Japanese encephalitis (JE) is a disease caused by an arbovirus that is spread by marsh birds, amplified by pigs, and mainly transmitted by the bite of infected *Culex tritaeniorhynchus* mosquitoes. The estimated annual incidence and mortality rates are 30,000–50,000 and 10,000, respectively, and the estimated global burden in JE in 2002 was 709,000 disability-adjusted life years lost. Here, we discuss the contextual determinants of JE, and systematically examine studies assessing the relationship between irrigated rice agriculture and clinical parameters of JE. Estimates of the sizes of the rural population and population in irrigated areas are presented, and trends of the rural population, the rice-irrigated area, and the rice production are analyzed from 1963 to 2003. We find that approximately 1.9 billion people currently live in rural JE-prone areas of the world. Among them 220 million people live in proximity to rice-irrigation schemes. In 2003, the total rice harvested area of all JE-endemic countries (excluding the Russian Federation and Australia) was 1,345,000 km². This is an increase of 22% over the past 40 years. Meanwhile, the total rice production in these countries has risen from 226 millions of tonnes to 529 millions of tonnes (+134%). Finally, we evaluate the effect of different vector control interventions in rice fields, including environmental measures (i.e. alternate wet and dry irrigation (AWDI)), and biological control approaches (i.e. bacteria, nematodes, invertebrate predators, larvivorous fish, fungi and other natural products). We conclude that in JE-endemic rural settings, where vaccination rates are often low, an integrated vector management approach with AWDI and the use of larvivorous fish as its main components can reduce vector populations, and hence has the potential to reduce the transmission level and the burden of JE.
1. Introduction

Japanese encephalitis (JE) is a mosquito-borne viral disease. The JE virus is a member of the family Flaviviridae. Clinically apparent infection takes place in one out of 200–300 infected patients (Pugachev et al., 2003). The disease is characterized by a wide range of presentations, as both the symptoms and the clinical course can differ broadly among patients. They range from mild flu-like symptoms to considerable neurologic symptoms, such as rashes, convulsions, polio-like flaccid paralysis, seizures or encephalomyelitis. Severe clinical cases are likely to have life-long neurological sequelae. Mostly children and young adults are affected (Solomon et al., 2000; Tsai, 2000; Ding et al., 2003; Halstead and Jacobson, 2003). The annual incidence and mortality estimates for JE are 30,000–50,000 and 10,000, respectively (Solomon, 2004). However, there is considered to be severe under-reporting of JE and one study estimated the annual incidence at 175,000 per year (Tsai, 2000). JE outbreaks occur in cycles that may be linked to climatic patterns and the immune status of the populations. The great majority of cases and death occur in World Health Organization (WHO) regions of South-East Asia and the Western Pacific. In 2002, the estimated global burden of JE was 709,000 disability-adjusted life years (DALYs) lost (WHO, 2004). At present there are no established antiviral treatments against JE. Interferon alpha was the most promising drug in small open-label trials, but it failed to affect the outcome in children with JE (Solomon et al., 2003).

There has been a changing pattern in the epidemiology of JE. On the one hand, primarily due to extensive vaccination campaigns, JE has been almost eliminated in many economically advanced countries of East Asia and South-East Asia (i.e. Japan, Republic of Korea and Taiwan) and the burden of JE has been substantially reduced in many other endemic countries (Halstead and Jacobson, 2003). On the other hand, intensified transmission has been observed in other parts of South-East Asia and the Western Pacific, most likely due to an expansion of irrigated agriculture and pig husbandry, as well as changing climatic factors. Water resource development and management, in particular flooded rice production systems, are considered among the chief causes for several JE outbreaks (Amerasinghe and Ariyasena, 1991; Akiba et al., 2001). Conversely, the occurrence of the disease has changed considerably over the past 50 years (Solomon et al., 2000). At present, the geographical distribution of JE ranges from Japan, maritime Siberia and the Republic of Korea in the North, to most parts of China and the Philippines in the East, Papua New Guinea in the South, and India and Nepal to the West (Broom et al., 2003). Recent outbreaks of JE have been reported Southward in Australia, and westward in Pakistan (Solomon et al., 2000).

Currently, approximately 90% of the world’s rice is produced in Asia (Consultative Group on International Agricultural Research et al., 1998). In most of the countries, where JE outbreaks have been reported, rice is not only a staple food, but rice growing also is a major economic activity and key source of employment and income generation (Consultative Group on International Agricultural Research et al., 1998). Efforts to further enhance the high annual rice production in these areas are essential to maintain food security. It has been estimated that in the next 25 years the demand for rice will rise by 65% in the Philippines, 51% in Bangladesh, 45% in Viet Nam and 38% in Indonesia (http://www.biotech-info.net/rice_expert.html). Thailand, for example, is already in the planning stages of designing new rice-irrigation schemes for year-round irrigation (Consultative Group on International Agricultural Research et al., 1998). Hence, there is considerable concern in public health circles, as the intensification of rice production systems as well as the extension of the flooded surface area, particularly in semi-arid areas, contributes greatly to increased frequencies and intensities of JE outbreaks.

