Leptospirosis is a serious disease with high mortality, mainly transmitted by the rat and pig, and it is an important public health problem in Jingzhou District, Jingzhou City, Hubei Province, China. Jingzhou District is situated in the middle reaches of the Yangtze River, in the western part of the Jianghan plain, where rice is the main crop. In the rural areas, the peasants and students are mainly infected by contacting water contaminated by the urine of infected rats in waterlogged paddy rice fields. The first epidemic of leptospirosis in Jingzhou District happened in 1985, and since then the cases of leptospirosis have been reported every year, with two epidemic peak years of 1986 and 1997 (mortality 582.80/100,000 population and 81.99/100,000 population, respectively), with mortality of 1.23/100,000 and 1.48/100,000, respectively.1

**Background.** The Shanghai Institute of Biological Products manufactured successfully a new outer envelope vaccine for leptospira in 1978. It has been confirmed by using animal tests and a number of human body trials that this vaccine is safe and effective. However, results regarding serological and epidemiological effects of the vaccine in population has not been reported. The objective of this study was to evaluate serological and epidemiological effects of this vaccine.

**Methods.** From May 1998 to May 1999, an evaluation of the serological and epidemiological effects of the outer envelope vaccine for leptospira made by Shanghai Institute of Biological Products was conducted among agricultural population aged 5–60 years in an epidemic area of leptospirosis, in Jingzhou District, Jingzhou City, Hubei Province, China by microscopic agglutination test (MAT), cohort study, matched case-control study and screening method.

**Results.** The serological surveillance results of 77 students immunized showed that the successful rates of vaccination with the outer envelope vaccine of *L. icterohaemorrhagiae* sero-group and *L. hebdomadis* sero-group leptospira were 79.22% and 77.92%, respectively, the antibody geometric mean titer (GMT) of the two groups were 1:106.71 and 1:63.31, respectively, with 6.7-fold and 4.7-fold rises comparing with the group of those of the antibody before inoculation, and the antibody protective rates of the two groups of outer membrane protein vaccine of leptospira all were 100% at one month after injection. Higher level antibody against *L. icterohaemorrhagiae* sero-group leptospira was still maintained at one year after injection. The results of cohort analysis showed that the protective rate of the outer envelope vaccine of leptospira for the same serological groups of leptospira was 75.17%, and the effective index of the vaccine was 4.03. The protective rate of vacines obtained from 1:2 matched case-control studies was 81.25%, while that estimated by screening method was 75%.

**Conclusions.** The study results showed that the serological and epidemiological efficiencies of leptospira vaccine all were ideal. In addition, this vaccine had partly protective effect against other sero-groups of leptospira.

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**Key Words**
- cohort study;
- epidemiological effect;
- leptospira outer envelope vaccine;
- matched case-control study;
- serological evaluation;

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Preventive oculation with leptospira whole cell vaccine is a specific and effective method for controlling epidemic of leptospirosis. However, this vaccine of ten is not accepted by the majority of the at-risk population because it includes toxic components of leptospira which can induce some serious side effects after vaccination, and basic immune requires injection of two doses of vaccine.

Since 1986, the vaccine of the whole cell vaccine of leptospira has been conducted in Jingzhou District every year among the agricultural population aged 7 to 60 years, but due to the serious side-effects of the vaccine commonly influencing the life and labor of the people immunized, its immune coverage age was very low (from 3.33% to 36.78%) in the majority of years excluding the year 1986 (73.56%). Also, the incidence of fever one month after injection was 4-fold or higher compared to before immunization. Leptospira antibody titers of the former (1:98.37) at one month after injection was significantly higher than that of the latter (1:72.26%).

It has been confirmed by using animal test and a small number of human body trials that this vaccine is safe and effective and has good serological effect and protective role for animals. Out of 61 healthy peasants vaccinated with leptospira outer envelope vaccine, 7 had a slight fever (37.1~37.5 °C), and its rate of fever (11.48%) was lower than that of the whole cell vaccine group (28.33%); also, the GMT of Leptospira icterohaemorrhagiae antibody level of the former (1:98.37) at one month after immunization was higher than that of the latter (1:72.26). However, data regarding epidemiological effect of the vaccine in population have not been reported. In order to further evaluate serological and epidemiological effects of the outer envelope vaccine for leptospira, an on-the-spot epidemiological study was carried out in Jingzhou District from May 1998 to May 1999.

