Update on diagnosis and chemotherapy of malaria

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Malaria, a disease acquired from "bad air of marshes", is known as early as 1700 BC. Worldwide there are 300 - 500 million cases annually, accounting for 1.5 to 2.1 million deaths each year, over 90% of these deaths occur in sub-Saharan Africa, most of them are children under five years of age and pregnant women.

Malaria was endemic in Hong Kong before and soon after the Second World War. Local transmission of malaria was successfully interrupted by 1969. Hong Kong is geographically surrounded by malaria endemic countries. Imported cases are unavoidable. A mosquito vector Anopheles minimus is present in parts of the New Territories making Hong Kong vulnerable and receptive to malaria.

DON'T MISS MALARIA

While over-diagnosis and unscrupulous use of anti-malarial agents leading to drug resistance is the problem of malaria endemic countries, under-diagnosis and late recognition leading to mortality are recurrent medico-legal stories in non-endemic countries. In developed countries, medical practitioners should suspect and actively exclude malaria in any ill patient returning from malaria endemic country. Falciparum malaria may not exhibit classic symptoms such as rigor, sweats or palpable spleen. Fever may not be marked. Cough or diarrhoea is common. The illness is not respiratory tract infection nor gastroenteritis until proven not to be malaria. An apparently clinically stable patient may deteriorate within hours to confusion, coma, multi-organ failure and death.

RAPID DIAGNOSTIC DEVICES

Thick and thin blood films are standard methods for laboratory diagnosis. Negative smears should be repeated every 12 hours for 48 hours if malaria is suspected. Malaria rapid diagnostic devices have been developed in recent years.

Immunochromatographic detection of Plasmodium falciparum histidine-rich protein 2 (PfHRP-2) present on surface of infected red cells is the basis of dipstick test such as ParaSight-F (Becton Dickinson, Sparks, Maryland, USA). Immunochromatographic detection of plasmodial genus-specific lactate dehydrogenase (pLDH) is the basis of another dipstick test OptiMal (Flow Laboratories, Portland, Oregon, USA) which detects also P. vivax. The dipstick tests are suitable for field conditions where microscopes and skilled technicians may not be available. Rapid diagnosis is available within 10 minutes. The Parasight F test takes up to 14 days to revert to negative after curative therapy and false positives are reported in patients with rheumatoid

factor. The OptiMal test reads negative when parasitaemia resolves, allowing treatment evaluation.

The limitations on self-use of rapid tests for malaria diagnosis have been described. One limitation is the impaired performance when done by sick or delirious patients. A healthy companion should be asked to do the test. The other limitation is the prozone event. All immunological tests are hampered by false-negative results at high antigen concentrations. A 1 in 10 0.9% sodium chloride dilution of the sample is needed in such situation to obtain a correct positive result. Diluting a negative sample in the case of a severely sick patient is recommended to exclude the prozone phenomenon.

NEW TREATMENT

A concept that is receiving increasing support is the use of combination therapy of drugs with independent modes of action. The potential benefits include synergistic effects resulting in improved efficacy, and reduced exposure time of parasite populations (biomass) to drugs thus reducing occurrence of drug-resistance mutations. The combinations that appeared promising are artemisinin derivatives together with longer acting, slowly eliminated compounds. Artemisinin drugs exert a rapid effect on malaria parasites. A 1*0"* - 10* fold decline of Plasmodium falciparum / vivax load per 48-hour replication cycle is common. Recrudescence can be prevented by combining drugs with a longer residence time.

Artesunate plus mefloquine

The best-tolerated and most efficacious regimen for treating uncomplicated Southeast Asian multi-drug resistant falciparum malaria is 3 days of oral artesunate plus mefloquine. Artesunate 4 mg/kg/day for 3 days plus mefloquine 25 mg/kg total given as a divided dose 8-24 h apart. Efficacy rates of 94% are reported in Thailand. In complicated and severe malaria with vomiting or impaired consciousness which preclude use of oral drugs, intravenous artesunate at 1.2 mg/kg IV twice daily on first day then 1.2 mg/kg daily until oral therapy is possible, or artesunate supportories 1200 - 1600 mg total in adults are effective.