The objectives of this paper were (i) to evaluate the effect of irrigated rice agriculture on the burden of JE; and (ii) to review different vector control interventions and discuss challenges and opportunities for integrated vector management (IVM). The remainder of this paper is structured as follows. First, we put forward contextual determinants of JE, highlighting the important role of rice agroecosystems on JE vector populations. Second, we review studies assessing the relationship between irrigated rice growing and clinical parameters of JE. Third, we present estimates of the population living in proximity to irrigation and rice-irrigation schemes in JE-prone areas, stratified by relevant WHO sub-regions of the world (WHO, 2004). Fourth, we quantify the changes of the rice-irrigated area, rice production, and rural population sizes over the past 40 years, in countries, where JE is currently endemic. Finally, we re-
viewed studies that employed different environmental
and biological control interventions to reduce larval JE
vector populations, which have been implemented in
irrigated rice production systems.

2. Contextual determinants

Fig. 1 depicts the contextual determinants of JE.
The most important epidemiological features that gov-
ern the transmission of JE include (i) environmental
factors, i.e., agricultural practice, altitude, climate and
the presence of pigs and marsh birds; (ii) human im-
munization rates and vector control measures; and (iii)
socioeconomic parameters.
The principal vector of JE is *Culex tritaenio-
rhynchus*. Female specimens are infective 9–10
days after having taken the viraemic blood meal,
having undergone three gonotrophic cycles (Gajanana
et al., 1997). Other culicine mosquitoes that can trans-
mitt JE include *Cx. bitaeniorhynchus*, *Cx. epidesmus*, *Cx.
fuscocephala*, *Cx. gelidus*, *Cx. pseudovishnui*, *Cx. si-
tiens*, *Cx. vishnui* and *Cx. whitmorei* (Sehgal and Dutta,
2003). In Australia, *Cx. annulirostris* was found to be
the major JE vector species (Hanna et al., 1996). In
a recent study in Kerala, South India, JE was isolated
from *Mansonia indiana* (Arunachalam et al., 2004).

Although JE vectors are able to breed in ground wa-
ter habitats, sunlit pools, roadside ditches, tidal marshes
of low salinity, or man-made containers, one of their
major preferred larval habitats are rice fields (Mogi,
1984; Sucharit et al., 1989). The ecology of *Culex*
sp. in rice fields has been studied and reviewed in
great detail (Suzuki, 1967; Lacey and Lacey, 1990).

JE vector abundance is closely related to agro-
climatic features (Peiris et al., 1993; Phukan et al.,
2004), most notably temperature and monthly rain-
fall (Suroso, 1989; Solomon et al., 2000; Bi et al.,
2003). In addition, potential JE vectors were rarely
found at altitudes above 1200 m (Peiris et al., 1993).
However, the most important causative factors of JE
is the management of paddy water, and the peak peri-
ods of mosquito abundance are associated with cycles
in local agricultural practices. In Thailand, the highest

Fig. 1. Contextual determinants of Japanese encephalitis.
The second category of contextual determinants comprises vaccination and transmission interruption strategies. Later in this paper, we review the effects of different environmental and biological control interventions in rice fields. Though JE vectors tend to bite and rest outdoors, self-protection behaviour, such as sleeping under insecticide-treated nets (ITNs), using insect repellants, and insect-proofing homes and work places, might also assist in reducing JE transmission (WHO, 1997). For example, a population-based case-control study in China, which evaluated the protective effect of ITNs against JE, showed that the risk of infection among children below 10 years was greatly reduced (Luo et al., 1994). On the other hand, all 187 serologically confirmed JE cases in recent outbreaks in Northeast India reported that they had slept under a bed-net (Phukan et al., 2004). Furthermore, application of deet-permethrin soap (“Mos-bar”) led to an 89–100% reduction in man-vector contact, including vectors transmitting JE (Mani et al., 1991b).

The third broad category of determinants that govern JE transmission is people’s socioeconomic status. In Central China, more JE cases were observed among children living in poor quality houses, and whose parents had lower income. However, the sample size of the study was small, which might explain why the associations were not statistically significant (Luo et al., 1995). Religion, exposure to domestic animals, and household crowding were also described as risk factors associated with JE (Halstead and Jacobson, 2003).

3. Rice irrigation and JE incidence

As summarized in the previous section, the management of paddy water strongly influences the transmission of JE. Hence, we were motivated to conduct a systematic literature review to identify published work with an emphasis on the relationship between rice irrigation and JE. We searched Biosis previews, Ovid Technologies, Medline and the Web of Science applying the following keywords: “Japanese encephalitis” and “water”, or “rice”, or “irrigation”, or “rice irrigation and JE. We searched Biosis previews, Ovid Technologies, Medline and the Web of Science
Four studies in India analyzed the presence of rice irrigation and vector abundance in relation to the incidence of JE. In the Gorakhpur district in Uttar Pradesh, and the Mandya district of Karnataka, areas extensively developed for irrigated rice agriculture, the occurrence of JE was closely associated with high vector densities, breeding in the fields or the canal system. The highest numbers of JE cases were observed shortly after the mosquito densities peaked (Mishra et al., 1984; Kanojia et al., 2003). In addition, in the Mandya district, a high incidence of JE was found in extensively irrigated areas, while few cases occurred in villages with less irrigation or no irrigation systems (Geervarghese et al., 1994). In Assam, 78.6% of the JE cases occurred in families practicing rice cultivation (Phukan et al., 2004).

