Chapter 11

SARS Coronavirus: An Animal Reservoir?

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Introduction

The emergence of SARS and its subsequent rapid global spread caused alarm in public health circles worldwide. The unknown aetiology of this novel, atypical pneumonia was one reason for the alarm. Several putative aetiological agents were considered initially, with a previously undescribed novel coronavirus (*Coronaviridae*) finally shown to be responsible (Chapter 7). The origin of the outbreak and the source of the infection were both major focuses of attention, the latter even more so once the outbreak in human populations was under control. Was this a previously avirulent human coronavirus or another example of an emerging zoonotic infection? What were the risk factors for exposure? This chapter addresses these questions.

The concept of reservoir

Simplistically, there are two plausible explanations of the origin of SARS coronavirus. It is either a previously unidentified human coronavirus whose virulence and/or infectivity increased as a result of genetic change or it is an animal coronavirus that jumped to an immunologically naïve human host. Consideration of the concept of reservoir is useful at this point. Haydon *et al.*[1] define a reservoir as 'one or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained, and from which infection is transmitted to the defined target population'. Thus, a discrete human population, an animal population, or an ecological community can be regarded as a reservoir. It is also important to recognize that, for any pathogen, multiple reservoirs are possible. An understanding of these concepts is necessary to grasp the potential complexity of the task of identifying the source of human infection, and thus for the identification of risk factors for exposure and the formulation of risk management strategies.

The search for the reservoir

Epidemiological investigations indicate that the SARS outbreak originated in Guangdong Province, China, with the earliest identified case in November 2002.[2,3] The search for the reservoir has therefore focused on Guangdong and has comprised both human and animal epidemiological studies. Human studies have relied heavily on case report and case interview data collected by the Guangdong Centre for Disease Control. Preliminary analysis of the early Guangdong data suggested an epidemiologic association between occupation and infection, with restaurant chefs being over-represented (*Report of the First WHO Mission to China*, April 2003). A more detailed analysis indicated that people working in the food industry and people living close to markets were over-represented in early cases (*Report of the Second WHO Mission to China*, May 2003). None of the early cases

lived close to livestock or farms, discounting an association with domestic animals. These findings were interpreted as best supporting a wildlife reservoir hypothesis, with the putative mode of transmission being exposure to infectious body fluids of the live or recently slaughtered animal.

Genetic comparison of SARS CoV with known coronaviruses of humans and domestic animals revealed that each gene of SARS CoV had only 70% or less identity with the corresponding gene of the known coronaviruses. Phylogenetic analysis also showed that SARS CoV does not fall into any of the three known groups of coronaviruses (Chapter 8). These findings suggested that SARS CoV did not arise by mutation of human coronaviruses or by recombination between any known coronaviruses.

Subsequent phylogenetic analysis by the authors revealed that human CoV isolates 229E (group 1) and OC43 (group 2) clustered with coronaviruses isolated from domestic mammals, such as pigs and mice. From an evolutionary point of view, viruses 229E and OC43 resulted from two previous independent interspecies transmission events from domestic animals to humans. This indicates that all human coronaviruses are zoonotic from domestic animals. However, SARS CoV is not closely related to other known coronaviruses but is derived from the root of group 2 coronaviruses. Thus, the plausible explanation is that SARS CoV was derived from an unknown coronavirus that existed in an unknown host, very likely a wild animal, before it jumped to humans.

In contrast to recent human infections with influenza H5N1 'bird flu' where transmission is so far limited largely to bird to human transmission, SARS CoV adapted to efficient human to human transmission. It was reasonable to hypothesize that the possible host of SARS CoV may be a mammal since a similar replication system and cellular factors made it much easier for SARS CoV to adapt to humans. For an RNA virus to be present as a viable population, there must be a host species with sufficient population size to support the virus prevalence. Therefore, when investigating the possible source of SARS CoV, the author mainly focused on those wild mammalian species as exotic food in the markets but with relatively large market populations, or domestic species that were known to harbour other coronaviruses.

The preliminary investigation was carried out at an animal market in Shenzhen on 7 May 2003. SARS CoV infection was found in throat and faecal swabs from three of eight species of wildlife sampled in a Shenzhen animal market (*Paguma larvata*, *Nyctereutes procyonoides*, *Melogale moschata*).[4] The animal isolates are phylogenetically distinct from the human isolates, making it improbable that the animals were infected from humans. Interestingly, a 29-nucleotide sequence present in all animal isolates is absent from the human isolates, except for an early Guangdong isolate. Another study also documented SARS CoV-like viruses from faecal swabs from farmed *P. larvata* (Himalayan palm civet) in Hubei Province (Hu ZH, personal communication). Independent studies carried out in several Chinese research institutions returned positive PCR results from *P. larvata* faecal swabs. The findings of these animal studies indicate that several wildlife species found in markets excrete a SARS-like coronavirus. Thus it is improbable that these are dead-end hosts.

