# Seroprevalence of fasciolosis and the difference of fasciolosis between rural area and city center in Isparta, Turkey

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## ABSTRACT

**Objectives:** To investigate the seroprevalence of fasciolosis and the possible causes of differences between rural and city center.

**Methods:** We undertook a multi-stage sampling analysis of data from Isparta, Turkey, between March and June 2004. Four hundred and fifteen individuals participants from Isparta center and 171 from Asagi Gokdere village were included in the study. *Fasciola hepatica* (*F. hepatica*) specific antibodies were analyzed using excretory-secretory (ES)-enzyme-linked immunosorbent assay (ELISA) method.

**Results:** *Fasciola hepatica* antibodies were detected as positive in 10 (2.4%) of 415 people whose sera were collected from the city center and 16 (9.3%) of 171 people from Asagi Gokdere village. The positivity rates between village and city center were found statistically significant. A statistical difference was noted for fasciolosis positivity between individuals who have ingested water cress and who have not. Fasciolosis was not detected in the individuals who used to wash vegetables with water containing vinegar.

**Conclusion:** Most of the patients in this region reported consumption of uncooked or unwashed water cress. Watering channel is one of the major risk factors of fasciolosis. Therefore, it is essential to determine the watering systems in this region. Moreover, ES-ELISA would be useful in investigating the laboratory diagnosis of fasciolosis.

**Saudi Med J 2006; Vol. 27 (8): 1152-1156**

Fasciolosis is a zoonotic infection, which is caused by *Fasciola hepatica (F. hepatica)*. It is suggested that approximately 2.4 million people are affected from fasciolosis in the world.[1,2] It is reported that herbivorous mammalians play an important role in the transmission of *F. hepatica*.[3] *Fasciola hepatica* inhabits in areas close to rivers and lakes. *Fasciola hepatica* has been described as being transmissible by ingestion of water plants or drinking water carrying metacercaria, and of poorly cleaned vegetables, and by using contaminated kitchen tools.[3,4] Water sources, which are necessary for both the development of intermediate host, Lymnaea truncatula and of water cress which is important for the transmission to human being, exist very common in our region.[5] Unfortunately, in our country, there are no enough data on the epidemiology of this disease. Due to the serological diagnostic methods were not applied

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Received 7th February 2006. Accepted for publication in final form 17th April 2006.

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in routine, the human fasciolosis cases have been determined generally during surgical operations as sporadic both in our country and throughout the world.[6-9] In our region, among individuals who have eosinophilia, fasciolosis cases have been determined at a significant rate, confirmed by serological methods.[10] In the light of the data mentioned above, Goller region (have many lakes) is likely to be one of the regions where fasciolosis is most commonly observed. In our region, the rural areas are rich from watering channel and water cress. The difference between the incidence of this disease in rural areas and city center is still remain unknown. In our study, we aimed to investigate the seroprevalence of fasciolosis and the possible causes of differences between rural and city center.

**Methods.** Isparta city center, which is at the South-west region of Turkey, and Asagi Gokdere Village, which belongs to Isparta city were included in the study. The sample size that should be taken to ensure a 95% confidence interval was considered to be 753 subjects, assuming the prevalence of *F. hepatica* as 2% and an error rate of 1%. One hundred sixty-seven (22.2%) samples were excluded from the study for various reasons, such as hemolysis, loss of sample sera, absent questionnaire and so forth. A total of 586 (77.8%) samples were included in the study. Eighteen health centers in Isparta city center were separated into regions according to their population number. The health centers were separated into groups as follows: 15-20 thousand population (Yedisehitler with 3 health centers); 10-15 thousand (Kurtulus and Karaagac with 6 health centers) and 5-10 thousand (Yenice and Cunur with 6 health centers).The population percentage was as follows: 35% from Yedisehitler, 25% from Gulistan, 18% from Kurtulus, 8% from Yenice, 4% from Cunur region. Between March and June 2004, 415 people (303 female, 112 male, age mean: 26.6 ± 20.9) from Isparta center, and 171 people (91 female, 80 male, age mean: 38.4 ±2 0) from Asagi Gökdere village, a region where watering channel exist, whose inhabitants deal with sheep and goat, and frequently consume water cress, were included in the study using the multi-stage sampling method. The people included in the study were asked for where they obtain water and bought the water cress or vegetables and how they clean vegetables. Their hand washing behavior and habit of eating water cress were also questioned.

Blood samples were taken from the study group by venipuncture into polystyrene tubes. Sera were separated and stored at -20$^{O}$C until used. All cases were examined for antibodies against Fasciola by modified ELISA employing as antigen the excretory-secretory (ES) product of the Fasciola (ES-ELISA) according to Espino et al,[11] and Carnevale et al.[12] In our hands, the sensitivity and specificity of this method were 100% and 95.3%.