Based on an epidemiological, serological and clinical study of 54 JE patients in the Northern Philippines, 41 cases (76%) were associated with irrigated rice fields and only one patient (2%) could not be linked to rice irrigation (Barzaga, 1989). In Taiwan, JE was monitored over 4 years and the highest numbers of cases were observed in the counties with the highest number of rice paddies (e.g. in Ilan county in 1969 a morbidity rate of 5.5 per 100,000 inhabitants). Fewer cases occurred in areas where dry farming had been adopted (e.g. in Yunlin county in 1969 a morbidity rate of 0.64 per 100,000 inhabitants) and no cases were reported in non-rice cultivated provinces (Okuno et al., 1975). In 1985–1986 in the Mahaweli System H, Sri Lanka, an epidemic of JE occurred, resulting in more than 400 cases and 76 deaths. In 1987–1988 a second outbreak took place with >760 cases and 138 deaths.

The promotion of smallholder pig husbandry was suspected to be responsible for these outbreaks. The highest number of cases occurred in areas with irrigation and pig husbandry, while no cases were reported from non-irrigated areas with few pigs (Amerasinghe, 2003).

4. Population at risk

In order to estimate the current population at risk of JE, we first compiled a list of all countries where JE epidemics or sporadic cases have been reported (CDC, 2004; http://www.cdc.gov/ncidod/dhdd/jencephalitis/risk-table.htm). The countries were grouped into different epidemiological sub-regions of the world, according to recent classifications of WHO, which is based primarily on child and adult mortality rates (WHO, 2004). The JE-endemic countries are located in seven of the 14 WHO sub-regions (Table 1).

Though sporadic JE cases have been reported from non-rice cultivated provinces (Okuno et al., 1994). In Assam, 78.6% of the JE cases occurred in families practicing rice cultivation (Phukan et al., 2004). The JE-endemic countries are located in seven of the 14 WHO sub-regions (Table 1).

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The promotion of smallholder pig husbandry was suspected to be responsible for these outbreaks. The highest number of cases occurred in areas with irrigation and pig husbandry, while no cases were reported from non-irrigated areas with few pigs (Amerasinghe, 2003).
Table 1
Rural population/population in irrigated areas in JE-endemic countries stratified by relevant WHO sub-regions of the world (all numbers in thousand; n.d.: no data currently available)

<table>
<thead>
<tr>
<th>Region</th>
<th>WHO sub-region</th>
<th>Population in irrigated areas</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Americas</td>
<td>United States of America: Guam (150/n.d.) and Saipan (40/n.d.)</td>
<td></td>
</tr>
<tr>
<td>Eastern Mediterranean</td>
<td>Pakistan* (16,704,001)</td>
<td></td>
</tr>
<tr>
<td>Europe</td>
<td>Russian Federation (614/n.d.)</td>
<td></td>
</tr>
<tr>
<td>South-East Asia</td>
<td>Indonesia (119,589,033), Sri Lanka (15,057,146), Thailand (42,796,014)</td>
<td></td>
</tr>
<tr>
<td>Western-Pacific</td>
<td>Australia (15/n.d.), Brunei Darussalam (85/n.d.), Japan (25,113,276), Singapore (480/n.d.)</td>
<td></td>
</tr>
</tbody>
</table>

| WHO sub-region 7         | Pakistan (16,704,001) |
| WHO sub-region 10        | Russian Federation |

| WHO sub-region 11        | Indonesia (119,589,033), Sri Lanka (15,057,146), Thailand (42,796,014) |
| WHO sub-region 12        | Pakistan* (16,704,001) |
| WHO sub-region 13        | Australia (15/n.d.), Brunei Darussalam (85/n.d.), Japan (25,113,276), Singapore (480/n.d.) |
| WHO sub-region 14        | Cambodia (115,414,72), China (766,757,276), Laos People’s Democratic Republic (448,930), Malaysia (814,949), Papua New Guinea (405,000), Philippines (11,000), Republic of Korea (9,395,108), Viet Nam (6,441,546) |


c Bangladesh and Bhutan are potential JE-endemic countries, but due to lack of data the situation has to be clarified (CDC, 2004, http://www.cdc.gov/ncidod/dvbid/jencephalitis/risk-table.htm).


e Hyperendemic in Southern Terai lowlands (BBIN, 2004).

f Rare sporadic cases on all islands except Hokkaido; population estimates from (United Nations, 2004) and (Microsoft Corporation, 2004).

g Cases in all provinces except Xizang (Tibet), Xinjiang, Qinghai (CDC, 2004, http://www.cdc.gov/ncidod/dvbid/jencephalitis/risk-table.htm); population estimates taken from (United Nations, 2004) and (Microsoft Corporation, 2004).

According to our estimates 1,025,000–1,080,000 km² land is irrigated in JE-prone areas. We find that currently 180–220 million people are living in proximity to irrigation or rice-irrigation schemes in the JE-endemic regions, and thus are at risk of contracting the disease (Table 2). Irrigated agriculture is most pronounced in WHO sub-region 12. According to our estimates 132–167 million people live in JE-endemic irrigated areas in this WHO sub-region (Table 2).