Co-artemether (CGP 56697)

Co-artemether is a fixed-dose combination of lumefantrine (benflumetol) and artemether. Lumefantrine is an amino-alcohol with structural similarities to mefloquine and halofantrine. Artemether is a semi-synthetic artemisinin derivative. The combination originated in China and is in advanced clinical development by Novartis. Four doses of co-artemether over 24 hours achieved cure rates of 94 - 98% at 28 days. One study demonstrated that co-artemether resulted in much lower gametocyte levels in infected patients than did sulphadoxine-pyrimethamine, confirming the effect of artemether component in reducing malaria transmission. The cost of this product is likely to be high. There is a potential for cross-resistance with mefloquine because

of structural similarities. It was suggested that the cure rate could be further improved with higher and prolonged dosing.

Artesunate plus sulphadoxine-pyrimethamine (SP)

Resistance to cheap efficient antimalarial drug poses an increasing threat in Africa. SP is widely used in sub-Saharan Africa as a replacement for chloroquine. The emergence of resistance to SP, already seen in East Africa, is likely to increase childhood mortality in many regions where no obvious replacement for SP is available. The combination of an artemisinin derivative with SP might delay or prevent the emergence of resistance to SP analogous to the combination of artesunate and mefloquine in Thailand to combat emergence of mefloquine resistance.

A double blind, randomised, placebo-controlled trial in Gambia comparing SP alone versus artesunate plus SP for uncomplicated P. falciparum malaria in children have demonstrated faster clearance of parasitaemia and reduced gametocytaemia when artesunate was added. The combination may reduce drug-resistance mutations during treatment because of reduced biomass, and may reduce transmission of potentially resistant parasites due to reduced gametocytaemia.

Atovaquone-proguanil (Malarone)

Combination of a novel agent atovaquone with an old drug proquanil in fixed dose as Malarone appeared in 2 tablet sizes. The adult tablet is atovaquone 250 mg / proquanil 100 mg and the paediatric tablet is atovaquone 62.5 mg / proquanil 25 mg. Malarone is widely registered for treatment of uncomplicated falciparum malaria. Atovaquone inhibits mitochondrial electron transport and also parasite pyrimidine biosynthesis. Proquanil is a prodrug of cycloguanil, a highly effective inhibitor of dihydrofolate reductase, also modulates pyrimidine biosynthesis.

The combination should be used only for acute uncomplicated falciparum malaria. The dose of malarone is 4 tablets daily for 3 days in adults. To enhance absorption, it should be taken within 45 minutes after eating. To decrease nausea and vomiting the dose can be divided into two.

Malarone is expensive (US$42 for 12 tablets). It is affordable for wealthy travellers and the military. It is made available to some African countries through a donation programme. There is worry of drug resistance after widespread use. Combination with artemisinin derivatives is suggested for use in endemic countries.

Reversal of chloroquine resistance

Chloroquine was developed half a century ago as a powerful weapon against P. falciparum malaria. It was only after a decade of widespread use that resistance appeared. Chloroquine has an unusual mechanism of action. After invasion of a red blood cell, the malaria parasite feeds on intracellular

proteins. The digestion of globin chains of haemoglobin releases heme which is toxic to the parasite. The parasite converts heme to an inert crystal haemozoin. Chloroquine binding to heme prevents its crystallisation, allowing heme concentration to rise and kill the parasite.

Mutation of a single gene (pfcrt) or multiple genes (pfcrt and pfmdrl) have been hypothesised to be responsible for altering the transport of chloroquine across the membrane of parasites' digestive vacuole, the basis of chloroquine resistance.

Many drugs in clinical use have demonstrated ability to chemosensitise chloroquine resistant parasites, providing hope for cheap and effective ways of restoring usefulness of chloroquine.

Chlorpheniramine, a histamine HI receptor blocker, seems to have a clinical impact in reversing chloroquine resistance. In one study chlorpheniramine-chloroquine combination out-performed chloroquine in an area of high drug resistance and cured 77% of children with chloroquine treatment failure. In another study, chlorpheniramine-chloroquine combination compared favourably with SP in acute uncomplicated falciparum malaria in Nigerian children. Promethazine, which is used clinically to treat chloroquine-induced pruritus, was shown to enhance chloroquine efficacy in monkey malaria model infected with chloroquine-resistant strains. In vitro synergising effects were also reported with verapamil, praziquantel, cimetidine, amitriptyline and others.