The *F. hepatica* adults were incubated in phosphate-buffered solution (PBS) containing 0.8 mol/l phenylmethylsulfonyl fluoride, 400 U of aprotinin/ml and 0.1 mM dithiothreitol (one worm/5 ml) (Sigma Chemicals, St. Louis, USA) at 37$^{O}$C for 3 hours. The suspension containing ES antigen of *F. hepatica* was centrifuged at 4$^{O}$C (13,000 $\mu$g) for 2 hours and was filtered through a 0.2 $\mu$m pore size filter.[11,12]

Excretory-secretory-enzyme-linked immunosorbent assay antigen was coated onto immuno plates (Nunc-MaxiSorp Immunoplate, Roskislde, Denmark) at a concentration of 12.8 $\mu$g/ml. Human sera (100 $\mu$l) were used at 1:100 dilution and alkaline phosphatase conjugated anti-human IgG (100 $\mu$l) (Sigma Chemicals, St. Louis, USA) was used at 1:10,000 dilution. One $\mu$g/ml of 4-nitrophenyl phosphate disodium salt (Merck, Darmstadt, Germany) was used as the substrate. Plates were read on a microplate reader (Bio-Tek Instruments, ultra microplate reader ELX 808, Winooski, USA) at an absorbance of 405 nm. Test serum, antigen and conjugate titrations were determined with checkerboard titration. The cutoff point was calculated as the average of the absorbance values of negative sera +3 SD.[11,12]

Stool examination was carried out for *F. hepatica* eggs for 3 times. Stool samples were examined by direct saline and Lugol preparation. Stools were also concentrated to examination by use of a formalin-ethyl acetate sedimentation technique. Concentrates were also examined as wet mounts native saline and Lugol preparation by examining the entire area under a 22- by 50-mm cover slip, using a 10 times the objective for screening and 40 times the objective for parasite identification.[13]

The statistical methods used to show the difference between the groups were Chi square tests and a *p*-value less than 0.05 was considered statistically significant.

**Results.** *Fasciola hepatica* antibodies were detected as positive in 10 (2.4%) of 415 people whose sera were collected from the city center and 16 (9.3%) of 171 people from Asagi Gökdere village. *Fasciola hepatica* eggs were detected in 2 of 415 (0.4%) among city center inhabitants and 3 of 171 (1.7%) among village inhabitants. We detected *F. hepatica* in 26 of all study group (4.4%) using serological methods, while egg examination could detect 5 of those (0.8%), which was statistically significant ($p$<0.001, $\chi^2$: 13.254). (**Table 1**)

**Table 1** - Evaluation and answer of questionnaire.

| Characteristics of study group | Positivity N (%) | Negativity N (%) | Total | Statistic |
|---|---|---|---|---|
| ***Domicile*** | | | | |
| Village | 16 (9.3) | 155 (90.7) | **171** | $p<0.001$ |
| Centrum | 10 (2.4) | 405 (97.6) | **415** | |
| ***Gender*** | | | | |
| Male | 5 (2.6) | 187 (97.4) | **192** | $p>0.05$ |
| Female | 21 (5.3) | 373 (94.7) | **394** | |
| ***Source of the water which is used*** | | | | |
| Network | 19 (4.2) | 430 (95.8) | **449** | $p>0.05$ |
| Non-network | 7 (5.1) | 130 (94.9) | **137** | |
| ***Hand washing behavior before meals*** | | | | |
| Yes | 24 (5.3) | 433 (94.7) | **457** | $p>0.05$ |
| No | 2 (1.6) | 127 (98.4) | **129** | |
| ***Grow their vegetables themselves*** | | | | |
| Yes | 13 (8.8) | 135 (91.2) | **148** | $p<0.05$ |
| No | 13 (3) | 425 (97) | **438** | |
| ***Grow their water cress themselves*** | | | | |
| Yes | 10 (11.1) | 80 (88.9) | **90** | $p<0.05$ |
| No | 16 (3.2) | 480 (96.8) | **496** | |
| ***Ingestion of water cress*** | | | | |
| Yes | 21 (6.7) | 292 (93.3) | **313** | $p<0.05$ |
| No | 5 (1.8) | 268 (98.2) | **273** | |
| ***Vegetable washing behavior*** | | | | |
| Vinegar-water | 0 (0) | 77 (100) | **77** | $p<0.05$ |
| Water | 26 (5.1) | 483 (94.9) | **509** | |

We found the positivity rates of village and city center to be statistically significant ($p<0.001$, $\chi^2$: 12.195). There were no statistically significant difference between the 2 gender (21 females, 5 males) for fasciolosis seropositivity ($p>0.05$, $\chi^2$: 2.262). No difference was noted for Fasciola seropositivity between usage of network water and natural spring water or between individuals who wash hands before meal and who does not ($p>0.05$, $\chi^2$: 0.191, $\chi^2$: 3.25). We determined the higher positivity rate in individuals who grew their water cress or vegetables themselves. These findings were also statistically meaningful ($p<0.05$, $\chi^2$: 11.171) (**Table 1**). Moreover, a statistical difference was noted for fasciolosis positivity between individuals who have consumed water cress and have not ($p<0.05$, $\chi^2$: 8.182). Fasciolosis was not reported among individuals who used to wash vegetables with water containing vinegar and it was also statistically significant ($p<0.05$, $\chi^2$: 4.116). Inhabitants of our region usually use the network or spring water as drinking or cleaning water. Channel or small lake water which run open are not used for cleaning.

