SPATIAL AND TEMPORAL VARIABILITY IN SCHISTOSOME CERCARIAL
DENSITY DETECTED BY MOUSE BIOASSAYS IN VILLAGE IRRIGATION DITCHES
IN SICHUAN, CHINA

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Abstract. A mouse bioassay was used monthly over the infection season of 2001 to determine the temporal and
spatial variability of schistosome cercarial density in irrigation ditches in five villages in southwestern Sichuan Province
in the People’s Republic of China. Analysis of variance showed that approximately half of the variability was due to the
village and site within the village, with little contribution from air temperature, weekly average rainfall, or the month
within the infection season in which the bioassay was performed. The location-specific variability in these data suggest
that epidemiologic studies will generally have low power to detect the influence of water-contact intensity on human
parasite burden without taking account of variations in cercarial density at sites of water contact.

INTRODUCTION
In 2000, we carried out an extensive cross-sectional study of schistosomiasis transmission in 20 villages in southwestern Si-
chuan, People’s Republic of China aimed at identifying agricul-
tural and environmental factors predictive of infection risk.1 In each of these villages between 6 and 16 sites were
monitored for cercariae in surface water in July, the middle of
the transmission season, using a mouse bioassay. Regression
of the village disease prevalence in mice (averaging over all
bioassay sites in a village) against prevalence in humans was
highly significant ($R^2 = 0.85$, $P < 0.001$). A similar result was
obtained between village mean worms per mouse and human
egg secretion per gram of feces as measured by the Kato-Katz
method ($R^2 = 0.80$, $P < 0.001$). Thus, in this earlier work, we
used the July bioassay data as an index of season-long infec-
tion risk to humans. However, while the July data gave insight
into the spatial variation of the cercarial risk within and be-
tween villages, it did not, of course, measure temporal varia-
tion. Likewise, in the earlier study, we did not determine how
the bioassay results might be influenced by environmental
factors such as temperature and rainfall during or just prior to
the exposure of the mice. Here we report results from five of
these villages in 2001 to quantify the relative contributions of
site, month, and weather to variations in cercarial risk as mea-
sured by the bioassay over one infection season. While our
primary interest in the bioassay is as an index of infection risk
in the context of disease control interventions, a side issue
concerns the association of water contact and transmission intensity. In particular, we are interested in the influence of
spatial and temporal variability in cercarial risk on estimates
of that association.

The villages in which these experiments were conducted
are near Qionghai Lake (elevation = 1,520 meters), the predomi-
nant crops are rice and wheat, whereas in villages in the ter-
raced foothills (elevation = 1,810 meters), tobacco, veg-
etables, and corn are more common. In all of these villages an
important factor to sustaining the disease cycle is that fertil-
ization practices make extensive use of human and animal
manure that is moved from residential pit latrines to field
storage pits without treatment and with minimal holding
times.

MATERIALS AND METHODS
The five villages that were selected for these bioassay studies
cover the range of topographic, agricultural, and infection-
related variables observed in the 20 villages of our 2000 study,
but focus on the high prevalence villages. Table 1 summarizes
the status of these villages in 2000 including the human infec-
tion data, snail data, and the village mean worm burden per
mouse determined from the bioassay described in this report.
All individuals diagnosed as infected were treated in the win-

In China, a bioassay using laboratory mice has been used to
detect the presence of cercaria in surface waters in preference
to alternative methods because of water turbidity and the
sticky nature of *Schistosoma japonicum* cercariae that makes
them difficult to recover from container surfaces or from the
tubing of sampling apparatus. The bioassay involves the sus-
spension of a metal screen cage containing five laboratory
mice so that the floor of the cage is just below the water
surface.2–3 Thus, the tails, paws, and portions of the lower
abdomen of the mice are wetted. In these studies, exposure
was for five hours per day at mid-day for two days. The mice
were then returned to the laboratory and held for six weeks to
allow for maturation of the parasite in vivo. The mice that
survived the six week period were then killed and dissected,
and worms were counted.

Four to six sites were selected per village from among the
sites used in the 2000 study. Each of the sites was monitored
during the same two-day period at the end of each month
from May to October. Air temperature and rainfall data were
collected daily in two villages that were selected to be repre-
sentative of all five. These were Xinmin 7 (representing Xin-
min 7 and Shian 5) and Minhe 3 (representing Minhe 3, Xin-
However, the monitoring stations were not fully functional until June 1 so that only five months of complete village weather data were available. Comprehensive year-long data were obtained from the Xichang Airport Weather Station approximately 15 km from the villages.

