Cuban Medical Literature

**Vax-Spiral ®. Trivalent Antileptospirosis Vaccine For Human Use; Research, Development And Impact On The Disease In Cuba**

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**ABSTRACT:** Leptospirosis is one of the most widely spread zoonosis in the world, with a larger incidence in tropical regions. Its causing agents are pathogenic spirochetes belonging to the Leptospiras. In 1989 this disease started to show up in our country with a growing tendency, until it reached the magnitude of an epidemic at the end of the 1990-1995 five-year period. In search of a solution to this health problem, research was initiated for the production of a vaccine from autochthonous strains. The preconceived goals included formulating variants of a vaccine from inactivated cells, which is either not adsorbed or adsorbed in aluminum hydroxide; evaluating immunogenicity, power and innocuousness of the formulated vaccine variants by means of trials with hamsters, achieving its scaling and productive consistency, and demonstrating its safety, immunogenicity and efficacy in human beings. The preclinic results made it possible to choose the adsorbed vaccine variant, which proved innocuous, immunogenic and protectogenic. Productive scaling was achieved through fermentation, as well as in-situ sterilization of the protein-free cultivation medium that was used in the fermentator. The product’s concentration was achieved by means of tangential micro filtering for its aseptic processing. The clinical evaluation of the vax-SPIRAL ® adsorbed vaccine showed its safety, low reactogenicity, immunogenicity and a specific serogroup efficacy of 78.1%. Vax-SPIRAL ® was registered in 1998, was included within the National Leptospirosis Prevention and Control Program, and demonstrated its contribution to a morbilethality reduction of 82.1%.

**INTRODUCTION**

Leptospirosis is one of the most widely spread zoonosis around the world. It is considered to be re-emergent, to have an endemic behavior and has had outbreaks in several continents. It affects domestic and wild animals, which get rid of the microorganism by their urine. Human beings are accidental guests who can contract a slight and self-limited disease to a mortal disease with multiorganic insufficiency. Leptospirosis is important due to its worldwide distribution, menace to human and animal life, and economic repercussions. Leptospirosis is highly frequent in rice producing countries.

The World Health Organization has estimated its prevalence in human beings between 4 and 100 cases per 100,000 inhabitants in those countries, and described an outbreak in China with 1,300 cases per 100,000 inhabitants. In October 1995, 2,000 cases were recorded in Achuapa, Nicaragua, with 40 people dying from a hemorrhagic febrile disease that was later diagnosed as leptospirosis. The first case of leptospirosis in Cuba was reported in 1945. The Leptospirosis Struggle and Control Program was started in 1983, coordinated by the Institute of Veterinarian Medicine and the Ministry of Public Health. In addition, prophylaxis of risk groups was started with the administration of a Soviet vaccine. Starting in 1989, this disease showed a growing tendency, reaching epidemic magnitude at the end of the 1990-1995 five-year period, with its peak in 1994, when 2,828 cases were reported for a rate of 25.6 per 100,000 inhabitants.

The main difficulties that were faced in preventing and controlling leptospirosis were associated with a high incidence of rodents, presence of dogs and pigs in the city, deficient treatment of cattle wastes, and limited
availability of protection means. All of this, together with the fact that the the Soviet vaccine was no longer available, made the disease a serious health problem for our population from that moment on. Immunoepidemiologic studies concluded that the most frequent serogroups were then *L. icterohaemorrhagiae, L. pomona, L. canicola and L. hebdomadis* (serologic diagnosis), whose circulating serovars were unknown. *L. pomona* was predominant in the epidemic outbreaks.

The vaccines that have been produced against human leptospirosis in the world are mono or polyvalent cellular suspensions, with concentrations per vaccine dose between 100 and 500x10^6 cells per serovar. These cells are inactivated by chemical agents like formaldehyde and phenol, or by physical agents like heat. These bacterines are inoculated subcutaneously or intramuscularly, with a scheme of two 0.5-2.5 mL doses with a 7-21 day interval in between doses. In the international market there is currently only a French vaccine from Pasteur Merieux, whose commercial name is SPIROLET. Suspension of inactivated cells (*L. icterohaemorrhagiae*), 2x10^8 lept/mL. Subcutaneous injection (2 doses, 15 day interval, third dose 4-6 months after the first dose, with biannual revaccination).