**Discussion.** Human fasciolosis infection is assumed to be a negligible infection type resulting from its low frequency, but in recent years according to the development of new diagnostic methods and newly documented findings in the pathogenesis, fasciolosis becomes a more important and more often diagnosed disease.[2,3,10] In 2000, Demirci et al[10] have investigated the serologic diagnosis of fasciolosis in patients with eosinophilia, which is one of the most common findings of fasciolosis in Isparta region. They reported the fasciolosis seropositivity as 6.1%. Turhan[14] have carried out the first seroepidemiologic study which included 597 people selected from 10 villages in Antalya city which belongs to Göller region. They reported seropositivity rate of 3.01%.[14] Several studies indicated that hypo and meso endemic places can be found in Göller region including Antalya, Isparta, Burdur, Afyon, Konya.[5,10,14] Yilmaz and Godekmerdan[15] reported the rate of egg determination as 1.8% in Van, a city in east of Turkey, and Kaplan et al[16] carried out a *F. hepatica* seroprevalence study in Elazg and they were reported the rate as 2.78%.[16] According to the results of previous studies, we suggested that fasciolosis can be seen in every region of Turkey with an increasing rates. The factors supporting this hypothesis can be enumerated as follows: water cress and similar plants are consumed in rural areas very often and there has been an increase in puddles and watering channels in recent years.[6,7,17] The main source of human infection is fresh water plants. The most important source is water cress in fresh water plants. Various water cress species such as Nasturtium officinale, N. sylvestris

(wild water cress) and Roripa aniphibia (wild water cress) are found in different regions of the world.[18,19] In Isparta region, water cress which is grown by farmers or collected from watering channels is sold in the local bazaar. In our region weed water cress can be eaten in February, March, April, May according to the annual climate conditions as same as in Spain.[20] After May, the plant gives flowers and is no longer collected. Aside from water cress other fresh water plants such as Valerianella olitora, Mentha viridis, and Taraxacum densleonis can also play an important role in the transmission of this infection.[1,8] In our study, water cress consumption rate was found to be higher in seropositive individuals compared with seronegative. Individuals who consume and grow both water cress, other fresh water plants including Romaine lettuce, Mentha viridis, Valerianella olitora, Taraxacum densleonis and some vegetables in their own garden were found to have higher positivity rates than those which were not consumed and grow water cress and other vegetables. The risk factors can be explained in 2 manners; the first one is; people would not wash the vegetables they pick up from their own garden. The second one is; the usage of manure due to those people has goats or sheep's which pasture in natural environment. The eggs of *F. hepatica* can be found in those manure and metacercariae can be grown up from miracidium in appropriate environmental conditions.[1,14] However, the infection rate is found low in the people who buy their vegetables in the market, also it can be explained; professional vegetables producer is generally using inorganic manure that does not contain metacercaria. The disease can be seen in rural areas and suburban regions. There are some studies reporting that the disease is more common among individuals who have close contact with sheep and goat than other rural residential area. Due to the main source in the transmission of this infection is water cress and infected water, reservoir animals play more important role in a region. Therefore, all rural area residents are under at the same risk for the disease. The transmission of the disease occurs basically by intake of water cress and infected water.[2,9,21] It is also reported that people in endemic regions do not follow hygiene rules sufficiently, and they take infected/dirty water and consume vegetables without cooking or washing it.[2,8]

In our study, we found a higher positivity rate in individuals living in the rural areas than living in city center. The causative factors can be explained as follows: in rural areas there are more herbivorous animals, which play an important role in the natural life cycle of the parasite and the individuals consume wild water cress and similar plants, responsible for the human transmission of the disease. In this study, no relationship could be detected between seropositivity and hand-washing habit. However, seropositivity was not detected in individuals who wash vegetables with vinegar-water mixture. This finding is very significant and it shows that prevention of the disease can be provided by simple disinfection methods. We believe that the education of people would be very important in this term. Therefore, we suggest that serological methods, especially ES-ELISA are more efficient and reliable than parasitological examination in the diagnosis of fasciolosis.

