Introduction

Typhoid and paratyphoid fever, collectively referred to as enteric fevers, are serious systemic infections caused by *Salmonella enterica* serovar Typhi (*S. Typhi*) and *Salmonella enterica* serovar Paratyphi (*S. Paratyphi*), respectively. Humans are the reservoir of infection and transmission occurs through food and water contaminated by acute cases or chronic carriers. The illness is treatable but life-threatening complications can occur.\(^1\)

In Singapore, typhoid was a serious endemic disease with case-fatality rates as high as 12% reported in the early 1950s.\(^2\) It was described as a ‘menace’ and a ‘scourge’\(^3\) with large localised outbreaks due to food, in particular, iced drinks contaminated by carriers occurring regularly.\(^4\) The disease was highly prevalent in communities not accessible to potable water supplies and modern sewage disposal facilities. The main national strategy implemented to bring this major public health problem under control was provision for a high standard of environmental hygiene and sanitation under a new Ministry of the Environment formed in 1972. Other measures taken included identification of the large pool of undetected carriers, estimated to be around 1500 - 2000,\(^5\) licensing of food establishments, relocation of street vendors to modern food centres, screening and vaccination of public foodhandlers, and health education.\(^6\) The impact of these efforts was seen in the decline in the incidence rate per 100,000 population of typhoid fever from 22.8 in 1975 to 5.9 in 1980 and to 1.2 in 1989.\(^7\)

We present the findings of an epidemiological review of enteric fevers in Singapore during the last 20 years from 1990 to 2009, and review current prevention and control measures.
Findings

Epidemiological trends

A total of 2464 laboratory confirmed cases of enteric fevers (typhoid and paratyphoid) were reported in Singapore from January 1990 to December 2009 (Fig 1). Of these, 1850 (75%) were imported cases and 614 (25%) were indigenous cases (i.e. those without recent travel history outside Singapore). While the incidence rate of indigenous cases showed a declining trend (from 4.30 per 100,000 population in 1990 to 0.26 per 100,000 in 2009 ($p < 0.005$), the proportion of cases classified as imported had been increasing significantly from 71% during the period between 1990 and 1993 to 92% between 2006 and 2009 ($p < 0.0005$) (Fig 2). Three deaths from typhoid were reported, one in 1990 and 2 in 1991, giving a case-fatality rate of 0.4% in 1990 and 1.5% in 1991. All three cases sought medical treatment late.

Indigenous cases

Among the indigenous cases, 311 (50.6%) were due to typhoid; 259 (42.2%), paratyphoid A; 43 (7.0%), paratyphoid B; and 1 (0.2%), paratyphoid C. The unusually high proportion of paratyphoid A among the indigenous cases was due to a nationwide outbreak of 167 cases in 1996.

Among the three main ethnic groups, the mean annual ethnic-specific incidence rate for indigenous enteric fevers between 2000 and 2009 was highest for Indians (0.36 per 100,000 population) followed by Malays (0.21 per 100,000 population) and Chinese (0.18 per 100,000 population). Singapore Chinese residents constituted more than half (53%) of the 60 indigenous cases of enteric fevers reported between 2000 and 2009, while foreigners made up 18% (Fig 3). The mean annual age-specific incidence rate among indigenous enteric fevers between 2000 and 2009 was...
highest in the 0 to 4 years age group (0.42 per 100,000 population) followed by the 15 to 24 years age group (0.25 per 100,000 population) and the 25 to 34 years and 55 years and above age groups (0.24 per 100,000 population). About 60% of the indigenous cases of enteric fevers reported between 2000 and 2009 were adults aged 15 to 54 years (Fig 4). Overall, there were more males than females among the indigenous cases.

**Imported cases**

Among the imported cases, 1388 (75.0%) were due to typhoid; 448 (24.2%), paratyphoid A; 12 (0.7%), paratyphoid B; and 2 (0.1%), paratyphoid C. Between 2000 and 2009, there were 6 cases of paratyphoid B from India, Bangladesh, Sri Lanka, Indonesia and Malaysia while no cases of paratyphoid
Figure 4
Age distribution (%) of 60 reported indigenous cases of enteric fevers in Singapore, 2000-2009

Below 5 years old 15%
5 - 14 years old 15%
15 - 24 years old 22%
25 - 34 years old 22%
35 - 44 years old 10%
45 - 54 years old 6%
55 years or older 10%

C were reported (Fig 5). The two main population groups with imported infections were local residents and foreign contract workers. While the proportion of local residents with imported enteric fevers declined from 52.9% during the period between 1990 and 1993 to 33.9% between 2006 and 2009, there was a corresponding increase in the proportion of foreign contract workers with imported enteric fevers from 12.8% to 40.4%, respectively (p <0.0005) (Fig 6). Most local residents contracted the disease through travel in Southeast Asia (64%) and the Indian subcontinent (28%) (Table 1). One significant trend was the increase in the proportion of imported cases acquired by local residents from India with a corresponding decline of that from Indonesia (p <0.0005). The reasons for travel to the endemic countries were primarily for vacation/social visit (71%) followed by employment or business (26%).

Outbreaks of typhoid and paratyphoid

A total of 5 typhoid and 3 paratyphoid A outbreaks were detected during the last 20 years. The first typhoid outbreak occurred in a psychiatric institution from April to September 1990. It involved 95 patients (47 symptomatic and 48 asymptomatic). All the S. Typhi isolates were of the same Vi-phage type D1 and of the same antibiogram, indicating a common source of infection. However, the source of infection could not be established. No infected foodhandler or contaminated food or water could be implicated. Transmission was mainly through close person-to-person contact. Three infected ‘worker patients’ who were deployed to assist in handling soiled laundry and other miscellaneous jobs could also have contributed to the spread of infection. To prevent clinical cases from appearing, mass immunisation with 2 doses of heat-phenol inactivated typhoid vaccine was also implemented. The vaccine was found to have an efficacy of 65.8% in preventing clinical illness but it was the maintenance of a high standard of environmental sanitation, and the identification and isolation of both symptomatic and asymptomatic cases that eventually brought the outbreak under control.8

The second outbreak of typhoid fever was reported in a family at Delta Road (3 cases and 2 carriers, phage type B1) in October 1990, the third in
Figure 5
Distribution (%) of 814 imported cases of typhoid and paratyphoid A and B in Singapore, 2000-2009

Figure 6
Mean distribution (%) of imported enteric fevers by population group, 1990-1993 and 2006-2009
Table 1

Distribution (%) of Singapore residents who contracted enteric fevers overseas by country of origin, 1990 to 2009

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A family at Telok Blangah (2 cases, phage type D2) in March 1992, the fourth in a family at Punggol (2 cases, 1 carrier, phage type USV1) in August 1992, and the last in the same psychiatric institution (2 cases) in November 1992.