Because the worm count data are highly skewed and we wanted to fit a linear regression model, a square-root transformation of the worm counts/mouse was used as an outcome variable. To examine the relative contribution of progressively more local factors (village, location in the village) when accounting for the more regional variables (rain, temperature, month) an analysis of variance sequential (type III) was performed to derive partial R² values. To account for the possibility of residual correlation of worm counts within a specific cage in a particular month, a random effects model was used.4

RESULTS

Based on data from the Xichang Airport Weather Station, the daily maximum temperatures in 2000 and 2001 over the May–October period were similar, ranging from 15°C to 35°C in May, 20°C to 35°C in July, and 15°C to 30°C in October. Figure 1 shows the daily maximum temperature and Figure 2 shows the weekly running average of the daily precipitation data from the village stations and the weather station at the Xichang Airport Weather Station for the five months in 2001.

In general, the weather in the area in both 2000 and 2001 was typical of the region and without notable weather abnormalities. The rainfall data show only minor differences between the stations, except for September in which amounts were markedly higher at the Daxing Station than at the other two stations. The daily maximum temperature was generally lower in Daxing in the summer and higher in the fall than the other two stations.

Table 2 summarizes the bioassay results from 2001 by month, village, and site within a village. As can be seen, mortality in the mice was high for some months in some sites. June in Shian 5, Minhe 3, and Xinlong 7 was the worst month with typically only one of the five mice surviving. Subjectively, the season long data in 2001 are consistent with the July 2000 data for Xinmin 7, Minhe 3, and Tuanjie 2, whereas the data from Shian 5 and, most obviously Xinlong 7, indicate a reduced cercarial risk, a finding of some interest as discussed later in this report. Other obvious features of the data are the site-specific variability in some villages. For example, in Xinmin 7, almost all sites in May have high worm counts as does site 5 in almost all months.

Exploratory analyses were conducted in an attempt to determine what function of the temperature and rainfall time series would be best for use in regression models. Because rainfall is episodic, we investigated the predictive value of various individual days and of three-day to one-week average rainfall on the square root of worms/mouse using a random and fixed effect model. The best predictor was the average rainfall in the week preceding the bioassay experiments. Thus, the one-week average rainfall and temperature were used in subsequent analyses.

The regression results shown in Table 3 were sequentially adjusted for those variables entered already, i.e., adjusted for

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### Table 1

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</thead>
<tbody>
<tr>
<td>Xinmin 7</td>
<td>73</td>
<td>104</td>
<td>86</td>
<td>75</td>
<td>35.4</td>
<td>37.9</td>
<td>47.3</td>
<td>0.6</td>
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<tr>
<td>Shian 5</td>
<td>68</td>
<td>91</td>
<td>74</td>
<td>55</td>
<td>21.4</td>
<td>25.0</td>
<td>37.8</td>
<td>1.6</td>
<td>1.1</td>
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<tr>
<td>Minhe 3</td>
<td>62</td>
<td>84</td>
<td>63</td>
<td>54</td>
<td>6.5</td>
<td>13.5</td>
<td>15.6</td>
<td>1.0</td>
<td>0.5</td>
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<tr>
<td>Xinlong 7</td>
<td>65</td>
<td>110</td>
<td>54</td>
<td>19</td>
<td>31.7</td>
<td>20.9</td>
<td>18.7</td>
<td>2.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Tuanjie 2</td>
<td>13</td>
<td>1</td>
<td>18</td>
<td>13</td>
<td>0.3</td>
<td>1.6</td>
<td>5.1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* Prevalence in mice for 2000 is for July only while that for 2001 is for all six months of the season.

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**FIGURE 1.** Maximum daily air temperature at three stations in or near the five villages in Sichuan, People’s Republic of China.

**FIGURE 2.** One week running average rainfall at three stations in or near the five villages in Sichuan, People’s Republic of China.
the effects of those variables in the list above the variable in question. First, the effects of temperature and rainfall were investigated. Neither was a significant predictor at the 5% level and both showed quite small sequential/partial R² values as shown in Table 3. The next variable entered was month, which also showed a very modest partial R² value and a P value of 0.44.

The last two variables investigated were village and site within village. Even taking into account the variables related to weather and seasonal changes represented by month, both village and site-within-village were highly significant and together account for approximately half of the total variability in the data. This may reflect limited power to detect the influence of month, despite the total number of cages of mice deployed in this study.

Because all of the predictor variables used in our analysis account for only approximately 50% of the variability in the bioassay results, and season, temperature, and rainfall explain almost none of this, the precision of estimation of cercarial risk is largely an issue of sample size once sampling locations are fixed. Barring extreme weather events, in this environment the timing of surveys within the main part of the infection season appears to be largely a matter of logistical convenience.

