Project no.	**SP22-CT-2004-511063**
Project acronym	**EPISARS**
Project title	**Prevention of future SARS epidemics through the control of animal and human infection**

Instrument: STREP

Thematic Priority: 8.1 Policy-oriented research (SSP)
Call identifier: FP6-2003-SSP-SARS

SPECIFIC TARGETED RESEARCH OR INNOVATION PROJECT

FINAL ACTIVITY REPORT

Period covered:
from April 2004 to March 2007

Date of preparation:
14 May 2007

Start date of project: **April, 1st 2004**

Duration: 36 months

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Project execution

Prevention of future SARS epidemics
through the control of animal and human infection:
the EPISARS project

In November 2002, a previously unrecognized animal coronavirus (CoV) emerged from the "wet markets" of China to become a worldwide threat to public health systems. Eight months later, more than 8000 people had been infected in 25 countries spread over five continents, and 774 had died from the disease. Although the number of fatalities was small compared to deaths related to HIV or malaria, the short-term impact of SARS was much wider. Researchers and public health specialists around the world were mobilised to develop drugs and vaccines, to contribute to surveillance of emerging diseases, and to propose ways of preventing future SARS pandemics. At the time this project proposal was submitted (September 2003), it was very difficult to predict what the future of this new emerging disease would be: Would a new pandemic restart during the 2003/4 winter season? Would the elimination from the markets of masked palm civets, the animals which most likely infected men during the first epidemic, prevent future epidemics? Was there another animal source for the virus? Were there other "human" CoV able to induce respiratory diseases? Would we have the appropriate diagnostic tools to differentiate SARS from the flu, during acute respiratory infections? Would neutralising antibodies play a role in protecting against re-infection; if yes, would such immunity be long-term, knowing the rapid decline in protective immunity associated with other human CoVs?

Three years later, much progress has been made in the understanding of the emergence and spread of this new viral disease. Several of these important discoveries involved partners of the EPISARS collaborative network (EPISARS publications in bold): horseshoe bats were identified as a natural reservoir for SARS-like coronavirus (SARS-CoV) **(Li et al, Science, 2005,** Lau et al, PNAS, 2005**)**; masked palm civets were again at the source of the small 2003/4 human outbreak in Guangdong, confirming the role of the palm civets as intermediate hosts, and allowing the study of human SARS-CoV right after transmission from animals **(Song et al, PNAS, 2005)**; two amino-acid substitutions in the SARS-CoV spike protein appeared sufficient for a virus adapted to palm civets to cross species and to bind to human

cellular receptors angiotensin converting enzyme-2 (ACE-2) **(**Li W, Nature, 2003**; Nie et al, Biochem Biophys Res Commun, 2004; Qu XX et al., J Biol Chem, 2005)**; neutralising antibodies, induced by an antigenic determinant on the S2 domain of the spike glycoprotein (**Zhang H et al., J Virol 2004**), could be detected 5-10 days after infection, and persist for two to three years, although titers declined markedly at the 24-month follow-up visit **(Nie et al, J Infect Dis, 2004; Liu W et al, J Infect Dis, 2006; Li T et al., PLoS One, 2006; Cao WC et al;, N Engl J Med, 2007)**. New human coronaviruses were described, HCoV-NL63 (Van der Hoek L. et al, Nat Med, 2004) and HCoV-HKU1 (Woo PC et al, J Virol 2005), and new diagnostic tools developed **(Vabret A et al., Emerg Infect Dis, 2005; and Vabret et al., Clin Infect Dis, 2006)**. Other important findings of the network included the study of long-term excretion of virus in sputum and stools of convalescent patients **(Liu W et al, Emerg Infect Dis, 2004)**; the comparison of the molecular switches in key nucleotides of the human SARS-CoV between the Beijing and the worldwide epidemics **(Liu W et al, Emerg Infect Dis, 2005)**; the quantitative detection of SARS-CoV by a multi-target real-time Taqman reverse transcription-PCR (RT-PCR) assay **(Hu W et al. J Clin Microbiol, 2005)**; and the use of a two-step screening method to identify small molecules derived from Chinese herbal medicine interfering with viral entry **(Yi L et al., J Virol, 2004)**.

