

## Sparganum infections in normal adult population and epileptic patients in Korea: A seroepidemiologic observation

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**Abstract:** A seroepidemiologic observation of anti-*Spirometra erinacei* plerocercoid (sparganum) antibody (IgG) in serum was made in normal adult and epileptic patients in Korea from February, 1987 to September, 1990. Sera were tested by enzyme-linked immunosorbent assay (ELISA) for anti-sparganum antibody together with anti-*Taenia solium* metacestode, and anti-*Paragonimus westermani* antibodies. Sera reacted positively to sparganum antigen only were considered. Positive rate for anti-sparganum antibody in 850 normal adults was 1.9% (standardized rate by provincial population was 1.7%). In 2,667 randomly selected patients of epilepsy at 28 local centers of the Changmi Club, positive rate was 2.5% (standardized rate: 2.3%). In both normal adult and patient groups, the higher antibody rates were observed in Kangwon and Chonnam Provinces. Positive rates were 10 times higher in male than in female in normal adults and 4.5 times in male epileptic patients. The rates were elevated especially with age over 30-year. Odd ratio of the antibody was 1.32 which indicated an ambiguous etiologic factor for epilepsy.

**Key words:** Sparganum, sparganosis, seroepidemiology, epilepsy

### INTRODUCTION

Diagnosis and treatment of human sparganosis depend on removal of the worm from an infected tissue. Though the antibody test for human sparganosis by ELISA is now available and is proved to be sensitive and specific (Kim *et al.*, 1984), its clinical application in preoperative diagnosis is still limited. The screening test for anti-sparganum antibody by ELISA has been demonstrated to be useful in confirming or in excluding the

cerebral sparganosis (Chang *et al.*, 1987; Moon *et al.*, 1993). A limitation of the antibody test is cross reactions with human cysticercosis. Unless antibody test for cysticercosis and sparganosis is performed simultaneously, non-specific positive reactions of sparganosis can not be completely ruled out (Cho *et al.*, 1992).

Up to present, epidemiological data on human sparganosis have been accumulated and analysed on the bases of case records in literature (Cho *et al.*, 1975; Min *et al.*, 1990). The prevalence of sparganosis in a population can not be figured out even by autopsy because of limitations such as many dormant infections, unpredictable location of the worm in the body and a probable low infection rate. Therefore, the antibody test for human sparganosis is the only feasible technique in undertaking a large scale epidemiologic evaluation of the infection (Kim *et al.*, 1984; Rim *et al.*, 1991).

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This study is seroepidemiological observation on prevalence of human sparganosis by applying the antibody test (ELISA) in normal adults and epileptic patients in Korea.

## MATERIALS AND METHODS

### 1. Subjects

The subjected population are the same with those described by Kong *et al.* (1993); normal population in Pusan (50) and Taegu (50) were included additionally.

As representation of normal adult population, 850 sera samples of farmers, university students, employees of firms, workers in factories and a few children were tested. The sera were collected at Cheju General Hospital (March, 1987), Chung-Ang University Hospital (July, 1990), Provincial Branch Laboratories of the Korea Association of Health, Inc. (August-September, 1990) for biochemical tests and serologic tests for hepatitis in periodic physical check-ups. The tested sera were a random series of 49-161 samples.

The epileptic patients who registered at the Changmi Club were subjected to the antibody tests. The Changmi Club is a voluntary, civilian, charity activity supported by Protestant Churches and volunteer neurologists, neurosurgeons and nurses in Korea (Kang *et al.*, 1979). Out of 93,121 members at 108 local centers of the Club (as of July 1990), 2,667 sera samples were collected at 28 local centers over the country considering provincial population. The subjects for the test were randomized by persuading patients to participate the survey without prior announcement of the test. After the survey purpose was explained individually, over 95% of patients agreed voluntarily to participate. This survey was carried out on February 1987 at a local center in Cheju Province and from October 1989 to July 1990 at 27 centers in peninsular Provinces and Cities in Korea (Table 3).