The marked difference between the 2000 and 2001 cercarial density of these villages with upstream sources of cercaria external to these villages. Recall that infected individuals in all five villages were treated in the winter of 2001 – 2002. This

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sequential R²</th>
<th>Sequential P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rain (past 7 days average)</td>
<td>0.0013</td>
<td>0.47</td>
</tr>
<tr>
<td>Temperature (past 7 days average)</td>
<td>0.034</td>
<td>0.07</td>
</tr>
<tr>
<td>Month</td>
<td>0.027</td>
<td>0.44</td>
</tr>
<tr>
<td>Village</td>
<td>0.21</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Location within village</td>
<td>0.32</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Among the variables measured in these villages, the variability in cercarial risk is principally explained by the village itself and the locations within the village at which the bioassay was conducted, rather than the month in which the assay was conducted or short-term fluctuations in temperature and rainfall. However, the bioassay data for October clearly indicate that the infection season was essentially over in four of the five villages, yet month did not account for a significant fraction of the total variability in the data. This may reflect limited power to detect the influence of month, despite the total number of cages of mice deployed in this study.

Because all of the predictor variables used in our analysis account for only approximately 50% of the variability in the bioassay results, and season, temperature, and rainfall explain almost none of this, the precision of estimation of cercarial risk is largely an issue of sample size once sampling locations are fixed. Barring extreme weather events, in this environment the timing of surveys within the main part of the infection season appears to be largely a matter of logistical convenience.

The marked difference between the 2000 and 2001 cercarial risk in Shian 5 and Xinlong 7, and the absence of a similar difference in Xinmin 7, may relate to the degree of connectedness of these villages with upstream sources of cercaria external to these villages. Recall that infected individuals in all five villages were treated in the winter of 2001–2002. This treatment presumably lowered the internally generated miracidial risk to snails in the spring of 2001. The treatment effect appears to have been substantial in Shian 5 and Xinlong 7 and negligible in Xinmin 7. This is consistent with the topographic setting and hydrologic situation of Shian 5 and Xinlong 7, as well as with the proximity of Xinmin 7 to an upstream village known historically to have a high prevalence of infection and which has not been the focus of recent treatment or intervention.
The within and between village variability in cercarial risk also has implications for the relative importance of water contact as a determinant of infection intensity in humans. In our studies in this area in 2000, we did not find exposure time, as determined by a retrospective water contact survey, to be a significant predictor of individual infection intensity, a finding that has been reported by others using a variety of methods of estimating water contact intensity.\(^5,7\) Conversely, and as noted earlier, we did find a strong relationship between the bioassay results and both human prevalence and infection intensity. In exploring the reasons underlying these observations in the light of the results presented here, consider the regression equation of the log of individual worm burden, \(w\), predicted by the log of total water contact time, \(T\):
\[
\log w = \beta_0 + \beta_1 \log T + \epsilon
\]

In general, the variance of the estimate of the regression parameter \(\hat{\beta}_1\) is proportional to the variance of the model residual error and inversely related to the variance of the covariate \(T\):
\[
\text{var}(\hat{\beta}_1) \propto \frac{\sigma^2(\epsilon)}{\text{var}(\log T)}
\]

The power to reject the null hypothesis that \(\beta_1 = 0\), i.e., to conclude that water contact is a predictor of worm burden, increases as var(\(\hat{\beta}_1\)) decreases. Thus, for a given distribution of \(T\), the power to reject the null depends on the variance of the residual error, \(\epsilon\).

To identify the component parts of the residual error, we assume infection intensity in humans to be an increasing function of exposure, and define the latter by the product of water contact duration, \(t_{ij}\), and the time-weighted cercarial concentration, \(c_i\), summed over site, \(i\), and activity, \(j\). That is,
\[
E = \sum_i \sum_j s_f t_{ij}
\]
where \(s_f\) accounts for differences in water contact intensity by activity, for example, washing clothes versus playing in the water. The effect of differences in exposure due to activity can be incorporated in an activity-adjusted contact time as is often done.\(^6,8,9\) With that adjustment, the residual error, \(\epsilon\), includes the effects of site-specific cercarial concentration, the fraction of the total contact time spent by the individual at the various sites, differences in individual susceptibility, and measurement errors.

We have calculated rough estimates of the var(\(\log T\)) and of \(\sigma^2(\epsilon)\) to allow power calculations specific to the environment we have studied. Using the data presented here to estimate only the component of \(\sigma^2(\epsilon)\) contributed by the variance of cercarial concentration suggests that we would have marginal power to detect the influence of water contact with a sample size of approximately 200 if all individuals spent equal contact time at each of the sites within a village. However, we know that the individual sites of exposure are highly variable within the village population as has been reported in other settings.\(^10\) Furthermore, ascertainment of water contact time is subject to error. These additional sources of variability suggest that, in our studies in 2000, we had low power to detect the effect of water contact time on infection intensity in the absence of site-specific cercarial risk data even if there were no significant differences in human susceptibility to infection at constant exposure. In that regard, we suspect that only in rare and fortuitous circumstances will a practical epidemiologic study design possess the power to detect differences in human susceptibility to schistosome infection without accounting for environmental variability in cercarial risk.

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