaer Spezialgeraete, Germany). The subjects were identified to species level as previously described [17]. Each baboon was weighed with a hanging Salter scale, sexed by visual inspection of the external genitalia, age classified and examined physically for presence or absence of ectoparasites and signs or lesions

of diseases. The subjects were age classified based on age-related changes in body weights and other physical traits of free-living hamadryas baboons as previously described [18]. The reproductive status of the adult females was determined by abdominal palpation and examination of the mammary glands for evidence of pregnancy and lactation, respectively. To prevent repeated sampling, each baboon was assigned a unique identification code and tagged with indelible ink. Between 5 and 8 ml of blood was collected from each baboon by aseptic venipuncture of the long saphenous vein and immediately transferred into labelled, sterile, plastic Vacutainer® tubes without anticoagulant. The blood collection tubes were placed on ice packs to facilitate clot formation. A piece of dry gauze was placed on the venipuncture site and digital pressure applied to control bleeding. Anesthetized baboons were monitored to full recovery and allowed to rejoin their groups.

Handling and storage of diagnostic samples

The blood samples were centrifuged at 2500 rpm for 15 min. Serum was transferred from each tube with a sterile disposable micropipette into labelled Eppendorf® cryovials, leaving behind the blood pellets. The serum samples and blood pellets were stored at $-70\text{ }^{\circ}\text{C}$ until the time of analyses.

Laboratory investigations

Direct enzyme-linked immunosorbent assays (ELISAs)

The procedure used is as previously described [19], but with some modifications. Ultracentrifuged whole-virus lysates were used as antigens in ELISAs, to screen for antibodies reactive or cross-reactive to CCoV mix (genotypes 1–3) and HCoV mix (HCoV 043/-NL63/-229), (Source: EVL, Woerden, the Netherlands). Virus lysate solution (1.0 m virus/ml Tris–NaCl–EDTA buffer and 0.25% Triton X-100), was added to each well of ELISA plates (675061, Half area, 96 well, Greiner Bio-One, Germany), at a concentration of 2 µg/ml in phosphate-buffered saline (PBS) and incubated overnight at room temperature (RT). The plates were washed (5×) with PBS, 0.1% Tween 20 and blocked for 1 h at RT with a mixture comprising 1.0% casein (SC5890, Sigma-Aldrich, St. Louis, MO, USA) in PBS. Serum samples, heat inactivated for 30 min at 56 °C were diluted 1 : 100 in

PBS, 1% casein, 0.1% cell lysate of uninfected cells, and 0.1% Tween 20 and added to the wells and incubated for 1 h at 20 °C. Wells were washed (5×) and conjugate solution comprising 1/1000 Protein G (539305, Calbiochem, San Diego, USA) was added followed by incubation for 1 h at RT. Plates were washed again and the substrate, BluePhos Phosphatase substrate (50-88-05/-6, KPL, Gaithersburg, MD, USA), was added to the wells. The reaction was stopped with 2 N NaOH and the plates read at 595 nm wavelength on a Bio-Rad model iMARK™ microplate absorbance reader (Hercules, CA, USA). Blank values were subtracted by the pre-programmed microplate reader from all optical density (OD) values. OD values greater than four times the mean of the negative controls were considered positive. Values less than two times the mean of the negative controls were considered negative. All values in-between were regarded as intermediate or equivocal responses.

Serum neutralization test (SNT) for MERSCoV

The test was performed using Vero B4 (MERSCoV) cells as previously described [20], with recent modifications [21]. Neutralization tests were performed in a 96-well format to reduce the required volumes of sera. Reactions contained 50 PFUs of MERSCoV (EMC/2012 strain) in 25 µl of medium mixed 1:1 with baboon serum diluted in 25 µl serum-free Dulbecco minimum essential medium. The starting dilution was 1:40. After incubation for 1 h at 37 °C, each well was infected for 1 h at 37 °C with a 50 µl virus–serum mixture. Supernatants were removed and fresh complete Dulbecco minimum essential medium was added. Assays were terminated by fixation with 8% paraformaldehyde for 30 min and stained with crystal violet after 3 days. Neutralization titres were defined as serum dilutions reducing cytopathic effects in two parallel wells.

Statistical analyses

Results were tabulated using Microsoft Excel 2013 software and analysed using IBM Statistical Package for the Social Sciences Version 20 program (SPSS v.20). Descriptive statistics of the body weights of the different age classes of baboons were summarized as means ± s.d., while that of positive, negative and equivocal sera were expressed as percentages, calculated according to the age class and sex of the baboons. To estimate the variation in prevalence of

HCoV, CCoV and MERSCoV antibodies in the sampled baboons, two explanatory variables (age class and sex) were tested for statistically significant associations with serological status of the animals, using χ^2 test or Fishers exact test. The *P*-value for statistical significance was set at <0.05.

RESULTS

Age and sex attributes of commensal baboons sampled at Ta'if, KSA

The sexes, age estimates and body weights of the subjects are presented in Table 1. A total of 50 hamadryas baboons were sampled and these included 22 males (16 Juveniles and 6 adults) and 28 adult females. Of the six adult males, four were group leaders or alpha-males. None of the female baboons was perceptibly pregnant, but two were lactating while four showed oestrus signs such as vulval swelling and reddening.