### 2. Antibody test

Serum antibody levels, specific to sparganum, were measured by ELISA (Kim *et al.*, 1984) concomitantly with those to *Taenia*

*solium* metacestode (Cho *et al.*, 1986) and *Paragonimus westermani* (Cho *et al.*, 1981). Used antigens were crude saline extracts of sparganum, cystic fluid of *T. solium* metacestodes and crude saline extracts of adult *P. westermani*. They were diluted in carbonate buffer (pH 9.6) in protein concentration of 2.5 µg/ml and were coated in wells of polystyrene microtiter plates (Costar, Cambridge, MA) at 4°C overnight. After washing, 1:100 diluted sera in phosphate buffered saline/0.05% Tween 20 (PBS/T, pH 7.4) were reacted for 2 hours at 37°C. After washing, 1:1000 diluted peroxidase-conjugated anti-human IgG (light- and heavy-chain specific, Cappel, West Chester, PA) in PBS/T was reacted for 2 hours at 37°C. After washing, substrate, composed of 1 ml of 1% *o*-phenylene diamine, 10 µl of 30% H<sub>2</sub>O<sub>2</sub> and 99 ml of distilled water was reacted at 37°C for 24 minutes. Absorbance (abs.) was read at 490 nm using Microplate Reader (Bio-Rad M3550, Richmond, CA). Cut-off abs. of positive reaction were 0.22 for anti-sparganum antibody, 0.25 for anti-*Paragonimus* antibody and 0.18 for anti-*T. solium* metacestode antibody as described by Cho *et al.* (1992). All tests were duplicated and mean abs. was used as an antibody level. Sera reacted positive only to sparganum were regarded as positive. The sera reacting positively for two or three antigens were excluded.

## RESULTS

### 1. Antibody positive rates in normal adult population

Out of 850 sera samples tested, 16 sera (1.9%) showed positive levels of the anti-sparganum antibody (Table 1). By administrative districts, Kangwon Province (8.0%), Chonnam Province (8.0%), Kyongbuk Province (4.0%), Chonbuk Province (3.9%) and Kyongnam Province (3.6%) showed higher rates of the positive reactions while 3 metropolitan cities (Seoul, Pusan, Taegu) and 3 provinces (Kyonggi, Chungnam and Cheju) revealed no positive reactors. When the rate was standardized by provincial population in 1990 (Economic Planning Board, Republic of Korea, 1990), it was 1.7%. When high positive rates in

**Table 1.** Positive rate of anti-sparganum antibody (IgG) in sera from normal adult population (students, factory workers and employees of firm) in their periodic check-up

| City/<br>Province | No.<br>examined | No. (%)<br>positive |
|-------------------|-----------------|---------------------|
| Seoul             | 124             | 0 (0)               |
| Pusan             | 50              | 0 (0)               |
| Taegu             | 50              | 0 (0)               |
| Inchon            | 50              | 1 (2)               |
| Kyonggi           | 50              | 0 (0)               |
| Kangwon           | 50              | 4 (8)               |
| Chungbuk          | 49              | 1 (2)               |
| Chungnam          | 50              | 0 (0)               |
| Chonbuk           | 51              | 2 (3.9)             |
| Chonnam           | 50              | 4 (8)               |
| Kyongbuk          | 50              | 2 (4)               |
| Kyongnam          | 55              | 2 (3.6)             |
| Cheju             | 161             | 0 (0)               |
| Total             | 850             | 16 (1.9)            |

**Table 2.** Age and sex distribution of tested cases and number of positive reactors in normal adult population

| Age<br>(year) | No. positive/No. examined (%) |             |              |
|---------------|-------------------------------|-------------|--------------|
|               | Male                          | Female      | Total        |
| 0-9           | 0/3                           | 0/2         | 0/5 (0)      |
| 10-19         | 0/47                          | 0/90        | 0/137 (0)    |
| 20-29         | 1/93                          | 0/107       | 1/200 (0.1)  |
| 30-39         | 5/169                         | 0/83        | 5/252 (2.0)  |
| 40-49         | 5/109                         | 0/35        | 5/144 (3.5)  |
| 50-59         | 1/52                          | 1/32        | 2/84 (2.4)   |
| over 60       | 3/20                          | 0/8         | 3/28 (10.7)  |
| Total         | 15/493 (3.0)                  | 1/357 (0.3) | 16/850 (1.9) |

Kangwon and Chonnam Provinces were standardized by age distribution of samples, the rates were 4.1% in Kangwon and 6.9% in Chonnam Provinces.

As shown in Table 2, the antibody positive sera were not found in children and adolescent under 19 years old. The positive rate was 2.0% in age group of 30-39 years old, and increased up to 10.7% in the age group over 60 years. By sex, male (3.0%) showed 10 times higher antibody-positive rate than female population (0.3%).