Meanwhile, SARS has left the political agenda, to be replaced by avian flu, despite four SARS outbreaks in 2003/4, one from its natural source, and three from laboratory accidents. In addition, the threat of a new epidemic is still here, and major questions remain concerning the prevention of future SARS epidemics. With regard to the animal origin of SARS, it is important to understand how the virus reached the markets of South China from horseshoe bats, and studies on the ecology of bats, and the epidemiology of infection among bats, will be the key to this analysis. Detailed analysis of the spike proteins of SARS-like CoV obtained from bats have shown divergences in the S1 domain, and particularly the receptor binding domain, suggesting that bat SARS-like CoV may use a vastly diverse ACE2 or even a different molecule as the entry receptor (**Shi & Hu, Virus Research, 2007**). Masked palm civets were at the origin of the two known human outbreaks, making it an important player in the chain of transmission from the natural reservoir to humans. How did masked palm civets get infected, and where, is still unknown. While most of the literature suggests that masked palm civets are found infected in markets only, our studies revealed that some could be already infected in the farms (**Hu et al., J Clin Microbiol, 2005; Shi & Hu, Virus Research, 2007).** On-going studies of the systematics, ecology, and farming of

masked palm civets suggest a complex scheme of animal breeding in farms, transportation, and release after the ban, limiting the interpretation of phylogeographic analysis (**Chen JP et al., submitted**). Molecular studies of viral adaptation to new hosts can now be extended to the new animal species identified as potential reservoirs (the horseshoe bats), and contribute to our general understanding of viral emergence and adaptation to humans. Natural history studies in convalescent patients should be continued, to document the persistence (or decline) of neutralising antibodies with time, and to select the immunological targets of vaccines against SARS. One should however be cautious about the risk of antibody-dependent enhancement of virus entry as shown in our experimental model (**Kam et al, vaccine, 2006**). Finally, the fear of an avian flu pandemic has reminded us of the importance of hospital preparedness, particularly with regard to diseases transmitted via the respiratory route and with a potential for nosocomial spread. This project gave us a unique opportunity to work on appropriate hospital preparedness plans that may be useful beyond the control of SARS re-emergence (**Puro et al, Infection, 2006**).

References (those related to EPISARS are in bold)

Cao WC, Liu W, Zhang PH, Zhang F, Richardus JH. Disappearance of Specific Antibodies to SARS-CoV in Recovered Patients. N. Engl. J. Med. (letter), in press

Chen JP., Andersen DH., Veron G., Randi E., Zhang SY. Isolation and characterization of polymorphic microsatellite markers for masked palm civets (Paguma larvata). Submitted.

Hu W, Bai B, Hu Z, Chen Z, An X, Tang L, Yang J, Wang H, Wang H. Development and evaluation of a multitarget real-time taqman reverse transcription PCR for detection of Severe Acute Respiratory Syndrome-associated coronavirus and surveillance for an apparently related coronavirus found in masked palm civets. J. Clin. Microbiol. 2005, 43;2041-6.

Kam YW, Kien F, Roberts A, Cheung YC, Lamirande EW, Vogel L, Chu SL, Tse J, Guarner J, Zaki SR, Subbarao K, Peiris M, Nal B, Altmeyer R. Antibodies against trimeric S glycoprotein protect hamsters against SARS-CoV challenge despite their capacity to mediate FcgammaRII-dependent entry into B cells in vitro. Vaccine. 2007;25:729-40. Epub 2006 Aug 22.

Kan B, Wang M, Jing H, et al. Molecular evolution analysis and geographical investigation of severe acute respiratory syndrome coronavirus-like virus in palm civets at an animal market and on farms. J Virol 2005;79:11892-900.