NEW CHEMOPROPHYLAXIS

Development in this area may help to overcome compliance problem associated with long duration regimens currently recommended.

Malarone

Malarone is active against both asexual and sexual forms of malarial parasite, as well as against liver stages. It is recently registered for prophylaxis of malaria. It is highly effective for prophylaxis of P. falciparum malaria with 95 - 99% efficacy. Efficacy against P. vivax may be lower at 70 - 90%. Efficacy against P. ovale and P. malariae is not well studied. The adult prophylactic dose is one tablet daily with meals. The activity against exo-erythrocytic stage of parasite allows travellers to discontinue Malarone one week after leaving a malarial endemic area. It is better tolerated than mefloquine or chloroquine-proquanil. Malarone may help to overcome compliance problem associated with long duration regimens currently recommended.

Primaquine

A synthetic 8-aminoquinoline, has long been used as causal prophylaxis in P. vivax and P. ovale malaria and for eradication of gametocyte after treatment of P. falciparum malaria. It is active on liver-stage parasite and gametocytes. It is a useful prophylactic agent for P. falciparum and P. vivax where chloroquine resistance is a problem. At a daily dose of 0.5 mg/kg (adult dose

30 mg daily), the prophylactic efficacy is comparable to doxycycline or mefloquine. Because the prophylactic activity of primaquine is primarily against infected hepatocytes rather than erythrocytes, travellers only need to take it for 2-3 days after leaving a malaria endemic area instead of the 4 - 6 weeks required for doxycline or mefloquine. Mild methaemoglobinaemia (< 13%) is an anticipated side effect. In a healthy adult, up to 25% methaemoglobinaemia can be tolerated well. Primaquine is a safe and effective short-term prophylactic agent in G6PD normal non-pregnant visitors to malaria endemic areas.

Tafenoquine

Tafenoquine (WR 238605) is a new synthetic analogue of primaquine, with an improved therapeutic index and safety profile. Currently it is the only agent active against all stages of the malarial parasite. It has a much longer half-life than primaquine (14 days vs 6 hours). Study in Thailand showed that it is safe and effective against P. vivax relapse. At 300 mg daily for 7 days, it was 100% effective in radical cure of P. vivax. Study in Gabon showed that it is a safe and effective prophylactic agent for P. falciparum. After 250 mg daily for 3 days, none of 84 subjects had positive blood smear when observed up to 11 weeks. It has potential to replace the 14-day primaquine regimen for eradication of latent P. vivax or P. ovale hepatic stages. The risk of haemolysis in G6PD deficient individuals needs further evaluation.

Azithromycin

Several antibiotics are active against malaria. They target bacterial protein synthesis which is present in a plastid-like organelle in malaria and related parasite. This explains the use of doxycycline in therapy and prophylaxis of malaria. Azithromycin daily was compared with doxycycline daily in prophylaxis of malaria in an indigenous Kenyan population. The protective efficacy was 82% for Azithromycin versus 93% for doxycycline. Although not registered for malaria prophylaxis, Azithromycin might find a use in high-risk groups unable to take doxycycline, such as children or pregnant women.

Rhodococcus equi infection

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Originally isolated from granulomatous lung infection in horses in 1923 by Magusson, *Corynebacterium* (now *Rhodococcus) equi* remains an important pathogen of foals. After the first human case reported in 1967 by Golub et al.[1] *R. equi has* emerged as an increasingly important opportunistic pathogen, primarily affecting severely immunocompromised patients. Over 100 cases have now been reported worldwide[2], the majority of which are HIV infected patients. Other immunocompromised patients at risk include those on long term steroid therapy, those with haematological malignancies, organ transplant recipients, patients with renal failure (especially those on peritoneal dialysis), chronic alcoholics and patients with diabetes. Other patient groups at risk include those with trauma and major wounds, intravenous drug users, and those with chronic lung diseases. Nevertheless, even immunocompetent patients without predisposing factors may be affected[3].