There were 3 outbreaks of paratyphoid A, one in March 1990 (9 cases in the Central Business District), one in August 1990 (3 cases at Kay Siang Road), and a large nationwide outbreak (167 cases) that occurred between February and May 1996.

In the 1996 outbreak, the attack rate was highest among the Indians. Cases were distributed all over Singapore and not clustered in any particular locality. As extensive epidemiological investigations based on a large variety of food items consumed and food outlets patronised by the cases 1 to 3 weeks prior to onset of illness did not provide any leads as to the source of infection and vehicle of transmission, special attention was given to the food history of the cases in 5 small clusters (2 to 5 cases each) identified. Cases from these clusters had patronised 9 different food establishments. On further investigations into the food supplies and methods of preparation of the various food items in these premises, a common link was the use of imported coconut as an ingredient. The coconuts were dehusked and deshelled in the country of origin and then transported to Singapore daily by lorries without proper packing and refrigeration. Some were sent to food factories for the production of pasteurised coconut milk and other products, and the others were distributed by several traders to the markets, food centres and restaurants where they were stored at ambient temperatures.

A case-control study based on the first 69 reported cases and 203 controls showed that consumption of iced ‘Chendol’ with black sugar and coconut milk, iced sago with black sugar and coconut milk, iced ‘Ais delima’ with coconut milk and other food items in which unpasteurised or uncooked coconut
milk or partially-cooked coconut was used as an ingredient was significantly associated with the illness ($p<0.001$, $p<0.04$, $p<0.04$ and $p<0.01$, respectively). No S. Paratyphi A was isolated from deshelled coconut and other coconut-based products at the point of import and at various retail outlets. With the banning of the import of deshelled coconuts, no further cases were reported.9

**Enteric fevers carriers**

A total of 54 typhoid carriers, including 48 inmates detected during the typhoid fever outbreak at the psychiatric institution in 1990, were recorded in the enteric fevers carrier registry. These comprised 49 temporary carriers and 5 chronic carriers. None of them were public foodhandlers. No paratyphoid carrier was detected.

**Comments**

With vast improvements in environmental sanitation, especially personal and food hygiene, and the universal accessibility to potable water supplies and sewage disposal facilities, the incidence rate of indigenous enteric fevers (typhoid and paratyphoid) in Singapore has declined to 0.26 per 100,000 in 2009. In the case of indigenous typhoid fever, its incidence has fallen to 0.14 per 100,000, comparable to that of other developed countries such as the USA (<1 per 100,000),10 UK (<1 case per 100,000),11 Canada (<1 per 100,000),12 and Australia (0.4 per 100,000).13 No death has been reported since 1991.

As in other industrialised countries, more than 90% of the reported cases of enteric fevers in Singapore are now imported, mainly from India and Indonesia. A substantial number of typhoid fever cases imported into USA14 and UK15 were from the Indian subcontinent. Of particular concern is the increasing proportion of imported S. Typhi strains from the Indian subcontinent with decreased susceptibility to fluoroquinolones.15,16 In Singapore, of 158 strains of S. Typhi tested at the Department of Pathology, Singapore General Hospital from 2002 to 2009,17 100% remained sensitive to ciprofloxacin and ceftriaxone with varying sensitivity to ampicillin and co-trimoxazole (73% to 100%). In the case of 50 S. Paratyphi strains tested from 2002 to 2009, 93.5% to 100% were sensitive to ampicillin, 93.8 % to 100% to ciprofloxacin and 100% to co-trimoxazole and ceftriaxone. Nevertheless, close vigilance over the antibiotics sensitivity pattern of S. Typhi and S. Paratyphi strains imported into Singapore should continue.

Imported cases of enteric fevers involved two main population groups: foreign contract workers and local residents travelling to the endemic countries for vacation, social visit, business or employment without taking adequate personal protective measures. The increasing proportion of foreign contract workers with imported enteric fevers during the last 20 years could be accounted by the 4-fold increase in the number of foreign contract workers in Singapore from 248,000 in 1990 to approximately one million in 2008.18 In the case of local residents, in a study conducted in 2002, only 20% of Singapore travellers sought pre-travel advice as it was perceived that there is a low risk of acquiring infectious diseases while overseas.19 In the UK, visit-friends-and-relatives (VFR)-related travel comprised 88% of all travel-associated enteric fevers in 2007, with vast majority of cases following travel to the Indian subcontinent.20 In the United States, VFR-related travel accounted for 40% of all travel-related enteric fevers in 1996.21 This category of travellers has a higher risk for illness because, unlike tourists, these VFR travellers are less likely to take precautions.
with regard to hygiene, food and pre-travel prophylaxis while visiting home countries. We strongly recommend that local residents travelling to endemic countries, especially those visiting friends and relatives, should be advised on food and personal hygiene and vaccinated against typhoid with either the live oral S. Typhi Ty21a vaccine strain or the parenteral Vi polysaccharide vaccine. In the case of foreign contract workers in Singapore, particular attention should be given to those working as domestic maids and those engaged in the preparation and handling of food for public consumption. Employers of foreign domestic maids are given the option to have their maids tested for enteric fevers carrier status.

The last outbreak of typhoid was reported in 1992. Since then, all the reported cases who had no recent travel history occurred singly and sporadically. This is different from the situation in the past when typhoid fever was prevalent and the high endemicity of the disease was maintained by the large pool of chronic carriers. With the natural attrition of these carriers over the years, spread of infection to susceptible contacts through contaminated food within households and in the community has become infrequent. Moreover, the routine follow-up schedules for all acute cases among local residents after discharge from hospital ensures that any carrier detected is adequately treated. Public foodhandlers found to be carriers will have their personal particulars recorded in the enteric fevers carrier registry and prohibited from food handling until permanently free from infection (either through antibiotics therapy or cholecystectomy).

The occurrence of typhoid in the psychiatric institution is a matter of concern. The confirmation of a case in such an institution should be considered as a public health emergency because of the rapidity with which the disease could spread, as demonstrated in the 1990 outbreak. Despite the extensive control measures implemented, infection spread from the male to female wards and transmission was only interrupted 5 months after the first case was detected. Based on the experience gained from this outbreak, a cluster of 2 cases from the same institution in 1992 was promptly aborted. We recommend that besides psychiatric institutions, all long-term healthcare facilities should also maintain a high degree of vigilance, as outbreaks of gastrointestinal illness, including cholera have been reported periodically from these institutions.

