Food and Agriculture Organization of the United Nations

FMM/RAS/298: Strengthening capacities, policies and national action plans on prudent and responsible use of antimicrobials in fisheries

China: Development of National Action Plans on AMR: Aquaculture Component, Project Accomplishments and Impacts

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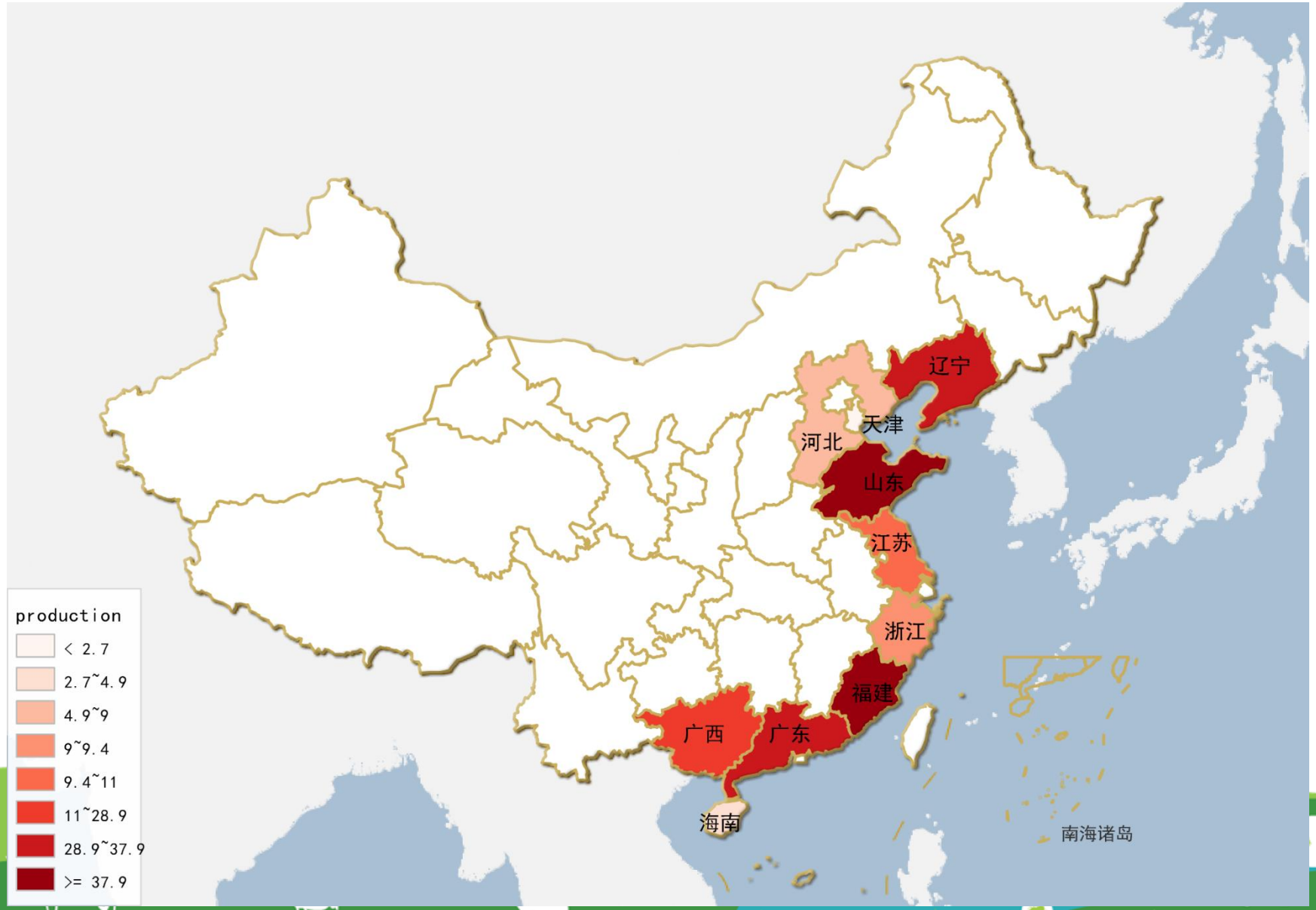
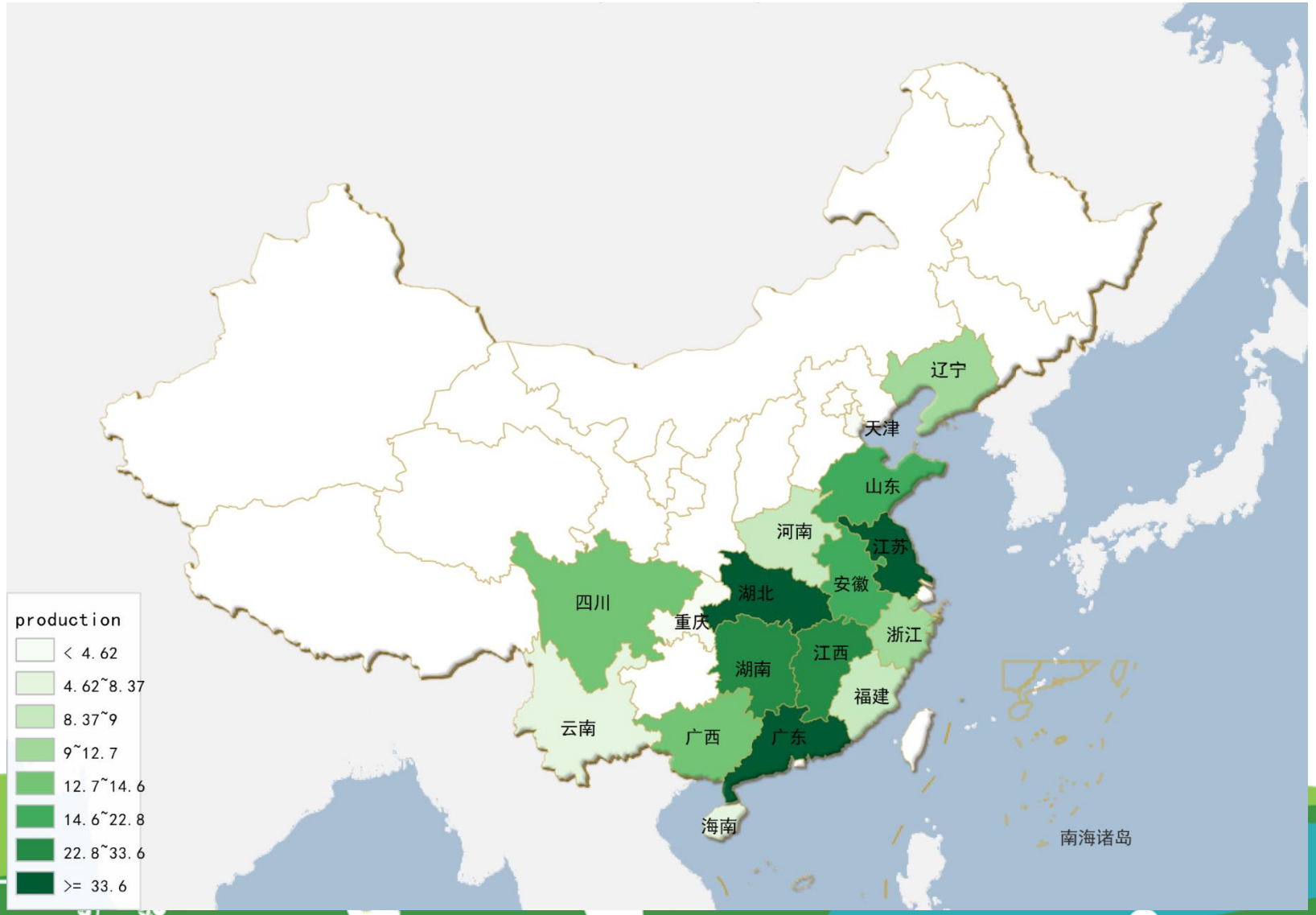
Major mariculture producers by province



Major freshwater aquaculture producers by province



Major freshwater fish and crustacean species cultured in China

many species of carp
Tilapia (*Oreochromis* spp.)
Snakehead （*Channa argus*）
largemouth catfish (*Silurus meridionalis* Chen)
Rice field eel (*Monopterus albus*)
largemouth bass (*Micropterus salmoides*)
Chinese mitten crab(*Eriocheir sinensis*)
Pacific white shrimp（ *Litopenaeus vannamei* ）
Red swamp crayfish(*Procambarus clarkii*)

