Vivax Malaria in the Republic of Korea: Trends & Health Implications for US Military and Civilian Populations

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Temperate zone vivax malaria – relapses + latent liver stages
Transmitted by *Anopheles* spp.
Patients are infectious to mosquitoes 1-2 days prior to the onset of symptoms
Mosquitoes become infectious, under ideal conditions, 8-10 days after blood-feeding on a malaria patient
Not all “malaria-positive” mosquitoes are equal
Implement effective strategies to interrupt the malaria cycle
Chemoprophylaxis, easy military fix, but difficult to monitor
Vivax malaria was endemic on the Korean peninsulae.

1945, after WWII, Korea divided into North and South Korea.

1950-53 (Korean War):
- >6,000 cases of vivax malaria in US Forces in Korea
- >12,000 cases of returning US military
- Mostly *P. vivax*, also *P. falciparum* and *P. malariae*
- Present day much different
1960’s: National Campaign for Malaria eradication initiated
1979: Korea declared malaria free
1993: vivax malaria re-emerged along the DMZ
1997: North Korea reports malaria outbreaks
   1998: 25,000 estimated cases
   2001: WHO estimates 300,000 cases

Possible causes for outbreak
   Introduction from China to North Korea
   Introduction by US soldiers or South Korean travelers
   Never eradicated
   Several genotypes of *P. vivax*, perhaps several introductions

Exhibits temperate zone characteristics of latent infections
Vivax malaria reemerged in 1993 when a ROK soldier stationed near the DMZ with no history of travel outside of the ROK developed malaria.

Malaria cases rapidly increased and in 1997, to reduce the spread of vivax malaria from the ROK military, the ROK Army initiated a chemoprophylaxis program including approximately 16,000 soldiers.

As a result of increased use of chemoprophylaxis, malaria peaked in 2000 with 4,142 cases, and then declined to a low of 826 cases in 2004.

From 2004, malaria increased to 2,180 cases in 2007, decreased to 1,054 cases in 2008 and then continued to increase to 1,722 cases by 2010.

Currently (2011), there are fewer than 900 cases of malaria due to heavy rainfall in the early summer and drying conditions, which resulted in reduced *Anopheles* populations.

As of 20 September 2011
1993 – 1st US malaria case in returning US soldier, acquired in 1992, before 1st reported ROK case

Initially, most cases reported from JSA, Cp Greaves, and Warrior Base

≈ 60% Latent malaria

1999 JSA and Cp Greaves initiated chemoprophylaxis program

Chemoprophylaxis failure due to non-compliance
Distribution of Vivax Malaria Among ROK Populations

- Vivax malaria high-risk areas parallel the DMZ
- Most cases from Gyeonggi and Gangwon Provinces
- Relatively high numbers of cases reported for Seoul and Incheon - medical facilities
- Low numbers of malaria attributed to exposure south of Seoul – most cases from veterans returning to their hometowns
- Predicted that malaria would rapidly spread south
Understanding the Vector: How does the DMZ Differ?

- Rear progeny broods to clarify *Anopheles* spp.
  - PCR techniques for species ID required
  - Costly, time consuming, often retrospective
- Determine geographical and temporal distributions
  - Determine annual changes affected by environmental factors
  - Determine daily biting patterns
  - Identify habitats, i.e., algal mats and rice paddies
- Identify *Anopheles* spp. vector species
  - Determine vector competence
    - Oocysts, sporozoites, Ross’ spores
  - Vector-borne disease surveillance
    - Methods?
    - Problems with malaria assays?
Taxonomy of *Anopheles* spp.

