

Presence of antibodies but no evidence for circulation of MERS-CoV in dromedaries on the Canary Islands, 2015

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In 2012, a new betacoronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV), was identified in humans. Several studies confirmed dromedary camels to be a potential reservoir and a source for human infection. Camels located on the Canary Islands were included in those studies and ca 10% of them were positive for MERS-CoV-specific antibodies. However, these findings could not be correctly interpreted because epidemiological information was not provided. Thus, further investigations were necessary to clarify these results. A total of 170 camels were investigated in this survey, of which seven (4.1%) were seropositive by ELISA. Epidemiological information revealed that all seropositive camels had been imported from Africa 20 or more years prior. We conclude that seropositive camels had contact with MERS-CoV in Africa and that there is no shedding of the virus among camels or people around the farms on the Canary Islands. However, the presence of antibodies in the camel herds should be monitored.

Introduction

In 2012, a new betacoronavirus, Middle East respiratory syndrome coronavirus (MERS-CoV), was identified in patients suffering from severe respiratory disease in the Middle East. As of 11 September 2015, 1,545 cases of laboratory-confirmed MERS have been reported to the World Health Organization (WHO) [1].

MERS-CoV has been shown to be phylogenetically related to bat coronaviruses [2,3] but the origin of MERS is still unclear. Dromedary camels were found to harbour specific antibodies against MERS-CoV, identifying them as the main potential reservoir, at least among the animal species investigated [4]. Comparison of genome sequences between viruses isolated from infected humans and camels indicated that there is transmission of MERS-CoV from camels to humans [5,6]. Moreover, in a nationwide serological survey evaluating 10,009 patients in Saudi Arabia, 15 were seropositive. Compared with the general population,

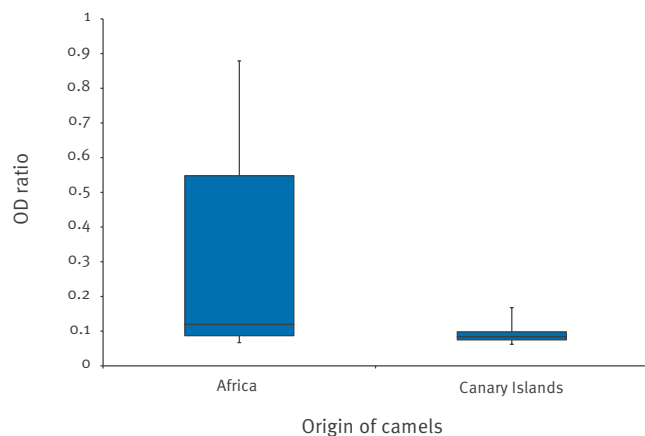
seroprevalence was 15-fold higher in shepherds and 23-fold in slaughterhouse workers [7]. However, a recent study failed to find a correlation between exposure to infected camels and serology in humans, reporting that zoonotic transmission of this virus from dromedaries is rare [8]. MERS-CoV and related genetic material were identified in camels in countries in the Middle East and North Africa. The implications of these findings for management and control recommendations would need further investigation [9].

A serological survey investing camels as the likely source of zoonotic transmission of MERS-CoV included sera from dromedary camels from Oman as well as the Canary Islands to compare regions affected and not affected by MERS [4]. All of the Omani camels had antibodies to MERS-CoV. Surprisingly, also ca 10% of the Canary camels were seropositive, which confirmed that they must have had previous contact with MERS-CoV. However, from an epidemiological viewpoint, the significance of these findings was limited because the animals were not well identified. The sampled camels came from the same farm and epidemiological information was not provided. Nevertheless, the media impact was enormous and drastically affected the camel trade in the Archipelago. These findings could potentially have a serious impact on human and animal health on the Canaries and, given the continuous movement of people from and to the Islands (ca 14 million tourists in 2014), also by extension, in Spain and the rest of Europe. Thus, further investigations seemed to be needed to clarify the presence of antibodies in camels on the Canaries.

The purpose of this study was to investigate the presence of MERS-CoV in camels on the Canary Islands, evaluating the prevalence and the epidemiology of the disease in order to implement sanitary and/or control measures if necessary.

FIGURE

Anti-MERS-CoV ELISA (IgG) in camels, by their origin, on the Canary Islands, 2015 (n = 170)



OD: optical density.

Photometric measurements at 450 nm, Camels born in Africa (n = 17): min: 0.067; median: 0.1195; max: 0.879. Camels born on the Canaries (n = 153): min: 0.062; median: 0.084; max: 0.168.

Methods

Animals

We carried out a transversal study between January and February 2015 including 170 dromedary camels from the four islands (Gran Canaria, Lanzarote, Fuerteventura and Tenerife) on which camels are kept. Per island, we selected 40 to 45 camels from at least two different farms. Sampling size was calculated as a minimum of 138 individuals, based on the 95% confidence level, 5% of desired absolute precision and 10% of expected prevalence [10]. With a current census of ca 1,500 camels, the sampling size of 170 individuals was ca 11% of the total camel population of the Islands.

Information taken at farm level was the following: island, farm location, owner, animal identification, place of birth (Canary Islands vs Africa), age and sex. We selected camels with a placid temperament in order to avoid unnecessary suffering of the animals or possible accidents of the handling personnel. Camels showing clinical signs of any disease or without official identification were excluded from this study.

Serological analysis

Blood sera were analysed using the anti-MERS-CoV ELISA Camel (IgG) manufactured by EUROIMMUN AG (Lübeck, Germany) to detect specific antibodies. This test is based on the S1 antigen of MERS-CoV and has successfully been used by other authors evaluating MERS-CoV in camels [11].

