

A comparative study of three methods for detecting *Fasciola* infections in Nigerian cattle

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ADEDOKUN, O. A., A. B. AYINMODE, B. O. FAGBEMI: A comparative study of three methods for detecting *Fasciola* infections in Nigerian cattle. Vet. arhiv 78, 411-416, 2008.

ABSTRACT

Qualitative examination of *Fasciola gigantica* eggs in faeces and bile were compared with the detection of precipitating antibodies in sera by agar gel precipitation test (AGPT) in 1000 cattle slaughtered at the Bodija municipal abattoir in Ibadan, Nigeria. Faecal and bile examination methods detected (196) 33.5% and (389) 38.9% of the animals as positive for fasciolosis, while (474) 47.4% were positive by AGPT. Both direct bile examination and faecal egg detection methods have high specificity and positive predictive value (100%) when compared with AGPT. However, lower values for sensitivity and negative predictive value were observed for both faecal egg examination (66.5% and 67.9% respectively) and bile examination (81.0% and 78.9% respectively). Faecal and bile examination failed to detect 33.5% and 19.0% of the cases detected by AGPT. The results of this study revealed that the AGPT could become a better test for the herd diagnosis of bovine fasciolosis for veterinarians and other investigators in Nigeria.

Key words: fasciolosis, cattle, agar gel precipitation test, faeces, bile

Introduction

The diagnosis of fasciolosis in ruminants caused by *Fasciola* sp. has been solely by the detection of *Fasciola* eggs in the faeces of infected animals (BORAY, 1985). Although the procedure is simple and confirmatory, it is however not a useful diagnostic tool at low levels of adult fluke burden. Also, it cannot detect infection at the prepatent period, because eggs are found in faeces when the flukes are already matured (usually between 10 and 14 weeks of infection) (URQUHART et al., 1996). Hence the need for methods other than faecal egg examination for the diagnosis of infection with fasciolosis has been obvious for decades (HORCHNER, 1973; HENRIKSEN, 1974).

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Serologic diagnoses have been developed as an alternative approach to faecal egg detection. Serological methods can test a large number of sera at a time and also detect infection earlier than faecal egg examination. There are evidences to show that serodiagnosis can detect the presence of infection as early as 2 weeks after infection (SANTIAGO and HILLYER, 1988; FAGBEMI and GUOBADIA, 1995).

Furthermore, serological methods like Enzyme Linked Immunosorbent Assay (ELISA) and western blots can detect serum antibodies to specific antigens of *Fasciola* sp. using adult fluke extracts or excretory/secretory (ES) materials (WESCOTT et al., 1983; HILLYER et al., 1992; FAGBEMI and GUOBADIA, 1995; CLERY et al., 1996; SAMPAIO-SILVA et al., 1996). Methods that directly measure *Fasciola* sp. antigen shed into serum or faeces of infected animals or human has also been developed (HILLYER, 1993). Other serological methods like AGPT and agar gel diffusion test (AGDT) have also been demonstrated to be simple and valuable for detection of *Fasciola* sp. antibodies particularly where there are less diagnostic facilities (BUI KHANH LINH et al., 2003).

This study is aimed at evaluating AGPT, faecal egg examination and bile examination in the determination of prevalence of bovine fasciolosis in Nigerian cattle.

Materials and methods

Faecal materials were collected directly from the rectum of each animal meant for slaughter at the Bodija municipal abattoir, Ibadan between December 2003 and October 2004. Jugular blood samples from each of the animals were collected at slaughter, allowed to clot and serum extracted. Bile was collected from the gall bladder after opening each carcass. Samples were labeled for identification per animal.

Faecal examination for *F. gigantica* egg was carried out using the sedimentation method described by (URQUHART et al., 1996). Bile was examined for eggs using a modification of the method described by THIENPONT et al. (1979). This was carried out by mixing equal volumes of bile and water, straining through a tea strainer before centrifuging at 3000 rpm. After obtaining a clear supernatant by repeated mixing of sediment with water and centrifuging, the sediment was examined under the microscope.

Test for precipitating antibodies to *F. gigantica* was done using the AGPT described by BUI KHANH LINH et al. (2003). The antigen used was prepared by homogenising 0.1 g of adult *F. gigantica* worms for 30 minutes in 5 mL of physiological saline. The emulsion was frozen and thawed twice before it was centrifuged at 5000 rpm at 4 °C. The supernatant was used as antigen and stored frozen. 1% agar solution in 10% saline was prepared and 5 mL poured into 6 cm diameter petri dishes. Serum was used undiluted and untreated.

The sensitivity, specificity, positive and negative predictive values of the fecal and bile examination tests was determined according to a general method (GALEN and GAMBINO, 1975; ROGAN and GLADEN, 1978) using AGPT as the gold standard.

