

Searching for the natural reservoir of the SARS virus

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Abstract

This paper presents an epidemiological perspective to our identification of species of bats as the natural hosts of SARS-like coronaviruses. Earlier evidence of infection with SARS coronavirus in masked palm civets (*Paguma larvata*) in 'wet markets' in southern China was widely interpreted as indicating they were the natural host of SARS. We hypothesized that while the outbreak may have originated from civets in markets, these species were not the natural reservoir of the virus. We prioritized bats for surveillance because of their known association with a broad taxonomic spectrum of viruses and with a number of recently emerged zoonotic viruses in the region. We found serologic and genetic evidence of infection with SARS-like coronaviruses in five species. Positive findings over space and time suggest that the genus *Rhinolophus* plays an important role in the maintenance of SARS-like coronaviruses in the wild. Prioritization can improve the efficiency of surveillance of uncontrolled wildlife populations.

Introduction

A number of successive findings pointed to SARS having a wildlife origin: the genetic 'unrelatedness' of SARS Co-V to known human coronaviruses (Ksiazek *et al*, 2003); early cases were associated with restaurants and wet markets, but not with domestic livestock or farms (Xu *et al*, 2004); a survey of market wildlife recovered SARS-like viruses from masked palm civets (*Paguma larvata*) and raccoon dogs (*Nyctereutes procyonoides*) (Guan *et al*, 2003); an antibody survey of market workers showed a significantly higher SARS CoV antibody prevalence in wildlife traders and animal slaughterers than in market and community controls (Yu *et al*, 2003). A WHO-led mission (which included Field and Wang) in August 2003 reviewed initial animal reservoir studies in China. It found uncorroborated and conflicting findings of SARS infection (based on ELISA or PCR) in an implausible list of wildlife, including civets (*P. larvata*), raccoon dogs (*N. procyonoides*), Chinese ferret-badgers (*Melogale moschate*), monkeys (*Macaca mulatta*), Chinese water snakes (*Enhydryis plumbea*), Chinese pangolin (*Manis pentadactyla*), domestic cats (*Felis domesticus*), pigeons (*Columba livia*) and bats (species unidentified) (unpublished Formenty, Field, Wang *et al*). This paper presents an epidemiological perspective to our subsequent identification of species of bats as the natural hosts of a cluster of SARS-like coronaviruses (reported in Li *et al*, 2005), including hypothesis generation and a prioritized approach to wildlife surveillance.

Materials and Methods

Bats at four locations across four provinces were sampled between March and December 2004. Locations were selected on the basis of spatial disparity and the presence of known and easily accessible bat roosts. The study population comprised bats roosting at the locations during the study

period. Individual bats at each location were non-randomly captured using mist-nets or scoop nets. Bats were physically restrained in dorsal recumbency by a rabies-vaccinated leather-gloved handler. Duplicate blood, and faecal and throat swabs were collected from each bat, after which it was released. Sera and swabs were independently analysed at the Wuhan Institute of Virology in China, and the CSIRO Australian Animal Health Laboratory in Geelong, Australia (Li *et al*, 2005). The target sample size was 35 individuals per species, enabling detection of a minimum species population antibody prevalence of 10% (assuming 90% test sensitivity and specificity).

Results

Evidence of previous (serology) or current (PCR amplification) infection with SARS-like coronaviruses was found in five species of bats from two locations (Li *et al*, 2005). The target sample size was reached for two species, one from each sub-order of bats; *Rousettus leschenaulti* (*Megachiroptera*) and *Rhinolophus pearsoni* (*Microchiroptera*). Univariate analysis showed an association between a positive SARS serostatus and all three explanatory variables (Table 1), but the association with province and sampling time disappeared on stratification by sub-order, leaving a strong association between a positive serostatus and sub-order (OR 43.3; 95% CI 9.8-387.6).

Discussion

The findings of Guan *et al* (2003) in wildlife market surveys were widely interpreted as identifying civets as the natural host of SARS. But Field and Wang hypothesized that while the human outbreak may have originated from contact with infected market wildlife such as civets, these species were not the natural reservoir of the SARS

virus, and that infection spilled from the natural reservoir to civets and other immunologically naïve species in the market environment (or somewhere along the wildlife supply chain), and thence to humans. This hypothesis was subsequently supported by studies showing an absence of infection in wild and farmed civet populations and by experimental studies showing clinical disease in experimentally infected civets (Wang *et al*, 2006). Field had previously successfully used a targeted

Table 2: Univariate association between independent variables and SARS CoV serostatus in bats non-randomly sampled in disparate locations in China between March and December 2004.

Variable	Number of bats testing ¹		P value ²
	Positive	Negative	
Sub-order			<0.001
Megachiroptera			
<i>Rousettus leschenaulti</i>	2	182	
<i>Cynopterus sphinx</i>	0	17	
Total	2	199	
Microchiroptera			
<i>Rhinolophus ferrum</i>	0	4	
<i>Rhinolophus macrotis</i>	5	2	
<i>Rhinolophus pearsoni</i>	13	33	
<i>Rhinolophus pussilus</i>	2	4	
<i>Miniopterus schreibersi</i>	0	1	
<i>Myotis altarium</i>	0	1	
<i>Nyctalus plancyi</i>	0	1	
Total	20	46	
Province			<0.001
Guangxi	17	177	
Guangdong	0	59	
Hubei	5	8	
Tianjin	0	0	
Sampling time			<0.001
March-July	1	142	
November-December	21	103	

¹ Tests and methodology described in Li *et al* (2005) supporting online material

<http://www.sciencemag.org/cgi/data/1118391/DC1/1>

² P value of chi square statistic

approach to identifying the natural reservoir of Hendra virus (a novel zoonotic paramyxovirus first described in Australia in 1994). Species or groups were prioritized for surveillance using relevant criteria and circumstantial evidence, an approach which led to the identification of species of fruit bats as the natural host of Hendra virus (Young *et al*, 1997). We applied this same approach in our search for the natural host of SARS CoV. Bats were again prioritized for surveillance because numerous viruses from multiple families have been isolated from bats (Hoar *et al*, 1998); species of bats have been associated with a number of recently emerged zoonoses in the Asian region, including Hendra and Nipah viruses whose disease ecology several of our group were then researching; and bats were on the list of uncorroborated SARS-positive wildlife compiled by the August 2003 WHO mission.

The high seroprevalence found in several *Rhinolophus* species (Li *et al*, 2005) indicates that infection was cycling in these populations at the time of sampling. Further, the evidence of infection in the genus across location and time indicates an important role in the maintenance of SARS-like coronaviruses in the wild.

The August 2003 WHO mission also highlighted unknown serologic test validity and a lack of rigour in PCR testing as problematic in some of the early wildlife surveys. The initial wildlife serosurveillance in China used an indirect ELISA developed for humans, and the lack of corroboration, either by a second testing method or by a second institution, limited confident interpretation of results. The samples from the Li *et al* (2005) survey were shared between two laboratories (one a WHO reference laboratory) and independently analysed.

Conclusion

Prioritization can improve the efficiency of surveillance of uncontrolled wildlife populations, allowing either confident rule-in or rule-out of species/genera in a systematic manner. The alternative non-prioritized approach often results in small numbers of many taxa, limiting meaningful interpretation of negative results. Species of *Rhinolophus* bats appear to play an important role in the maintenance of SARS-like coronaviruses in the wild. An understanding of the dynamics of infection both in the natural system and in wet markets is essential if we are to manage the risk of future SARS outbreaks.

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