**Table 3.** Positive rates of anti-sparganum antibody (IgG) in epileptic patients of the Changmi Club shown by local center and administrative district

| City/<br>Province | No.<br>examined | No.<br>positive | Positive<br>(%) |
|-------------------|-----------------|-----------------|-----------------|
| Seoul             | 204             | 2               | 0.9             |
| Chonngong         | 98              | 1               |                 |
| Yongdungpo        | 106             | 1               |                 |
| Inchon            | 124             | 1               | 0.8             |
| Kyonggi           | 306             | 7               | 2.2             |
| Ansong            | 42              | 3               |                 |
| Kangwha           | 14              | 2               |                 |
| Kimpo             | 44              | 0               |                 |
| Pochon            | 3               | 0               |                 |
| Pyongtaek         | 71              | 1               |                 |
| Suwon             | 132             | 1               |                 |
| Kangwon           | 80              | 6               | 7.5             |
| Chunchon          | 77              | 6               |                 |
| Chorwon           | 3               | 0               |                 |
| Taejon            | 101             | 0               | 0               |
| Chungbuk          | 89              | 3               | 3.4             |
| Chungju           | 42              | 2               |                 |
| Umsong            | 47              | 1               |                 |
| Chungnam          | 312             | 2               | 0.6             |
| Hongsong          | 96              | 2               |                 |
| Kumsan            | 29              | 0               |                 |
| Nonsan            | 86              | 0               |                 |
| Chonbuk           | 171             | 1               | 0.6             |
| Iri               | 110             | 1               |                 |
| Namwon            | 61              | 0               |                 |
| Chonnam           | 356             | 25              | 7.3             |
| Huasun            | 217             | 15              |                 |
| Hampyong          | 139             | 10              |                 |
| Kyongbuk          | 276             | 9               | 3.3             |
| Andong            | 107             | 2               |                 |
| Munkyeong         | 97              | 3               |                 |
| Sangju            | 41              | 4               |                 |
| Yechon            | 31              | 0               |                 |
| Kyongnam          | 344             | 10              | 2.9             |
| Kimhae            | 152             | 3               |                 |
| Chinyang          | 192             | 7               |                 |
| Cheju             | 405             | 0               | 0               |
| Total             | 2,667           | 66              | 2.5             |

## 2. Antibody positive rates in epileptic patients

The antibody positive rates in epileptic patients of 28 local centers (The Changmi Club) were shown in Table 3. The positive rate

was 2.5% in the sample population. When the antibody positive rate was standardized by the provincial population, the rate was 2.3%. By City/Province, the rate was the highest in Kangwon Province (7.5%), followed by Chonnam Province (7.3%), Chungbuk Province (3.4%), Kyungbuk Province (3.3%), Kyongnam Province (2.9%) and Kyonggi Province (2.2%). Standardized positive rate by age in sample population was 5.3% in Kangwon and 7.2% in Chonnam Province. In Taejon City and Cheju Province, no positive reactors of the antibody were found.

The antibody positive rates were shown in Table 4, analysed by age and sex of the epileptic patients. The rates were below 1.0%

in age groups under 19 years old, increased with age up to 5.2% in epileptic patients of 50-59 years old. By sex, the rate in male patient was 4.8 times higher than that in females.

### 3. Antibody levels in the surveyed population

The anti-sparganum antibody levels ranged from abs. 0.01 to 1.55 in the surveyed population. The cut-off abs. of positive reaction was 0.22. The positive antibody levels were divided arbitrarily into borderline positive (abs. 0.22-0.49), moderate positive (abs. 0.50-0.79) and highly positive (abs. 0.80 and over). The case numbers in each abs. range were exhibited in Table 5 according to the normal

**Table 4.** Positive reactors of anti-sparganum antibody in 2,667 epileptic patients analysed by age and sex

| Age<br>(year) | No. positive/No. examined (%) |                |                |
|---------------|-------------------------------|----------------|----------------|
|               | Male                          | Female         | Total          |
| 0-9           | 0/49                          | 0/31           | 0/80 (0)       |
| 10-19         | 1/187                         | 0/125          | 1/312 (0.3)    |
| 20-29         | 6/363                         | 3/303          | 9/665 (1.4)    |
| 30-39         | 17/435                        | 4/381          | 21/816 (2.6)   |
| 40-49         | 15/214                        | 1/196          | 16/410 (3.9)   |
| 50-59         | 11/146                        | 2/105          | 13/251 (5.2)   |
| over 60       | 6/81                          | 0/52           | 6/133 (4.5)    |
| Total         | 56/1,474 (3.8)                | 10/1,193 (0.8) | 66/2,667 (2.5) |