In conclusion, fasciolosis is an important health problem in our region. Most of the patients in this region reported consumption of uncooked or unwashed water cress. Watering channel is one of the major risk factors of fasciolosis. Therefore, it is essential to determine the watering systems in this region. Moreover, ES-ELISA would be useful in investigating the laboratory diagnosis of fasciolosis.

## References


1. Mas-Coma MS, Esteban JG, Bargues MD. Epidemiology of human fascioliasis: a review and proposed new classification. ***Bull World Health Organ*** 1999; 77: 340-344.
2. Mas-Coma S, Angles R, Esteban JG, Bargues MD, Buchon P, Franken M, et al. The Northern Bolivian Altiplano: a region highly endemic for human fascioliasis. ***Trop Med Int Health*** 1999; 4: 454-467.
3. Arjona R, Riancho JA, Aguado JM, Salesa R, Gonzalez-Macias J. Fascioliasis in developed countries: a review of classic and aberrant forms of the diseases. ***Medicine*** 1995; 74: 13-23.
4. Mas-Coma MS, Bargues MD, Esteban JG. Human fasciolosis. In: Dalton JP, editor. Fasciolosis Wallingford: CABI Publishing; 1999. p. 411-434.
5. Demirci M, Korkmaz M, Sakru N, Kaya S, Kuman A. Diagnostic importance of serological methods and eosinophilia in tissue parasites. ***J Health Popul Nutr*** 2002; 20: 352-355.
6. Bengisun U, Ozbas S, Sarıoglu U. Fascioliasis observed during laparoscopic cholecystectomy. ***Langenbecks Arch Surg*** 1999; 384: 84-87.
7. Kabaalioglu A, Apaydin A, Sindel T, Luleci E. US guided gallbladder aspiration: a new diagnostic method for biliary fascioliasis. ***Eur Radiol*** 1999; 9: 880-882.
8. Esteban JG, Bargues MD, Mas-Coma S. Geographical distribution, diagnosis and treatment of human fascioliasis: a review. ***Res Rev Parasitol*** 1998; 58: 13-42.
9. Farag HF. Human fascioliasis in some countries of the Eastern Mediterranean Region. ***Eastern Mediterranean Health Journal*** 1998; 4: 156-160.
10. Demirci M, Korkmaz M, Kaya S, Kuman A. Fascioliasis in eosinophilic patients in Isparta region of Turkey. ***Infection*** 2003; 31: 15-18.
11. Espino AM, Dumenigo BE, Fernandez R, Finlay CM. Immunodiagnosis of human fascioliasis by enzyme-linked immunosorbent assay using excretory-secretory products. ***Am J Trop Med Hyg*** 1987; 37: 605-608.

12. Carnevale S, Rodriguez MI, Santillan G, Labbe JH, Cabrera MG, Bellegarde EJ, et al. Immunodiagnosis of human fascioliasis by an enzyme-linked immunosorbent assay (ELISA) and a micro-ELISA. ***Clin Diagn Lab Immunol*** 2001; 8: 174-177.
13. Markel EK, John DT, Krotoski WA. Markel and Woge's Medical Parasitology. Philadelphia (PA): WB Saunders Company; 1999. p. 431-455.
14. Turhan OA. Antalya yoresinde Fasciola hepatica seroepidemiyolojisi. [MD Thesis]. Antalya: Medical Faculty of Akdeniz University. 2002.
15. Yilmaz H, Godekmerdan A. Human fasciolosis in Van province, Turkey. ***Acta Trop*** 2004; 92: 161-162.
16. Kaplan M, Kuk S, Kalkan A, Demirdag K, Ozdarendeli A. Fasciola hepatica seroprevalence in the Elazig region. ***Mikrobiyol Bul*** 2002; 36: 337-342.
17. Guclu F, Dik B, Agaoglu M. A case of fascioliasis in a woman. ***Acta Parasitologica Turcica*** 1995; 19: 370-374.
18. Rondelaud D, Vignoles P, Abrous M, Dreyfuss G. The definitive and intermediate hosts of Fasciola hepatica in the natural water cress beds in central France. ***Parasitol Res*** 2001; 87: 475-478.
19. Antoniou M, Lionis C, Tselentis Y. A focus of Fasciola hepatica in Crete without human cases. ***Eur J Epidemiol*** 1997; 13: 99-101.
20. Luzon-Pena M, Rojo-Vazquez FA, Gomez-Bautista M. Seasonal availability of Fasciola hepatica metacercariae in a temperate Mediterranean area (Madrid, Spain). ***Zentralbl Veterinarmed B*** 1995; 42: 577-585.
21. Esteban JG, Gonzalez C, Bargues MD, Angles R, Sanchez C, Naquira C, et al. High fascioliasis infection in children linked to a man-made irrigation zone in Peru. ***Trop Med Int Health*** 2002; 7: 339-348.