- Prior to 1999, there were 6 *Anopheles* spp. recorded in the ROK
- 1999 – Progeny broods of *Anopheles* spp. were reared for taxonomic purposes and sent to the Walter Reed Biosystematics Unit, WRAIR – *Reported to WRBU that individuals of broods of *An. sinensis* had characteristics of both *An. sinensis* and *An. lesteri*
- 2001 – *An. yatsushiroensis* was synonymized as *An. pullus*
- 2003 – *An. anthropophagus* was synonymized as *An. lesteri*
- 2005 – *Anopheles* spp. were determined to be different from *An. sinensis* and other species present in the ROK using PCR techniques
- 2005 – Rueda identified two new species, *An. belenrae* and *An. kleini*, that were morphologically indistinct from *An. sinensis*
Evaluation of Adult Collection Methods

**Differences – lower populations + higher density of An. sinensis (zoophilic??)**

**Number Anopheles spp. Collected**

- June 2000
- September 2000

**Mean Number Mosquitoes**

**Collection Period – Shannon Trap**

- 1800
- 1900
- 2000
- 2100
- 2200
- 2300
- 2400
- 0100
- 0200
- 0300
- 0400
- 0500
Larval Collections – Rice Paddy Maturation

- Collections may not reflect adult populations based on abundance of larvae
- Larval habitats vary with ecological features and human activities
  - DMZ – unmanaged lands
  - S. Korea – rice paddies
  - N. Korea – rice paddies, less pesticides (??)
- Identify larval habitats/preference
  - Rice paddy maturation population changes
  - Algal mats protect mosquitoes from predators and increase populations during low rainfall
  - Pools, depressions, ponds, etc.
Collect Larvae. Then What?

- Larvae are collected by various techniques, using pans, dippers, suction devices (tree holes), etc.
- Larvae are placed in Whirl-Pac containers, sealed with air, labeled, and then transported to a laboratory where they are counted, instar recorded, and then reared to adult.
- 4th instar larvae are separated into individual rearing vials.
- Larval skin is removed and placed in 80% ETOH.
- After adults emerge, the pupal skin is removed and placed in 80% ETOH, while adults are placed in dry curing vials.
- After 24 hrs, the adults are killed, mounted on paper points, and labeled with a unique collection number, etc.
- Adults and larval/pupal skins are sent to WRBU where they are stored until mounted and identified (morphological).
- Adults belonging to species groups where morphological techniques are not useful, a leg is removed and assayed by PCR to determine species.
Larval Collections – Warrior Base

- **An. pullus** appears early in May and then declines.
- **An. kleini** appears early declining from July.
- **An. sinensis** with low populations in the spring, increases through August and makes up >85% of all larvae collected by October.
- **An. sinensis** is the predominant mosquito collected in all habitats surveyed, except stream margins (few collected).
- Mostly 3rd/4th instars collected in ditches, indicating flushing of most larvae from other habitats during flooding.
- What do unmanaged lands contribute? Dangerous to sample!

**RP** = Rice Paddy; **D** = Ditch; **P** = Pond; **GP** = Ground Pool; **M** = Marsh; **SM** = Stream Margin
Proportion of *Anopheles* Species Larvae, Mar-Oct 2005

**Munsan Area**
(Tongilchun/Gimpo/IlSan/Majung-ri/Cheongpyeong)
N=2830

- An. sinensis: 1802 (63.7%)
- An. Pullus: 247 (8.7%)
- An. kleini: 704 (24.9%)
- An. belenrae: 54 (1.9%)
- An. sineroides: 1 (0.6%)
- An. Lesteri: 22 (0.8%)

**Jechun/ Ttaebaek/Cheongsong**
N=190

- An. kleini: 1 (0.5%)
- An. sinensis: 179 (94.2%)
- An. Lesteri: 1 (0.5%)
- An. sineroides: 7 (3.7%)
- An. Pullus: 1 (0.5%)

**Anmyeon-do/Jeonju**
N=359

- An. sinensis: 358 (99.7%)
- An. kleini: 1 (0.3%)
- An. sineroides: 7 (1.9%)

**Gwangju/Boseung**
N=170

- An. sinensis: 167 (98.2%)
- An. kleini: 2 (1.2%)
- An. sineroides: 1 (0.6%)

**Busan/Geoje/Sacheon**
N=200

- An. sinensis: 191 (95.5%)
- An. kleini: 5 (2.5%)
- An. Pullus: 1 (0.5%)
- An. sineroides: 3 (1.5%)