Two separate studies have undertaken serologic studies of humans working in animal markets.[2,4] Both studies show significantly higher SARS CoV antibody prevalence in wildlife traders and animal slaughterers than in market and community controls. None of the seropositive individuals has a reported history of SARS-like symptoms, suggesting exposure to either an avirulent SARS or SARS-like virus.

A second SARS outbreak averted

Even though coronaviruses genetically highly related to SARS CoV were isolated from wild animals, particularly from *Paguma larvata*, not all investigating laboratories were able to detect the virus in these market animal species. The resulting controversy led to the lifting of the ban on wildlife trade in food markets of Guangdong in September 2003, soon after SARS was controlled in humans in July 2003. As a result, different species of wild animals, including civet, were once again traded in the markets of Guangdong.

To attempt to resolve the question of the role of live animal markets in the origin of human SARS CoV, surveillance studies in Guangdong food markets were re-initiated by our group soon after the wildlife ban was lifted in September 2003. This surveillance focused on five species of wild animals including *Paguma larvata*, *Arctonyx collaris* (hog badger), *Melogale moschata* (Chinese ferret badger), *Nyctereutes procyonoides* (raccoon dog) and *Meles meles* (Eurasian badger). As in previous market surveillance, a SARS-like virus was also detected in each of these species. However, *P. larvata* still provided the main body of SARS CoV isolates, with the highest positive rate (about 76%, authors' unpublished data). At the end of December 2003, while these surveillance efforts were still ongoing, the first suspected SARS patient re-emerged in Guangzhou, the capital city of Guangdong Province. Within a short interval, the second and third suspected SARS CoV cases appeared; SARS was resurgent in the region. Genetic analysis revealed that all of these most recent SARS cases were caused by interspecies transmission that had occurred recently, and the counterpart of human SARS CoV was detected from *P. larvata* in the markets (authors' unpublished data). These findings convinced the authorities in Guangdong that they had to take action for a second time and remove all wild animals from the food markets. After this action was taken, no new SARS case was found in humans, providing convincing evidence that the wet markets in Guangdong were the infectious source of human SARS CoV. In conclusion, the culling of civets and other wild animals in Guandong markets during early 2004 possibly averted another SARS outbreak.

A putative reservoir

The repeated investigations and animal studies support the hypothesis that a wildlife market (or markets) was the origin of the SARS outbreak in humans. The findings of serological studies further support this hypothesis by providing a plausible explanation for the under-representation of wildlife traders in early cases—that is, at-risk traders had previous exposure to an avirulent SARS or SARS-like virus which provided protection against exposure to the outbreak strain.

Two plausible scenarios could explain the mechanism for the emergence of SARS CoV. These are: i) increasing demand resulted in animals from previously unexploited populations (unidentified reservoir species in which SARS CoV is likely to be asymptomatic) entering markets and/or ii) a genetic change in the circulating wild animal strain, possibly as a result of passage in animals or humans in the market, resulted in increased capacity for introduction into humans.

The first scenario is certainly true. In the last decade, China has undergone rapid economic growth, especially in Guangdong and other coastal areas. The rising prosperity of the people in these regions led to increased demand for exotic foods that was met by the farming of wild species, and before the SARS outbreak, China had 700 raccoon dog and 1000 civet cat farms. This generated an ecosystem change allowing those coronaviruses resident in wildlife increased contact with the human population. The second scenario is difficult to

prove because the SARS CoV precursor virus and its animal reservoir have not been identified yet, but is plausible given the first scenario and the high mutation rates of RNA viruses. Both scenarios are consistent with the clustering of the early cases in the Pearl Delta region.[3]

A putative causal model

While a SARS or SARS-like virus maintained in wildlife markets is the probable source of the human outbreak, it is implausible that this cycle exists in isolation. Indeed, the emerging picture is of a virus able to infect a wide range of hosts, suggesting a complex ecology. A causal model with interacting natural, market, human, and peri-human animal components has been proposed (Fig. 11.1). Such a model is a useful tool not only for conceptualizing the likely complexity of the system, but also for identifying possible transmission control points. For example, regulation (or elimination) of the trade in wild-caught wildlife might control transmission to market and farm populations, and thus to humans; elimination of infection in the farmed wildlife population and ongoing monitoring might control transmission within this group, and thus to wildlife markets, and to humans.