**Table 5.** Distribution of cases in different interval of the antibody levels in the surveyed groups

| Group                      | No.<br>examined | No. of cases in abs. (antibody level) interval |           |           |               |
|----------------------------|-----------------|--|-----------|-----------|---------------|
|                            |                 | 0.01-0.21                                      | 0.22-0.49 | 0.50-0.79 | 0.80 and over |
| Normal adult               | 850             | 834  | 7         | 5         | 4             |
| Male                       | 493             | 468  | 6         | 5         | 4             |
| Female                     | 357             | 356  | 1         | 0         | 0             |
| Kangwon                    | 50              | 46   | 0         | 1         | 3             |
| Chonnam                    | 50              | 46   | 3         | 1         | 0             |
| Other cities and provinces | 750             | 742  | 4         | 3         | 1             |
| Epileptic patients         | 2,667           | 2,601  | 29        | 16        | 21            |
| Male                       | 1,473           | 1,417  | 24        | 14        | 19            |
| Female                     | 1,193           | 1,184  | 5         | 2         | 2             |
| Kangwon                    | 80              | 74   | 1         | 1         | 4             |
| Chonnam                    | 356             | 331  | 10        | 8         | 7             |
| Other cities and provinces | 2,231           | 2,196  | 18        | 7         | 10            |

**Table 6.** Odds ratio of anti-sparganum and anti-*Taenia solium* metacestode antibodies for epilepsy between normal adult and epileptic patients

| Antibody                            | Odds ratio | Confidence interval (95%) |
|-------------------------------------|------------|---------------------------|
| Anti-sparganum                      | 1.32       | 0.76-2.30                 |
| Anti- <i>T. solium</i> metacestode* | 1.94       | 1.14-3.29                 |

\*Based on the data in Kong *et al.* (1993)

adult and epileptic patients, sex and administrative district. Distribution of cases in positive categories of abs. was not statistically significant between normal adult and epileptic patients ( $\chi^2 = 0.4511$ ,  $p > 0.1$ ). The difference of case distribution between male and female patients of epilepsy was also not significant ( $\chi^2 = 0.5923$ ,  $p > 0.1$ ). By province, Kangwon Province, where higher rate of the antibody was revealed, majority of the positive reactors were highly positive (abs. 0.80 and over) in both normal adult and epileptic patients. On the contrary, in Chonnam Province with equally high rate of the antibody, higher frequency in category of highly positive antibody was not observed.

#### 4. Odds ratio of anti-sparganum antibody positive reactions for epilepsy

For epilepsy, odds ratio of the anti-sparganum antibody positive reactions was calculated between normal adult and epileptic patients. As shown in Table 6, it was 1.32 while that of anti-*T. solium* metacestode was 1.94.

### DISCUSSION

The antibody test is the only feasible epidemiologic tool so far in obtaining a large scale prevalence data of human sparganosis (Rim *et al.*, 1991). Even by the most careful physical examination or autopsy, the prevalence of the infections can not be figured out. Ultrasonographic findings in soft tissue sparganosis (Park *et al.*, 1993) awaits evaluation for its applicability and significance in population surveys.

Feasibility and significance of the anti-sparganum antibody test have been studied since the 1980s. The sensitivity of the antibody test was 85.7% and the specificity was 97.5% when undertaken by ELISA (Kim *et al.*, 1984).

Elevation of the antibody levels has been proved in experimental sparganosis in rabbits (Ohnishi *et al.*, 1986) or mice (Hong *et al.*, 1989). Declining pattern of the antibody levels after surgical removal was also described (Kim *et al.*, 1993). The antibody test was found cross-reactive with some cases of *Taenia saginata* infections (Kim *et al.*, 1984) and cysticercosis (Choi *et al.*, 1988). Cho *et al.* (1992) described that all the neurologic patients, whose sera and/or cerebrospinal fluid (CSF) reacted positively to both sparganum and *T. solium* metacestode antigens, revealed brain CT findings of cysticercosis. Therefore, the anti-sparganum antibody test was undertaken together with anti-*T. solium* metacestode and anti-*P. westermani* antibody tests. All the cases reacting positively to sparganum antigen together with *T. solium* metacestode (1 in normal adults and 8 in epileptic patients) or *Paragonimus* antigens (1 in normal adult samples) were excluded from consideration. By the multi-antigen screening, the frequency of non-specific reactions to sparganum antigen was minimized. Chang *et al.* (1987, 1992) and Moon *et al.* (1993) described brain CT or MR findings of the neurologic patients with positive anti-sparganum antibody reactions in their serum and/or CSF. In all operated patients by brain surgery, cerebral sparganosis were confirmed except a case of brain angioma. In cases of incidental combination of intracranial bleeding with extracranial sparganum infection, exudation of the antibody into CSF is inevitable (Cho *et al.*, 1988). As Holodniy *et al.* (1991) commented, the antibody test for human sparganosis need to be characterized especially in its relations with the stages of infection.