R. equi is a water and soil organism which is most commonly found in environment associated with domesticated animals, whose manure provides nutritional support for these organisms[4]. Human infection is believed to occur secondary to exposure of a susceptible individual to the appropriate environment, through the process of inhalation, ingestion or direct inoculation of the organism. Indeed, a history of animal or manure contact can be elicited in up to 50% of reported cases[5]. Part of the phylogenetic group of nocardioform actinomycetes, *R. equi* is a Gram positive, partial acid fast, facultative intracellular organism that is characterized in part by rod-to-coccus growth cycle variation and the presence of tuberculosteric acid and cell wall mycolic acids. The pathogenic potential of *R. equi* results from its ability to persists in and destroys macrophages[6]. It can be easily grown on unselective media, producing typical red coloured colonies from which the organism derives its name. It can be easily identified in the modern microbiology laboratory by a commercially available panel of biochemical tests (API Rapid CORYNE).

Clinically, the lung is the most frequent site of involvement, although other sites including the eyes, lymph nodes, soft tissue and bone have also been reported[7]. In one large review of 72 cases, pneumonia occurred in 76% of patients, and the lung was the sole site of infection in 82%[8]. Pulmonary infection with the organism typically leads to development of necrotizing pneumonia, lung abscesses, empyema, pleural effusions and even pneumothoraces. In addition, secondary haematogenous spread causing brain abscess is not uncommon in immunocompromised patients. Patients usually present with insidious onset of fever, chills, weight loss, dyspnea and cough. Chest radiographs often reveal thick-walled cavitating lung lesions, sometimes with air-fluid levels within the cavities, and hilar lymphadenopathy and pleural effusions may also be found. Although it is overall an uncommon opportunistic infection in HIV-infected patients, its propensity to cause upper lobe cavitary lesions in this patient group may be the cause for diagnostic confusion with *Mycobacterium tuberculosis* infection. However, *R. equi*

typically affects patients with advanced HIV disease (CD4 count < 200, or those patients that has progressed to AIDS already)[9], whereas *M. tuberculosis is a* more likely cause for pulmonary disease in less advanced patients. Also, *M. tuberculosis* is less likely to cause cavitary lung lesions in AIDS patients than in patients in early stage of HIV infection[10]. Other differentials for cavitary pulmonary lesions in HIV-infected patients include *Pneumocystis carinii* pneumonia, Cryptococcus, Coccidioidomycosis, Histoplasmosis, Aspergillosis, *Mycobacterium kansasii, Pseudomonas aeruginosa, Nocardia asteroides,* Kaposi's sarcoma and lymphoma[11].

Diagnosis of the infection relies on culture of the organism from blood (positive in > 50% of HIV patients infected), sputum, and other tissue samples. Bronchoscopy with bronchoalveolar lavage and lung biopsy is indicated for investigation of cavitary lung lesions. The lung biopsy typically shows granulomatous reactions, and the presence of matacoplakia with typical Michelis-Gutmann bodies in macrophages is highly suggestive of the condition. *R. equi* is not difficult to grow, but is frequently regarded as "contaminating diphtheroids" in the laboratory. In addition, there has been reports of misdiagnosis of the organism as mycobacterium due to its staining properties. Therefore, clinicians and microbiologists must have vigilance and prompt communication to alert each other whenever *R. equi* infection is suspected, in order to ensure a rapid and accurate diagnosis of the condition.

Treatment for R. equi infection is often difficult and no firm recommendations can be made due to paucity of data from large clinical trials. In general, multiple antibiotics should be used for prolonged courses in immunocompromised patients, and repeated culture and sensitivity monitoring should be performed during therapy. The organism has been shown to be susceptible in vitro to erythromycin, extended-spectrum macrolides, rifampicin, fluoroquinolones, aminoglycosides, glycopeptides and imipenern[11]. Beta-lactams should be avoided even if the organism is susceptible, as resistance has been shown to develop during therapy[12]. Several combinations of antibiotics have been shown to have synergistic activity against *R. equi, and* these include erythromycin-rifampicin, erythromycin-minocycline, and imipenam-amikacin[13]. Treatment is divided into 2 phases, and in the initial bacteraemic phase antibiotics with proven bactericidal effect in serum and tissue should be used in combination; the choices include aminoglycosides (especially gentamicin), imipenam and glycopeptides (vancomycin and teicoplanin). In the maintenance phase, in which the organisms residing in macrophages need to be eliminated, antibiotics that reach high concentrations inside macrophages should be employed; the choices include macrolides, quinolones and rifampicin[9]. Treatment in immunocompromised patients with pneumonia must span at least 2 months using several agents in combination, whereas immunocompetent patients may only need single agent therapy. Patients should be carefully monitored during therapy with serial cultures and imaging to ensure that the treatment response is satisfactory. In HIV patients, due to the high chance of relapse, indefinite secondary prophylaxis therapy with macrolides is recommended. Surgery may be indicated for resection of lung abscess and pseudotumor and has been associated with improved survival in some cases. The prognosis of *R. equi* infections in HIV patients is