The estimated global burden of JE in 2002 was 709,000 DALYs. WHO sub-regions 12 and 14 currently bear 84% of this burden (597,000 DALYs). The remaining 16% are thought to occur in WHO sub-regions 7.
<table>
<thead>
<tr>
<th>WHO sub-region</th>
<th>DALYs lost in 2002</th>
<th>Total area (km²)</th>
<th>JE-endo areas (km²)</th>
<th>Irrigated land in JE-endo areas (km²)</th>
<th>Rice paddies in JE-endo areas (km²)</th>
<th>Total population in 2003 (×10³)</th>
<th>Rural endemic population (×10³)</th>
<th>JE-endemic irrigated areas (×10³)</th>
<th>Population in JE-endemic areas (×10³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>n.d.</td>
<td>663</td>
<td>n.d.</td>
<td>663 (100%)</td>
<td>n.d.</td>
<td>190 (100%)</td>
<td>190 (100%)</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>7</td>
<td>83,000</td>
<td>296,089</td>
<td>140,114 (45.7%)</td>
<td>31,507 (10.5%)</td>
<td>3912 (0.5%)</td>
<td>153,278</td>
<td>16,730 (10.9%)</td>
<td>4,661 (2.6%)</td>
<td>487 (0.3%)</td>
</tr>
<tr>
<td>50</td>
<td>n.d.</td>
<td>17,075,400</td>
<td>n.d.</td>
<td>165,790 (1.0%)</td>
<td>n.d.</td>
<td>143,246</td>
<td>614 (0.4%)</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>11</td>
<td>29,000</td>
<td>2,483,300</td>
<td>177,420 (7.1%)</td>
<td>104,100 (4.2%)</td>
<td>31,507 (4.0%)</td>
<td>301,782</td>
<td>31,507 (4.0%)</td>
<td>8,689 (2.9%)</td>
<td>18,468</td>
</tr>
<tr>
<td>12</td>
<td>277,000</td>
<td>6,893,622</td>
<td>5,881,672 (86.7%)</td>
<td>632,349 (11.7%)</td>
<td>65,146 (11.7%)</td>
<td>1,046,246</td>
<td>65,146 (11.7%)</td>
<td>34,971 (6.5%)</td>
<td>104,203 (10.1%)</td>
</tr>
<tr>
<td>13</td>
<td>n.d.</td>
<td>8,528,628</td>
<td>3,087,734 (41.1%)</td>
<td>220,917 (7.2%)</td>
<td>12,921 (0.4%)</td>
<td>151,396</td>
<td>20,906 (1.4%)</td>
<td>1,537 (0.1%)</td>
<td>29,370 (2.1%)</td>
</tr>
<tr>
<td>14</td>
<td>320,000</td>
<td>11,539,400</td>
<td>9,902,400 (86.3%)</td>
<td>767,580 (8.2%)</td>
<td>308,120 (3.3%)</td>
<td>1,301,000</td>
<td>308,120 (3.3%)</td>
<td>56,897 (4.4%)</td>
<td>37,749 (2.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>709,000</td>
<td>44,457,730</td>
<td>13,425,469 (30.1%)</td>
<td>0.908,850 (2.0%)</td>
<td>1,080,999 (2.4%)</td>
<td>3,623,815</td>
<td>1,080,999 (2.4%)</td>
<td>320,879 (0.1%)</td>
<td>320,879 (0.1%)</td>
</tr>
</tbody>
</table>

- n.d.: no data.
- Data for the whole country obtained from the Food and Agriculture Organization 2004 and multiplication by the endemic fraction.
- The size of the endemic irrigated population and endemic population in rice areas was estimated by multiplying the average national rural population densities (United Nations, 2006) by the total area under irrigation of rice paddies in JE-endemic areas.
- Omitting Singapore and Australia.
- Omitting Papua New Guinea.
Meanwhile, the rice-irrigated area has decreased in until 1993, but remained stable over the past 10 years. In WHO sub-region 7 (Pakistan) the rice area grew a substantial increase in the rice-irrigated area is apparent in regions, where JE has been reported to be a significant problem. The total rice production has risen from 226 million tonnes in 1963 to 529 million tonnes in 2003 (+134%). The most significant increases of rice production occurred in WHO sub-regions 7, 11, 12 and 14, as shown in Fig. 2. On the other hand, in WHO sub-region 13 (mainly Japan) a reduction from 16.6 to 9.7 million tonnes of tonnes (~43%) has taken place.

We also depict in Fig. 2 the change of the rural population of the five most relevant WHO sub-regions, which overall increased from 1325 million inhabitants in 1963 to 2197 million inhabitants in 2003 (+66%). In the past decade a decrease in the rural population has been observed in WHO sub-regions 11 and 14.

6. Intervention strategies in rice fields

Our aim was to systematically review the literature to identify published work on biological control strategies against Cx. tritaeniorhynchus larvae in rice fields. We did not include studies on the application of synthetic larvicides and insecticides against Cx. tritaeniorhynchus in rice fields, as the use of chemical control was found to be of no operational value due to the short activity of these products, high costs and resistance development (Wada, 1988). We searched the same electronic databases as mentioned in Section 3 and used the following keywords: “Japanese encephalitis” or “Culex tritaeniorhynchus” in combination with “alternate wet and dry irrigation”, or “Azolla”, or “Bacillus”, or “biological control”, or “bacteria”, or “control”, or “fungi”, or “intermittent irrigation”, or “invertebrates”, or “larvivorous fish”, or “larvicides”, or “natural products”, or “nematodes”, or “predator”, or “water management”.