Indigenous paratyphoid fever was uncommon in Singapore until the large nationwide outbreak which occurred in 1996. The vehicle of transmission was subsequently traced to deshelled coconut imported into Singapore without proper sanitary control. This was not the first time that imported food was responsible for outbreaks of food-borne diseases in Singapore. Imported chilled shucked oysters from the Philippines caused nationwide outbreaks of paratyphoid A in 1979 and hepatitis A in 1980. Imported cockles from Malaysia were responsible for several nationwide outbreaks of hepatitis A. Frozen half-shelled oysters imported from Shandong, China was the vehicle of transmission of several outbreaks of norovirus gastroenteritis from December 2003 to January 2004, and contaminated dried anchovy imported from Southeast Asia caused an outbreak of multidrug-resistant S. Typhimurium involving mainly infants and toddlers from July to October 2000. The Agri-Food Veterinary Authority, the licensing agency for importation of food into Singapore, has taken various measures to further strengthen its surveillance, including microbiological testings, and import control to prevent recurrences of food-borne disease outbreaks.
In a concerted effort to curb the rising incidence of typhoid in the 1970s, a mass screening and typhoid vaccination programme for public foodhandlers was implemented between 1977 and 1980. The screening programme was subsequently discontinued as no typhoid carrier was detected from 48,884 foodhandlers and their assistants. However, typhoid vaccination was discontinued since September 2010 as the current incidence of indigenous typhoid in Singapore is similar to that in other developed countries. Foodhandlers are also not routinely vaccinated against typhoid in other developed countries. In addition, the vaccine does not prevent the carrier state which is far more important than clinical cases from the public health point of view. Moreover, the World Health Organization (WHO) had recommended that routine vaccination should only be undertaken in areas of high endemicity among high risk groups or for interrupting outbreaks. However, sustained efforts in educating foodhandlers to practise a high standard of personal and food hygiene should continue.

In conclusion, Singapore has experienced a marked decline in the incidence of enteric fevers that is now comparable to that of other developed countries, despite being situated in a region where enteric fevers remain endemic. Of particular concern is the high prevalence of multidrug-resistant S. Typhi and S. Paratyphi in the region. Through prompt epidemiological surveillance and strict food import control with the population practising a high standard of personal and food hygiene, enteric fevers in Singapore can continue to be maintained at a level comparable to other developed countries. Proactive measures that address the changing epidemiology of enteric fevers in Singapore is necessary to sustain the milestone accomplished in the past two decades.

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References


Vaccination against human papillomavirus (HPV)

HPV and cervical cancer

HPV is one of the most common causes of sexually transmitted infection (STI). More than half of all sexually active adults in the USA will become infected with HPV during their lifetime and it is the major cause of cervical cancer.1,2

There are more than 100 different types of HPV, most of which are harmless. About 40 types of HPV are spread through sexual contact. Many people infected with HPV have no symptoms. There are high-risk and low-risk types of HPV. High-risk HPV may cause abnormal Pap smear results, and could lead to cancers of the cervix, vulva, vagina, anus or penis. Low-risk HPV may also cause abnormal Pap smear results or genital warts. In general, about 10-20% of sexually active women are infected with the high-risk types of HPV.

About 70% of high grade cervical dysplasia and cervical cancer are caused by HPV types 16 or 18. More than 90% of genital warts are caused by HPV types 6 and 11. The HPV that is most commonly linked with cervical cancer is HPV 16, which causes 50% of cervical cancers. HPV 18 causes about 20% and the other HPV types cause the rest.

As with many viral diseases, infection with HPV will stay with the human host until it is eliminated by the host immune system. HPV infection in many young females is usually transient and most will be eliminated by the age of 40-50 years old. A persistent infection with one of the high-risk oncogenic HPV types is necessary for the development of precancerous lesions (cervical in situ neoplasia (CIN) lesions) and ultimately, invasive cervical cancer after years or even decades.

Cervical cancer in Singapore

Cervical cancer is the sixth most common cancer among Singapore women, with an age-standardised rate of 8.5 per 100,000 population per year in the period of 2003 to 2007. It is the eighth most frequent cause of cancer deaths among Singapore women with an age-standardized mortality rate for cervical cancer of 3.4 per 100,000 population per year (2003-2007).

The incidence rate of cancer of the cervix in Singapore has consistently declined over the last three decades. The age standardized rate has declined steadily from 18 per 100,000 population per year (1968-1972) to 8.5 per 100,000 population per years (2003-2007).

A local study conducted by Chow and colleagues found that HPV types 16 and 31 were the most common HPV types causing cervical cancer in the general community. Another study conducted on female sex workers in Singapore by Chan and colleagues found that the most common HPV types were 16, 18 and 58.

HPV vaccine

Until recently, regular screening with subsequent treatment of CIN was the only way to prevent cervical cancer in Singapore. CervicalScreen Singapore, the national cervical cancer screening programme, encourages women aged 25-69 who have...
ever had sexual intercourse to go for a Pap smear once every 3 years. However, there is no centrally organised, comprehensive system in place to remind women to go for regular Pap smears. In 2007, approximately 59 percent of females in Singapore, aged between 25 and 69 years old, underwent a recent Pap test. However, screening alone will not prevent all cases of cervical cancer due to a less than 100 percent sensitivity in the tests used (risk of false negatives) and limited coverage rate.

Studies suggest that HPV vaccines have almost 100% efficacy in preventing cervical dysplasia and CIN in women aged 16-25 years unexposed to HPV type 16 or 18. The overall efficacy in the general population is much lower because some of these individuals would already have been exposed to HPV 16 or 18. Furthermore, currently available vaccines would not prevent cervical adenocarcinoma or squamous cell carcinoma due to other HPV types. In a population of unscreened women, the current vaccines are estimated to have an overall efficacy of about 60% in preventing cervical cancer. Regular Pap smear screening is therefore still crucial post-vaccination.

There is currently little data about the long-term efficacy of the vaccines, and it is not known if booster doses are required. Recent data of immunological response at 4-5 years post-vaccination and the phenomenon of innate human immune memory suggest that the long-term efficacy could be quite good. Population-level long-term efficacy trials are currently being conducted. It is not known if widespread use of the vaccines will result in replacement by other high-risk HPV types. Such a phenomenon will further reduce the overall efficacy of the vaccines. It is also not clear if there is cross-protection against non-vaccine HPV types.

Notwithstanding, experience with these vaccines to date has shown a good safety profile, with minor local side-effects being the most common. While there have been reports of serious adverse events post-vaccination, no pattern to suggest causality has been found on review.