grim before the era of HAART, with >80% of infection leading to chronic progression and death; a mortality rate of up to 50% has been reported in recent series. In contrast , the mortality rate in non-HIV immunocompromised patients is 20-25% and that in immunocompetent patients as around 10%. It remains to be seen whether the widespread use of HAART will result in better survival and reduced incidence of this infection among HIV patients in future.

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An update on travellers' diarrhoea
(Highlight of the 7th Conference of the International Society of Travel Medicine, 27 - 31 May 2001, Vienna)

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Travellers' Diarrhoea (TD) is defined as 3 loose stools per day coupled with at least one abdominal symptom. It is considered the most frequent health complaint in travellers, with 2-week incidence ranging from 20% to 50% according to provenance and destination (Castelli F.)

The main risk factors for TD were reviewed by Dr. Peltola H. They include a) a travelling history to certain countries including tropical Africa, Latin America and Asia, where up to 60% of visitors from industrialized countries contract TD; b) “risk behaviour” in alimentation (negligence of food and drink hygiene) especially in the group of backpackers; c) travel during the summer season; and d) being age > 30 years. TD is bacterial in origin in most cases, though the etiology of 20 - 30% of the cases remains unknown. During the journey, travellers may acquire orally different bacteria which include *Enterotoxigenic E. Coli, Campylobacter jejuni, Salmonella spp.* non-cholera *Vibrios, Aeromonas spp.* and *Shigella spp.* according to geographic area. Bacterial drug resistance is an increasing problem in many parts of the developing world. For example, there is a rising trend of quinolone-resistant Campylobacter in Thailand.

Most travellers with diarrhoea can be hydrated by drinking soups and sugar flavored mineral waters with salty crackers without oral rehydration solution. Nonspecific symptomatic therapy may decrease frequency of diarrhoea and improve stool form. Nonspecific therapy of TD includes a) bismuth subsalicylate which will decrease diarrhoea through salicylate-mediated anti-secretory effects; b) anti-motility agents which transiently improve symptoms without producing a clinical cure. Loperamide is the more effective anti-motility agent in reducing stool number.

Because of the importance of bacterial agents, antibacterial drugs play the central role in the therapy of TD where cure (passage of no further unformed stools) is expected. Antibacterial drugs have become the standard therapy for moderate to severe cases of TD. The older antibacterial drugs such as ampicillin and septrin are of limited value because of increasing bacterial resistance in most regions of the world.

Fluoroquinolones, given in single dose or daily up to 3 days depending on the persistence of symptoms or the presence of bloody diarrhoea, remain the drugs of choice for adult travellers to areas where quinolone-resistant Campylobacter are uncommon (Dupont H.L). In other areas, and in all high-risk areas for children and pregnant women, a single dose of azithromycin may be the preferred agent.

Because of the growing resistance to fluoroquinolones in many parts of the world and its limitation of use in children and pregnant women, new

therapeutic agents are needed. Poorly absorbed orally administered antibacterial drugs such as bicozamycin and aztreonam have been shown to shorten diarrhoea of travellers even when the patients have shigellosis or are passing fecal leukocyte-positive stools (Dupont H.L.). Recently, rifaximin is another promising new drug in the treatment of TD.