Statistical analysis

Data were entered in a database and statistically processed using Epi info version 3.031, an open source epidemiological statistics software for public health (http://www.openepi.com/Menu/OE_Menu.htm).

Two-by-two tables were used to evaluate the association between a possible risk factor (exposure to the virus) and the presence of circulating antibodies. Chi-square and exact measures of association tests were also used.

Results

Of 170 camels analysed, 17 originated from Africa and 153 were born on the Canaries; 101 were male and 69 female, and the mean age was 14 years, ranging between 2 and 26 years. Seven camels (4.1%) were seropositive, with photometric measurements ranging from 0.378 to 0.879 (Figure). These camels belonged to three different farms located on three different islands. Positive samples were re-assessed using the same ELISA and all showed positive reactions again. Epidemiological information revealed that all seropositive camels originated from Africa, all were female and between 20 and 26 years-old. In contrast, 10 other camels born in Africa and all camels born on the Canaries (n = 153) were negative. The likelihood to have had contact with the virus was 0.41 for camels born in North-West Africa, while it was 0 for those born on the Canaries. Chi-square and other measures of association between camels born in Africa or on the Canary Islands and presence or absence of antibodies were statistically significant ($p < 0.0000001$).

Farmers and the veterinarians in charge were interviewed about the medical records of the seropositive camels. They informed that clinical signs related to MERS such as respiratory distress or nasal or ocular discharges had not been observed in these animals.

Discussion

This study intended to further assess the presence of specific antibodies against MERS-CoV in camels on the Canary Islands, as previously reported [4]. The main purpose of this work was to assess the whole camel population using a significant sampling size and to interpret the results according to the epidemiological information.

Firstly, the efficacy of the serological test used in this survey needs to be considered. The manufacturer's information indicates that sensitivity and specificity are almost 100%, although there may be cross-reaction with some coronaviruses affecting ruminants and camels and therefore possible false positive results. Indeed, many coronaviruses affect livestock, particularly ruminants [12] and camels [13] worldwide. Thus, immunogenic proteins of closely related coronaviruses that share common structural or linear epitopes can elicit cross-reactive and cross-neutralising antibodies in the host [14]. In fact, in the previous study about MERS on the Canaries, camel sera reacted with human coronavirus OC43 and bovine coronavirus antigen [4]. Nevertheless, none of the camels born on the Canaries, including some young animals, showed circulating anti-MERS-CoV antibodies. Other CoV are common in young animals, causing infections and peaks of

antibodies: Therefore the fact that we did not find antibodies in these animals would indicate high sensitivity and specificity of the kit detecting specific anti-MERS-CoV antibodies based on S1 antigen.

All seven seropositive camels had been introduced from the near North-West African coast. None of them had shown any clinical signs related to MERS infection, but we know that infected camels do not usually show any signs of infection [1]. Serological evidence of the circulation of MERS-CoV among dromedary camels was found in African countries like Egypt (81.4%), Sudan (86.7%), Somalia (83.7%) [11], Kenya (9.8% in farm animals, 57.9% in nomadic animals) [15], Nigeria (94%), Ethiopia (97%) or Tunisia (54%) [16], the latter in the north of the continent and closer to the Canary Islands. All 17 camels imported to the Canaries came from the Western Sahara, a region not previously studied for MERS-CoV; 10 of them did not have antibodies in our study. Mean values obtained for the camels born in Africa vs those born on the Canaries showed statistically significant differences (ANOVA, $p < 0.005$). The photometric values in the positive camels from North-West Africa (mean: 0.302) can be considered low compared with those obtained from Middle East countries in which MERS-CoV is actively circulating (ca 2,500–3,000, EUROIMMUN database (data not shown)). These findings indicate that MERS-CoV is not circulating among camels on the Canary Islands. The fact that no camel born on the Canaries was seropositive indicates that there is no shedding of the virus among the camels nor, by extension, among the people working in close contact with these animals (personnel, farmers, veterinarians) or tourists who have occasional contact riding camels. No human cases of MERS have occurred on the Canaries.

Given that the last import of live camels from Africa was authorised in 1995, we can assume that the animals originating from Africa have not had any contact with the virus for more than 20 years. This could be indicative of MERS-CoV being a highly immunogenic virus in camels, generating and maintaining a specific immune response (IgG) for at least 20 years. Studies carried out in camels in Somalia and Sudan using archived serum samples accumulated during the past 30 years detected seropositive animals by neutralisation assay, suggesting long-term virus circulation in these animals [11], which was also confirmed in Kenya [15]. MERS-CoV seems to have been present in North-West Africa before 1995 and its MERS-CoV-specific antibodies may not be detectable in Canary camels in the future.

In conclusion, we propose that MERS-CoV does not present a threat for humans or animals on the Canary Islands. However, surveillance measures should be taken to monitor the presence of antibodies in the camel herds. Further studies including more animals and herds not only on the Canary Islands would be needed to evaluate the whole camel population, which

is not large, and the focus should be on the imported camels.

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Conflict of interest

EL and SR are employees of EUROIMMUN AG, the company producing the anti-MERS ELISA used in this paper.

Authors' contributions

CG: Designing the survey, providing samples, processing samples, drafting the manuscript; MT T-J: Designing the survey, processing samples, performing ELISA tests, drafting the manuscript; MG: Designing the survey, performing ELISA tests; EL: Designing the survey, drafting the manuscript; SR: Designing the survey, critical review of the manuscript.

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