The cost of the vaccine is high, ranging from $450 to $600 for a course of 3 doses.

There are currently two vaccines approved for sale in Singapore. Gardasil (marketed by Merck) is a recombinant quadrivalent vaccine that protects against HPV 6, 11, 16 and 18. The other is Cervarix (marketed by GlaxoSmithKline), a bivalent HPV vaccine which protects against HPV 16 and 18.

**Economic evaluation**

As the cost of HPV vaccination is high, information on its cost-effectiveness was needed to aid in health policy related decisions, including the evaluation of the addition of this vaccine in the national immunisation programme. The health gains and cost-effectiveness of including HPV vaccination to the current screening recommendation in Singapore was assessed by the Health Services Research and Evaluation Division, Ministry of Health (MOH), in collaboration with the Workgroup for Economic Evaluation of HPV vaccination. They developed a Markov model to investigate the possible gains in health outcomes (cervical cancer, cervical cancer deaths) and the incremental cost-effectiveness of adding HPV vaccination to the current screening programme for cervical cancer. Combining data from several sources, and using local information whenever possible, estimates relevant for health policy making were obtained.
Only the vaccination of 12 year old girls was considered. Vaccination of boys was not investigated, as current models have clearly shown that it was not cost-effective. Herd immunity effects were also excluded from the analyses, as insufficient local data were available to populate a model including herd immunity. In the model, a situation with screening plus vaccination was compared to the current situation of screening alone. For both situations, a screening coverage of less than 100% was assumed (59% in base-case). The analysis was from a direct medical costs perspective. Indirect costs, such as productivity losses were not included. Lifetime costs and effects were estimated. Costs were expressed in 2008 Singapore Dollars (S$).

The study showed that implementation of a HPV vaccination programme alongside the current cervical screening programme in Singapore would provide important health benefits to the population. Based on a coverage rate of 80% and assuming lifetime duration of protection of the vaccine, the implementation of HPV vaccination among a local cohort of 25,000 girls aged 12 years would avoid 122 incremental cases of cervical cancer and 49 related deaths compared to screening alone. The incremental cost-effectiveness of HPV vaccination, using national cost data, was S$19,500 per quality-adjusted life-year (QALY) gained, compared to a base case of Pap smear screening without vaccination. Sensitivity analysis gave a range of S$8,500-S$43,900. A societal perspective was used in the analysis for both costs and effectiveness. Using WHO’s standard comparison for cost-effectiveness, these values are below Singapore’s per capita GDP of S$53,000, and as such would be considered highly cost-effective. These findings are consistent with other cohort-based cost-effectiveness analyses that have generally shown that vaccination of 12-year old girls can be considered cost-effective.

When implementing a national programme of HPV vaccination, it is crucial to establish an educational campaign highlighting the need for continued screening and clarify the role of the vaccines within the existing programme. Otherwise, the gains from adding vaccination to screening might be diminished by a decreased adherence with screening.

**Socio-cultural considerations**

HPV type 16 and 18 are sexually transmitted. As such, vaccination is useful only in women who are sexually active and therefore may become exposed to these HPV types. However, the vaccine is most effective in an unexposed individual. As such, the vaccination should therefore be given before a person becomes sexually active for better efficacy. In most countries this would be in early-mid adolescence. Low parental acceptance of the vaccine has been an issue in the US, because of fears that it could encourage early sexual activity amongst teenagers. The Student Health Survey conducted in 2006 in Singapore found that prevalence of sexual intercourse among secondary three and four students was 4%, with 15 years of age as the median age of initiation. There was no gender difference noted.

**Vaccination policies in other countries**

In view of the good safety profile, WHO has recommended that the HPV vaccine be included as part of the national immunization programme for all countries, provided that a) prevention of cervical cancer or other HPV-related diseases, or both, constitutes a public health priority; b) vaccine introduction is programmatically feasible; c) sustainable financing can be secured; and d) the cost-effectiveness of vaccination in the country or region is considered.
United States

The US Centers for Disease Control and Prevention’s (USCDC) Advisory Committee on Immunization Practices (ACIP) have recommended that the HPV vaccine be given to girls at age 11 or 12, with catch-up vaccination of girls aged 13 to 18 years. HPV vaccination is incorporated into the recommended immunization schedule for persons aged 0 through 18 years.

United Kingdom

The HPV vaccine was incorporated into the national immunization programme in 2008, and vaccination is performed for girls at 12-13 years of age. It is not compulsory, and parental consent is required. From September 2008, a three-year “catch-up” campaign was started in older girls aged 14–17 years and the aim was to complete the catch-up programme within two years.

Australia

The HPV vaccine was incorporated into the national immunization programme in 2006, and vaccination is performed for girls at 12-13 years of age. It is not compulsory, and parental consent is required. A school-based catch-up programme was implemented for girls aged 12-18 years, and this was completed in 2008. A community-based catch-up programme was available through general practice and community immunisation services for 13 to 26 year old women and finished on 31 December 2009.

European Union

Denmark, Germany, Greece, Italy, Luxembourg, Portugal, Sweden, Spain and the Netherlands have implemented publicly-funded vaccination programmes and Belgium and France offer HPV vaccination on a co-payment basis. Ireland has included HPV vaccination into its immunisation recommendations, but a national programme has been deferred. Some EU countries such as, the Czech Republic and Slovenia, have not included HPV into their immunisation recommendations nor implemented nationwide vaccination programmes.

Recommendations of the Expert Committee on Immunisation

MOH’s Expert Committee on Immunisation (ECI) had recommended the use of HPV vaccines, which are clinically safe and effective, for females in the age range as specified by the manufacturers (between 9 and 26 years), in order to prevent cervical cancer.

For full benefit, the vaccine should be given before the onset of sexual activity, as it does not protect against pre-existing HPV. However, females who are sexually active can still be vaccinated, as the risk for acquiring HPV infection would continue as long as they are sexually active.

The ECI recommended that even after HPV vaccination, all sexually active women aged 25-69 years should continue to have regular Pap smears. This is because HPV vaccination does not protect against all types of cancer-causing HPV.

Vaccination of males is not recommended.

Standard of care

MOH had accepted the recommendation of the ECI to include HPV vaccination in the national childhood immunisation programme (NCIP). HPV
vaccination is therefore positioned as a standard of care for females aged 9 to 26 years. Young females as well as parents are encouraged to discuss the HPV vaccination with their family doctor, so that they can make an informed decision on whether to be vaccinated against HPV.

The characteristics of the two vaccines available in Singapore are shown in Table 2.