Prevalence of HCoVs, CCoVs and MERSCoV in commensal baboons of Ta'if

Of the 50 hamadryas baboons, 22% (*n* = 11) were seropositive to HCoVs (Types -043/-NL63/-229) and 10% (*n* = 5) to CCoV mix (Types -1/-2/-3). None of the subjects was found to be positive for MERSCoV neutralizing antibodies (Table 2). There was no significant association between age class and seropositivity of the baboons to HCoVs (Fisher's test: *P* = 1.0) or CCoVs (*P* = 0.163). There was also no significant association between sex and seropositivity of baboons to HCoVs (Fisher's test: *P* = 0.734) or CCoVs (Fisher's test: *P* = 0.059), although the ELISA results appeared to suggest a higher predisposition of female baboons to CCoV infections (Table 3).

DISCUSSION

This study was designed to generate baseline information about the exposure of hamadryas baboons in Taif, Saudi Arabia to pathogenic human and animal coronaviruses. A sample of 50 baboons was screened for serum antibodies to the human coronaviruses HCoV-043, HCoV-NL063 and HCoV-229E, canine coronavirus genotypes 1–3 (CCoV 1–3) and MERSCoV. We found serological evidence of coronavirus activity in the baboon population in the study area. To the best of our knowledge, this is the first report of naturally-occurring coronavirus infections of the indigenous baboons of KSA. The subjects

Table 1. Characteristics of commensal hamadryas baboons (n = 50) sampled at Ta'if in February 2013

Age class	Traits*	Number (percentage of total sample)	Body weight (mean \pm s.d.) [†]
Juvenile two males (3.0–4.8 years)	Brown pelage; dolichocephalic (face with long, dog-like snout unlike that of infant baboons); body shape similar to adults	4 (8)	6.75 \pm 0.5
Juvenile three males (4.8–6.8 years)	Nearly adult size; very early stages of a mantle evidenced by growth of longer hairs at the sides of the head	12 (24)	10.33 \pm 2.06
Adult males (>10.3 years)	Mantle and hair at the sides of the head fully developed and silvery grey	6 (12)	18.92 \pm 3.53
Adult females (>5.6 years)	Evidence of regular oestrus cycle, pregnancy or lactation	28 (56)	10.46 \pm 2.12

* Age-related morphological changes observed in free-living Hamadryas baboons (Jolly [17]; Sigg *et al.* [18]).

[†] Calculated from values recorded during the fieldwork in Ta'if, KSA.

Table 2. Coronavirus infections detected in commensal hamadryas baboons (n = 50) sampled at Ta'if, Saudi Arabia, in February 2013

Diagnostic test	No. of baboons testing positive (%)	No. of baboons testing equivocal (%)	No. of baboons testing negative (%)	Total (%)
Anti-CCoV 1–3 ELISA *	5 (10)	0 (0)	45 (90)	50 (100)
Anti-HCoV mix (043/NL63/229) ELISA *	11 (22)	0 (0)	39 (78)	50 (100)
MERSCoV-SNT *	0 (0)	0 (0)	50 (100)	50 (100)

ELISA, enzyme-linked immunosorbent assay; SNT, Serum Neutralization Test; CCoV 1–3, canine coronavirus genotypes 1–3; HCoV 043/NL63/229, Human Coronaviruses 043, NL63 and 229; MERSCoV, Middle East Respiratory Syndrome Coronavirus

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were drawn from a large group of commensal baboons that mingled freely with feral dogs at the vicinity of a dam that served as a tourist site in Ta'if. This environment might have allowed exposure of the baboons to pathogenic coronaviruses of human beings and dogs and also MERSCoV.

The adoption of opportunistic sample collection in this study was consistent with disease surveys in free-living wildlife, when there is insufficient data to guide the use of systematic sampling methods [22]. We used specially designed immunoassays that could detect antibody to more than one pathotype of HCoV or CCoV in NHP sera. To confirm MERSCoV exposures in the baboons, the specific and confirmatory MERSCoV-SNT assay [20, 21] was used. The results constitute baseline health assessment data on pathogenic coronavirus infections of commensal hamadryas baboons that are indigenous to KSA and justify the need for a comprehensive survey to evaluate the health risk to baboons, human beings and dogs where the habitats of all three groups meet and overlap.