Mariculture

Large yellow croaker （*Larimichthys crocea*）
Turbot (*Scophthalmus maximus*)
Sea bass (*Lateolabrax japonicus*)
Grouper fish (*Epinephelus* spp.）
Red drum (*Sciaenops ocellatus*)
Pacific white shrimp（*Litopenaeus vannamei* ）
Swimming crab (*Portunus trituberculatus*)
mud crab(*Scylla paramamosain*)
Giant tiger prawn (*Penaeus monodon*)
Chinese white shrimp (*Fenneropenaeus chinensis*)

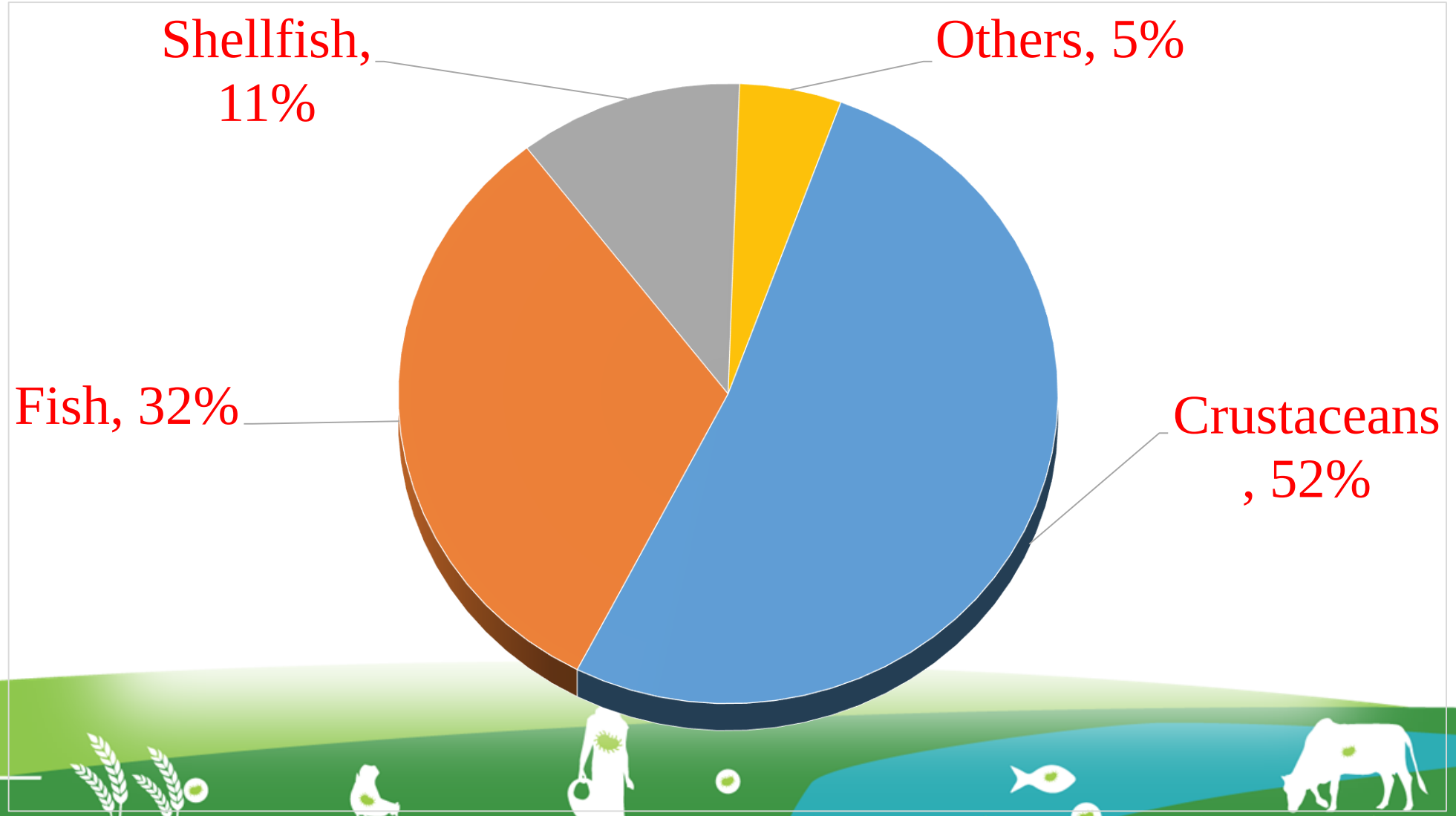
List of major fish pathogenic Gram-negative bacteria in China

Pathogen	Host of pathogen
Aeromonas hydrophila	Catfish, carp, trout, eel, sturgeon, tilapia and bass, etc.
Aeromonas salmonicida	Salmon, trout, carp and catfish
Other motile *Aeromonas* species	Carp, catfish, eel, sturgeon, tilapia, etc.
Edwardsiella ictaluri	Catfish, yellow catfish
Edwardsiella tarda	Turbot, flounder, carp, catfish, eel and tilapia
Flavobacterium columnare	Carp, mandarin fish trout, tilapia , catfish and salmon
Citrobacter spp.	Carp, sturgeon, crab, crayfish, softshell turtle,
Acinetobacter spp.	sturgeons
Photobacterium spp.	Sturgeon, sea bream, yellow catfish, sea bass, snakehead
Pseudomonas spp.	Carp, catfish, eel, salmon
Vibrio spp.	Most of the marine fish species, crayfish
Yersinia ruckeri	Trout and salmon

List of pathogenic Gram-positive bacteria reported in China

Pathogen	Host of pathogen
Lactococcus garvieae	flounder, soft-shell turtle, crayfish
Nocardia sp.	Snakehead, large yellow croakers, seriola, largemouth bass, *Trachinotus ovatus*
Streptococcus agalactiae	Tilapia, Grouper
Streptococcus iniae	Tilapia, sea bream, flounder, hybrid striped bass
Streptococcus dysgalactiae	Sturgeon
Weissella sp.	Trout
Mycobacterium spp.	sturgeon

Distribution of economic loss caused by diseases by group of species in 2015



Allowed to use in aquaculture	Not allowed to use in aquaculture
Neomycin Sulphate	Norfloxacin
Doxycycline Hydrochloride	Ciprofloxacin
Thiamphenicol	Erythromycin
flofenicol	chloramphenicol
Sulfadiazine(SD) ,	Tylosin
Sulfamethoxazole(SMZ)/TMP ,	Bacitracin Zinc
Sulfadimidine(SM2) , Sodium	Nitrofurans (Furazolldone,
sulfamonomethoxine (SMM-Na)	Nitrofurazon, Nitrofurantoin, etc)
Enrofloxacin	Olaquindox
Flumequine	
Oxolinic Acid	
Oxytetracycline	

Development of NAPs on AMU/AMR in China

◆National Action Plan to Contain Antimicrobial Resistance (2016-2020),published on August 5,2016.

http://en.nhfpc.gov.cn/2016-08/26/c_70850.htm

◆National action plan to contain antimicrobial resistance of animal origin (2017—2020),(official) published on June 22,2017 by MoA.

http://www.moa.gov.cn/zwllm/tzgg/tz/201706/t20170623_5726086.htm

The major objectives of MoA’s NAP

- The proportion of sales with veterinary prescription of antibacterial agents in animal sector will be realized in 50% in provinces (autonomous regions and municipalities).
- To optimize the surveillance networks of AMU and AMR. To set up reference laboratories of antimicrobial resistance and bacterial strain banks. To establish evaluation system for AMU and AMR.
- The antimicrobials shared by humans and animals or easily producing cross-resistance should be gradually withdrawn from the market of animal growth promoter. To effectively control the increasing trend of the main animal origin antimicrobial-resistant bacteria.
- To develop and implement educational efforts to ensure that medical staff, veterinarians and animal producers receive information and training of rational use of antibacterial agents.

- Five objectives to be achieved:
  - The proportion of antibiotics sold by the veterinarian prescription will be up to 50%.
  - The type structures of veterinary antibiotics have been optimized.
  - Antimicrobial agents currently used by human and veterinary, or antimicrobial agents susceptible to develop cross resistance will be gradually quitted as animal growth promotion agents.
  - Veterinary Antimicrobial Testing System will be further perfected.
  - The capacity of antibiotic use scientifically will be improved.