Its conferred protection in human beings is known to be no longer than one year, while there are cases that need revaccination six months after the scheme conclusion during epidemic periods. It is also known that induced immunity is serovar-specific, though to a lesser degree it is possible to find protection in serovars of the same serogroup as well as in different serogroups.

Providing an answer to this health problem, “Finlay Institute” produced the first Cuban vaccine against human leptospirosis, a trivalent vaccine (*canicola, copenhageni, mozdok*) of inactivated cells and adsorbed in aluminum hydroxide, whose vaccination scheme includes deep intramuscular application of two 0.5 mL doses in the deltoid with a 6-week interval in between doses. In 1998 the vax-SPIRAL ® vaccine was registered, and was included in the National Leptospirosis Prevention and Control Program. Until 2001, a total of 1,730,632 people exposed to a risk of this disease had been vaccinated.

**MATERIAL & METHODS**

Strains were chosen of *Leptospira interrogans* belonging to the *canicola, icterohaemorrhagiae and pomona* serogroups, which were the most common serogroups affecting the country at that time [1]. These strains were classified to serovar by means of the micro agglutination technique (MAT) [2], using referential polyclonal and monoclonal antibodies as well as the polymerase chain reaction technique [3]. Its pathogenicity was characterized in the Golden Syrian Hamster model [4], and its growth conditions were standardized in a free environment [5], as well as its conservation in Fletcher semisolid environment [7].

Variants of non-adsorbed and adsorbed vaccine were formulated in aluminum hydroxide gel, and the process and final controls of the chosen variant were defined as well. Its immunogenicity and power were evaluated in hamsters, while its innocuousness was assessed in the Duncan Hartley Guinea Pig and the Balb/c Mouse. Induced homologous protection was evaluated by means of the challenge versus 10,000 DL 50 of virulent strains of *L. canicola, L. copenhageni and L. mozdok* serovars.

The productive scaling was standardized for the culture of *L. canicola, L. copenhageni and L. mozdok* vaccine strains to achieve the vaccine’s active principle in an aired fermentator.

A clinical evaluation was made of the formulated vaccine’s safety, reactogenicity, immunogenicity and efficacy.

**MAIN RESULTS OF THE RESEARCH STAGE**

The optimal adsorption conditions (temperature, agitation, number of cells per mg of aluminum hydroxide gel) were defined for the variants of adsorbed vaccines. The non-adsorbed variants of the vaccine represented the trail controls. The process and final controls were established for the preparations of the formulated vaccine.

- The pathogenicity of the *Leptospira* vaccine strains was characterized in the Golden Syrian Hamster animal model [4].
• Three protein-free cultivation means were optimized to obtain vaccine antigens.
• The research demonstrated the innocuousness, immunogenicity (IgG specific in each of the serovars involved in the vaccine formulation), and power of the formulated variants. The adsorbed variant proved best on the basis of the level of induced protection in hamsters after the application of a dose, and a 100% protection to the challenge with 10 000 DL 50 of highly virulent strains of the serovars implied in the vaccine.
• A vaccination scheme was defined with two 0.5 mL doses and a 6-week interval in between doses, by means of a deep intramuscular inoculation [8].

Brand-Included Product Design: 1994

Vax-SPIRAL ® trivalent antileptospirosis vaccine.

Request for Clinical Trials: 1994

• A document was submitted with the product’s Chemical-Pharmaceutical Information, pre-clinical results, and Phase I (safety) and Phase II (Immunogenicity plus dose and scheme study) clinical trial protocols.

Productive Scaling

• The productive scaling was achieved in the fermentator.
• In-situ sterilization of the protein-free cultivation means used in the fermentator was implemented.
• The product’s concentration was implemented by means of tangential micro filtering for aseptic processing.

MAIN RESULTS OF THE CLINICAL TRIALS STAGE

Phase 1 Clinical Trial. Product Safety Evaluation

• No serious adverse events were reported during the studies.
• Adverse reactions did not last longer than 72 hours.
• Signs and symptoms were slight and did not increase with a second dose.
• Pain was the most frequent local reaction, occurring during the first 12 hours, with spontaneous relief.
• The most frequent general reactions were fever, general discomfort and cephalgia in a very low percentage of the vaccinated volunteers.
• None of the volunteers needed to interrupt their daily activities or their job.