6.1. Environmental control strategies

6.1.1. Alternate wet and dry irrigation (AWDI)

Traditionally, rice fields are flooded, which provides ideal breeding places for several mosquito species, including those that transmit JE. The management of irrigation water that leads to the alternate wetting and drying of fields, including the canals and ditches, can be active or passive. An important feature of this tech-
Fig. 2. Changes of rice growing area, rice production and rural population at risk of JE in 5 WHO sub-regions between 1963 and 2003.
The technique is that the soil can dry out, which in turn curtails the life cycle development of the mosquito from larvae and pupae to adult. In order to achieve a significant reduction of mosquito larvae, AWDI (also termed intermittent irrigation) has to be applied during the entire cropping season and should cover all rice fields that are connected by irrigation canals over a large area. This method is particularly feasible in places where control of the water supply and drainage is possible, hence where land and climatic conditions are suitable (Mogi, 1988). Growing water shortages in many areas create an incentive to better control irrigation water management. AWDI is one such strategy. The potential of AWDI is, however, limited in areas where there is a threat of insufficient resources to re-flood the fields and where farmers perceive a risk of reduced yields by letting their fields dry out.

The effects of AWDI on the abundance of Anopheles, which are the vector species of malaria, rice yield, water consumption and methane emission have been reviewed recently (van der Hoek et al., 2001; Keiser et al., 2002). In brief, this method had been introduced in Asia about 300 years ago, primarily to obtain higher rice yields. The first study of AWDI as a potential tool to reduce mosquito vectors was conducted in Bulgaria on mosquitoes of the An. maculipennis complex in the 1920s (Keiser et al., 2002). AWDI was compulsory in Portugal in the 1930s, when malaria was still a problem there.

We found four studies analyzing the effect of AWDI on densities of JE vectors and the key findings are summarized in Table 3. Overall, Cx. tritaeniorhynchus immatures were reduced by 14–91% in rice fields applying AWDI. One study investigated the effect of AWDI on the Cx. tritaeniorhynchus adult population, which decreased by 55–70%. The crop yield was examined in two trials and increases between 4 and 13% were observed in AWDI rice fields. The effect this method has on the incidence of JE remains to be investigated.

### Table 3: Alternate wet and dry irrigation (AWDI) against Culex tritaeniorhynchus larvae in rice fields

<table>
<thead>
<tr>
<th>Study site</th>
<th>Specificity of AWDI</th>
<th>Outcome</th>
<th>Rice yield</th>
<th>Water consumption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Henan, China (Lu-Baulin, 1988)</td>
<td>Irrigation interval: 5 days</td>
<td>Decrease: 81–91%</td>
<td>Increase: 13%</td>
<td>Decrease: 50%</td>
</tr>
<tr>
<td>Tamil Nadu, India (Rajendran et al., 1995)</td>
<td>Irrigation interval: 3–5 days</td>
<td>Decrease: 75–88%</td>
<td>Increase: 4%</td>
<td>Not known</td>
</tr>
<tr>
<td>Kinryu, Japan (Mogi, 1993)</td>
<td>Irrigation interval: Several days</td>
<td>Decrease Not known</td>
<td>Not known</td>
<td>Not known</td>
</tr>
<tr>
<td>Tsu City, Japan (Takagi et al., 1995)</td>
<td>Midseason drying</td>
<td>Decrease of fourth instars: 14.3–48.2%</td>
<td>Not known</td>
<td>Not known</td>
</tr>
</tbody>
</table>

### 6.2. Biological control strategies

#### 6.2.1. Bacteria

The larvicidal activity of the spore forming bacteria Bacillus sphaericus and B. thuringiensis israelensis have been discovered in 1965 and 1976, respectively (Mittal, 2003). Subsequently, these bioicides have been evaluated in various formulations against...
mosquito vectors worldwide (Lacey and Lacey, 1990).

- *B. thuringiensis* produces four key insecticidal proteins, while *B. sphaericus* produces a single binary toxin (Federici et al., 2003). These protein toxins bind to cells of the gastric caecum and posterior midgut of the mosquitoes causing intoxication, which eventually leads to death (Mittal, 2003). The advantages of these bacterial insecticides in terms of efficacy, specificity and environmental safety are well documented (Zahiri et al., 2004). A major drawback of these products, however, is their high cost of production, the labor-intensive delivery, as well as first reports of resistance (Sundararaj and Reuben, 1991; Federici et al., 2003). The effect of the individual biolarvicides depends on water temperature, pH, aquatic vegetation, the formulation applied, type of habitat, and the target mosquitoes (Mittal, 2003). *B. sphaericus*, for example, is known to exhibit a high activity against *Culex* mosquitoes, while certain *Aedes* species are not affected (Mittal, 2003; Zahiri et al., 2004). Hence, no general conclusion can be drawn about whether *B. sphaericus* and *B. thuringiensis israelensis* formulations are suitable for JE vector control in rice fields. This results on the application of these bacterial formulations in rice fields against *Culex tritaeniorhynchus* are summarized in Table 4. In Tamil Nadu, India, the application of 4.3 kg/ha of a microgel droplet formulation of *B. sphaericus* 1593M resulted in a 44–79% reduction of early instar and 82–100% reduction of late instar culicinae larvae (*C. fuscans, C. pseudovishnui* and *C. tritaeniorhynchus*) for at least 5 weeks (Sundararaj and Reuben, 1991). Similarly, up to 95–98% of *C. tritaeniorhynchus* larvae were reduced in three other field sites evaluating *B. sphaericus* or *B. thuringiensis* formulations (Balaraman et al., 1983; Rhee et al., 1983; Kramer, 1984). However, the larvicidal activity did not persist in these rice fields beyond a couple of days. In the Republic of Korea the residual effect of *B. thuringiensis* H-14 was found to last only 24 h (Rhee et al., 1983).