- Six actions will be carried out

  - Regulatory actions: Strengthened veterinary drug quality control.
  - Monitoring actions: Intensified the surveillance of AMR of animal bacteria. The establishment of a national veterinary drug residues and AMR control consultant expert team. Adjusted and optimized the annual surveillance plan of animal-borne AMR, and accelerate the construction of animal-derived AMR monitoring network.
  - Supervisory actions: Reinforced veterinary antibiotic residue monitoring.

- Six actions will be carried out (cont.)
  - Demonstration actions: Established demonstration farming enterprises and counties to practice the action plan to reduce the use of antimicrobials, and to promote the use of alternatives of antimicrobials with high efficiency and low residue.
  - Propaganda and Education Actions: Strengthen the training of practitioners and public awareness and education. For example, have launched a series of public welfare activities of "Scientific use of veterinary antibiotics", it will be expected to cover hundreds of counties, thousands of aquaculture enterprises, million farmers within a year.
  - Withdrawal actions: the gradual withdrawal of growth-promoting antibiotics. The approved use of veterinary antibiotics and drug feed additives to conduct a risk assessment, planned to complete the risk assessment by 2020, the eliminating the species of security risks.

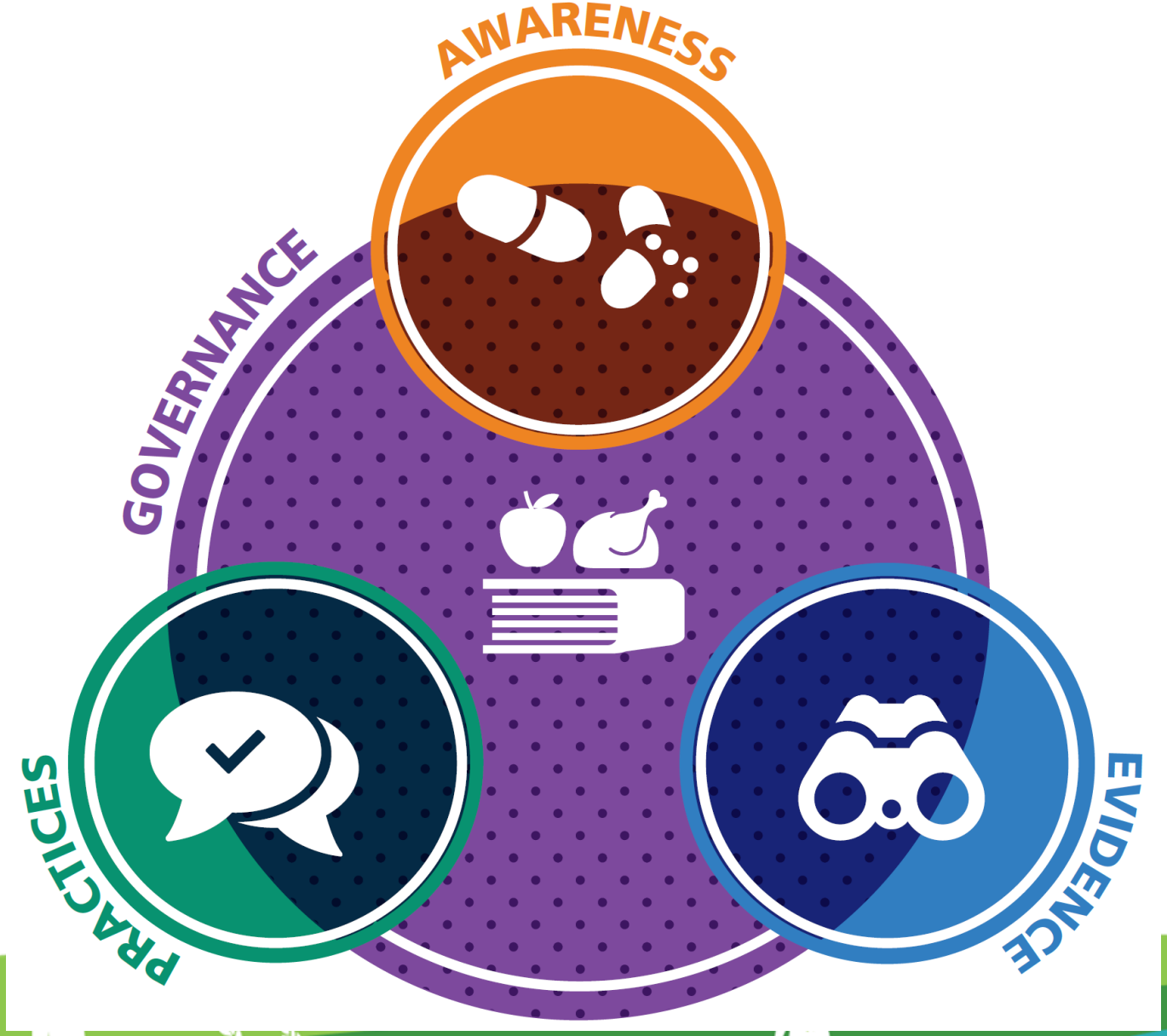


Figure 1. Four Focus Areas of the FAO Action Plan on AMR

Focus Area 1 : Improve Awareness on Antimicrobial Resistance and Related Threats

We have conducted the following activities in this aspect:

- Technological training for aquaculture farmers and technicians are hold at various scales and types around the year.
- AMU and AMR awareness propaganda for aquaculture professionals and the general public via conventional media, social media, and network social software.
- Editing a “Guidance for the Clinical Use of Veterinary Antimicrobials” .
- Conducting a demonstration building activity of safe use of veterinary antimicrobials.
- Others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2017年水产品质量安全培训班

Focus Area 2 : Develop Capacity for Surveillance and Monitoring of AMR and AMU in Food and Agriculture

- MoA has established a National Consultative Expert Commission of Antimicrobial Resistance Containment and Veterinary Drug Residues

- NFTEC has established an aquaculture AMU/AMR expert team

  This team are responsible of making up detailed AMR monitoring program, investigating the use status of aquatic drugs, providing technical protocol and instructions on antimicrobial susceptibility testing, evaluating the results, reporting the conclusions to the authority.

- The annual nationwide aquatic AMR surveillance and monitoring program organized by NFTEC. Almost all of the biggest provincial aquaculture producers are involved in the project.
- CLSI’s breakpoints were directly used to interpret the results for fish bacteria in most studies.
- Online Analysis System of AMR Surveillance Data of Pathogenic Bacteria Isolated from Aquaculture Animals developed by NFTEC and supported technically by Suzhou Jie’An Info Technology Co., LTD

- A comprehensive aquatic animal epidemic prevention system has been established in China
    - The regional aquatic animal epidemic prevention technology laboratories
    - 13 provincial aquatic animal disease control center
    - 628 county aquatic animal disease prevention station
    - Remote diagnosis system for aquatic animal diseases
    - Epidemic monitoring system (network) consisting of more 4210 monitoring and reporting spots cross the country
- Have established a large and perfect monitoring system for drug residues.

    MoA issues regular monitoring statistic information of quality and safety of agricultural products quarterly.