Phase II Clinical Study. Immunogenicity

The vaccine’s immunogenicity was demonstrated, as well as its response to each of the serovars involved in the formulation.

Tables 1 and 2 show the results of the IgG response in different vaccinated populations.

Table 1: ELISA Evaluation of IgG Antibody Response Against vax-SPIRAL ® Serovars in Two Populations in San José de Las Lajas Municipality, Havana Province. 6-Week Interval Between Doses.

<table>
<thead>
<tr>
<th>Seroconversion</th>
<th>Canicola Copenhageni mozdok</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>T1/T0*</td>
</tr>
<tr>
<td>UAH**</td>
<td>≥2</td>
</tr>
</tbody>
</table>
Table 2: ELISA Evaluation of IgG Antibody Response Against the Serovars in vax-SPIRAL® Induced in Vaccinated Volunteers During an Efficacy Trial in Several Municipalities in Villa Clara Province. Interval in Between Doses: 6-8 weeks.

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Strain and time</th>
<th>N</th>
<th>n</th>
<th>%</th>
<th>-0.95</th>
<th>0.95</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB</td>
<td>CANT1</td>
<td>422</td>
<td>7</td>
<td>1.7</td>
<td>0.8%</td>
<td>3.4%</td>
</tr>
<tr>
<td>Vax-SPIRAL®</td>
<td>CANT1</td>
<td>460</td>
<td>176</td>
<td>38.3</td>
<td>33.9%</td>
<td>42.8%</td>
</tr>
<tr>
<td>HB</td>
<td>COPT1</td>
<td>422</td>
<td>12</td>
<td>2.8</td>
<td>1.6%</td>
<td>4.9%</td>
</tr>
<tr>
<td>Vax-SPIRAL®</td>
<td>COPT1</td>
<td>460</td>
<td>146</td>
<td>31.7</td>
<td>27.7%</td>
<td>36.1%</td>
</tr>
<tr>
<td>HB</td>
<td>MOZT1</td>
<td>422</td>
<td>14</td>
<td>3.3</td>
<td>2.0%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Vax-SPIRAL®</td>
<td>MOZT1</td>
<td>460</td>
<td>143</td>
<td>31.1</td>
<td>27.0%</td>
<td>35.5%</td>
</tr>
</tbody>
</table>

* IgG concentration 21 days after the second doses / IgG concentration before vaccination, ** Agrarian University of Havana, *** National Center of Agropecuarian Sanitation.

Phase III Clinical Study. Efficacy

- 101,708 volunteers aged between 20 and 64 were vaccinated.
- They were observed for 12 months.
- Case control:
  - 12 cases in vaccinated individuals (2.38 x 100,000)
  - 56 cases in non-vaccinated individuals (6.69 x 100,000)
- A 78.1% specific serogroup efficacy was achieved.
- A 60.4% efficacy was obtained in other serogroups not included in the vaccine.

Phase IV Clinical Study. Effectiveness (1996-1997)


Medical Sanitary Registry

Vax-SPIRAL® trivalent antileptospirosis vaccine

REPUBLIC OF CUBA. MINISTRY OF PUBLIC HEALTH ERM: 846/98

Date of registration: Dec. 11, 1998

Registration number: 1050
Impact on the disease in Cuba

Total of exposed individuals who were vaccinated (1996-2001) = 1,730,632

Contribution to morbidity reduction in the period = 81.2%

Contribution to reducing the number of deaths = 31.7%

Incidence rate decrease from 25.6 x 100,000 in 1994 to 4.7 x 100,000 in 2001

DISCUSSION

From its first epidemic outbreak in 1910 among the workers building Havana’s sewerage system, until the 1970’s, when several outbreaks of this disease appeared, leptospirosis was not a serious health problem in our country. Starting in 1978, conditions were created in the National Institute of Hygiene and Epidemiology for the diagnosis of this disease in all of the provinces. Cuban sanitary authorities became concerned with diagnosing and controlling this disease in human beings and animals [9].