Research priorities

There are two key research priorities in investigating the reservoir of SARS. The first is a simple, inexpensive serological test that has been validated for the target species. The second is a comprehensive understanding of the ecology of the reservoir.

Serology is well-established as a surveillance tool. Serological testing allows us to screen large numbers of animals in a relatively short time, to guide the direction of further surveillance efforts. However, serological data can be problematic since there is a lack of a suitable detection conjugate for use in the relevant wild animal species.

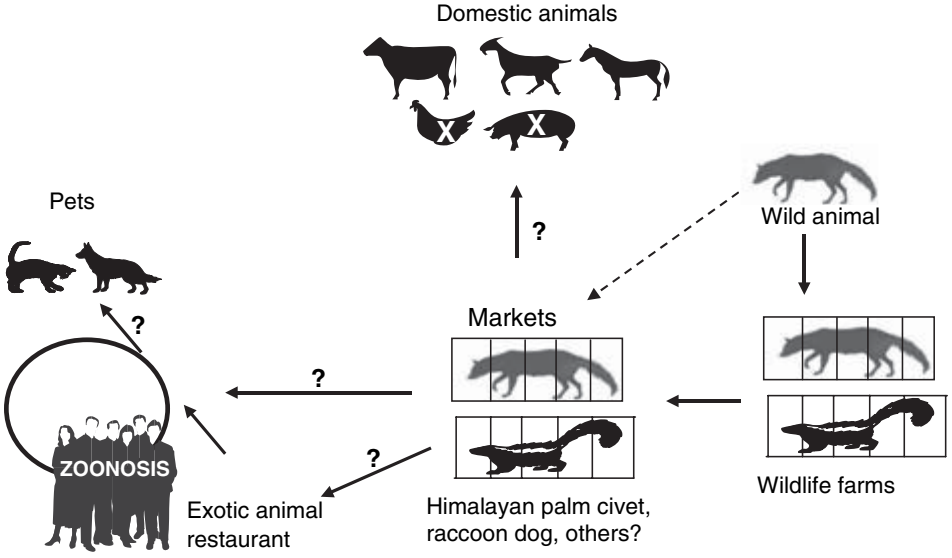

Figure 11.1 Ecology of SARS.

There is also cross-reactivity between known coronaviruses in the Western blot test.

Systematic virological surveillance is the only way to gain insight into the ecology of the SARS CoV. The findings will provide information to identify the reservoir of SARS and understand the risk factors of re-introduction to the human population. This requires a systematic approach that includes prevalence studies, longitudinal studies, and modelling. Avian influenza surveillance programmes, similar to what is needed for SARS, have been conducted successfully in Hong Kong for pandemic preparedness. In fact, the detection and prevention of the second SARS outbreak through wild animal culling was possible because of the lessons learnt from the H5N1 avian influenza in Hong Kong in 1997.

In a situation where the wildlife reservoir is a trade commodity, an extension of understanding the ecology of the reservoir is an understanding of the trade. We know that the wildlife trade and farms still exist in southern China, leaving the possibility of the re-emergence of SARS.

Understanding the wild animal trade is critical to its effective management, and directly related to this is an understanding of what drives the wildlife trade—a complex mix of economic, social and cultural factors. Wildlife is expensive (US$30 per kg, compared to US$1 for chicken), and there is evidence that demand and consumption have increased in recent years as economic conditions in China have improved. Why do people eat wildlife? Usually it is for perceived health benefits. For example, *Paguma larvata* is typically eaten in winter when fresh fruit is often unavailable. It is believed that eating the animal (also known colloquially as the *fruit fox* or *flower fox* because of its dietary preferences) provides the same health benefits as eating fruit. In markets, wild-caught *P. larvata* meat attracts a price premium because people believe it is more health-giving and tastes better than its grain-fed farmed counterpart.

Conclusions

Our understanding of this novel disease is not complete; however, accumulated evidence suggests that SARS resulted from a zoonosis, very probably from wildlife. *Paguma larvata*, and other wild animals found in markets, were the infection source for the two recognized SARS outbreaks. Since the reservoir and ecology of SARS are not well-defined the re-emergence of this disease cannot be excluded. Research efforts need to concentrate on identifying the SARS CoV reservoir and understanding its ecology better.

References

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