Doctors should notify the National Immunisation Registry (NIR) of HPV vaccinations through online or via notification forms that can be requested by email hpb_nir@hpb.gov.sg or telephone 64353676/64353267.

**Use of Medisave for HPV vaccination**

Medisave may be used to pay for HPV vaccination, like the pneumococcal and hepatitis B vaccinations. From 1 Nov 2010, MOH extended the $300 Medisave annual withdrawal limit that covers vaccinations and outpatient treatment under the Medisave300 scheme to cover HPV vaccinations for females aged 9-26 years. Under this scheme, patients can use up to $300 per Medisave account per year to pay for HPV vaccines. Patients may use their own Medisave or that of their immediate family members (e.g. parents or spouse) to help pay for the vaccination. Needy females or parents with insufficient Medisave balances can seek special financial assistance at the polyclinics.

(Reported by the Epidemiology and Disease Control Division, Communicable Diseases Division and Health Services Research and Evaluation Division, Ministry of Health)

**References**


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</table>
Introduction

Singapore is located in the tropics and influenza viruses circulate perennially with peaks in influenza activity observed in April to July and November to January. With the global alert on the emergence of the novel influenza A (H1N1-2009) virus by the World Health Organisation in late April 2009, it became important for Singapore to ensure adequate capability and capacity to detect and monitor the activity of the pandemic H1N1-2009 virus in the community, as well as its impact on the local healthcare services.

In order to ensure timely detection and tracking of the activity of the pandemic H1N1-2009 virus in Singapore, biosurveillance for influenza activity was stepped up. Other existing indicators of influenza activity, including emergency department (ED) and polyclinic attendances for acute respiratory infections (ARI), as well as ED attendances and hospital admissions for pneumonia, were also closely monitored.

Materials and methods

Biosurveillance for influenza activity was stepped up in May 2009, and surveillance samples (nasopharyngeal swabs or throat and nasal swabs) were obtained from patients with influenza-like illness (ILI) who attended the public sector polyclinics, private general practitioner clinics and public hospital EDs. For the purpose of surveillance, ILI in a patient was defined as one having fulfilled the criteria of (a) fever or history of fever (> 38°C), and (b) the presence of cough, and/or sore throat. The surveillance samples were tested at designated satellite laboratories and the National Public Health Laboratory using a series of polymerase chain (PCR) reaction assays to screen for influenza A and subtype for seasonal or pandemic influenza. Trends in the weekly biosurveillance data from May to September 2009 were then compared with other indicators of influenza activity (ED and polyclinic attendances for ARI, ED attendances and hospital admissions for pneumonia) over the same period.

Results

The first imported case of influenza A (H1N1-2009) in Singapore was detected on 26 May 09, and the pandemic H1N1-2009 virus was subsequently detected in 1% of all the ILI biosurveillance samples in the week from 14 - 20 June 09. The activity of the pandemic influenza (H1N1-2009) strain increased swiftly, and the weekly proportion of influenza A (H1N1-2009) peaked at 58% during the week of 2 - 8 August 09, and remained at high levels until the week 20 – 26 Sept 09 (Fig. 7). The H1N1-2009 strain also almost completely replaced the seasonal flu strains in the community.

The other indicators of influenza activity were noted to have peaked at around the same period - the weekly polyclinic attendance for ARI increased rapidly from 9137 in the week from 14-20 June 09 to peak at 24477 in the week from 26 July-1 August 09 (Fig. 8). This was more than 1.5 times that of the usual seasonal trend for ARIs. The weekly ED attendances for ARI and pneumonia was also noted to increase from the week between 5 and 11 July 09 to peak in the week between 19 and 25 July at 5005 (about 3
times the seasonal trend) and in the week 26 July-1 August at 639 (about 1.5 times the seasonal trend), respectively (Fig. 9).

**Conclusion**

Biosurveillance of the influenza A (H1N1-2009) virus during the pandemic allowed the Ministry of Health to monitor the activity of the virus together with other quantitative indicators of influenza activity to assess the spread of the pandemic influenza A (H1N1-2009) strain in the local population. It also allowed the Ministry to monitor the demand for healthcare services so that medical surge capacity and capability could be triggered early during times of increasing healthcare need.

**Figure 7**
Weekly proportion (%) of influenza A (H1N1-2009) among influenza-like illness samples and 95% confidence intervals, 2009

**Figure 8**
Weekly polyclinic attendances for acute respiratory infection, 2009
Figure 9
Weekly Emergency Department attendances for acute respiratory infection and pneumonia, 2009

(Reported by Tey SH, Ang IW, Mak TM, Cutter J, James L, Communicable Diseases Division, Ministry of Health, Singapore)

References


Laboratory test results on *Plasmodium knowlesi* in clinical malaria positive cases registered in 2009 in Singapore

Introduction

*Plasmodium knowlesi* is a simian malaria parasite that naturally infects long-tailed macaques (*Macaca fascicularis*), pig-tailed macaques (*Macaca nemestrina*) and mitred leaf-monkeys (*Presbytis melalophos*) native to forested areas in south-east Asia. It is thought to have been observed first time by Franchini in 1927¹, and subsequently characterised in greater detail by Knowles and Das Gupta in 1931².
The pair described malarial blood stages from a long-tailed macaque imported to Calcutta from Singapore and performed transmission experiments on human volunteers. At the same time, Napier and Campbell worked on characterising the haemoglobinuria induced by this parasite in the monkey. Interestingly enough, none of these experimenters attempted to name the parasite. It was only in 1932 that Sinton and Mulligan, studying parasite samples from Knowles and Das Gupta, as well as their own parasite isolate from an infected macaque (M. fascicularis) in Singapore, named the parasite *Plasmodium knowlesi* in honour of Dr. Knowles. In 1935, Mulligan completed his work and wrote a more detailed and illustrated description of the parasite. *P. knowlesi* human infection was considered rare despite of some reports in peninsular Malaysia, until a large number of patients from Sarawak, Malaysia, were screened by nested-polymerase chain reaction (nt-PCR) and found to be infected with *P. knowlesi*. Since this study, many *P. knowlesi* infections have been reported across Southeast Asia (SEA). Countries such as peninsular Malaysia, Borneo Malaysia, Borneo Indonesia, Thailand, China, Philippines, Myanmar, Vietnam and Singapore have reported local cases of zoonotic transmission to humans, and non-endemic regions of the world such as Europe, the United-States and Australia have reported imported cases of *P. knowlesi* infection originating from SEA.

Naturally occurring *Plasmodium knowlesi* human infections are now commonly recognized as being widely distributed across SEA and potentially life-threatening, due to an abundance of natural reservoir species in forested areas of the region capable of coming into close contact with humans residing in rural, semi-sylvatic settlements.