Leptospirosis control measures included mainly the use of protection means (boots, gloves, etc.), as this is an occupational disease, and environmental sanitation (deratization, residue treatment, etc). The prophylactic measures include vaccination and treating the exposed personnel with Doxiciclin [10].

Prophylactic control of the disease was first carried out in Cuba in 1983 by means of the administration of a Soviet vaccine to at-risk personnel. This vaccine contained 4 serovars (icterohaemorrhagiae, pomona, grippotyphosa and hebdomadis), and was used until the late 1980’s.

As a result of the collapse of the socialist bloc, the Soviet vaccine was no longer being received in Cuba. This fact, along with the advent of the Special Period and the consequent deterioration of hygienic and sanitary conditions in the country, made it possible for leptospirosis to have an ever-growing tendency in all of our provinces [9]. It then became a serious health problem for our population in general, as it had stopped being a disease affecting risk groups only.

Research was started then at the “Finlay Institute” towards producing “as soon as possible” a vaccine against human leptospirosis from autochthonous strains.

As there were no strains in stock that had been isolated from clinical cases in human beings, which could supply strains to produce vaccine variants for evaluation, it was decided to use strains in conservation from isolations in animals, coming from CENEDI (Institute of Veterinarian Medicine).

The state of the art about vaccines for human use against leptospirosis confirmed that it was not a topic intensely worked on around the world, and so it was only possible to obtain important information of the results achieved in China [11] and Israel [12, 13]. The information that was gathered allowed us to design a strategy to follow for the formulation of the Cuban vaccine against human leptospirosis.

A hypothesis was outlined to conceive an inactivated cell vaccine with an antigenic concentration per vaccine dose that would be lower than that reported for other vaccines, and that would be adsorbed in aluminum hydroxide gel, thus ensuring response potentialization and reactogenicity decrease [14, 15].

The vaccine strains needed to be responsive to the serogroups with higher circulation in the country, besides being highly virulent, which was a necessary condition to consider them as vaccine candidates. Characterizing their pathogenicity in the Golden Syrian Hamster animal model [4] would allow us to have strains that would be able to express those virulence factors responsible for the disease’s clinical frame, and against which protection should be directed.

Leptospira’s subcultures are known to cause biological activity loss, which is also greatly influenced by the medium composition as well as the cultivation and maintenance conditions. To ensure biological stability in
vaccine strains, their maintenance and conservation conditions were characterized, evaluating the main growth factor of the microorganism. Growth standardization was achieved in the TA semi-synthetic medium, while MLP differential formulations were obtained for the production of vaccine antigens. This result guaranteed the achievement of protein-free biomass, as in the vaccine antigen production process, all the washings were able to eliminate traces of albumin that could become reactogenic elements [16].

Several variants of the adsorbed and non-adsorbed vaccines were formulated in response to our working hypothesis. The results obtained showed that the adsorbed variant induced an IgG antibody level that was remarkably higher than that with the non-absorbed vaccine, and a greater immunological response as well [14, 17]. Recent results have demonstrated that vaccine formulations of inactivated cells adsorbed in aluminum hydroxide for animals, are capable of inducing cellular response [18]. Innocuousness trials with Balb/c Mouse and Duncan Hartley Guinea Pig demonstrated the innocuousness of the adsorbed formulation.

The preclinical trials demonstrated the innocuousness, immunogenicity and protective capacity of the adsorbed variant of the vaccine.

Once the technological production flow of the proposed vaccine was defined up to filtering, it was passed on to the Institute’s production plant, where production scaling was developed in a 35 L aired fermentator. Consistent production was then achieved to guarantee different vaccine lots for clinical trial evaluation.

An ELISA system was mounted, standardized and validated for humoral response evaluation. Taking into account that the vaccine’s active principle consists of whole cells, the same principle was used as a covering antigen. LPS is the major antigen against which protective antibodies are induced [19]. It would then be logical to think of it as the covering antigen to make an accurate evaluation of induced protective immune response. Present literature, however, reports the identification of other structural components of a proteinic nature which are involved in protecting, and more than that, inducing crossed immunity [20-23]. All of these elements confirm that the best antigen is the vaccine’s active principle, which allows a more integral response measure.