Results

Out of the 1000 cattle examined for *Fasciola gigantica* (585) 58.5% were positive by AGPT, (474) 47.4% by bile examination and (389) 38.9% by fecal egg examination (Table 1). Fecal egg examination failed to detect (196) 33.5% of the positive samples detected by AGPT, while bile examination failed to detect 19.0% cases (Table 1). All positive results with fecal egg examination and bile examination were also positive with AGPT.

The specificity and positive predictive value of both fecal egg detection and bile examination in comparison with AGPT were 100% respectively (Table 2). Fecal egg detection has a lower sensitivity (66.5%) than bile examination (81.0%) and their negative predictive value was 67.9% and 78.9% respectively in relation to AGPT (Table 2).

Table 1. Comparison of AGPT with other parasitological methods of detecting *Fasciola gigantica*

AGPT		Fecal egg examination		Bile examination	
		Positive (%)	Negative (%)	Positive (%)	Negative (%)
Positive	585 (58.5%)	389 (66.5%)	196 (33.5%)	474 (47.4%)	111 (19.0%)
Negative	415 (41.5%)	0	415	0	415
Total	1000	389 (38.9%)	611 (61.1%)	474 (47.4%)	526 (52.6%)

Table 2. Comparison of the sensitivity and specificity of various methods of *Fasciola gigantica* detection with AGPT

	Fecal egg detection (%)	Bile examination (%)
Sensitivity (%)	66.5	81.0
Specificity (%)	100	100
PPV (%)	100	100
NPV (%)	67.9	78.9

PPV- Positive Predictive Value; NPV- Negative Predictive Value

Discussion

AGPT and Ouchterlony precipitation in gels, has been considered to be one of the valuable diagnostic methods for fasciolosis (BUI KHANH LINH et al., 2003) and has been used to diagnose *F. gigantica* infection in sheep in Iraq (KADHIM and AL-ATTAR, 1974) and buffaloes in India (SWARUP et al., 1987).

This study, while comparing the traditional parasitological methods used in the detection of *Fasciola gigantica* egg with AGPT showed that the sensitivity of faecal egg examination was the lowest (66.5%), which may be due to low numbers of egg in the faecal samples, possibly as a result of low worm burden or occlusion of the gastrointestinal tracts by debris (URQUHART et al., 1996). Low sensitivity could also be as a result of inability of the method to detect of *Fasciola sp* eggs in animals at the early stages of infection. However, the procedure for faecal egg examination for *Fasciola sp.* egg is a simple, confirmatory and very valuable method when the infection is patent (DORSMAN, 1956).

Direct bile examination for *Fasciola sp.* egg showed higher sensitivity than faecal egg detection method, but lower when compared to AGPT. This observation could possibly be due to intermittent emptying of the gall bladder into the digestive tract (SHAH-FISCHER and SAY, 1989). Although the direct bile examination proved to be more sensitive than faecal egg examination in this study, it is however only useful as a diagnostic procedure at slaughter or necropsy, because of the difficulty of accessing the gall bladder in a live animal.

Faecal egg detection and direct bile examination methods are well correlated, both with high specificity (100%) and high positive predictive value (100%); they also showed lower sensitivity and negative predictive values, probably due to the zero egg counts from some animals with positive serum antibodies detected by AGPT. The two egg detection methods would certainly have detected current infection status in the animals examined, since eggs would be detected only when the fluke is present and the infection is patent.

This study also showed that apart from the fact that AGPT detected more animals with *Fasciola gigantica* infection than the two egg detection methods; the test was also able to detect the entire samples that were positive with the other two methods (AGPT had no false positive). This is envisaged, since AGPT being a serological test, will detect precipitating antibodies to the flukes in the sera of infected animals both in past and present infections. Furthermore the ability of precipitating an antibody test like AGPT to detect fasciolosis in the acute phase of infection earlier than egg detection methods, and even from calves with very low egg counts, has been demonstrated by previous investigators (VAN TIGGELE and OVER, 1976; HILLYER and DE WELL, 1981).

Although, AGPT is less sensitive than other serological tests like ELISA, the procedure in general has been demonstrated to be more specific than either the indirect

hemagglutination or indirect fluorescent antibody test (SADUN, 1976). The use of the precipitating antibody test in the diagnosis of *Fasciola gigantica* has also been shown not to cross-react with antigen of other trematodes (BUI KHANH LINH et al., 2003).

In conclusion, while the traditional parasitological methods of egg detection in fasciolosis remains valuable, the results of this study showed that AGPT could serve as a useful technique for herd diagnosis of fasciolosis in cattle by veterinarians and investigators that lack suitable equipment for faecal examination and also have no access to an expensive serological ELISA kit, particularly in developing nations like Nigeria.