Lau SK, Woo PC, Li KS, Huang Y, Tsoi HW, Wong BH, Wong SS, Leung SY, Chan KH, Yuen KY.. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Proc Natl Acad Sci U.S.A. 2005. 102 :14040-10405.

Li T, Xie J, He Y, Fan H, Baril L, Qiu Z, Han Y, Xu W, Zhang W, You H, Zuo Y, Fang Q, Yu J, Chen Z, Zhang L. Long-Term Persistence of Robust Antibody and Cytotoxic T Cell Responses in Recovered Patients Infected with SARS Coronavirus. PLoS ONE. 2006 Dec 20;1:e24

Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JS, Luzuriaga K, Greenough TC, Choe H, Farzan M.Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 2003, 426, 450-454.

Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, Wang H, Crameri G, Hu Z, Zhang H, Zhang J, McEachern J, Field H, Daszak P, Eaton BT, Zhang S, Wang LF. Bats Are Natural Reservoirs of SARS-Like Coronaviruses. Science. 2005; 310, 676-679.

Liu W, Tang F, Fontanet A, Zhan L, Zhao QM, Zhang PH, Wu XM, Zuo SQ, Baril L, Vabret A, Xin ZT, Shao YM, Yang H, Cao WC. Long-term SARS coronavirus excretion from patient cohort, China. Emerg Infect Dis. 2004; 10:1841-3.

Liu W, Tang F, Fontanet A, Zhan L, Wang TB, Zhang PH, Luan YH, Cao CY, Zhao QM, Wu XM, Xin ZT, Zuo SQ, Baril L, Vabret A, Shao YM, Yang H , Cao WC. Molecular epidemiology of severe acute respiratory syndrome–associated coronavirus in a patient cluster from Beijing. Emerg Infect Dis. 2005;11:1420-4.

Liu W, Fontanet A, Zhang PH, Zhan L, Xin ZT, Baril L, Tang F, Lu H, Cao WC. Antibody responses in convalescent SARS patients : a two-year prospective study. J Infect Dis 2006;193:792-5.

Nie Y, Wang P, Shi X, Wang G, Chen J, Zheng A, Wang W, Wang Z, Qu X, Luo M, Tan L, Song X, Yin X, Chen J, Ding M, Deng H. Highly infectious SARS-CoV pseudotyped virus reveals the cell tropism and its correlation with receptor expression. Biochem Biophys Res Commun. 2004; 321:994-1000.

Nie Y, Wang G, Shi X, Zhang H, Qiu Y, He Z, Wang W, Lian G, Yin X, Du L, Ren L, Wang J, He X, Li T, Deng H, Ding M. Neutralizing antibodies in patients with severe acute respiratory syndrome-associated coronavirus infection. J Infect Dis. 2004;190:1119-26. Epub 2004 Aug 2.

Puro V, Girardi E, Daglio M, Simonini G, Squarcione S, Ippolito G. Clustered cases of pneumonia among healthcare workers over a 1-year period in three Italian hospitals: applying the WHO SARS alert. Infection. 2006;34:219-21

Qu XX, Hao P, Song XJ, Jiang SM, Liu YX, Wang PG, Rao X, Song HD, Wang SY, Zuo Y, Zheng AH, Luo M, Wang HL, Deng F, Wang HZ, Hu ZH, Ding MX, Zhao GP*, and Deng HK*. Identification of two critical amino acid residues of the severe acute respiratory syndrome coronavirus spike protein for its variation in zoonotic tropism transition via a double substitution strategy J. Biol. Chem. 2005; 280 (33): 29588-29595.

