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Current Status of Research on Surra in China

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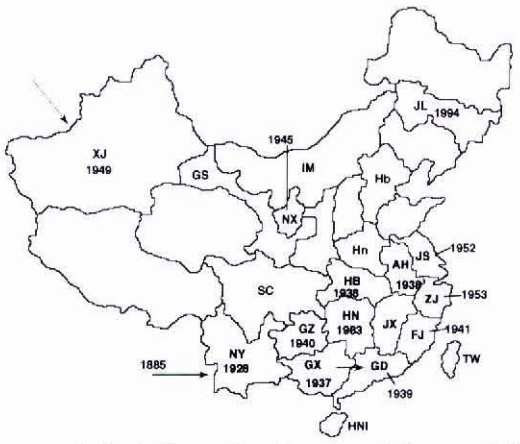
Trypanosoma evansi has a wider distribution in geography, which causes trypanosomiasis-*evansi*or surra in horses, asses, mules, cattle, camels, buffaloes and pigs etc. The Chinese veterinary parasitologists have done a lot of research on identification, mAb, in vitro cultivation and genes of *T. evansi*, epidemiology, diagnosic, treatment on surra since it had caused a great economic loses. Athough a number of achievements have been obtained, there are many questions to be solved. Main achievements made during the recent years in China are summarized below.

1. History of epidemiology

It is first recorded in 1940 that trypanosomiasis-*evansi* in mule occured in China. However, Luckins (1988) reported that there was trypanosomisis in 1891 and 1905. Since 1943, trypanosomiasis-*evansi* has been found in buffaloes, horses, camels, cattle, asses, pigs, dogs and deers etc. Trypanosomiasis-*evansi* can be divided into two epidemical parts in the geographic distribution in China, that is South and North parts. In South part, cattle and horses were sensitive to *T. evansi* but trypanosomisis-*evansi* occurred mainly in camels in North part. But we found some reports on trypanosomiasis-*evansi*in cattle in Northeast Part, Jilin Province. There is an obvious isolation from South and North parts. Trypanosomiasis-*evansi* is now reported from most Chinese provinces with exception of few provinces that are Shanxi, Shanxi, Shandong, Liaoning, Heilongjing, Tabit and Qinghai. According to the data of the national investigation on trypanosomiasis of domestic animals, the provinces considered to be the most important endemic regions are Anhui, Fujing, Guangdong, Guangxi, Hainan, Hubei, Hunan, Jiangsu, Jiangxi, Yunnan and Zhejiang (Fig. 1). In the recent years, we found that there is a widen tendency on ranges of hosts, which indicated that strain of *T. evansi* may be variation. For this, Chinese veterinary parasitologists have found that *T. evansi* in South and North parts in China came from two contrary isolation strains respectively by kDNA probe, isoenzyme and immunoblot etc. Moreover variation groups in antigenicity have been found in different epidemical areas and hosts. Additionally, we found



Figigure 1. Map of China showing the provinces with high prevalence of *T. evansi*. Keys to province and autonomous regions: AH, Anhui; FJ, Fujing; GD, Guangdong; GS, Gansu; GX, Guangxi Zhuang Autonomous Region; GZ, Guizhou; HB, Hubei; HN, Hunan; Hb, Hebei; Hn, Henan; HNI, Hainan Island; IM, Inner Mongolia Autonomous Region; JS, Jiangsu; JX, Jiangxi; NX, Ningxia; SC, Sichuan; TW, Taiwan; XJ, Xinjiang Uygur Autonomous Region; YN, Yunnan; ZJ, Zhejiang., JL., Jilin. The years given are those in which the first case of surra was reported in a particular region. The solid arrows indicate the paths of entry and the possibility of migration of *T. evansi* in China. The broken arrow shows the presumed entry of *T. evansi* from the northwest. (Lun et al. 1993)

that there are kinetoplast and dyskinetoplast strains that probably are *T. equinum* in China.