Rifaximin is a new oral semisynthetic rifamycin antimicrobial showing < 1 % gastrointestinal absorption. Its broad antimicrobial spectrum includes most gram-positive and gram-negative bacteria, both aerobes and anaerobes. The MIC90 of rifaximin for bacteria isolated from TD patients from Mexico, India, Kenya and Jamaica averaged 32 ug/ml, 250 times lower than the concentration of the drug in stool after three days administration (Dupont H.L.). Three randomized, double-blind studies were presented by Dr. Robert Steffen to show the promising value of rifaximin in the management of TD: 1) A trial done among 72 TD patients in Mexico showed that rifaximin reduced the duration of TD to 43 hours as compared to septrin (56 hours). Clinical failures occurred in 6 of 55 (11%) rifaximin patients as compared to 5 of 17 (29%) of septrin patients. 2) A second study compared the effect of rifaximin 400mg bid and ciprofloxacin 500mg bid, in 187 adult TD patients in Mexico and Jamaica. The median time to the last unformed stool was very similar in both groups (26 vs 25 hours) and equally there was no significant difference in the proportion of subjects who failed to respond to treatment. 3) The third trial compared rifaximin and placebo among 380 TD patients in Mexico, Guatemala and Kenya. The median time to the last unformed stool was significantly shorter in the active treatment group (33 hours) as compared to placebo controls (59 hours) ($p < 0.001$). The tolerance of this non-absorbable medication is good, with no significant difference in the overall incidence of adverse events between the rifaximin and the placebo groups.

Prevention is better than cure. However, there have been only a few enteric vaccines available. They include oral vaccines against cholera and typhoid, and parenteral vaccines against hepatitis and typhoid fever. There are now several promising new vaccines mentioned by Dr. Svennerholm A.M.: a) Both live oral and inactivated parenteral vaccines against shigellosis have been developed and tested for safety and immunogenicity in human volunteers and have demonstrated promising results. A parenteral *Shigella sonnei* conjugate vaccine has afforded approximately 70% serotype-specific protection for at least 3 months; b) An oral inactivated fimbriated *Enterotoxigenic E. coli* has been shown to be safe and to result in strong mucosal immune responses against the key antigens (i.e. the enterotoxin and the colonization factors) in human volunteers. In preliminary studies, this vaccine has afforded 80% protection against *Enterotoxigenic E. coli* diseases in travelers. Hopefully, a cocktail of vaccines against the most prevalent enteric infection may soon be available as immunoprophylaxis against TD.

The 2nd Current Topic in Infectious Diseases on Influenza Centre of infection. Faculty of Medicine, the University of Hong Kong

Introduction

The 2nd Current Topic in Infectious Diseases on Influenza organized by the Centre of Infection of the Faculty of Medicine, The University of Hong Kong, was held on March 26, 2001. The meeting was chaired by Prof. Y. L. Lau (Department of Paediatrics). Speakers included: Professor Ann Arvin (Department of Pediatrics, Division of Infectious Diseases, Stanford University) as well as Dr. J. S. M. Peiris and Professor Kennedy Shortridge (Department of Microbiology). The following were abstracts of the presentation made by the speakers.

Overview of influenza in Hong Kong

J. S. *M. Peiris*

The disease burden of influenza in Hong Kong (and in other tropical or semi-tropical settings) is unclear. This is, in part, due to the "broader" seasonality of influenza in Hong Kong where influenza occurs through January - August. The impact of influenza in elderly residential homes has been recognised and this group is now targeted for influenza vaccination. Data from centres in Hong Kong where viral diagnosis is intensively used has shown that influenza is a significant cause of morbidity and hospitalisation of children, and that its impact is no different to that seen in temperate regions. Similar data for the elderly in the community and other established risk groups are urgently required.

The H5N1 influenza virus - before and after 1997

Kennedy F. Shortridge

The recognition that the H3 antigen of the H3N2 pandemic virus that emerged through Hong Kong in 1968 was of avian origin provided the impetus for studying the ecology of influenza viruses worldwide. Ecological studies commenced in Hong Kong in 1975 and led to three core hypotheses:
Pandemic influenza is a zoonosis.
Southern China is a hypothetical epicentre for the emergence of pandemic influenza viruses.
The domestic duck is the principal reservoir of influenza viruses in the region and the domestic pig a 'mixing-vessel' for reassortment with a prevailing human virus for the onward transmission of a 'full-blooded' pandemic influenza virus.

Information of this type provided a momentum of understanding of the interrelationships amongst non-human influenza viruses and their various hosts on the one hand and their relationship to the human host on the other, providing a foundation for Hong Kong's recognition of untoward influenza activity in chicken and humans in the H5N1 "bird flu" incident in 1997.