METHODS

Observation subjects and immunization program

Agricultural population aged 5-60 years from three townships (Chuandian, Jilan and Baling) of Jingzhou District with epidemic of leptospirosis were chosen as observation subjects. Out of those subjects, about 40% were randomly selected as the immunization group by random numerical table, and the others as control group. During the last ten days of May (1 month before epidemic), 1998, regardless of their immunization status, all subjects of the immunization group were subcutaneously inoculated with 1 mL (0.5 mL for subjects aged ≤ 13 years) leptospira outer membrane vaccine which included 200 μg of the outer envelope protein of Leptospira icterohaemorrhagiae and 200 μg of L. hebdomadis sero-group made by Shanghai Institute of Biological Products.

Observation of side effects

Four hundred and twenty-two students of Tuendian Primary School in Chuandian Township were surveyed every day for temperature and local reaction for one week after immunization by two doctors of the Anti-epidemic Station of Jingzhou District.

Test for antibody against leptospira

Ve nous blood samples of the students immunized in Tuendian Primary School were respectively collected before and after (30 d and 1 yr) immunization. Sera were stored in chest freezers (-20 °C) and tested simultaneously for specific antibodies against the L. icterohaemorrhagiae sero-group and L. hebdomadis sero-group of leptospira by microscopic agglutination test (MAT) in Hubei Provincial Preventive Station. Sera at one year after vaccination were only tested/denatured for an antibody of Leptospira icterohaemorrhagiae. Serum samples were put into a water bath at 56 °C for 30 min, diluted double from 1:10 to 1:1280 with physiological saline, mixed with same-capacity leptospira cultured Korthof’s culture solution at 37 °C for 7-10 days, and then cultured at 37 °C for 2 hours and observed for the sample. Fifty percent of leptospira agglutinating (++) was regarded as the criterion of the antibody titer. It was considered to be a successful vaccination if the antibody titer at one month after injection was 4-fold or higher compared to that before immunization. Leptospira antibody ≥ 1:20 was decided as a protective titer.
Surveillance of the leptospira cases

Because the epidemic of leptospirosis had an obvious seasonal feature in Jingzhou District, surveil lance and report of the pa tients of leptospira were strictly demanded from all the health centers and clinics in the study area from June to October of 1998. Based on the leptospirosis diagnostic standard promulgated by the Ministry of Public Health of China, patients with all or some of the following clinical symptoms, including fever, dizziness, headache, cough, bloody sputum, tiredness, lumbago, leg pains, conjunctivitis, lymph node enlargement and musculus gastrocnemius tenderness, and with a history of contact with water contaminated by leptospira, were diagnosed as clinical leptospirosis. The sera samples in the acute and/or convalescent stages of the clinical cases were collected and tested for specific antibody of common sero-groups leptospira by MAT. The clinical cases with 4-fold or greater rise of antibody level in the double blood samples (or titer ≥ 1:400 in the single blood samples) were regarded as confirmed cases. In the population vaccinated, the leptospira cases relating to vaccine sero-groups who suffered from illness at one month or longer after vaccination were classified as immune failure cases.

Evaluation methods of the epidemiological effect

Cohort study

Incidence of leptospirosis relating to vaccine sero-groups were compared between the immunization group and control group, and the vaccine efficiency (VE) and the effect index (EI) were calculated according to the methods recommended by the literature. The VE was calculated as:

\[
VE(\%) = \frac{(ARU-ARV)}{ARU} \times 100\%
\]

where ARV is the attack rate of vaccinated population and ARU is the attack rate of unvaccinated population.

The EI was calculated as:

\[
EI = \frac{P_1}{P_2}
\]

where \(P_1\) is the incidence of the control group and \(P_2\) is the incidence of the immune group.

Screening method

On the basis of vaccination rates of the leptospira cases and all other vaccination subjects, the VE was obtained from the curve figure of vaccine efficiency presented in the literature.