Transmission dynamics of coronavirus infections in commensal baboons cohabiting with human beings and dogs at Ta'if, KSA

The susceptibility of macaques and baboons to infections caused by autologous coronaviruses and the antigenically related human coronavirus HCoV-043 (anthroponosis) was reported [12, 13]. The high degree of genetic, anatomical and physiological similarities that exist between baboons and human beings predispose to cross-transmission of pathogens between the two groups [23]. We detected serum antibodies to HCoVs in 22% of the free-ranging commensal baboons sampled at Ta'if (Table 2). However, a previous study [9] reported a much higher HCoV seroprevalence (about 50%) in caged macaques and baboons concluding that close and prolonged exposure or contact between the infected and non-infected members held together in confinement can trigger higher transmission rates of coronavirus infections. HCoVs are spread efficiently by the respiratory route [24]. Therefore

Table 3. Distribution of selected coronaviral infections in commensal hamadryas baboons (n = 50) sampled at Ta'if, Saudi Arabia, in February 2013

Virus	JM-2 (%)	JM-3 (%)	ADM (%)	ADF (%)	Total (%)
CCoV 1–3	0 (0)	0 (0)	0 (0)	5 (10)	5 (10)
HCoV-043/ NL63/229	2 (4)	1 (2)	1 (2)	7 (14)	11 (22)
MERSCoV	0	0	0	0	0 (0)

JM-2, juvenile 2 male; JM-3, juvenile 3 male; ADM, adult male; ADF, adult female; CCoV 1–3, canine coronavirus type 1–3; HCoV 043/NL63/229, human coronavirus strain 043, 063 and 229; MERSCoV, Middle East Respiratory Syndrome Coronavirus; %, number of seropositive baboons belonging to the group expressed as a percentage of the total number of baboons sampled.

close exposure to humans rather than direct contact may sufficiently predispose NHP to anthroponotic HCoV infection. In contrast, animal coronaviruses are spread more efficiently by direct contact and the faecal-oral route [25]. We detected serum antibodies to CCoVs only in adult female baboons (Table 3). No juvenile females were sampled throughout the duration of the fieldwork at Ta'if. Female baboons at Ta'if were observed to be more directly involved in the adoption and grooming of puppies that were kidnapped by errant baboons, usually the alpha males. It has been reported that young puppies <2 months of age are particularly prone to CCoV infections [25] and that female baboons, especially paternal or maternal relatives rather than unrelated females, tend to groom one another more closely than their male counterparts [26]. These reports [25, 26] may explain the high frequency of CCoV infections we detected in female baboons.

Significance of the findings for baboon conservation, public health and canine health

Although the baboons that we screened had no overt clinical signs or lesions of disease, the results of this study imply health risks to susceptible human beings and domestic dogs that are exposed to or come in direct contact with the baboons. In children, the elderly and immunocompromised individuals, HCoV-043, HCoV-NL63 and HCoV-229 can cause severe upper and lower respiratory tract disease [24, 27]. According to surveys, between 5% and 30% of all human respiratory tract infections are caused by HCoV-NL63 and

HCoV-229 [27, 28]. Clinical and *in vitro* studies have revealed that HCoVs may represent an important aetiological factor in the pathogenesis of demyelinating disease such as multiple sclerosis and acute disseminated encephalomyelitis in humans [29, 30].

Phylogenetic analysis of the spike (S) gene sequence of group 2 coronaviruses such as CCoV-3 point to a common ancestry of these viruses and multiple host-species shifts, some zoonotic [31]. However, there is currently no evidence that immunocompetent human adults are at risk of contracting CCoV infections [32]. Canine coronaviruses are enzootic in dog populations across the world and dogs of all age groups and breeds are susceptible. Dogs infected with CCoV-1 or CCoV-2 (group 1 or alphacoronaviruses) may develop clinical signs including anorexia, diarrhoea, emesis and dehydration. Although CCoV-1 is typically self-limiting and results in few mortalities in dogs, in conjunction with CCoV-2, it runs a severe and rapidly fatal course in young puppies <2 months of age [33] and for older dogs with concurrent Canine distemper virus and Canine parvovirus type 2 infections [34, 35]. Canine coronavirus genotype 3 (CCoV-3) alone or in conjunction with parainfluenza virus, adenovirus, distemper virus, herpesvirus, influenza virus, *Bordetella bronchiseptica*, *Mycoplasma* spp. and *Streptococcus zooepidemicus*, can cause an acute respiratory tract infection and pneumonia of dogs [36]. To the best of our knowledge, this study is the first to report specific antibodies against CCoVs in free-living baboons. Because the subjects were apparently healthy, further investigations are required to demonstrate the status of infection of CCoVs in NHP and to determine if CCoVs evoke clinical illness or mortalities in NHP, or if infected primates shed the viable virus.

Although baboons have the DPP4 receptor that confers susceptibility to this MERSCoV as previously reported [8, 37], we found no evidence of MERSCoV infection in the baboons that we screened. This might not be unconnected with the sample size and limited geographical area covered in this exploratory study.

CONCLUSIONS

As a follow-up to this study, we recommend further screenings of the baboons in KSA and detailed molecular characterization of their viral pathogens. To safeguard human health in communities that are contiguous with the habitats of commensal baboons in KSA, it is imperative to educate members of the public on the health risks associated with human

exposure or contact with baboons and renew efforts at enforcement of extant legislations aimed at reducing baboon provisioning and commensalism. In places such as Ta'if, homes should be adequately fortified to prevent incursions by commensal baboons and dog owners should be encouraged to vaccinate their pets against canine distemper, canine parvovirus enteritis and canine infectious hepatitis, since CCoV infections are usually more severe in dogs that already harbour these diseases.

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CONFLICT OF INTEREST

None.

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