During the incident, the H5N1 virus had multiplied to the extent that around one-in-five chicken in the live poultry markets were infected, Hong Kong being in the midst of what was considered to be an incipient pandemic, the first time it had been possible to recognize one. The slaughter of chicken and other poultry across the SAR eliminated the source of the virus for humans, the last of the 18 cases being recognized the day before the slaughter began, and a pandemic averted.

Since 1997, studies on isolates obtained in 1997 and from subsequent surveillance have indicated that the H5N1 virus of 1997 was a reassortant involving three precursor influenza viruses - H5N1 from the goose and H9N2 and H6N1 from quail to produce a reassortant highly pathogenic for chicken and humans. More importantly, these three precursor viruses in varying states of evolution are still present in poultry in the region, one lineage of H9N2 having been isolated from two children in Hong Kong with mild influenza-like illness and another from pigs while the quail is thought to be an avian 'mixing-vessel' facilitating the generation of reassortant avian influenza viruses. This situation gives rise to the possibility that the H5N 1, H9N2 or the H6N1 virus has the potential to emerge as a pandemic influenza virus in its own right or in a different guise especially if any one of them undergoes further reassortment with the many avian influenza viruses in nature. While the H5N1 virus of 1997 was highly pathogenic for humans it had low human-to-human transmissibility. It remains to be seen whether the H5N1, H9N2 and H6N1 viruses are presently in a 'smouldering' phase awaiting activation to full-blooded pandemicity through reassortment with a prevailing human influenza virus, most likely in the pig or possibly in a human. Thus, in the case of the H5N1 virus, while it may have been beaten in 1997, it remains to be seen if it has been vanquished.[1,2,3]

The WHO has provided diagnostic reagents for the recognition of H5 viruses to its Collaborating Laboratories worldwide and H9 and H6 diagnostic reagents are currently being evaluated for distribution as a step toward influenza pandemic preparedness. Again, this is something that had never been possible before.

More than likely, human influenza epidemics long time past and pandemics of the last 1000 years or so are probably the result of the domestication of the duck in China about 4500 years ago. This has brought a 'harmless' virus from its natural, aquatic avian reservoir into the farmyard with economic consequences for the poultry industry of today and as a cause of significant infectious disease for humans.

Cold-adapted live attenuated influenza vaccine
Ann M. Arvin

Influenza infections are caused by influenza A and influenza B strains. The increased morbidity and mortality that is experienced by elderly people during the annual outbreaks of influenza A and B is well-recognized and can be documented as excess mortality during the epidemic period among those over 65 years of age. While it is not as widely appreciated, the morbidity of

influenza infections in children is also significant, even when there is no underlying disease that predisposes to complications. Younger children who are experiencing primary infection with either influenza strain type are more likely to have lower respiratory disease, presenting as acute pneumonia, and initial and subsequent infections are also associated with otitis media. Secondary bacterial infections, particularly pneumococcal pneumonia, are a serious and potentially life-threatening complication of influenza A and B infections during the first few years of life.[4]

Influenza infections can be prevented by immunization with inactivated influenza vaccines. The standard vaccine is a killed split virus vaccine, updated annually to contain circulating viral strains. Inactivated flu vaccines are safe and immunogenic in all age and risk groups and have an overall efficacy of 70 to 90%. These vaccines are currently recommended for patients with cardiopulmonary disease and the elderly but not for otherwise healthy children. Cold-adapted live attenuated vaccines (CAIV) are a new option for immunization against influenza. These vaccines are made by exchanging the influenza haemagglutinin and neuraminidase genes from epidemic strains for the HA and NA genes of cold-adapted A and B viruses; the remaining six genes carry mutations which make the resulting reassortant attenuated. The current CAIV candidate vaccine being tested in the United States and elsewhere is FluMist, made by Aviron, Inc. The vaccine characteristic is that it is temperature sensitive: CAIV type A will not grow above 39 C and CAIV type B will not grow above 37 C and are cold-adapted, that is, the vaccine virus replicates well at 25 C. The live attenuated vaccine strains induce a broad immune response, including cellular and mucosal immunity as well as influenza specific antibodies. These vaccines have been shown to be highly effective in clinical trials when given to healthy adults and children. Adults were protected when challenged with wild type influenza strains. In the original efficacy trial of FluMist in children, the vaccine was generally safe and well tolerated; one and two dose regimens were efficacious against the circulating influenza A/H3N2 and B strains.[5,6] Annual re-vaccination was also safe and well tolerated and protection against a new influenza A strain was demonstrated.[7] CAIV was safe in children with moderate-severe asthma and in patients with HIV infection as well as when given in a combined regimen with inactivated flu vaccine to elderly patients. The genetic stability of FluMist component virus strains was established by showing that all isolates recovered from vaccinated children remained cold-adapted and temperature sensitive and that all isolates were 6/2 reassortants with the cold-adapted/temperature sensitive 'backbone' genes. CAIV vaccines are given by intranasal spray which may be better accepted for use in healthy children than
annual injections.