### 6.2.2. Nematodes

*Romanomermis culicivorax* is probably the best-studied nematode parasite of mosquitoes. The preparasitic nemas are applied to the fields, where they locate a host, penetrate the cuticle and develop within the mosquito larvae (Lacey and Lacey, 1990). Although this method has not been widely studied, we identified three publications assessing the effect of nematodes on JE vectors, which are summarized in Table 5. In Taiwan, application of 7000 nematodes per m² rice field yielded a reduction of 11–18% *C. tritaeniorhynchus* (Lacey and Lacey, 1990). In two studies in China the nematode *R. yunnanensis* has been distributed in rice fields.

### Table 4

<table>
<thead>
<tr>
<th>Study site: period (if known); reference</th>
<th>Intervention strategy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>India, 1983–1984 (Kramer, 1984)</td>
<td>Application of <em>Bacillus sphaericus</em> and <em>Bacillus thuringiensis</em> H-14 in rice fields</td>
<td>91–99% reduction with doses of 0.5–1.5 kg/ha <em>Culex tritaeniorhynchus</em> and <em>Aedes</em> subpictus; however, activity did not sustain beyond a few days</td>
</tr>
<tr>
<td>India, 1982 (Balaraman et al., 1983)</td>
<td>Application of <em>Bacillus thuringiensis</em> H-14 in rice fields</td>
<td>100% reduction with doses of 27×10⁶ spores/ml, but first and second instars reappeared 3 days after application</td>
</tr>
<tr>
<td>India (Sundararaj and Reuben, 1991)</td>
<td>Application of microgel droplet formulation of <em>Bacillus sphaericus</em> in rice fields</td>
<td>44–79% reduction of early instar and 82–100% reduction of late instar of <em>Culex tritaeniorhynchus, Cx. vishnui</em> and <em>Cx. pseudovishnui</em> for at least 5 weeks applying a dose of 4.3 kg/ha</td>
</tr>
<tr>
<td>Korea (Rhee et al., 1983)</td>
<td>Application of <em>Bacillus thuringiensis israelensis</em> H-14 in rice field</td>
<td>95–98% reduction of <em>Culex tritaeniorhynchus</em>. The residual effects lasted only 24 h at doses of 0.6–1.2 kg/ml of <em>Bacillus thuringiensis israelensis</em> H-14. SAN 402 suspension concentrate containing 600 IU/ml</td>
</tr>
<tr>
<td>Rourkela city, India, June 1993 to October 1994 (Yadav et al., 1997)</td>
<td>Application of <em>Bacillus sphaericus</em> (strain B-101, serotype H5a, H5b) in rice field</td>
<td>Significantly reduced larval and pupal counts (P &lt; 0.0001) in rice fields. Duration of effect could not be determined as fields received periodically <em>Bacillus sphaericus</em> treated wastewater</td>
</tr>
</tbody>
</table>

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fields (2000–4000 nematodes/m²), which resulted in parasitism rates of 52.2–96.7% and 60.8–95.5%, respectively, of C. tritaeniorhynchus larvae (Song and Peng, 1996; Peng et al., 1998).

6.2.3. Invertebrate predators
Invertebrate predators, i.e. Coleoptera, Hemiptera or Odonata, though less common than the use of fish, are also known to substantially reduce mosquito larval populations in rice fields (Lacey and Lacey, 1990). However, they are highly sensitive to temperature, presence of vertebrates, growth of rice and chemical pollutants (Lacey and Lacey, 1990). In India, the presence of notonectids was negatively associated with larval abundance of Cx. pseudovishnui, Cx. tritaeniorhynchus and Cx. vishnui (Sunish and Reuben, 2002).

6.2.4. Larvivorous fish
The use of larvivorous fish to reduce mosquitoes has a history of over 100 years (Lacey and Lacey, 1990). The mosquitofish, Gambusia affinis, is the most widely used predator. Other fish species include Tilapia spp., Poecilia reticulata or Cyprindus (Lacey and Lacey, 1990). A detailed summary of the most common predatory fish is given in (Lacey and Lacey, 1990).

After stocking rice fields with 1–10 natural predators per m², larval populations of Cx. tritaeniorhynchus were reduced by 55.2–87.8% (Table 6).

Larvivorous fish cannot be applied in rice fields, where irregular irrigation is practiced. It should also be noted that predator populations are strongly influenced by temperature, rice growth, vegetation, size of pesticides or chemical pollutants (Lacey and Lacey, 1990). In addition, recent research has shown that ovipositing mosquitoes may move to other breeding sites in response to the stocking of rice fields with predatory fish (Angelone and Potranka, 2002). Furthermore, the introduction of exotic predators such as Gambusia might displace the native fish populations to reduce their natural value, as being observed in Japanese rice fields (Wada, 1988). Therefore, the compatibility of the chosen fish with local fauna and flora is of high importance (Lacey and Lacey, 1990).

6.2.5. Fungi
Fungi that have been studied extensively for their potential as biological mosquito control agents include Coelomomyces spp. and Lagenidium giganteum. The former have been investigated how they impact the development of JE vectors in China. Field observations showed a strong effect of the fungus Coelomomyces indicus on Cx. tritaeniorhynchus, as infected larvae were unable to develop into adults (Liu and Hou, 1982). However, fungi have not been applied for biological control of JE vectors on a large scale so far, as practical problems, for example their production, have yet to be solved (Lacey and Lacey, 1990).