Matched case-control study

According to the fundamentals of matched case-control study, we regarded vaccination as a factor influencing in infection of leptospirosis. Therefore, odds ratio (OR) was used to express the influence of leptospira outer envelope vaccine on the leptospirosis in infection. On the contrary, the value opposite to the OR value (1-OR) demonstrated the vaccine efficiency (VE) on the leptospirosis. In this study, the protective rate of the leptospira outer membrane protein vaccine was evaluated by 1:2 matched case-control study conducted by health care workers of the Health Bureau and Anti-epidemic Station of Jingzhou District. The 40 cases relating to leptospira outer membrane vaccine sero-groups and 80 healthy controls were surveyed simultaneously by six trained investigators, filling in a questionnaire. The controls were obtained randomly from healthy neighbors of the cases, and their sex and nationality being consistent with that of the cases, while the difference on age of the match was a range of ±5 years of age.

RESULTS

Side effects of vaccine

Out of 422 students observed, 3 had local edema within 48 hours after vaccination, and only 2 suffered from slight fever (37.1-37.5 °C) within 24 hours; no serious side effects had been discovered.

Serological efficiency

In 77 students with blood samples at three different times, Leptospira icterohaemorrhagiae antibody level of 26 was ≥ 1:20 before immunization, with a protective rate of 33.77%, and the antibody GMT was 1:15.83, while only 9 students had a protective antibody of Leptospira hebdomadis; their protective rate was 11.69%, and GMT was 1:13.34.

The successful rates of immunization with the leptospira outer envelope vaccine of L. icterohaemorrhagiae...
The antibody protective rates after vaccination were 100% in both sero-groups and were obviously higher than that before immunization ($t = 8.73$ and $5.52$, both $p$ values < 0.01) (Table 1, 2). Table 1 and 2 shows that the successful immune rates of the two sero-group leptospira vaccines were influenced by the basic antibody level, and all exceptone students with a higher antibody (≥ 1:80) of leptospira before injection hadn’t been successfully immunized.

At one year after vaccination, only leptospira antibody of the L. icterohaemorrhagiae group was detected. Out of 77 observation subjects, the antibody titters of 54 were ≥ 1:20 and the protective rate was 70.13%, which dropped 29.87%, compared with that at one month after injection. The antibody GMT was 1:32.23 and, although obviously declined by 70%, it was still two-fold that before vaccination.

**Table 1. Serological efficiency of the leptospiral outer envelope vaccine (icterohaemorrhagiae group)**

<table>
<thead>
<tr>
<th>Antibody before immunization</th>
<th>No. tested</th>
<th>No. antibody rise ≥ 4-fold</th>
<th>Success rate of immune % (95% CI)</th>
<th>GMT ± SD</th>
<th>No. antibody titer ≥ 1:20</th>
<th>Protective rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10</td>
<td>51</td>
<td>46</td>
<td>90.20 (82.04,98.36)</td>
<td>91.65 ± 0.052</td>
<td>51</td>
<td>100.00</td>
</tr>
<tr>
<td>1:20</td>
<td>13</td>
<td>11</td>
<td>84.62 (65.01,100.00)</td>
<td>122.56 ± 0.08</td>
<td>13</td>
<td>100.00</td>
</tr>
<tr>
<td>1:40</td>
<td>5</td>
<td>4</td>
<td>80.00 (44.94,100.00)</td>
<td>211.12 ± 0.12</td>
<td>5</td>
<td>100.00</td>
</tr>
<tr>
<td>1:80</td>
<td>5</td>
<td>0</td>
<td>0.00</td>
<td>105.56 ± 0.181</td>
<td>5</td>
<td>100.00</td>
</tr>
<tr>
<td>1:160</td>
<td>2</td>
<td>0</td>
<td>0.00</td>
<td>226.27 ± 0.150</td>
<td>2</td>
<td>100.00</td>
</tr>
<tr>
<td>1:320</td>
<td>1</td>
<td>0</td>
<td>0.00</td>
<td>320.00 ± 0.000</td>
<td>1</td>
<td>100.00</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>61</td>
<td>79.22 (70.16,88.28)</td>
<td>106.71 ± 0.041</td>
<td>77</td>
<td>100.00</td>
</tr>
</tbody>
</table>

95% CI = The 95% confidence interval; GMT = Geometric mean titer of the antibody; SD = Standard deviation.