**Jeju Is**
N=43

- An. sinensis: 43 (100.0%)

Legend:
- An. belenrae
- An. kleini
- An. Lesteri
- An. Pullus
- An. sinensis
- An. sineroides
- An. koreicus
Number/Proportion of Species from of Adult *Anopheles* Mosquitoes Collected by NJ Light Trap, ROK, Jun-Oct 2005

[Cp Bonifas/Warrior Base]

- *An. lesteri*: 1, 1.4%
- *An. pullus*: 64, 29.1%
- *An. kleini*: 113, 51.4%
- *An. belenrae*: 11, 5.0%
- *An. sinensis*: 45, 68.2%
- *An. sneroides*: 3, 4.5%

[Greater proportion of *An. kleini* and *An. pullus* adults due to trap type and collections near humans – Anthropophilic (??)]

[Rodriguez Range]

- *An. sinensis*: 37, 55.8%
- *An. Sinensis*: 1, 1.4%
- *An. Sinensis*: 22, 91.7%

[Cp Humphreys]

- *An. sinensis*: 467, 95.9%
- *An. sinensis*: 22, 91.7%

[Cp Long/Eagle]

- *An. Sinensis*: 45, 80.2%
- *An. Sinensis*: 62, 100.0%

[Gunsan AB]

- *An. sinensis*: 22, 91.7%
- *An. sinensis*: 619, 99.2%

[Cp Carroll]

- *An. Sinensis*: 62, 100.0%

**Legend**

- An. belenrae
- An. pullus
- An. kleini
- An. sinensis ss
- An. sinensis
- An. sneroides
Environmental Factors: *An. sinensis* Group at Daeseongdong

1st JUL (26-28 weeks), 2nd JUL (29-30)
Vivax Malaria in *Anopheles* spp. from Daeseongdong

- Mean number of mosquitoes collected, by species, and number positive for vivax malaria
- Overall rates: *An. belenrae* 25/348, 7.2%; *An. kleini* 7.9%, *An. lesteri* 1/8, 12.5%; *An. pullus* 21/276, 7.6%; *An. sinensis* 23/237, 9.7%
- Sequences of bands for positive mosquitoes were not always vivax malaria positive (possible fungal contamination)
- What effect does this have on previously reported data?
- As with larvae, more adult *An. sinensis* collected during the late season
- *An. pullus* adult populations do not correlate with larval collections (early mosquito) at Warrior Base
Of 1,174 *Anopheles sinensis* Group collected, number identified (% of total) by PCR to species and percent positive for vivax malaria (sporozoite rate)

- *An. lesteri*, 128 (49.8%), sporozoite rate 1.6%
- *An. sinensis*, 58 (22.6%), sporozoite rate 5.2%
- *An. pullus*, 48 (18.7%), sporozoite rate 0%
- *An. kleini*, 20 (7.8%), sporozoite rate 15%
- *An. belenrae*, 3 (1.2%), sporozoite rate 0%
Vivax Malaria Vector Competence

- Limited studies and inconclusive due to low numbers of specimens evaluated (difficult to rear and blood feed in the laboratory)
- Prior to, and even after, 2005 *An. sinensis* concluded as the primary malaria vector in Korea due to large populations
  - Unknown what species was evaluated
  - After 2005, known that identification could only be done by PCR
- Lee W-J, et al. 2007:
  - *An. kleini*: 8.8% oocysts
  - *An. pullus*: 7.5% oocysts
  - *An. sinensis*: 4.2% oocysts
- Joshi D, et al. 2009:
  - *An. lesteri*: 100% oocysts; 64% sporozoites – high numbers
  - *An. sinensis*: 68% oocysts; 11% sporozoites – low numbers
  - *An. pullus*: 67% oocysts; 0% sporozoites
- Rueda LM, et al. 2010: Implicates *An. belenrae* as a malaria vector
- Unpublished data: *An. sineroides*, considered as a non-vector found positive for sporozoites
- Additional studies needed
Previous to 2006-07, all US Soldiers were housed in ill-kept screened tents at Warrior Base, primary site of most malaria cases