Prevalence data in this study coincide very well with the previous epidemiologic findings of human sparganosis compiled on the bases of

case records in Korea (Cho *et al.*, 1975; Min, 1990; Chang *et al.*, 1992) especially in relation with age and sex of the surveyed population. Predilection of sparganum infections in males and adult age has been known. Unlike previous report on geographic distribution of clinical cases (Cho *et al.*, 1975), however, the antibody positive rates were higher in Kangwon and Chonnam Provinces than any other province. Exact cause of higher rates awaits proper explanations. But this finding suggest that ecology is well preserved and eating habits of intermediate hosts is relatively higher among people in these provinces.

Schantz and Sarti-Gutierrez (1989) described that different levels of specificity of anti-*T. solium* metacestode antibody tests affect positive predictive value when used in community based epidemiologic study of human cysticercosis. The positive predictive value is low in low endemic population even when specificity of the test is near to 100%. The specificity of the anti-sparganum antibody test can be assumed as 97.5% in this study. Positive rates of the antibody in the sample population are low in range of 1.7-2.3%. Then the positive predictive value of a positive antibody test would be 41% in this study. Additional confirmatory studies in the antibody positive cases are necessary in the future.

Odds ratio for epilepsy in sparganosis, obtained in this study, was not high as in cysticercosis (Sarti *et al.*, 1994). This finding suggests that frequency of the brain involvement of a sparganum is far less than that in cysticercosis. While cerebral involvement of *T. solium* metacestodes is seeded by oncospherical spreading through circulation, that of a sparganum has been speculated as a result of incidental invasion through soft tissues at skull base foramina (Chang *et al.*, 1987). Ambiguous result of odds ratio in this study also does not exclude cerebral sparganosis as a definite cause of epilepsy. In this connection, patients of chronic diseases in the past tended to eat snakes or frogs as a medicine for their disease. This tradition may be related with the higher rates of positive anti-sparganum antibody in epileptic patients.

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스파르가눔 감염 환자에 대하여 혈청학적으로 특이항체검사를 실시할 경우 그 민감도와 특이도가 높다는 것은 이미 알려진 바와 같다. 그러므로 특이항체 양성율은 이용할 경우 무중상 정상인 집단이나 특정 집단에서의 스파르가눔 감염 상태를 간접적으로 관찰할 수 있다. 이 연구는 우리나라 정상 성인 및 간질(癎疾) 환자의 혈청을 수집하고, 효소면역측정법으로 혈청내 항스파르가눔 항체, 항유구낭미충 항체 및 항폐흡충 항체를 동시에 측정하고, 항스파르가눔 항체양성자 중 항유구낭미충 항체 및 항폐흡충 항체와 교차반응한 예를 제외하고 오로지 항스파르가눔 항체가만 양성범위인 사람을 양성으로 하는 방법으로 양성자를 조사하였다. 1987년 2월부터 1990년 9월까지 중앙의대 부속병원, 한국건강관리협회 전국 각 지부 검사소 및 제주의료원에 신체검사를 목적으로 내원한 무중상 성인 850명의 혈청을 부작위로 선정하여 정상 성인 조사 대상으로 하였고 전국 장미회 108개 지부중 28개 지부에 등록된 간질 환자중 부작위로 2,667명을 조사대상자로 선정하였다. 항스파르가눔 항체의 양성 반응을 흡광도 0.22 또는 그 이상으로 하였을 때, 항체양성율은 정상 성인군에서 1.9% (1990년 전국 시도별 인구로 표준화하면 1.7%), 간질환자군에서 2.5% (표준화하였을 때 2.3%)이었다. 항체양성율은 남자에서, 30세 이상 연령군에서 높아 환자 증례를 기초로 조사한 기존 역학조사 자료와 일치하였다. 시도별로는 강원도와 전라남도의 대상자에서 항체양성율이 높았고 특히 강원도의 대상자에서는 항체가(흡광도)가 매우 높은 양성자의 비율이 높았다. 항체양성율을 기초로 간질에 대한 스파르가눔 감염의 상대위험도(odds ratio(교차비)로 표현)는 1.32로서 스파르가눔 감염은 유구낭미충 감염(교차비, 1.94)에 비하여 낮았다.

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