6.2.6. Other natural products
Natural products might also have high potential for reducing the proliferation of culicine mosquitoes in rice fields. Our literature search on the control of JE vectors in rice fields yielded in-depth investigation of only two natural products. In Tamil Nadu, India application of the floating water fern Azolla microphylla greatly reduced immature mosquito populations. However, the infestation of the rice field with Azolla was difficult to achieve, 80% coverage by Azolla was accomplished only 13–14 days after rice transplantation, limiting its wider use as a biological mosquito control agent (Rajendran and Reuben, 1991).

Neem cake powder, made from freshly harvested or stored neem kernels, from the neem tree (Azadirachta indica) rich in the active principle azadirachtin. At a

Table 5

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<tr>
<td>Predation efficacy of Romamonemertes yarni in rice fields</td>
<td>2000–4000 nematodes/m² yielded parasitism rates of 52.2–96.7% for Culex tritaeniorhynchus and 1000–3000 nematodes/m² resulted in parasitism rates of 60.8–95.5% of Culex tritaeniorhynchus and Cx. quinquefasciatus and Anopheles spp.</td>
</tr>
<tr>
<td>Predation efficacy of Romamonemertes carinivorus in rice fields</td>
<td>Larvae reduction of 11–18% of Culex tritaeniorhynchus</td>
</tr>
<tr>
<td>Application of nematodes against Cx. tritaeniorhynchus larvae in rice fields</td>
<td>Larvae reduction of 11–18% of Culex tritaeniorhynchus</td>
</tr>
<tr>
<td>Predation efficacy of Romamonemertes yunnanensis in rice fields</td>
<td>Larvae reduction of 11–18% of Culex tritaeniorhynchus</td>
</tr>
<tr>
<td>Predation efficacy of Romamonemertes indicus in rice fields</td>
<td>Larvae reduction of 11–18% of Culex tritaeniorhynchus</td>
</tr>
<tr>
<td>Predation efficacy of Coelomomyces indicus in rice fields</td>
<td>Larvae reduction of 11–18% of Culex tritaeniorhynchus</td>
</tr>
<tr>
<td>Predation efficacy of Lagenidium giganteum in rice fields</td>
<td>Larvae reduction of 11–18% of Culex tritaeniorhynchus</td>
</tr>
<tr>
<td>Predation efficacy of Azolla microphylla in rice fields</td>
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The effects of these water projects and accompanying ecological transformations are manifold, and inherently difficult to assess and quantify. Negative effects include increased frequencies and transmission dynamics of water-based (e.g. schistosomiasis) and water-related vector-borne diseases, including malaria (Keiser et al., in press), lymphatic filariasis (Erlanger et al., in press) and JE. On the other hand, such water projects are key for hydroelectric power production and food security; hence they can stimulate social and economic development. In this review, we have focused on irrigation schemes, with particular emphasis on rice growing. Currently, there is a paucity on high quality data on the effects of large and small dams on JE.

Over the last four decades rice production has expanded considerably in most countries where JE is currently endemic. This growth will most likely continue for food security reasons. We find that in 2003 approximately 180–220 million people live near irrigation schemes in JE-prone countries, hence have a potential JE endemicity of 180–220 million people living near irrigation schemes in JE-prone countries.

### 7. Discussion and conclusion

Water resource development and management, in particular the construction and operation of small and large dams and irrigation schemes, occurred at an enormous pace over the past 50 years (Gujja and Perrin, 1999). The effects of these water projects and accompanying ecological transformations are manifold, and inherently difficult to assess and quantify. Negative effects include increased frequencies and transmission dynamics of water-based (e.g. schistosomiasis) and water-related vector-borne diseases, including malaria (Keiser et al., in press), lymphatic filariasis (Erlanger et al., in press) and JE. On the other hand, such water projects are key for hydroelectric power production and food security; hence they can stimulate social and economic development. In this review, we have focused on irrigation schemes, with particular emphasis on rice growing. Currently, there is a paucity on high quality data on the effects of large and small dams on JE.