2. In vitro cultivation and preservation

In vitro cultivation is essential for the study on immunology, molecular biology and treatment of trypanosomiasis. Nie (1991) successfully cultured *T. evansi* for 60 days in the MEM medium in the presence of peritoneal cells of mice. Luo (1991) and Fang (1991) cultured *T. evansi* in cell free culture. Also Wang (1995) cultured *T. evansi* isolated from camel in chicken embryo. However, an inneglected question is whether there are differences in biology, biochemistry, immunology and molecular biology between in vitro produced *T. evansi* especially *T. evansi* cultured in media without feeder layer cells and native parasites and these difference can affect on the reliability of experiments. For preservation of *T. evansi*, Shang (1982) first reported that *T. evansi*, which was isolated from camel and preserved in liquid nitrogen for 150 days, still etained its infectivity to hosts. Xu (1991) found that *T. evansi* passaged in mice can be preserved for 1 year in liquid nitrogen and -80 ℃. However, it should be passaged in sensitive animals once a year. *Trypanosoma evansi* preserved in -20℃ should be passaged in animals every two months and *T. evansi* can be alive for 4 days in 4 ℃. Ba (1993) found *T. evansi*, which preserved in ultra low temperature or liquid nitrogen for 3200 days, also retained its infectivity to hosts.

3. mAb

Yang (1988) first reported mAb against *T. evansi*. Later, Fang (1990) established 9 mAb strains in which 4 strains are IgG1, one is IgG3 and others are IgM. He also found that 3 strains detected by 2 heterogeneous *T. evansi* antigens are strain specific. In 1989, 3 mAb strains against surface membrane antigen of *T. evansi* and 2 mAb strains against VSG (variable surface glycoprotein) of *T. evansi* were established by Dong and Jia. The specific test showed that 2 mAb strains only react specifically with the same type of VSG and did not react with different strains of *T. evansi* and different variated type of same strain. So far, over 16 strains of mAbs have been established. However, we must be caution when different strains were identified with mAbs since there are different VSGs and serum types in different organs even in the same host.

4. Diagnosis

For diagnosis of trypnosomiasis-*evansi* in China , it was divided into 4 kinds that were detection of parasites, circulating antigen, circulating antibody and allergy in 1970s and 1980s. In recent years DNA probe and PCR have been used in the diagnosis of trypanosomiasis-*evansi*. Actually, immunodignosis has being widely used. However, there are some problems to be solved, in which reagents are not standardized, procedure is not unification and comparability is poor.

5. Treatment

So far, control of trypanosomiasis in domestic animals still depands on chemotherapy. Bayer 205, Antricidum, Naganol, Berenil, Trypamidium and 914 etc. have been widely used in the treatment of trypanosomiasis-*evansi*. We also found reports on the treatment with Imidocarb and T46 which is a new anti-trypanosoma drug produced in China. However drug-resistant strains are a major problem recently. There are different drug sensitivities in different hosts and geographical strains or drug resistant trypanosome strains. The cure rates of Antricidum, Naganol, Berenil, Trypamidium to trypanosomiasis-*evansi*in mice infected

with Zhejing strain are 100%, 80%, 30% and 20% respectively. The cure rates of Antricidum to trypanosomiasis infected with Zhejing, Yunnan and Anhui strains are 80%, 0% and 10%, moreover. Berenil and Trypamidium are not effective to trypanosomiasis infected with these 3 strains. Fang (1994) developped Suramin-resistant clones of *T. evansi* in vitro by drug exposure. Three such clones were derived from 3 suramin-sensitive of *T. evansi* over a period of 550 days.