2007-Present, US Soldiers primarily housed in air-conditioned screened barracks

- Augmented with permethrin treated bed nets
- Soldiers often in PT uniform while outdoors during evening hours
Malaria-Risk Area - Korea

- Malaria high-risk areas based on ROK and US malaria case analysis and vector surveillance
- Both latent and non-latent malaria

* Provided by the National Center for Medical Intelligence Website: https://www.intelink.gov/ncmi/index.php
Malaria Risk Reduction

- Develop and implement efficient and effective vector reduction strategies:
  - Larval control –
    - Rice paddies – drain periodically
    - Eliminate water sources
  - Adult control
    - Pesticides
    - Application equipment
      - ULV
      - Hot/cold fogger
- Evaluate vector control strategies
  - Pesticide resistance
  - Equipment calibration and application effectiveness
ULV Evaluation

- ULV application of pesticides
  - Environmental factors:
    - Wind speed >5 mph – pesticide disperses too fast
    - Excessive temperatures during application – pesticide disperses upward
    - Wind direction – may miss target area
  - Pesticide resistance
    - Laboratory evaluation
    - Field evaluation
- Cold/Hot foggers
  - Pesticide does not disperse as rapidly
  - Petroleum base - increased environmental pollution
- Barrier sprays
  - Understand behavior
    - Mosquitoes land on vegetation and structures prior to feeding
    - Indoor/outdoor biting behavior
Evaluate Control Measures

Pre-ULV fogging

Post-ULV fogging

Site 1

Warrior Base

Day

log(Total)
Personal Protective Measures (PPM)

DOD REPELLENT SYSTEM

Permethrin on uniform + Deet on skin + Properly worn uniform = Maximum protection
Personal Protection and Education

- Public awareness
- Media:
  - Television
  - Newspaper
  - Health related pamphlets
  - Inform the public of their responsibilities
- Human surveillance
  - Comprehensive programs
  - Passive surveillance
  - Active surveillance – identify malaria reservoirs
- PMM
  - Treated ACUs, Repellents, Avoidance
  - Malaria patients: <20% treated uniforms, <50% repellents
1997 - ROK Army initiated chemoprophylaxis (16,000) hydroxychloroquine + primaquine

- Provided to ROK soldiers in malaria high-risk areas
- Increased annually to 196,000 by 2007, then decreased annually to 162,000 by 2011
- Hydroxychloroquine resistance reported
- Non-compliance rates high – increases potential for resistance
- Primaquine non-compliance and latent malaria increases risk of veterans developing malaria 1-2 years after retirement

1997 US Army initiated chemoprophylaxis (chloroquine/doxycycline + primaquine)

- Reduced usage to only JSA and Cp Greaves
- Deemphasized its use due to low numbers of malaria patients, no observed reduction of malaria, and increased numbers seen after departure from Korea (latent forms)
- Chemoprophylaxis delays detection of malaria
- Chemoprophylaxis increases risks for exporting malaria to the US or other areas where US soldiers are deployed
Armistice:
- Potential spread of malaria south of the DMZ present greater challenges for malaria eradication
- Improvement of training areas with screened barracks = reduced exposure to mosquito bites
- Continued emphasis to reduce malaria rates by ROK government (civilians and military)
- Reduce impact of border malaria – Provide North Korea with antimalarial drugs
- Malaria rates affected by climate (rainfall + onset of early spring or late fall periods (contracted or expanded mosquito season)
- Potential for development of chloroquine resistance
- Recommend chloroquine + primaquine on day 0 to reduce transmission from man to mosquito

Natural Disaster/Hostilities
- Malaria will be a significant health threat during the “malaria season”
- Recommend employment of chemoprophylaxis (chloroquine + primaquine) for all US soldiers deployed to the ROK
- ROK military ??