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### Table 6

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<tbody>
<tr>
<td>Buloki, Bosong-gun, Chollanam-do, Korea 1995–1996 (Lee, 1998)</td>
<td>Presence of M. mizolepis in rice fields</td>
<td>Coefficients of correlation between M. mizolepis and abundance of mosquito larvae showed negative correlations in A. sinensis (r = −0.66) and C. tritaeniorhynchus (r = −0.47).</td>
</tr>
<tr>
<td>Suwon, 1992–1993 (Kim et al., 1994)</td>
<td>Presence of M. mizolepis and M. anguilloideatus in rice fields</td>
<td>4,000 kg/m² gave a reduction of 55.2% of immatures of A. sinensis. Culex pipiens pallens and Culex tritaeniorhynchus compared to control field after 8 weeks</td>
</tr>
<tr>
<td>South Delhi, May–June 1980 (Mahur et al., 1991)</td>
<td>Predation efficacy of C. p. pipiens against C. p. pipiens in rice fields</td>
<td>10,000 m² gave a mean reduction of 63.9% of immatures of Culex tritaeniorhynchus in 4 weeks</td>
</tr>
<tr>
<td>India, June to October 1991 (Prasad et al., 1993)</td>
<td>Predation efficacy of Gambusia affinis in rice fields</td>
<td>78 days after treatment with 1.9 fish/m² a reduction of 80% Culex tritaeniorhynchus larvae was observed.</td>
</tr>
<tr>
<td>South Korea, 1979 and 1981 (Yu et al., 1982)</td>
<td>Predation efficacy of Aphyocypris chinensis</td>
<td>Fish stocking of 1.5 m² in Wondang-Ni rice paddy resulted in mosquito larval reduction of 98.8% in the third week after fish introduction against A. sinensis, Culex pipiens, Culex tritaeniorhynchus and Aphyocypris chinensis.</td>
</tr>
<tr>
<td>Jindo Island, Chollanam-do Province, South Korea, 1989 (Yu and Kim, 1993)</td>
<td>Predation efficacy of Aphyocypris chinensis in combination with Bacillus thuringiensis (H-14)</td>
<td>Aphyocypris chinensis (1.5 fish/m²) achieved a reduction of 60–80% against both, A. sinensis and Culex tritaeniorhynchus in the first 2 months. In the third month Bacillus thuringiensis (H-14) treatment was made, which yielded a satisfactory degree of control maintained above 93.1% for 2 weeks</td>
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boosts every 3–4 years (Pugachev et al., 2003). Minor booster doses after a year are required, with subsequent 100% seroconversion, 2–3 primary doses and one several Asian countries. However, in order to achieve JE-V ax), are licensed for immunization against JE by or Beijing-1 strains grown in adult mouse brain (e.g. inactivated vaccines, based on wild type Nakayama campaign will remain the mainstay of control. Formalin- and extensive government-supported vaccination campaigns will remain the mainstay of control. Formalin-
injected vaccines, based on wild type Nakayama or Beijing-1 strains grown in adult mouse brain (e.g. JE-Vax), are licensed for immunization against JE by several Asian countries. However, in order to achieve 100% seroconversion, 2–3 primary doses and one booster dose after a year are required, with subsequent boosts every 3–4 years (Pugachev et al., 2003). Minor side effects occur in 10–30% and allergic reactions occur in 0.6% of vaccinated adults (Jones, 2004). Furthermore, clinically significant neurological adverse events, such as a meningoencephalitis, have been reported. In addition, these vaccines are expensive and production can hardly keep up with demand (Jones, 2004). In China a live attenuated vaccine (SA14–14-
-2) was developed in 1988 and the efficacy of a single dose was estimated to be 99.3% (Bista et al., 2001). However, its use in other affected countries has been restricted by regulatory concerns over manufacturing and control (Solomon et al., 2003). There is ongoing research to develop improved JE vaccines. These efforts received a boost in December 2003, when the Bill and Melinda Gates Foundation donated US$ 27 million to the Children's Vaccine Program at the Program for Appropriate Technology in Health for this purpose (UNO, 2003). For example, a newly developed live attenuated vaccine (ChimeriVax-JE) that uses yellow fever 17D as a live vector for the envelope genes of SA14-14-2 virus has been tested in phase 2 clinical trials and appears to be well tolerated (Monath et al., 2003). It is currently undergoing a 2-year clinical study in Australia, to assess the duration of immunity and to gain further knowledge on safety and immunogenicity (Jones, 2004).

It is important to recognize that strategies other than vaccination may play an important role for prevention and control of JE, particularly in rural areas, where vaccination coverage are sometimes low or where there is no history of immunization against JE at all, as recently documented for Northeast India (Phukan et al., 2004). Vaccination of the far rural population is strategically difficult and costly, in particular as three doses are required to achieve adequate neutralizing antibody levels. As demonstrated in China self-protection behaviour, such as sleeping under insecticide-treated nets (ITNs) can significantly reduce the risk of infection (Luo et al., 1994). On the other hand, all JE patients of a recent outbreak in Northeast India reported that they had slept under a bed-net (Phukan et al., 2004). Consequently, irrigation schemes should be implemented and maintained in a way that adverse health effects to rice growers and residents of the area are minimized and social and economic development improved. We evaluated and discussed several environmental and biological vector control measures in rice fields: Bacillus sphaericus and B. thuringiensis israelensis.

We evaluated and discussed several environmental and biological vector control measures in rice fields: Bacillus sphaericus and B. thuringiensis israelensis.
found to greatly reduce JE mosquito larvae. However, it is currently not economically applicable to use these bacterial toxins in rice fields, as they are costly, labor-intensive and often only have a short duration of activity. Few studies are available on invertebrates, fungi, nematodes or the neem cake powder in rice fields, which allows assessing and quantification of their potential as JE mosquito control agents. Reviewing the literature has shown that in settings where the irrigation water in the rice fields can be managed, AWDI has considerable potential to reduce JE vector densities. A similar conclusion can be made for the use of larvivorous fish. An integrated vector management approach with AWDI and the use of larvivorous fish as its main components can reduce JE vector populations, and hence has the potential to reduce the transmission level and the burden of JE. It should be emphasized that these intervention strategies must be tailored carefully to a specific setting, which renders it difficult to generalize reported experiences and results.

Consequently, there is a pressing need to implement and monitor the performance of well-designed studies to further strengthen our understanding of the contextual determinants of environmental and biological control methods on vector abundance and clinical outcomes of JE in different ecological, epidemiological and socio-cultural settings.

Acknowledgements
This work was part of a project entitled “Burden of water-related vector-borne diseases: an analysis of the fraction attributable to components of water resources development and management”, which partially funded by the World Health Organization. J. Keiser (Project no. PMPDB-10622) and J. Utzinger (Project no. PPOOB-102883) are grateful to the Swiss National Science Foundation for financial support. We thank Dr. B. Kay for carefully reviewing this manuscript.

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