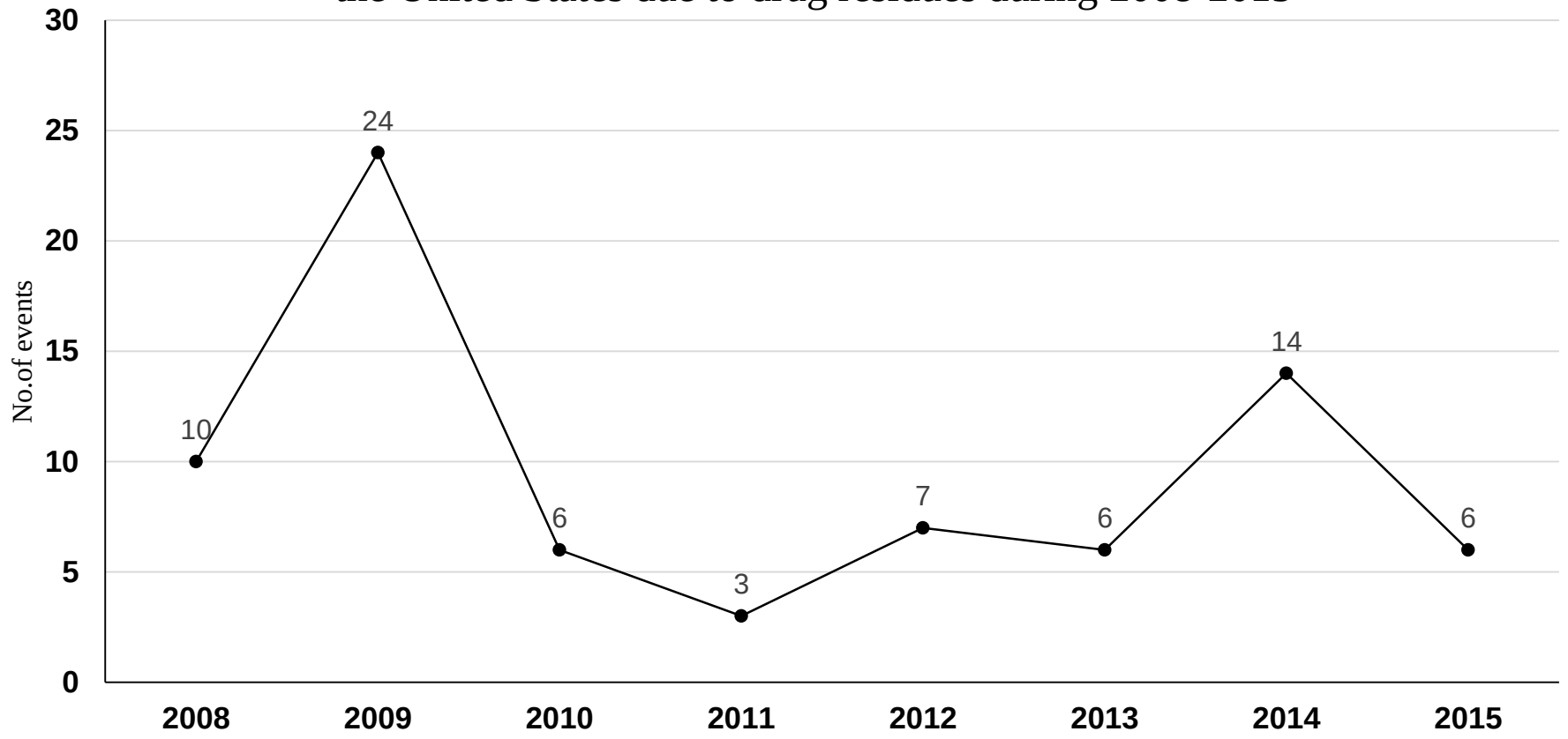
- Conducted surveillances of probiotics use in aquaculture carried by NFTEC.
- Conducted a questionnaire investigation for AMU in the selected farms in the key aquaculture provinces.
- Farm visit investigations conducted by the aquaculture AMR expert team.
- Various levels of laboratories which can perform antimicrobial susceptibility testing are all over the major aquaculture areas.

Currently used standards for detection of drug residues in aquatic products in China

Drugs	Criteria for detection	Detection method	Detection limit/μg.kg^{-1}
chloramphenicol	No. 958 Bulletin-14-2007,MoA	GC-MS	0.3
malachite green/crystal Violet	GB/T 20361-2006	HPLC	0.5
quinolones	SN/T 1751.3-2011	HPLC	0.2~50
erythromycin	GB 29684-2013	HPLC-MS	0.5
penicillin	GB 29682-2013	HPLC	3~10
cypermethrin, fenvalerate, deltamethrin	GB 29705-2013	GC	0.2
avermectin, ivermectin	GB-29695-2013	HPLC	2
trimethoprim	GB 29702-2013	HPLC	20
albendazole and its metabolite	GB 29687-2013	HPLC	0.5~10

GC-MS: gas chromatography-mass spectrometry; HPLC: high performance liquid chromatography

Numbers of FDA-notified incidents of Chinese aquatic product exports to the United States due to drug residues during 2008-2015



- Involved antimicrobials: chloramphenicol, sulfadiazine, sulfamerazine, sulfamethoxazole, trimethoprim, enrofloxacin and ciprofloxacin
- Involved aquatic product: crab(1), tilapia(4), leg of frog and others(9)

Focus Area 3: Strengthen Governance Related to Antimicrobial Use and Antimicrobial Resistance in Food and Agriculture

- Important laws related to the governance of food safety and AMU/AMR in agriculture
  - Animal Epidemic Prevention Law of the People's Republic of China
  - Agricultural Product Quality and Safety Law
  - Food Safety Law
  - Regulations on Administration of Veterinary Drugs

- Important policies related to the governance of food safety and AMU/AMR in agriculture

  - List of veterinary drugs banned by the Ministry of Agriculture of China
    - Bulletin No.176, No. 193 and 1519 of the Ministry of Agriculture
  - Decision on the Cessation of 4 Kinds of Veterinary Drugs (lomefloxacin, Ofloxacin, Norfloxacin, levofloxacin) in Food Animals
    - Bulletin No. 2292 Of The Ministry Of Agriculture （2015/9/1）
  - Cessation of use of colistin sulphate as premix for Animal Growth promotor
    -Bulletin No. No. 2428 of the Ministry of Agriculture （2016/7/26）
  - MRLs of Veterinary Drug in Animal Food (2002).
    - Bulletin No. 235 of the Ministry of Agriculture
  - Cessation of use of arsanilic acid, roxarsone (ROX) and olaquindox as drug feed additives in food animals was recommended (opinions are being collected)
  - Publication of “Management methods for clinical application of veterinary antimicrobial agents used in food animals (draft)”, Veterinary Bureau of MoA, Dec/2017

- Activities to enforce the implementation of the NAPs
    - The Whole Nation's Special Rectification Action Plan on Antibiotics, Banned Compounds and Veterinary Drug Residue Exceeding the Standards in Livestock and Aquatic Products (July, 2017)
    - MOA has established a National Consultative Expert Commission of Antimicrobial Resistance Containment and Veterinary Drug Residues (May,2017)
    - Annual national implementation program of veterinary drug residue monitoring plan (MoA)
    - New version of MRLs of veterinary drugs in animal products (including aquatic animals) is under revision.

- Activities to enforce the implementation of the NAPs (cont.)
    - Special rectification actions on veterinary drugs for 7 consecutive years since 2011.
    - Ministry of Agriculture issued "Five-year action plan for Veterinary Drug (antimicrobials) integrated governance " in 2015
    - Strengthening the punishment of illegal use of drugs in livestock and aquaculture.
    - Advocating and exploring antibiotics-free fish farming related technologies.

- Activities to enforce the implementation of the NAPs (cont.)
  - Standardized the development, approval, production, marketing and use of veterinary antimicrobial agents
    - Promote the implementation of the veterinary drug GLP, GCP, GMP and GSP.
    - Prudent approval of compound products
    - Antibacterial agents which are of human medical importance, are easy to produce accumulation and residue, or easy to develop cross-resistance will not be approved
    - Implement two-dimensional code management for the veterinary product labels and instruction manuals

FOCUS AREA 4 : Promote Good Practices in Food and Agriculture Systems and the Prudent Use of Antimicrobials

- Have issued a “pilot action plan for reduction of antimicrobial consumption in aquaculture”
    - Establish and implement operating procedures for disease prevention and control
    - Carry out surveillance of AMR of fish pathogenic bacteria
    - Advocate and guide precise medication technology: timely and accurate disease diagnosis; right selection of antibiotic based on the result of AST; using appropriate dose at the right time; avoiding overdose and extended treatment course; prohibiting prophylactic antibiotics use and being used as growth promoter.

- Alternatives to antibiotics are widely used in aquaculture
    - Probiotics
    - Bacteriophage
    - Prebiotics: Short-chain carbohydrates (oligosaccharides)
    - Herbal medicine
    - Vaccine
    - Dietary acidifiers, short-chain fatty acid
    - Egg yolk antibody(IgY)
    - Antimicrobial peptides
    - Bioflocs technology

- Innovating and popularizing new cultivation technologies. This is the key to reduce AMU and AMR in aquaculture
    - Integrated multi-trophic aquaculture (IMTA)
    - integrated ecological fishery such as Rice Field Integrated Farming of different aquatic animals, such as fish plus crayfish, and so on.
    - Application of microporous aeration technology
    - Industrialized recirculating aquaculture system (RAS)
    - Deep-sea cage farming technology
    - Bioflocs technology in shrimp culture
    - Multiple-trait selection technology of aquatic animals
    - Development of high-throughput quick diagnosis technology
    - Progress on fish vaccination

● ChinaGAP

Certification and Accreditation Administration of China **(CNCA)** was authorized to exercise administrative responsibilities of undertaking unified management, supervision and overall coordination of certification and accreditation activities including **Good Agricultural Practices (GAP)** across the country.