**Background**

**Techniques of diagnosis**

The recent renewal of interest in *P. knowlesi* has inspired the development of a number of laboratory tests to rapidly identify the parasite and distinguish it from the other human *Plasmodium* species.

**Morphology**: Classical morphology, considered to be the golden standard for *Plasmodium* identification, is widely used and most of the time the only technique available in clinical laboratories. Unfortunately, *P. knowlesi* morphological identification is challenging, because the early trophozoites stages resemble those of *Plasmodium falciparum* and the later stages resemble those of *Plasmodium malariae*. Due to these morphological similarities, many cases have been misdiagnosed. A 2009 study by Lee et al highlighted the minor differences between the blood stages of *P. knowlesi* and *P. malariae*, but concluded that it is difficult to base a diagnosis of *P. knowlesi* only on morphology.

**Rapid diagnostic test (RDTs)**: Detection of *P. knowlesi* by RDTs has been tested and shown to be possible. Pan-malarial immunochromatological reactions based on parasite lactate dehydrogenase (pLDH) or aldolase allow for genus-level detection of *Plasmodium* parasites. However, species-level identification can sometimes prove tricky because while there is no cross reaction with some tests using monoclonal antibody against histidin-rich protein 2 (HRP2), there are cross reactions with other tests based on *P. falciparum* or *P. vivax*-specific pLDH. In addition, infections with low parasitaemia are not detected. Thus these tests are not fully species-specific, they can be informative and useful for a rapid diagnosis of a
malarial infection, but sometimes yield results that seemingly conflict with diagnoses based on classical microscopy.

**Molecular diagnostics:** Ever since the identification of a cluster of human cases of *P. knowlesi* infection by nested-PCR (nt-PCR), this technique has been the most commonly used method for identifying *P. knowlesi*. Most studies on *P. knowlesi* infection in humans have targeted genes encoding the small subunit ribosomal RNA (SSUrRNA), or the circumsporozoite protein (CSP) as developed by Singh et al. Until recently, this protocol which uses a set of oligonucleotide primers (Pmk8 and Pmkr9) that amplify a small fragment (153 bp) of one of the SSUrRNA genes of *P. knowlesi*, was considered the best standard for *P. knowlesi* identification based on its high sensitivity and specificity. However, this protocol was recently found to yield a stochastic cross amplification of *P. vivax* SSUrRNA in view of this finding, some authors started to contest certain results previously obtained using this method, emphasising that all results obtained with the Singh et al protocol should be confirmed by sequencing. To avoid this problem of cross reactivity, Imwong et al designed another set of oligonucleotide primers (PkF1060, PkF1140 and PkR1550) targeting a different SSUrRNA gene of *P. knowlesi*. These primers gave reliable and reproducible results when tested.

At the same time, other PCR protocols consistently targeting SSUrRNA genes with few divergent techniques were developed. An example was the detection by real-time PCR (rt-PCR) using fluorescent transfer energy (FRET) technology. These authors updated the method developed for the four others human species, based on differences in the melting temperatures of a newly designed set of *P. knowlesi*-specific probes. This type of PCR furnishes reliable results in a short time and can be used for rapid diagnosis.

A different molecular tool, the loop-mediated isothermal amplification method (LAMP) has been evaluated by Iseki et al to diagnose *P. knowlesi*. They developed a *P. knowlesi*-specific primer set and tested their protocol on plasmid DNA and whole blood from infected monkey. This method showed no cross reaction with other primate *Plasmodium* species. It enabled an earlier detection of the parasite, when compared against PCR detection on plasmid DNA and similar results when compared against PCR detection on whole blood.

**Plasmodium knowlesi in Singapore**

**Registered cases:** As mentioned above, the history of *P. knowlesi* starts with Singapore and its macaques in 1932. However, in spite of natural human infections with *P. knowlesi* occurring in the Malayan peninsula over the years since, these remained undetected until 2007, when a soldier of the Singapore military was found to be infected with the parasite after a period of forest training. After this discovery, all soldiers camping in the same forest were screened for *P. knowlesi* infection, and five more human cases (four in 2007 and one in 2008) were recorded. In addition, a joint operation was performed by the Singapore military and the National Environmental Agency (NEA) to evaluate the epidemiological risk of infection by *P. knowlesi* in Singapore. Part of the results of this large project, detailed by Wong et al, showed that “wild” long-tailed macaques living in the heart of the forest (restricted areas) harboured *P. knowlesi* whereas the “peri-domestic” long-tailed macaques living in neighbourhood parks in closer proximity.
proximity to the human population were free of *P. knowlesi* infection.

In addition, this study illustrated that certain soldiers’ *P. knowlesi* infections were of the same genotypes as those in the monkeys. Thus, the study was instrumental in highlighting the preponderant role of disease vectors (mosquitoes) and the risk of acquiring *P. knowlesi* zoonosis by frequenting the sylvatic habitats (restricted forested areas) of “wild” monkeys in Singapore. Wong et al paired PCR-based molecular tests, following the Singh et al protocol, with cloning and sequencing to confirm the parasite species in malaria-positive samples. This demonstrated the usefulness of this method for confirming the presence of the parasite in monkeys which typically harbour low levels of mixed natural infection and for intra-species genotyping.

**Malaria-free country:** Singapore has officially been declared free from malaria since November 1982, not counting local *P. knowlesi* transmission because it is a zoonosis. Nevertheless, Singapore remains highly vulnerable to the re-emergence of malaria due to the natural presence of competent vectors and the large number of foreigners arriving from malaria endemic countries. This fragile balance explains the 29 outbreaks of malaria reported in Singapore between 1983 and 2007, as reported by Lee et al.

**Surveillance:** The surveillance and the epidemiological monitoring of the malaria cases are managed by the Ministry of Health (MOH), which works in collaboration with the NEA for mosquito vector surveillance and control. All positive malaria clinical samples are sent to the National Malaria Reference Centre (NMRC), which was based in and managed by the National University of Singapore (NUS) prior to 2009, but has since been relocated to the National Public Health Laboratories (NPHL) at Singapore General Hospital (SGH) and managed by MOH. After the move to NPHL, all samples received in 2009 were re-probed using molecular tools for species confirmation. The present study reports the results of these investigations.