**Table 2. Serological efficiency of the leptospiral outer envelope vaccine (hebdomadis group)**

<table>
<thead>
<tr>
<th>Antibody before immunization</th>
<th>No. tested</th>
<th>No. antibody rise ≥ 4-fold</th>
<th>Success rate of immune % (95% CI)</th>
<th>GMT ± SD</th>
<th>No. antibody titer ≥ 1:20</th>
<th>Protective rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10</td>
<td>68</td>
<td>58</td>
<td>85.29 (76.87,93.71)</td>
<td>55.43 ± 0.035</td>
<td>68</td>
<td>100.00</td>
</tr>
<tr>
<td>1:20</td>
<td>1</td>
<td>1</td>
<td>100.00</td>
<td>80.00 ± 0.000</td>
<td>1</td>
<td>100.00</td>
</tr>
<tr>
<td>1:40</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1:80</td>
<td>2</td>
<td>0</td>
<td>0.00</td>
<td>80.00 ± 0.000</td>
<td>2</td>
<td>100.00</td>
</tr>
<tr>
<td>1:160</td>
<td>5</td>
<td>1</td>
<td>20.00 (0.00,55.06)</td>
<td>242.51 ± 0.153</td>
<td>5</td>
<td>100.00</td>
</tr>
<tr>
<td>1:320</td>
<td>1</td>
<td>0</td>
<td>0.00</td>
<td>320.00 ± 0.000</td>
<td>1</td>
<td>100.00</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>60</td>
<td>77.92 (68.66,87.18)</td>
<td>63.31 ± 0.038</td>
<td>77</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Morbidit y of leptospirosis

During an epidemic period, 116 clinical cases of leptospirosis were reported in 72,629 observed subjects aged 5-60 years in the study area, and the general incidence was 159.72/100,000. The incidence of the immunized group was 56.78/100,000 (14/24,658), and it was significantly lower than that of the control group (212.63/100,000 or 102/47,971) \( \chi^2 = 24.81, p < 0.001 \).

Out of 116 cases, 98 cases had serological data, accounting for 84.48%, in which 53 cases were infected by the same sero-group leptospira as the outer envelope vaccine, 37 cases infected by the other sero-groups, and 8 cases were negative results of MAT. The incidences of leptospira which had nothing to do with the vaccine sero-group in study group and control group were 24.33/100,000 (6/24,658) and 64.62/100,000 (31/47,971), respectively, with a significant difference (\( P^2 = 5.19, p < 0.025 \)).

Epidemiological effect

The results of cohort study showed that the incidence of vaccine-related leptospirosis of the study group (24.33/100,000 or, 6/24,658) was significantly lower than that of the control group (97.98/100,000 or, 47/47,971) \( \chi^2 = 12.11, p < 0.005 \); VE was 75.17% (95% CI: 71.55%, 78.79%), and EI was 4.03 (95% CI: 2.79, 5.83).

The immune coverage rate of the leptospira outer envelope vaccine in all observation subjects was 33.95% (24658/72629). Of 53 vaccine-related cases confirmed by serology, 6 cases accepted immunization of the leptospira outer membrane protein, and immune rate was 11.32%. The es timated VE was 75% that obtained from the curve figure for vaccine efficiency using the above-mentioned two rates.

The result of 1:2 matched case-control study (40 matches) showed that the vaccine efficiency was 81.25% (Table 3).

DISCUSSION

The result of one serological study showed that the serovars of L. icterohaemorrhagia and L. hebdomadis were responsible for 56.41-95.29% of leptospirosis in Hubei, Zhejiang and Hunan provinces. Serological tests of 62 cases of leptospirosis revealed that the serogroup of L. icterohaemorrhagia ac counted for 96.77% in Jingzhou Dis trict, Hubei in 1986. How ever, the percent age of L. icterohaemorrhagia serogroup de creased to 60%, and that of serogroup of L. hebdomadis increased to 20.41% during an ep i demic of leptospirosis in Jingzhou Dis trict in 1997. Be cause the above-mentioned two serogroups were the main patho gens of leptospira in Jingzhou, we eval u ated the serological and ep i de mi o log i cal ef fects of their outer en ve lope vac cine in the field trial.