Shi Z. and Hu Z.. A review of studies on animal reservoirs of the SARS coronavirus. Virus Res. 2007;Apr 20 [Epub ahead of print]

Song HD, Tu CC, Zhang GW, Wang SY, Zheng K, Lei LC, Chen QX, Gao YW, Zhou HQ, Xiang H, Zheng HJ, Chern SW, Cheng F, Pan CM, Xuan H, Chen SJ, Luo HM, Zhou DH, Liu YF, He JF, Qin PZ, Li LH, Ren YQ, Liang WJ, Yu YD, Anderson L, Wang M, Xu RH, Wu XW, Zheng HY, Chen JD, Liang G, Gao Y, Liao M, Fang L, Jiang LY, Li H, Chen F, Di B, He LJ, Lin JY, Tong S, Kong X, Du L, Hao P, Tang H, Bernini A, Yu XJ, Spiga O, Guo ZM, Pan HY, He WZ, Manuguerra JC, Fontanet A, Danchin A, Niccolai N, Li YX, Wu CI, Zhao GP. Cross-host evolution of severe acute respiratory syndrome coronavirus in palm civet and human. Proc Natl Acad Sci U S A. 2005; 102:2430-5. Epub 2005 Feb 4.

Vabret A, Mourez T, Dina J, van der Hoek L, Gouarin S, Petitjean J, Brouard J, and Freymuth F. Human coronavirus NL63, France. Emerg Infect Dis 2005;11:1225-9

Vabret A, Dina J, Gouarin S, Petitjean J, Corbet S, Freymuth F. Detection of new human coronavirus HKU1 : a report of 6 cases. Clin Infect Dis 2006;42:634-9.

Van der Hoek L, Pyrc K, Jebbink MF, Vermeulen-Oost W, Berkhout RJ, Wolthers KC, Wertheim-van Dillen PM, Kaandorp J, Spaargaren J, Berkhout B. Identification of a new human coronavirus. Nat Med. 2004;10:368-

73.

Woo PC, Lau SK, Chu CM, Chan KH, Tsoi HW, Huang Y, Wong BH, Poon RW, Cai JJ, Luk WK, Poon LL, Wong SS, Guan Y, Peiris JS, Yuen KY. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol. 2005;79:884-95

Yi L, Li Z, Yuan K, Qu X, Chen J, Wang G, Zhang H, Luo H, Zhu L, Jiang P, Chen L, Shen Y, Luo M, Zuo G, Hu J, Duan D, Nie Y, Shi X, Wang W, Han Y, Li T, Liu Y, Ding M, Deng H, Xu X. Small molecules blocking the entry of severe acute respiratory syndrome coronavirus into host cells. J Virol. 2004; 78:11334-9.

Zhang H, Wang G, Li J, Nie Y, Shi X, Lian G, Wang W, Yin X, Zhao Y, Qu X, Ding M, Deng H. Identification of an antigenic determinant on the S2 domain of the severe acute respiratory syndrome coronavirus spike glycoprotein capable of inducing neutralizing antibodies. J Virol. 2004; 78:6938-45.

Dissemination and use

The SARS epidemic has been exemplary in that it gave the scientific and the public health community the opportunity to study the emergence of a virus in human populations at a time both epidemiological and virological tools were available for in-depth studies. The results were remarkable, with the control of the epidemic within six months. EPISARS has markedly contributed to this outcome, notably with the identification of the horseshoe bats as reservoirs for SARS-like coronaviruses, and with the documentation of the crucial role played by the masked palm civets in the spread of the disease to humans. Needless to say, our contribution, although very significant, is one piece of a gigantic effort putting together international organisations such as the World Health Organisation, national sentinel surveillance systems, and first-class laboratories. Our work had direct impact on public health policies, with the control of farming and selling of masked palm civets in China. It also showed the value of multidisciplinary approaches to emerging viruses, combining, among others, zoologists, veterinarians, virologists, epidemiologists, clinicians and public health specialists. Our findings have been disseminated through participation in international conferences and more than thirty scientific publications in peer-reviewed journals, including five editorial papers. More details are available on our website: http://www.pasteur.fr/recherche/episars/accueil/