**Materials and methods**

**Clinical malaria positive cases in Singapore in 2009**

**Cases:** A total of 172 malaria positives clinical cases identified by microscopy were reported to MOH in 2009, by hospital laboratories, clinicians and medical practitioners. The breakdown of these cases was 135 *P. vivax*, 36 *P. falciparum* and one case of *P. malariae*. Of the related material [slides and/or residues of ethylene-diamine-tetra-acetic (EDTA) blood] received in NMRC, 162 positive cases were accounted for out of the 172 total cases. The breakdown of these samples as reported by hospital laboratories, clinicians and medical practitioners was 132 *P. vivax*, 28 *P. falciparum*, 1 *P. malariae* and 1 “potential *P. knowlesi***.

**Preparation of the samples**

**DNA extraction from EDTA blood samples:** DNA extraction was performed on 200 µL of whole blood using the automated extractor EZ1 Advanced XL (Qiagen) and the EZ1 DNA Blood Extraction kit (Qiagen) following the manufacturers’ recommendations.

**DNA extraction from blood films:** In some cases only stained blood smears were provided. Before DNA extraction by the same method as outlined for the EDTA blood samples, blood was scraped from
the slide and digested by Proteinase K in ATL buffer (Qiagen) for 1 hour at 56°. Extracted DNA was stored in -30°C until use.

Molecular tests

We decided to proceed in several steps to re-investigate each sample.

Confirmation of the presence of Plasmodium parasites and identification of the two most common species registered in Singapore: *P. falciparum* and *P. vivax*. We used a real-time PCR (rt-PCR) derivate from the protocol of Safeuki et al\(^4\). This system used one set of oligonucleotides primers to amplify a 186 bp fragment of the 18S small subunit ribosomal RNA gene (ssrRNA), and different Taqman probes for rapid, sensitive and quantitative detection of pan-*Plasmodium* species. The system also allowed for the differentiation of *Plasmodium vivax* and *Plasmodium falciparum* from the other *Plasmodium* species.

Screening for the presence of the 3 others species *P. knowlesi*, *P. malariae* and *P. ovale*. We used a nested polymerase chain reaction (nt-PCR) to confirm the presence of pan-*Plasmodium* parasites and indentify all human *Plasmodium* species present. This system was adapted from the protocol of Snounou & Singh\(^4\) and Imwong et al\(^3\) and allowed for the unequivocal identification of parasite species. The sensitivity and specificity of the assay was markedly improved when a nested PCR strategy was adopted, because this involved two round of amplification, in which the longer amplification product of the first reaction served as the template for the more specific second reaction. All sets of oligonucleotides primers used targeted the 18S ssrRNA gene of *Plasmodium* species. One pan-*Plasmodium* set of oligonucleotide primers amplifying a large fragment (1820 bp) was used in the first reaction. This was followed by one pan-*Plasmodium* set of oligonucleotide primers amplifying a smaller fragment (235 bp) in the second reaction to confirm the presence of *Plasmodium*. Finally, several sets of oligonucleotide primers were used to amplify species-specific small fragments (size range: 140 to 500 bp), allowing for the selective detection of each *Plasmodium* species.

Sequencing confirmation. For each *P. knowlesi* infection found, we performed several other nested reactions. The fragments generated by these reactions were purified using the PCR Purification Kit (Qiagen), following manufacturers’ instructions, and sequenced in both directions with an ABI 3730XL sequencer. Combining the sense and antisense sequences of the same regions allowed us to reconstruct the large fragment (1820 bp) amplified during the first reaction of the nt-PCR. Alignments, comparisons and analyses of the sequences were performed using CLC Main Workbench 5.7 software.

Results

Morphological results vs. molecular tests

All the 162 samples received by the NMRC were tested and confirmed positive for *Plasmodium*. The results of the molecular tests are presented in Table 3. A remarkable thing is the discovery of 6 *P. knowlesi* pure infections in the total of 172 malaria positive cases; even though none of the diagnostic laboratories which handled the primary patient blood samples reported *P. knowlesi* infections to MOH and only one reported a “potential *P. knowlesi* infection” to the NMRC. The failure of diagnostic laboratories in Singapore to pick up and notify cases of *P. knowlesi* infection from blood smear examinations highlights the difficulties of correctly identifying *P. knowlesi*.
by classical morphology alone. Microphotographs of Fig. 10 present several stages observed on the slides corresponding to these pure *P. knowlesi* infections. Nevertheless, the reporting of a “potential *P. knowlesi* infection” by one of the laboratories shows that it is possible for a well-trained morphologist to correctly suspect *P. knowlesi* by thin smear examination. Of the 5 other cases of misdiagnosis, three cases were misdiagnosed as *P. vivax* infections and two were misdiagnosed as *P. falciparum* infections. In addition, the only *P. malariae* case reported was confirmed as *P. malariae*. There were no cases of *P. ovale* and no mixed infections out of the 2 cases with mixed infection *P. vivax* + *P. knowlesi* reported as *P. vivax* alone. For these two latest cases, the quality of the DNA extracted on slides was poor. The use of the Imwong et al protocol with a two step species-specific nested reaction to pick-up *P. knowlesi* positive samples was helpful for avoiding the problem of cross reactivity with *P. vivax*.

### Sequences comparison

For the 6 pure *P. knowlesi* infections, a large fragment (1820 bp) of the 18S ssrRNA gene has been sequenced. The comparison of our sequences with the references sequences by basic local alignment tool (BLAST) shows a high similarity (>99%) with the strain of *P. knowlesi* evolving in Malaysia both peninsular and Borneo.

To allow phylogenetic reconstruction with published *P. knowlesi* 18S ssrRNA genes, our sequences have been at the same size (1.5 kb) and several other published sequences of different *Plasmodium* species from primates, birds and rodents have been included in the analysis. On the phylogenetic tree depicted in Fig. 11, the 6 cases imported in Singapore clustered with the sequences of *P. knowlesi* confirming the identification. In addition, we note that our sequences show same range of variability than the sequences pre-

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**Table 3**

Results of the molecular diagnosis on the malaria positive cases reported to the MOH of Singapore in 2009 by the hospital laboratories, the clinicians and the medical practitioners

<table>
<thead>
<tr>
<th>Morphological diagnosis</th>
<th>Molecular diagnosis</th>
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<tr>
<td>Cases reported to MOH</td>
<td>NUS-NMRC</td>
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<td>Samples received</td>
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<tr>
<td><em>Pv</em></td>
<td>135</td>
</tr>
<tr>
<td><em>Pf</em></td>
<td>36</td>
</tr>
<tr>
<td><em>Pm</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Pk</em></td>
<td>0</td>
</tr>
<tr>
<td><em>Po</em></td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>172</td>
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</table>

*Pv* = *P. vivax*  
*Pf* = *P. falciparum*  
*Pm* = *P. malariae*  
*Pk* = *P. knowlesi*  
*Po* = *P. ovale*
Cases analysis

All the pure *P. knowlesi* infections discovered in these clinical samples were imported cases, brought into Singapore by travellers returning from neighbouring countries where *P. knowlesi* is endemic.