- GB/T 20014.13-2008 良好农业规范 水产养殖基础控制点与符合性规范
- GB/T 20014.14-2008 良好农业规范 水产池塘养殖基础控制点与符合性规范
- GB/T 20014.15-2008 良好农业规范 水产工厂化养殖基础控制点与符合性规范
- GB/T 20014.16-2008 良好农业规范 水产网箱养殖基础控制点与符合性规范
- GB/T 20014.17-2008 良好农业规范 水产围拦养殖基础控制点与符合性规范
- GB/T 20014.18-2008 良好农业规范 水产滩涂、吊养、底播养殖基础控制点与符合性规范
- GB/T 20014.19-2008 良好农业规范 罗非鱼池塘养殖控制点与符合性规范
- GB/T 20014.20-2008 良好农业规范 鳗鲡池塘养殖控制点与符合性规范
- GB/T 20014.21-2008 良好农业规范 对虾池塘养殖控制点与符合性规范
- GB/T 20014.22-2008 良好农业规范 鲆鲽工厂化养殖控制点与符合性规范
- GB/T 20014.23-2008 良好农业规范 大黄鱼网箱养殖控制点与符合性规范
- GB/T 20014.24-2008 良好农业规范 中华绒螯蟹围拦养殖控制点与符合性规范

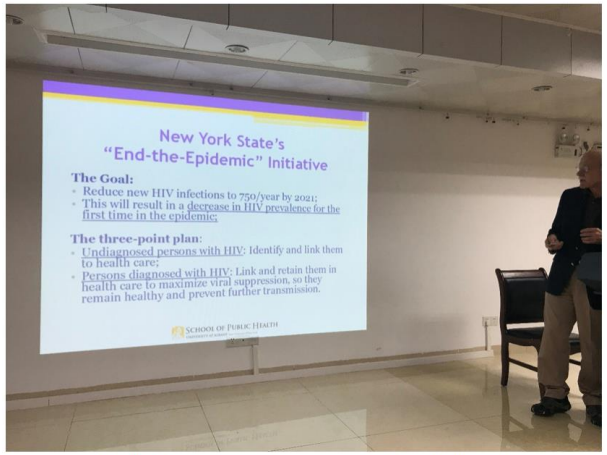
12 National Technical Standards for aquaculture GAP certification

Certified aquaculture product catalogue for GAP in China

Cage farming module	Large yellow croaker
Enclosure farming module	Chinese mitten crab (*Eriocheir sinensis*)
Pond farming module	mud crab (*Scylla serrata*), perch, softshell turtle, six species of carp, bream
Intertidal Mudflat Culture/ Hanging Culture/ Bottom-sowing Culture module	Shellfish, Acanthosis, Seaweed,

● “One health” in China

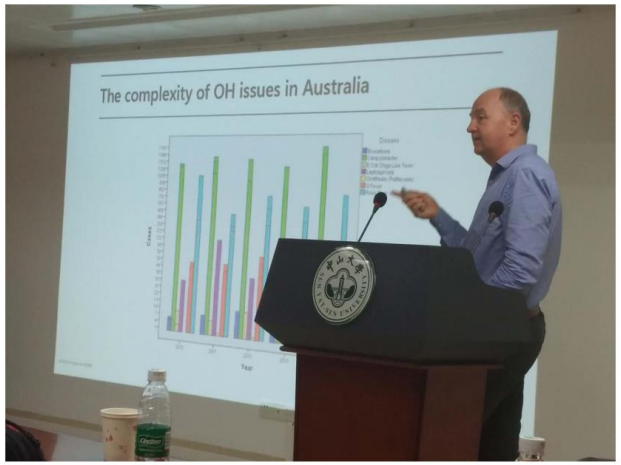
Major event 1:
The Second International “One Health Day” symposium Host by Sun Yat-sen University in Guangzhou



纽约州立大学阿尔巴尼分校Guthrie Birkhead教授作报告

One Health研究学术研讨会合影

中山大学陆家海教授作报告



昆士兰大学Simon Reid教授作报告

昆士兰大学Charles Gilks教授作报告

Major event 2

The construction of antimicrobial-resistant bacteria research platform based on "one Health“ is under consideration

基于“One Health”耐药菌研究平台建设论证会

时间：[2017-10-30] 来源：

10月19日，“基于‘One Health’耐药菌队列研究平台建设论证会”在综合楼316成功召开。会议由山东大学公共卫生学院环境卫生学系副教授李学文主持，中山大学陆家海教授、浙江大学郑焙文副教授、山东省动物疫病预防控制中心兰邹然教授、山东大学公共卫生学院徐凌忠教授等专家相聚一堂，就“‘One Health’耐药菌队列研究平台的建设”这一议题展开论证。公共卫生学院院长李士雪出席会议。

Major event 3

The first “One Health” training course in China

2016年1月11-15日，中国首届One Health 培训班在中山市举办。本次培训班由中山大学、昆士兰大学、昆士兰科技大学联合举办，由中山大学公共卫生学院One Health研究中心和中山市疾病预防控制中心承办。

培训班特邀澳大利亚昆士兰大学Simon Reid教授、澳大利亚昆士兰大学Maxine Whittaker教授、澳大利亚昆士兰科技大学Wenbiao Hu教授，以及中山大学陆家海教授等国内外著名One Health领域的专家前来授课。

Molecular Evolution of the SARS Coronavirus During the Course of the SARS Epidemic in China

The Chinese SARS Molecular Epidemiology Consortium*

Sixty-one SARS coronavirus genomic sequences derived from the early, middle, and late phases of the severe acute respiratory syndrome (SARS) epidemic were analyzed together with two viral sequences from palm civets. Genotypes characteristic of each phase were discovered, and the earliest genotypes were similar to the animal SARS-like coronaviruses. Major deletions were observed in the Orf8 region of the genome, both at the start and the end of the epidemic. The neutral mutation rate of the viral genome was constant but the amino acid substitution rate of the coding sequences slowed during the course of the epidemic. The spike protein showed the strongest initial responses to positive selection pressures, followed by subsequent purifying selection and eventual stabilization.

Severe acute respiratory syndrome (SARS) first emerged in Guangdong Province, China. Subsequently, the SARS coronavirus (SARS-CoV) was identified as the causative agent (*1*–*5*). It remains a challenge to establish the relationship between observed genomic variations and the biology of SARS (*4*–*8*). Recent molecular epidemiological studies have identified characteristic variant sequences in SARS-CoV for tracking disease transmission (*7*, *9*–*11*). Evidence suggests that SARS-CoV emerged from nonhuman sources (*8*, *12*). In this study, we sought epidemiological and genetic evidence for viral adaptation to human beings through molecular investigations of the characteristic viral lineages found in China (*13*).

On the basis of epidemiological investigations (*14*), we divided the course of the epidemic into early, middle, and late phases (Fig. 1). The early phase is defined as the period from the first emergence of SARS to the first documented superspreader event (SSE) (*13*). The middle phase refers to the ensuing events up to the first cluster of SARS cases in a hotel (Hotel M) in Hong Kong (*15*). Cases following this cluster fall into the late phase.

The early phase was initially characterized by a series of seemingly independent cases. Eleven index cases that had arisen locally in the absence of any contact history were identified from different geographical locations within Guangdong Province (fig. S1). This phenomenon was observed from the retrospectively identified SARS index patient from the city of Foshan (onset date, 16 November 2002) (*13*) through to an index patient from the city of Dongguan (onset date, 10 March 2003). All of these cases were confined to regions directly west of Guangzhou, the capital city of Guangdong Province, and to the city of Shenzhen in the south, with no cases being reported to the north or east of Guangzhou (Fig. 1) (fig. S1). This region, the Pearl River Delta, has enjoyed rapid economic development since the late 1970s, leading to the adoption of culinary habits requiring exotic animals. Seven of these 11 cases had documented contact with wild animals. In contrast to the apparently independent seeding of the earliest cases, the rest of the epidemic was characterized by SSEs and clusters of cases that were epidemiologically linked (Fig. 1) (fig. S1) (*10*, *11*, *13*, *15*, *16*).

The first major SARS outbreak occurred in a hospital, HZS-2, in the city of Guangzhou, beginning on 31 January 2003 where an SSE was identified to be associated with more than 130 primary and secondary infections, of which 106 were hospital-acquired cases. Doctor A, a nephrologist who worked in this hospital, visited Hong Kong and stayed in Hotel M on 21 February 2003. Other visitors to the hotel later became infected with SARS-CoV (*13*, *15*). This led to the transmission of SARS to Vietnam, Canada, Singapore, and the United States (*17*) with two further SSEs in Hong Kong, each resulting in the virus being transmitted to >100 contacts (*10*, *16*).