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HIV-infected man with a cavitary lesion in the lung

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A 28-year-old Chinese man was admitted to the Princess Margaret Hospital because of significant haemoptysis.

The patient enjoyed good past health till one year ago. He presented with on and off haemoptysis. He was followed up in Chest clinic. Serial CXR did not reveal any significant abnormality. Computed tomography of chest was performed. However, the formal report and films could not be traced. According to the patient, he was told to have mild bronchiectatic change only. Multiple sputum specimens were sent for microscopic examination and culture, acid-fast bacilli staining and culture, and cytology. All yielded negative results. In the past year, he had weight loss of more than 10 kg. He was unemployed for one year because of his illness. Previously he worked as waiter in restaurant. He had no recent travel history outside Hong Kong.

He presented to us with copious purulent sputum, increased haemoptysis and fever for few days before admission. The temperature was 38.9C, the pulse was 90 per minute, and the respiratory rate was 20 per minute. The blood pressure was 120/68 mmHg. On examination, the patient was pale and cachectic. He was not dyspnoeic at rest. Oral thrush was found. Chest examination revealed bronchial breathing and increased vocal resonance over the left upper zone. There was no cervical lymphadenopathy. The examination of the cardiovascular system and abdomen were unremarkable.

Laboratory tests were performed: haemoglobin 8.1 g/dL; MCV 96.8 fL; white blood cell 1.5 x 10"/L; neutrophil 1.2 x 10"/L: lymphocyte 0.1 x 10/L; platelet count 139 x 10/L; ESR 140 mm/hr: C-reactive protein 60.9 mg/L. The HIV status was confirmed by Western blot test. The CD4+ cell count was 5 cells/ul only. Blood for galactomannan antigen test, cryptococcus antigen test and CMV pp65 antigen test were all negative.

Multiple sputum specimens were sent for microscopic examination and culture. They were reported as contaminant or oral flora only. Sputum specimens sent for acid-fast bacilli staining and culture were also negative. Bronchoscopy did not reveal any endobronchial lesion. Bronchoalveolar lavage and transbronchial biopsy did not show any evidence of mycobacterial, Pneumocystis carinii or CMV infection. Blood culture yielded diphtheroid-like organism and it was commented as contaminants.

Radiography of the chest showed consolidation with cavitary lesion in left upper lobe. Computed tomography noted large consolidation mass with air bronchogram in anterior segment of left upper lobe. Multiple non-enhancing areas are seen within the mass, suggestive of necrosis. This necrotic mass was abutting on the aortic arch and surrounding the pulmonary trunk. There was no gross mediastinal or hilar mass seen. No definite pleural effusion was seen. The patient had persistent high swinging fever and copious sputum despite of different antibiotics, including amoxicillin-clavulanate and cefoperazone-sulbactam. There was no response to empirical anti-

tuberculosis therapy. The "contaminant" pathogen found in blood culture and sputum was reviewed and it was finally identified as Rhodococcus equi. There was no history of exposure to horse. Combination therapy of intravenous vancomycin and oral rifampicin were started. However, there was persistent fever, copious sputum, persistent positive culture in blood and sputum, and progression of consolidation radiologically. The antimicrobial therapy was switched to triple therapy of vancomycin, imipenam and clarithromycin. There was dramatic improvement in clinical condition, microbiology and radiology.