Clinical trials were started in December 1994 with the authorization of the Center of Clinical Trials, after a document had been submitted containing the results of the pre-clinical trials as well as the protocols of clinical trials I and II.

The clinical trials to evaluate reactogenicity and immunogenicity were carried out between 1994 and 1995. The results obtained demonstrated that the adsorbed variant, whose commercial name was vax-SPIRAL ®, was as little reactogenic and immunogenic as in the Golden Syrian Hamster animal model [24].

The high incidence of this disease in Holguín Province caused the relevant authorities of the Ministry of Public Health to make the decision to vaccinate the identified risk population (118,018 people). The effectiveness trial was carried out in this province, posing a challenge for the vaccine under evaluation, as results of immunoepidemiological studies indicated that the most relevant serogroup was L. Ballum, which had not been included in the vaccine formulation. The trial results indicated that the vaccine had been 97.3% effective, thus confirming the pre-clinical results. The vaccine’s power was demonstrated once again, but this time with human beings [25, 26].

The efficacy trial conducted in Villa Clara Province (1997-1998) reported 78.1% of efficacy with serogroups that were included in the vaccine, while 60.4% was reported with other non-included serogroups [26]. Preclinical results have reported cross protection induction in hamsters that were vaccinated with vax-SPITALR ® and challenged with virulent strains of the L. ballum serogroup [27].

Post-license Phase IV clinical trials developed with risk-exposed Personnel from UAH and CENSA allowed us to somehow characterize induced IgG response, which was different between different individuals in relation with the circulating antibody level resulting from exposure. This trial showed seroconversion levels of 70.04%, 64.22%, and 66.06% for the canicola, copenhagen and mozdok serovars respectively [28]. These results are coincidental with the 78.1% efficacy level found in Villa Clara province.

The demonstrated efficacy of vax-SPIRAL ® lead to its inclusion in the human leptospirosis control program through vaccination to different risk groups around the country. The results achieved have demonstrated the
impact on leptospirosis morbidity and lethality exerted by vaccination as a prophylactic measure and by sanitary hygienic controls as well. A decreasing tendency is currently observed with this disease in the country, which is expected to further decrease as the levels of immunity in the risk population grow higher.

The results of having researched, developed and ensured a stable production of this vaccine provide us with an important weapon to battle this terrible disease, and make Cuba one of very few countries to have a vaccine of this kind. It opens up interesting commercial perspectives, as vax-SPIRAL® is to be used in epidemiological conditions that favor this disease, mainly in very diverse regions in Latin America.

CONCLUSIONS

- A characterization was made of autochthonous strains chosen as vaccine strains, belonging to *L. canicola*, *L. copenhageni* and *L. mozdok* serovars. Their pathogenicity was characterized in the Syrian Hamster animal model.
- A definition was made of the optimal growth conditions for vaccine strains in a proteinic medium, and three protein-free media were optimized for the production of vaccine antigens.
- An inactivated-cell trivalent vaccine variant was formulated. Its optimal conditions for absorption in aluminum hydroxide were described.
- Process and final controls were defined for the chosen absorbed variant.
- The vaccination scheme was defined for two 0.5 mL doses, with a six-week interval between doses that are administered deeply intramuscularly.
- Immunogenicity, innocuousness and power of the adsorbed vaccine formulation were demonstrated in the pre-clinical trials.
- The production technology of the adsorbed variant was transferred to the production plant of “Finlay Institute”, where scaling was achieved at production level.
- The clinical trials carried out, both before and after the license was issued, demonstrated that the vaccine was safe, lowly reactogenic, immunogenic against all implied serovars and all other serovars in a crossed way, efficient (78%-98%) in terms of circulating serovars.
- As part of the relevant program of the Ministry of Public Health, it made an impact on morbidity (81.2%) and lethality (31.7%) reduction.
- Having a vaccine against human leptospirosis in Cuba makes an important economic impact, as it saves hard currency that would have to be used to import the vaccine for risk groups. It is also very beneficial in that it considerably reduces the negative economic impact of the disease and its social costs, while it constitutes a potentially exportable product.

The vaccine will be useful to contribute to controlling the disease in Cuba and other affected countries.

REFERENCES


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