Genomic sequence data for SARS-CoV were largely derived from isolates linked to the Hotel M cluster (*6*), hence they were predominantly from the late phase of the epidemic. We determined 29 SARS-CoV genomic sequences obtained from 22 patients from Guangdong Province with disease onset dates in all three phases of the epidemic, and from two patients from the late phase in Hong Kong. To eliminate mutational noise, we assumed that sequence variants associated with common ancestry, but not arising in cell culture, should be seen in multiple isolates (*7*). Meanwhile, critical genomic variations or complete genome sequences of certain virus isolates were verified by sequencing the reverse transcription polymerase chain reaction (RT-PCR) products derived directly from patient specimens (*14*). The genomic sequences obtained were compared with 32 human SARS-CoV sequences and two SARS-like coronavirus sequences from Himalayan palm civets (*Paguma larvata*) available at GenBank as of the end of September 2003 (Fig. 2).

Only two major genotypes predominated during the early phase of the epidemic. Five isolates were found to contain a 29-nucleotide (nt) sequence that is absent in most of the publicly available SARS-CoV

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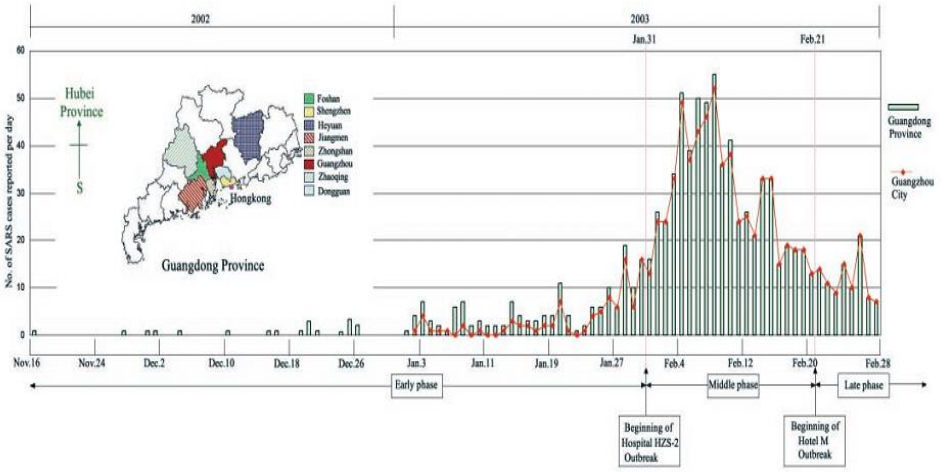

Fig. 1. The triphasic SARS epidemic in Guangdong Province, China. Shown are daily numbers of SARS cases reported in Guangdong Province, in particular the city of Guangzhou. The early, middle, and late phases of the epidemic are defined in the text. The map shows the geographical distribution of cases belonging to the early phase by administrative districts of Guangdong Province. The detailed data for individual cities are presented in fig. S1.

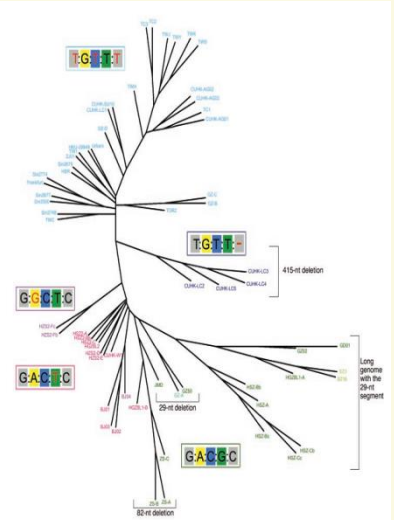

Fig. 2. Genotype clustering of SARS-CoV during the course of the epidemic. An unrooted phylogenetic tree of SARS-CoV is constructed from 61 human SARS-CoV genomes and two SARS-like coronavirus sequences from palm civets. Only those variant sequences (including deletions) that were present in at least two independent samples were used for tree construction (table S2). The map distance between individual sequences represents the extent of genotypic difference. The 5-nt motifs [see text] that characterized the phylogenetically related genotypes are boxed. The genomic sequences are named in concordance with their GenBank nomenclature and are represented in different colors according to the genotype clusters determined by our scoring method (table S2). Genotypes with major deletions are marked specifically (see text). All other genotypes (unmarked) had the 29-nt deletion. This 29-nt deletion was specifically marked for three genotypes, namely GZ-A, JMD, and GZ50, to indicate their special clustering within the early-phase isolates.

SARS related research

Physiol Genomics 18: 245–248, 2004.
First published May 25, 2004; 10.1152/physiolgenomics.00102.2004.

Report

Glycan arrays lead to the discovery of autoimmunogenic activity of SARS-CoV

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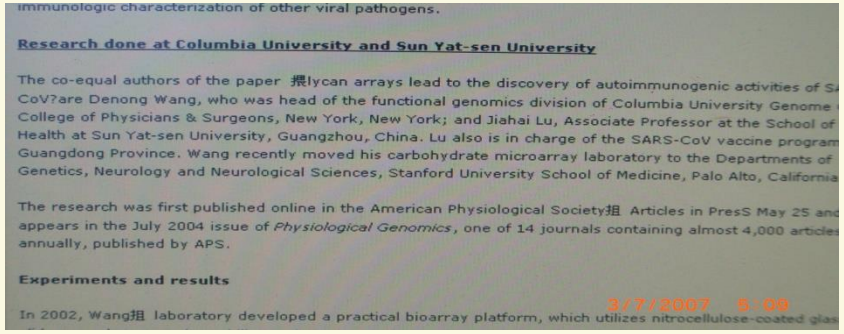
Wang, Denong, and Jiahai Lu. Glycan arrays lead to the discovery of autoimmunogenic activity of SARS-CoV. *Physiol Genomics* 18: 245–248, 2004. First published May 25, 2004; 10.1152/physiolgenomics.00102.2004.—Using carbohydrate microarrays, we characterized the carbohydrate binding activity of SARS-CoV neutralizing antibodies elicited by an inactivated SARS-CoV vaccine. In these antibodies, we detected undesired autoantibody reactivity specific for the carbohydrate moieties of an abundant human serum glycoprotein asialo-orosomucoid (ASOR). This observation provides important clues for the selection of specific immunologic probes to examine whether SARS-CoV expresses antigenic structures that mimic the host glycan. We found that lectin PHA-L (*Phaseolus vulgaris* L.), which is specific for a defined complex carbohydrate of

nology, we constructed a glycan array (Fig. 1, *A* and *B*)[1] to display a collection of 51 carbohydrate antigens, including both microbial polysaccharides and cellular glycan complex carbohydrates. Blood group substances A, B, O, Lewis, I, and i antigens, and their precursors and structural derivatives were specially included. These complex carbohydrates are covalently attached to cellular proteins by either *O*- or *N*-glycosylation linkages as protein posttranslational modifications or linked to a membrane-bound lipid molecule. Therefore, scanning the antibody fingerprints of immunized or infected subjects using this glycan array is a specific immunologic approach to exploring the evidence of viral expressio

We applied this strategy

preparation of anti-SARS


immunologic characterization of other viral pathogens.

Research done at Columbia University and Sun Yat-sen University

The co-equal authors of the paper 揝lycan arrays lead to the discovery of autoimmunogenic activities of SA
CoV?are Denong Wang, who was head of the functional genomics division of Columbia University Genome
College of Physicians & Surgeons, New York, New York; and Jiahai Lu, Associate Professor at the School of
Health at Sun Yat-sen University, Guangzhou, China. Lu also is in charge of the SARS-CoV vaccine program
Guangdong Province. Wang recently moved his carbohydrate microarray laboratory to the Departments of
Genetics, Neurology and Neurological Sciences, Stanford University School of Medicine, Palo Alto, California

The research was first published online in the American Physiological Society揾 Articles in PresS May 25 and
appears in the July 2004 issue of *Physiological Genomics*, one of 14 journals containing almost 4,000 articles
annually, published by APS.