6. Immunoprophylaxis

Many reports on vaccines included inactive vaccine, attenuated strain and anti-idiotypic antibody vaccines have been found. However, there are no effective vaccines to be used in field so far. Liu (1984) reported that the survival time of mice inoculated with attenuated trypanosomes, which were irradiated by gamma ray with 4, 6, 9 10k rad, prolongated 2-3 times compared with uninoculated mice. In 1997, Liu developped poly-factors attenuated vaccine against *T. evansi*. Through the following series of experiments, he found the vaccines were effective to immunoprophylaxis of trypanosomiasis: 1) 30 mice were inoculated with the vaccines. On days 30, 45 and 60, 10 mice were challenged with *T. evansi* respectively. The protective rates were 9/10, 8/10 and 6/10 but the mice without inoculated with vaccines died at day 4-7 after challenged with *T. evansi*; 2) 30 guinea pigs were immuned with the vaccine. Ten immunized guinea pigs were challenged with *T. evansi* on days 30, 60 and 90 respectively. The protective rates are 10/10, 9/10 and 8/10. However, the guinea pigs without immunization died at day 17-23; 3) Liu (1997) inoculated 9 horses with the vaccines and 3 horses were challenged with *T. evansi* on days 30, 60 and 90 respectively. All the horses were protected against infection with *T. evansi*, however, the control horses died at day 45-50 respectively. Through microscopioc examination, no parasites were found in blood smears and negative for animal experiment (Table 1). Zhou and Shan (1997) did the cross immunity with different geography strains. The result showed that the protective rates with same strain is higher than heterogeneous stains (Table 2).

Table 1. Immunoprophylax of trypanosomiasis with poly -factor attenuated vaccines

Animals	Group	Inoculation				Challenge		Results
		Ad	Quantity	Do (ml)	IT (d)	Quantity	PR	M
Mice	I	SI	2	0.1	30	$2X10^3$	9/10	1/10
	II	SI	2	0.1	45	$2X10^3$	8/10	2/10
	III	SI	2	0.1	60	$2X10^3$	6/10	4/10
Guinea pigs	I	SI	2	0.5	30	$4X10^3$	10/10	0
	II	SI	2	0.5	60	$4X10^3$	9/10	1/10
	III	SI	2	0.5	90	$4X10^3$	8/10	2/10
Horses	I	SI	2	10	30	$1X10^4$	3/3	0
	II	SI	2	10	60	$1X10^4$	3/3	0
	III	SI	2	10	90	$1X10^4$	3/3	0

* Ad: administration; Do: dose; IT: Interval time; PR: protective rate; M: mortality; SI: subcutaneous injection

Table 2. Cross immuno-protection with different geographical strains of *T. evansi*

Strains	Mice	Immuno-protection to challenge with different strains				
		Anhui	Guangxi	Yunnan	Xinjiang	Control (Anhui)
Anhui	5	5/5(30d) 0/5(14.6d)	---	---	2/5(16.3d)	0/5(6.9d)
Guangxi	5	0/5(7.8d)	0/5(21.5d)	---	---	0/5(5.6d)
Yunnan	5	0/5(5.7d)	---	0/5(19d)	---	0/5(5.4d)
Xinjiang	5	0/5(8.5d)	---	---	0/5(23.8d)	0/5(10.8d)

7. Others

Xu (1997) reported that ^{3}H-TdR and live *T. evansi* were added into the preserver and then preserved in -20℃ and -80℃. CPM of preserved *T. evansi* body was determined by liquid scintillation detector. The result showed that there were significant differences ($P<0.01$) between live *T. evansi* added with ^{3}H-TdR and *T. evansi* without adding ^{3}H-TdR or dead *T. evansi*. This demonstrated that ^{3}H-TdR could keep *T. evansi*, which remained the metabolism at low temperature, alive. Zhang (1993) detected the presence of TNF (Tumor Necrosis Factor) in serum of mice infected with *T. evansi* by mAb of mice against TNF with ELISA. Li (1996) confirmed the trypanocidal activity aganst *T. evansi* in human serum by in vitro or in vivo trypanocidal assays. Inocubation of *T. evansi* cells at 37℃ with human serum resulted in high-rate conversion of cells into ring-forms and finally lysed forms. Intraperitoneal injection of human serum resulted in clearance of all parasites from the blood stream in infected mice and protected them from the infection. In the mice infected i.p. with 10^2 *T. evansi* were given as small as 0.2 ml of human serum on the 5th day post-infection. The parasites in their blood stream gradually decreased and completely disappeared by day 10. Also, they found that trypanocidal activity of human serum against *T. evansi* may be related to natural antibodies of IgM class. Moreover, decomplemented human serum exhibited significantly lower in vitro trypanocidal activity whereas in vivo protection was still effective.

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