Four-hundred and twenty-two stu dents were con tin uously observed for seven days after inoculation in Tuendian Pri mary School, Chuandian Town ship, and a gen eral side-reaction (slight fe ver) was found in only 2 stu dents. Three stu dents had slight red ness and swell ing (0.71%) at the site of in jec tion, but re cov ered rap idly within 2 days. In view of the re sults, it ap pears that it is a very safe vac cine com pared with the whole cell leptospira vaccine which had high lo cal (15.33%) and gen eral side-re actions (1.26%) and al ler gic re ac tion (0.06~2.03%) re ported in the past.

By compar ing with 77 stu dents, antibod ies against L. icterohaemorrhagia Leptospira and L. hebdomadis Leptospira be fore and af ter in oc u la tion, a higher suc cess ful rate on im mu ni za tion with leptospira outer en ve lope vac cine was found, es pe cially in stu dents who had a lower ba sic an ti body level (1:10~1:40), with a very high im mune suc cess rate (80~100%). The an ti body GMT of two sero-groups were re spectively 6.7-fold (L. icterohaemorrhagia) and 4.7-fold (L. hebdomadis) com par ing with those be fore vac ci na-

Table 3. 1:2 matched case-control study on the epidemiological effect of the outer envelope vaccine of leptospira

<table>
<thead>
<tr>
<th>Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>-</td>
<td>4</td>
</tr>
</tbody>
</table>

Odds ratio (OR) = (1+2×1)/(2×4+8) = 0.1875,
Vaccine Efficiency (%) = (1 - OR) × 100% = (1 - 0.1875) × 100% = 81.25%
tion, and the protective rates of the two vaccines both rose to 100%. The above results showed that the outer envelope vaccine of leptospires made by Shanghai Institute of Biological Products had ideal serological efficiency, and its efficiency was similar to that reported elsewhere. At one year after vaccination, the antibody level of L. icterohaemorrhagiae had obviously declined, but it still remained on a higher level. However, our study on the serological endurance of leptospires vaccine wasn't sufficient, and should be followed up in the future.

We improved the serological diagnosis of cases during the epidemic period, and carried out an extensive survey on failing to report illness in the whole area after the epidemic (November 1998); it increased accuracy of evaluation. We simultaneously used cohort study, matched case-control study and screening method in the evaluation of epidemiological effect, and the results showed that the protective rates calculated by three methods were similar (75-81.25%), and also ideal. Cohort study is a classical method for evaluating vaccine efficacy, while screening method is a method roughly estimating VE that was developed in 1990s. Matched case-control study was used to explore the agents of disease in the past, and used in analyzing the VE of immunization in 1980s, with a reliable result only in investigating the cases and its controls in spite of the other observation subjects. Our re results of screening method and case-control study were basically consistent with that of cohort study, and they showed that the preceding two methods were simple, rapid and reliable evaluation methods.

This study found that the incidence of non-vaccine-relative serogroup leptospirosis such as Pomona, Ballum, Grippotyphosa and Bataviae, was quite un like between the immune group (24.33/100,000) and the control group (64.62/100,000), \( \chi^2 = 5.19, p < 0.025 \). It seems that the leptospires outer envelope vaccine could prevent non-vaccine-related serogroup leptospirosis, with a protective rate of 62.35%. In the past, scientists believed the outer envelope vaccine of leptospires could be some common antigens with serotype specificity. However, our study showed that in the outer envelope vaccine of unlike serogroup leptospires, there could be some common antigens shared by them. This has been verified in serological test by enzyme immune as say (EIA) in which the antigen covered with the outer membrane proteins of the L. icterohaemorrhagiae serogroup leptospires and L. hebdomadis serogroup leptospires found specific IgM antibody in the blood samples of the other serogroups leptospires cases confirmed by MAT. If this inference is true, it would be hopefully possible to prevent all serogroups leptospirosis using the outer envelope vaccine of some leptospires with a strong immunogenicity.

**REFERENCES**