Experiments and results

In 2002, Wang揾 laboratory developed a practical bioarray platform, which utilizes nitrocellulose-coated glas

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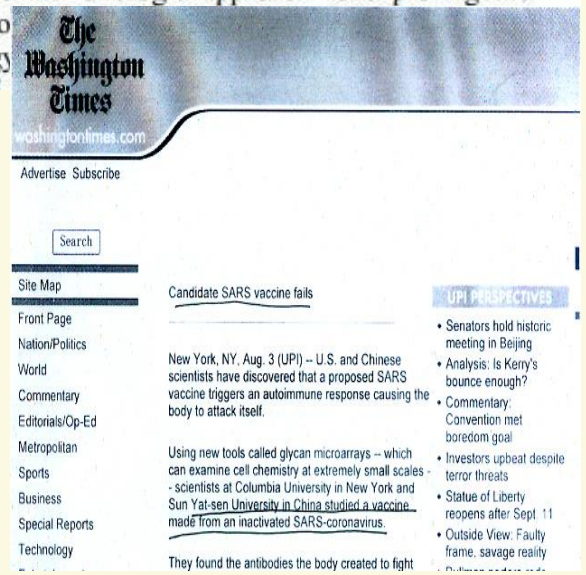
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Candidate SARS vaccine fails

New York, NY, Aug. 3 (UPI) -- U.S. and Chinese scientists have discovered that a proposed SARS vaccine triggers an autoimmune response causing the body to attack itself.

Using new tools called glycan microarrays -- which can examine cell chemistry at extremely small scales -- scientists at Columbia University in New York and Sun Yat-sen University in China studied a vaccine made from an inactivated SARS-coronavirus.

They found the antibodies the body created to fight

UPI PERSPECTIVES

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Brief report

A retrospective serological study of severe acute respiratory syndrome cases in Guangdong province, China

LIAO Jia-wei, LU Jia-hai, GUO Zhong-min, WANG Guo-ling, ZHANG Ding-mei, CHEN Liu-jing, ZHENG Huan-ying and ZHONG Nan-shan

Keywords: *severe acute respiratory syndrome; coronavirus; neutralizing antibody; epidemiology*

Severe acute respiratory syndrome (SARS) is a life threatening, upper respiratory disease. Its cause is a coronavirus, SARS-CoV. Since its emergence in 2003 in China, SARS has affected more than 8000 patients and caused 776 deaths in 26 countries.[1] A reemergence of SARS occurred in Guangdong province, in which the first case was confirmed on January 5, 2004 and three more reported the following month.

To better understand the immunological characteristics of the SARS pandemic, we studied SARS-CoV neutralizing antibody titres of four reemerging SARS patients using SARS-CoV strains (Z2-Y3 and F69) isolated from two previous cases. Their neutralizing antibody profiles were compared with those of fourteen SARS cases infected in Guangdong province prior to 2004, including the first identified case on November 16, 2002.

METHODS

Epidemiological investigation

Medical records and close contacts of the SARS patients, provided by Guangdong CDC, were analysed. Laboratory safety procedures were carefully reviewed.

Serum collection

Sera were collected from the four reemerging SARS cases (A, B, C and D) and from fourteen SARS cases before 2004 at different time points (Tables 1, 2).

SARS-CoV strains

SARS-CoV strain Z2-Y3 (NCBI/Genbank: AY394989) was previously isolated from the pharynx swab of a 35 years old female medical faculty member (Guangdong province) hospitalized on February 5, 2003, and diagnosed with the infection on February 12 of the same year. SARS-CoV strain F69 (NCBI/Genbank: AY313906) was previously isolated from sputum specimen of another case in Guangdong. The patient was hospitalized on April 3, 2003 and confirmed with SARS on April 9, 2003. Both strains were isolated from Vero-E6 cells and identified as SARS-CoV virus by electron microscopy, reverse transcription polymerase chain reaction and sequence analysis. SARS-CoV virus Z2-Y3 and F69 strains were sequenced and compared, showing certain differences (Table 3).

Determination of neutralizing antibody titre

TCID50 SARS-CoV was titrated by Reed-Muench method.[2] Titration results showed that Z2-Y3 and F69 strain titres reached 6.5log TCID50/25 μl and 6.6log TCID50/25 μl, respectively. Neutralizing antibody assay was carried out according to standard procedure (WHO, 1997. Manual for the virological investigation of polio. WHO/EPI/GEN/97.01). Sera from the four reemerging cases were inactivated at 56˚C for 30 minutes, and incubated with 100 TCID50 of both Z2-Y3 and F69 SARS-CoV strains at 36˚C for 2 hours. The same method was applied for sera from the fourteen earlier cases using Z2-Y3 strain isolated during the original epidemic. Vero-E6 cells (10^4 cells/ml) were added to the neutralizing mixture. Plates were incubated at 36˚C for 5−7 days and examined with an inverted microscope for the appearance of cytopathic effects.

RESULTS

To characterize the neutralizing antibody profiles of the four reemerging SARS cases, sera were collected at various times and incubated with two strains of SARS-CoV (Z2-Y3 and F69) isolated from patients infected in early 2003. The neutralizing antibody titres of 4 reemerging cases peaked within 11−13 days at a lower level (1: 160−1: 640), and then rapidly dropped after a short period of plateau (Fig). This observation is in sharp contrast to the neutralizing antibody titres of SARS

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This research was supported by the Science Foundation for SARS of Guangdong Province (No. 2003Z3-E0461).

Table 1. Human sera of 4 reemerging SARS cases in Guangdong province in 2004

Case	Sex	Age (years)	Date of onset	Date of diagnosis	Sample collection (Days after onset)	Epidemiology (contact)	
						Wild animal	Hospital
A	M	32	Dec. 28, 2003	Jan. 5, 2004	6,7,8,9,10,11,12,13,15,17	−	−
B	F	20	Dec. 28, 2003	Jan. 17, 2004	7,8,11,19,22	+	−
C	M	35	Dec. 31, 2003	Jan. 17, 2004	8,10,11,13,18	−	−
D	M	40	Jan. 7, 2004	Jan. 26, 2004	9,12,16	−	+

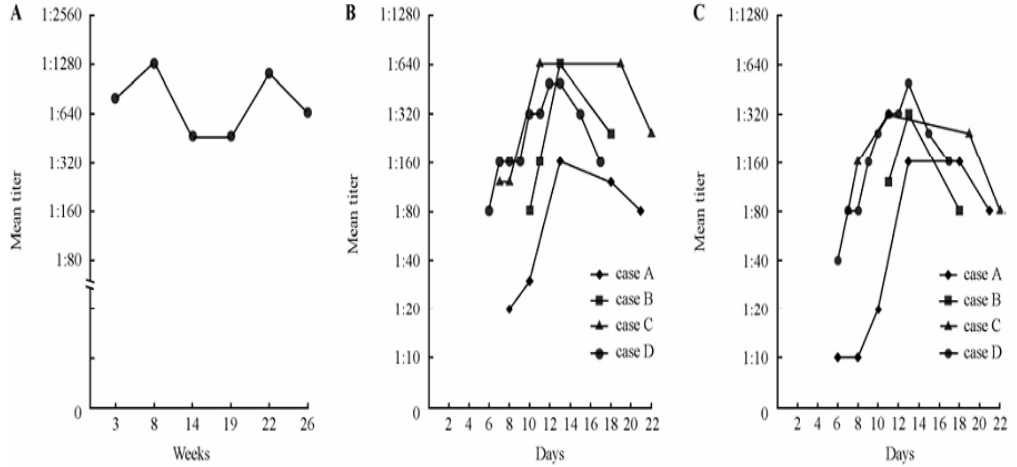

Fig. The neutralizing antibody titres of SARS cases. **A:** Neutralizing antibody titre of 14 SARS cases in retrospective screening; **B:** Neutralizing antibody titre of 4 reemerging SARS cases by SARS-CoV strain Z2-Y3; **C:** Neutralizing antibody titre of 4 reemerging SARS cases by SARS-CoV strain F69.

Table 2. Human sera of SARS patients in early 2003

Case	sex	Age(years)	Date of onset	Days from onset to sample collection
1	M	52	Jan. 7, 2003	17
2	M	23	Feb. 5, 2003	21
3	F	32	Jan. 2, 2003	50
4	M	44	Jan. 26, 2003	54
5	F	36	Feb. 7, 2003	94
6	F	36	Feb. 5, 2003	96
7	F	21	Feb. 2, 2003	99
8	M	31	Apr. 4, 2003	130
9	M	39	Jan. 5, 2003	137
10	M	32	Jan. 5, 2003	138
11	M	40	Dec. 16, 2002	151
12	M	35	Dec. 15, 2002	153
13	M	50	Nov. 27, 2002	174
14	M	45	Nov. 16, 2002	181

M: male; F: female.