Four cases were imported from peninsular Malaysia: one from state of Perak, two cases were twins from Endau Rompin Park on the border of Johor and Pahang states and one from Pahang state. Three of these patients were Singaporean citizens and the last a British citizen, all returning after several days of jungle trekking.

Two cases were imported from Brunei Darussalam: both patients were Singapore Civil Defence Force officers who acquired their infections separately on different dates, but from the same place, during a jungle training exercise in Temburong National Park.

In addition, there were two cases of mixed infections (*P. vivax* and *P. knowlesi* co-infections) borne by travellers coming from different parts of India. Unfortunately, the lack of information about their travel histories did not allow us to determine if their *P. knowlesi* infections were acquired abroad or locally.

Discussion

In our report, we have highlighted the presence of *P. knowlesi* infections among the clinical malaria positives cases imported into Singapore in 2009. This is the first report of imported *P. knowlesi* infections in Singapore in clinical samples. These cases add to the 6 locally transmitted cases recorded among soldiers.85,86
Figure 11

Phylogenetic tree based on a large fragment (1500 bp) of the 18S ssrRNA genes of different Plasmodium sp. produced by the maximum likelihood method. The highlighted samples are the 6 pure P. knowlesi infection imported in Singapore in 2009. Numbers closed to the nod are bootstrap percentages based on 1000 replicates and only those above 85% are shown.
The total number of 8 cases harbouring *P. knowlesi* (6 pure and two mixed infections) is significant, because it represents 4.9% of the malaria cases tested by the NMRC. An important point to note is that *P. knowlesi* was the third most common species recorded in Singapore after *P. vivax* and *P. falciparum* in 2009.

Six pure infections were imported from neighbouring countries in patients returning after jungle trekking or forest camping, confirming the broad and natural transmission of this zoonosis in the forested areas of the south-east Asia region. Four of these cases were imported from peninsular Malaysia from states already known for the transmission of *P. knowlesi*. The two other cases acquired in Brunei Darussalam confirmed the presence of local transmission of *P. knowlesi* within the country, in addition to the 2007 reported case.

*P. knowlesi* is a zoonotic disease and thus does not challenge malaria-free status of Singapore or Brunei Darussalam. Nevertheless, considering the spate of recent human infections by *P. knowlesi* acquired in both countries, this status might need to be reviewed or redefined.

Two mixed infections with *P. vivax* were detected in patients arriving from India over a short space of time. Apart from the knowledge that these patients came from different parts of India, no further details about their travel histories were furnished. This lack of information did not allow us to determine if they acquired their *P. knowlesi* infections locally or abroad. Furthermore, patient samples for these two cases were of very poor quality. More studies are thus required to investigate more fully these cases of infection. If these cases are imported, they might constitute the first reports of human *P. knowlesi* infection in India.

Natural hosts of *Plasmodium knowlesi* are present in India and could be a reservoir for natural transmission of the parasite from monkeys to humans.

Our data illustrate one more time the difficulties of identifying this parasite by its morphology – there were five misdiagnosed cases out of 6 that were positive for *P. knowlesi*. Although clinical or hospital laboratory morphologists are well-trained to identify the four human *Plasmodium* species, most do not know the morphology of *P. knowlesi* and are not particularly mindful of this human zoonosis. They are, however, able to see small morphological differences and record these differences in their reports as “atypical *P. vivax*”, “atypical *P. falciparum*” or “mixed *P. falciparum* and *P. malariae*”, concluding their identifications with one of the classical human *Plasmodium* species. We would recommend that malaria diagnostic laboratory staff be informed of the broad presence of *P. knowlesi* in South East Asian monkey species and the possibility of its zoonotic transmission to humans. Malaria diagnostic laboratory staff should also be educated in detail about the small morphological differences between *P. knowlesi* blood stages and those of the other four human *Plasmodium* species. In this way, malaria diagnostic laboratories will be informed enough to make accurate diagnoses of potential *P. knowlesi* infections, and request for complementary molecular identification to be performed for species confirmation. Finally, coupling morphological suspicions with patients’ clinical details (e.g. time interval between periodic fevers) and travel histories (e.g. forest activities) would build a robust body of information to aid in the accurate diagnosis of this parasite infection.

In the cases we received, there was no complementary diagnostic data furnished by RDTs, thus
we could not compare the efficacy of this diagnostic method against that of molecular tests. Although we could have tested our samples with RDTs to determine the effectiveness of this diagnostic method, we refrained from doing so because of the unclear results that these tests can potentially yield\textsuperscript{19,27,29}. We thought it prudent to wait for the development of an RDT which utilises \textit{P. knowlesi}-specific antibodies before testing it as an alternative diagnostic method.

In all cases, our data show the importance of the molecular confirmation for \textit{P. knowlesi} diagnosis. Currently, the most commonly used protocol based on the nt-PCR developed by Singh et al\textsuperscript{6} remains a good method, but must be followed by an additional step of sequencing to ascertain the result. This last sequencing step requires a few days. Thus, it might be sufficient to use the protocol proposed by Imwong et al\textsuperscript{30} which avoids the sequencing step and gives specific result in one day. The use of the rt-PCR or the LAMB methods gives interesting first results. These methods have the potential to be faster than nt-PCR at producing the same reliable results, and should be optimised using large-scale screening tests to simplify their protocols and improve their rapidity for this purpose.

In waiting for the development of a RDT specific for \textit{P. knowlesi} and for the latest molecular diagnostic tools to be more widely used, the combination of good morphological analyses with specific nt-PCR tests remains the best way to rapidly and accurately identify \textit{P. knowlesi}.

Our newly recorded cases of \textit{P. knowlesi} in Singapore were identified only from the clinical samples received in NMRC for the year 2009. Over this period, there was a high incidence of misdiagnosis recorded in archival blood films in Malaysian Borneo\textsuperscript{25}. Hence, it would be interesting to investigate the old material deposited in NMRC for the presence of \textit{P. knowlesi} in Singapore before the first official record in 2007.

(Reported by Quek DL\textsuperscript{1,2}, Zhang Y\textsuperscript{1}, La MV\textsuperscript{1}, Fernandez PP\textsuperscript{1} and Chavatte J-M\textsuperscript{1,2}, National Public Health Laboratory, Ministry of Health\textsuperscript{1} and National Malaria Reference Centre, Ministry of Health\textsuperscript{2})

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References


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