Warrior Base after 2006-07

Chemoprophylaxis (US Military During the “Malaria Season)
Conclusions

- Malaria high-risk areas parallel the DMZ
- ROK military provided chemoprophylaxis in malaria high-risk areas
- US military implemented chemoprophylaxis from 1997-1999

- Human cases based on area of diagnosis, not exposure
- Latent malaria cases decreases prediction of area of exposure
- Most malaria south of Seoul, veterans
- Hydroxychloroquine, compliance unknown
- Primaquine, compliance unknown, but large number of veteran soldiers develop malaria 1-yr after leaving the military suggests poor compliance (rumor that primaquine causes sexual dysfunction)
- Chloroquine, compliance unknown, one company treated daily with chloroquine until discovered
- Primaquine, compliance unknown, but large number of cases reported after departing Korea, suggests low compliance
- JSA only unit on chemoprophylaxis: malaria cases due to non-compliance
- Chemoprophylaxis gives sense of protection for other disease, i.e., JE
Conclusions – cont.

- Human – Mosquito Transmission
  - Members of the *Anopheles sinensis* Group identified 2005
  - *Anopheles* spp. geographical and temporal distributions defined for limited areas. In general:
    - Asymptomatic vivax malaria carriers unknown (passive surveillance)
    - Days from onset of symptoms to diagnosis and treatment reduced (≈3 days)
    - Patients infective 1-2 days prior to the onset of symptoms
    - PCR for identification costly and time consuming
    - Information reported as *An. sinensis* sensu lato
    - *An. sinensis* s.s., may be composed of two species
      - *An. sinensis*, >90% south of Seoul, 50-70% north of Seoul
      - *An. kleini*, <5% south of Seoul, 20-50% north and northeast of Seoul
      - *An. lesteri*, uncommon at Panmunjeom, predominate northwest of Seoul
      - *An. pullus*, <5% south of Seoul, 10-30% north and northeast of Seoul
Species temporal distributions based on larval collections

Habitat preference not defined

Biting behavior not described

Vector competence unknown

- At WB, the proportion of *An. sinensis* populations low during the spring (5%) but increase to 90% by fall
- At WB, the proportion of *An. kleini* populations increase through July, then decrease

- Monthly collections of larvae, including instar data at the time of the collection sitting in freezer at WRBU – no funding
- Indoor/outdoor and hourly biting behavior not determined
- Host preference not determined: Anecdotal data suggests that *An. kleini* is more anthropophilic than *An. sinensis* (larger proportion collected at WB)

- Limited data suggests that *An. kleini, An. pullus, and An. lesteri* are the primary vectors
- New evidence indicates that *An. belenrae and An. sineroides* are vectors
- Evidence suggests that *An. sinensis* is a secondary vector
Conclusions – cont.

- PCR of *Anopheles* spp. for diagnosis of vivax malaria
- Vector surveillance and control
- Personal Protective Measures

- New evidence indicates that some *P. vivax* positive mosquitoes using PCR methods are false positive (possible fungal contamination)
- Fungal contamination can also lead to false *Plasmodium* positive mosquitoes by ELISA
- PCR methods difficult to quantify estimated numbers of sporozoites
- ELISA difficult to assess often due to low background and false positives, but advantage of sporozoite quantification
- Surveillance tools not evaluated for each species, including attractants (light, CO₂, Octenol)
- Vector control methods of application and pesticide resistance not evaluated
- Directed to be used by US military – infrequently used
- ROK military say repellents are available to soldiers – use unknown
- Permethrin treated uniforms: Usually >90% usage
- Repellents: Infrequently used and often not available
Summary

- Malaria is preventable, but is dependent upon:
  - Understanding malaria high-risk areas
  - Understanding the vector
  - Developing sensitive and specific assays for the identification of *Plasmodium* spp. in mosquitoes
  - Understanding the effect of environmental factors
  - Education of populations, including medical providers
  - Development of effective vector and malaria control strategies
  - Cooperation with North Korea to eliminate malaria along the DMZ

- The key is to not get bitten by mosquitoes
QUESTIONS

Sunset over the DMZ

Collaborators

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And many others