Table 3. Complete genomic sequence comparison between F69 and Z2-Y3

Locus	1-15	2015	3852	5455	6247	6760	7347	7777	8094
F69	N1	C	C	T	C	G	A	G	T
Z2-Y3	−	T	T	C	T	A	C	A	C
Locus	8591	9333	10265	11493	13470	14186	16959	17565	20374 20383
F69	G	C	T	T	A	T	T	T	N2
Z2-Y3	A	A	C	C	G	A	C	G	−
Locus	21732	22233	24706	25275	25309	26488	27403	29358	
F69	G	T	G	G	G	G	T	G	
Z2-Y3	A	C	A	A	A	T	C	A	

N1: atattaggttttaca; N2: caagaatgta; −: no nucleotide.

Original article

Comparison of effectiveness of whole viral, N and N199 proteins by ELISA for the rapid diagnosis of severe acute respiratory syndrome coronavirus

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Keywords: *severe acute respiratory syndrome virus; N199; enzyme-linked immunosorbent assay*

Background Although severe acute respiratory syndrome (SARS) has been controlled, the subsequently emerging sporadic cases in 2004 emphasize the necessity of developing a rapid diagnostic method, which would be of great help in clinical diagnosis and also wild host screening. This study aims to establish an effective and rapid serological tool for the diagnosis of SARS-CoV by comparison among whole viral, N and N199 proteins by ELISA.

Methods SARS-CoV N and N199 (a truncated nucleocapsid gene) genes were cloned, expressed, identified by Western blotting, and applied in screening of human and swine samples. Sera of SARS convalescent-phase patients, normal human sera, sera of patients with other respiratory diseases, and swine sera were screened by ELISA, with whole SARS-CoV F69, N and N199 proteins as antigens.

Results The sensitivity and specificity of N and N199 proteins in human sera diagnosis were approximate (P=0.743), which was higher than whole viral protein but the difference was not significant (P=0.234). The N199 protein proved to be more specific in swine sera screening than whole viral and N protein (P<0.001).

Conclusion N199 protein is feasible in both clinical diagnosis and SARS-CoV reservoir screening.

Chin Med J 2007;120(24):2195-2199

Severe acute respiratory syndrome (SARS), an epidemic which triggered a worldwide panic in 2003,[1] is currently under control. SARS coronavirus (SARS-CoV), a novel coronavirus phylogenically un-related to previously identified coronaviruses, has been isolated and identified as the causative agent.[2-5] However, since the nosogenesis and immunogenesis of SARS-CoV have not been identified completely, effective treatment for SARS is still unavailable. The subsequently emerging sporadic cases emphasize the necessity of developing a rapid diagnostic method, which would be of great help in clinical diagnosis and also wild host screening. Unfortunately, such an effective method has not been established so far, due to the cross reaction between SARS-CoV and other viruses especially in pigs.[3,6,7]

The N protein of coronaviruses is highly conserved in each group, immunogenic, and abundantly expressed during infection. It has been identified as a suitable candidate for diagnostic applications for human and animal coronaviruses.

The sequence of the nucleocapsid gene of SARS coronavirus was found to have 26%–32% homology with nucleocapsid genes of various animal coronaviruses. To eliminate possible cross-reactions between the nucleocapsid protein of the SARS coronavirus and nucleocapsid proteins of various animal coronaviruses, in this study, a fusion protein named N199 was cloned, expressed, purified and applied for the sample screening by ELISA, and was compared with whole viral protein and N protein to evaluate the diagnostic capacity.

METHODS

Virus strains

SARS-CoV F69 (NCBI/Genbank AY313906) was isolated from a SARS patient from Guangdong Province, China in 2003.[8-10]

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GUO Zhong-min, LU Jia-hai, HAN Wen-yu and LIU Ze-yu contributed equally to this study.
This study was supported by a grant from the Science Foundation for SARS of Guangdong Province (No. 2003Z3-E0461).

Table 1. Human sera in ELISA screening

Groups	Samples (n)	Origin of serum samples	Date of collection
SARS convalescent-phase patients	36	Chest Hospital of Guangzhou	March, 2003
Healthy controls	50	School of Public Health, Sun Yat-sen University (volunteer blood donors)	September, 2003
Patients with other respiratory diseases	42	First Affiliated Hospital of Sun Yat-sen University	March, 2003

Table 2. Porcine sera in ELISA screening

Samples (n)	Origin of serum samples	Date of collection
14	Foshan, Guangdong	October, 2002
11	Zengcheng, Guangdong	November, 2002
12	Dongguan, Guangdong	October, 2002
11	Zhongshan, Guangdong	November, 2002
12	Ili, Xinjiang	December, 2003

Table 3. Primers facilitating N and N199 gene amplification

Gene	Primers
N	p1: 5'-GCACCATGGCTTCTGATAATGGACCCCAA-3' (NcoI)
	p2: 5'-CACGTCGACTGCCTGAGTTGAATCA-3' (Sal I)
N199	p1: 5'-GCAGAATTCATGCTAGACAGATTGAACCAGCTT-3' (EcoR I)
	p2: 5'-CACGTCGACTGCCTGAGTTGAATCA-3' (Sal I)

Table 4. The aminoacid sequence of N199 protein

LDRLNQLESKVSGKGQQQQGQTVTKKSAAEASKKPRQKRTATKQYNVTQAFGRRGPEQTQGNFGDQDLIRQGTDYKHWPQIAQFAPSASAFFGMSRIGM
EVTPSGTWLTYHGAIKLDDKDPQFKDNVILLNKHIDAYKTFPPTEPKKDKKKKTDEAQPLPQRQKKQPTVTLLPAADMDDFSRQLQNSMSGASADSTQA

Table 5. Results of indirect ELISA to human serous specimens (n(%))

Group	SARS convalescent-phase (n=36)		Serum of other respiratory diseases (n=42)		Normal sera (n=50)	
	Positive	Negative	Positive	Negative	Positive	Negative
Whole virus	27 (75.0)	9 (25.0)	2 (4.8)	40 (95.2)	2 (4.0)	48 (96.0)
N protein	31 (86.1)	5 (13.9)	0 (0)	42 (100)	0 (0)	50 (100)
N199 protein	30 (83.3)	6 (16.7)	0 (0)	42 (100)	0 (0)	50 (100)

Table 6. Results of indirect ELISA to swine sera (n(%))

Group	n	Positive	Negative
Whole virus	60	59 (98.3)	1 (1.7)
N protein	60	58 (96.7)	2 (3.3)
N199 protein	60	5 (8.3)	55 (91.7)

- Plans for the future: The Ministry of Agriculture (MoA) will further:
  - intensify the veterinary drug management measures;
  - improve the national veterinary drug information data platform;
  - promote the construction of two-dimensional code traceability system, in order to strengthen the traceability supervision of the whole process covering the production, management and use of veterinary drugs
  - expand the demonstration scope of antimicrobial use reduction.
  - reinforce monitoring of veterinary drug residues and AMR
  - increase the strength of risk assessment of AMU
  - carry out in depth supervision, inspection and rectification campaign of veterinary antimicrobials, especially in the feed production link.

How serious is the bacterial resistance of animal origin in China? and What measures were/will be taken to curb antimicrobial resistance of animal origin?

This was the topics of the presentation given by, Dr. Cai Xuepeng, the Director of China Institute of Veterinary Drug Control, an important official from Veterinary Bureau, MoA, on “2017 National Animal Health and Food Safety Summit Forum” which was held on Nov. 11,2017 in Beijing.
http://news.nm18.com/201711/17/207.html

- This is a kind of evidence that AMR has become the priority of the government or an important task of governmental work.

As a country delegates for this project, my contribution in term of initiatives/action taken

- Suggested that NFTEC set up a team of aquaculture AMU/ AMR experts, and conducted farm visits for AMU investigations with other expert members and officials.
- Distributed materials produced from the previous workshops to my colleagues and my students.
- Provided guidance for AMR survey for some laboratories.
- Delivered and emphasized the importance and necessity of prudent and responsible use of antimicrobials on training courses.

My future role on AMR work and my plans to achieve this role:

- As the member of aquaculture AMU/ AMR expert team, and the member of National Consultative Expert Commission of Antimicrobial Resistance Containment and Veterinary Drug Residues, I may have chance to provide some suggestions or advice for the implementation or modification of the aquaculture component of current NAP for AMU/AMR.
- Continue to provide guidance for AMU/AMR investigations
- Continue to use a variety of training courses to publicize the importance of prudent and responsible use of antimicrobials.
- As scientific researcher, I will conduct some studies on AMR issues, and will try to apply funding to do this work although it is not easy.

Thank you very much for your attention !

感谢您的关注 !