References

- BORAY, J. C. (1985): Flukes of domestic animals. In: Parasites, Pests and Predators. (S. M. Gaafar, W. E. Howard, R. E. Marsh, eds.). Elsevier, New York. pp. 179-218
- BUI KHANH LINH, D. T. T., M. Y. LE NGOC, S. OSAMU, Y. SHINOBU (2003): Application of agar gel diffusion test to the diagnosis of fasciolosis in cattle and buffaloes in the Red River delta of Vietnam. *Jpn. Agr. Res. Q.* 37, 201-205.
- CLERY, D., P. TORGERSON, G. MULCAHY (1996): Immune responses of chronically infected adult cattle to *Fasciola hepatica*. *Vet. Parasitol.* 62, 71-82.
- DORSMAN, W. (1956): A new technique for counting eggs of *Fasciola hepatica* in cattle faeces. *J. Helminthol.* 30, 165-172.
- FAGBEMI, B. O., E. E. GUOBADIA (1995): Immunodiagnosis of fasciolosis in ruminants using a 28-kDa cysteine protease of *Fasciola gigantica* adult worms. *Vet. Parasitol.* 57, 309-318.
- GALEN, R. S., S. R. GAMBINO (1975): Beyond normality-the predictive value and efficiency of medical diagnoses. John Wiley and Sons, New York.
- HENRIKSEN, S. V. A. A. (1974): Fascioliasis diagnostics. *Nord. Veterinarmed.* 26, 21-22.
- HILLYER, G. V. (1993) Serological diagnosis of *Fasciola hepatica*. *Parasitol.* 17, 130-136.
- HILLYER, G. V., M. SOLER DE GALANES, J. RODRIGUEZ-PEREZ, J. BJORLAND, M. S. DE LAGRAVA, S. R. GUZMAN, R. T. BRYAN (1992): Use of the falcon assay screening test-enzyme-linked immunosorbent assay (FAST-ELISA) and the enzyme-linked immunoelectrotransfer blot (EITB) to determine the prevalence of human fascioliasis in the Bolivian Altiplano. *Am. J. Trop. Med. Hyg.* 46, 603-609.
- HILLYER, W. G., N. S. DE WELL (1981): Serodiagnosis of experimental fascioliasis by immunoprecipitation test. *Int. J. Parasitol.* 11, 71-78.
- HORCHNER, H. W. (1973): Proceedings of Fasciolose Symposium 1973, Tecklenburg. pp. 14-18.
- KADHIM, J. K., M. AL-ATTAR (1974): Preliminary observations on antigen activity of *Fasciola gigantica* by immunodiffusion test in sheep. *Br. Vet. J.* 130, 1004-1006.
- ROGAN, W. J., B. GLADEN (1978): Estimating prevalence from the results of a screening test. *Am. J. Epidemiol.* 107, 71-76.

- SADUN, E. (1976): Application of immunodiagnostic tests. Blackwell Scientific Publications, London, UK.
- SAMPAIO-SILVA, M. L., J. M. CORREIA DA COSTA, A. M. VAIANA DA COSTA, M. A. PIRES, S. A. LOPES, A. M. CASTRO, D. L. MONJOUR (1996): Antigenic components of excretory-secretory products of adult *Fasciola hepatica* recognized in human infections. Am. J. Trop. Med. Hyg. 54, 146-148.
- SANTIAGO, N., G. V. HILLYER (1988): Antibody profiles by EITB and ELISA of cattle and sheep infected with *Fasciola hepatica*. J. Parasitol. 74, 810-818.
- SWARUP, D., S. P. PACHAURI, B. SHARMA, S. K. BANDHOPADHYAY (1987): Serodiagnosis of *Fasciola gigantica* infection in buffaloes. Vet. Parasitol. 24, 67-74.
- SHAH-FISCHER, M., R. SAY (1989): Manual of Tropical Veterinary Parasitology. CAB International, Wallingford, Oxon, U.K.
- THIENPONT, D., F. ROCHETTE, O. F. J. VANPARIJS (1979): Diagnosing helminthiasis through coprological examination. Janssen Research Foundation, Beerse Belgium.
- VAN TIGGELE, L. J. A., H. J. OVER (1976): Serological diagnosis of fascioliasis. Vet. Parasitol. 1, 239-248.
- URQUHART, G. M., J. ARMOUR, J. L. DUNCAN, A. M. DUNN, F. W. JENNINGS (1996): Veterinary Parasitology, 2nd ed., Blackwell Science, London. p. 110.
- WESCOTT, R. B., C. J. FARRELL, D. T. SHEN (1983): Diagnosis of naturally occurring *Fasciola hepatica* infections in cattle with an enzyme-linked immunosorbent assay. Am. J. Vet. Res. 45, 178-179.

Received: 17 September 2007

Accepted: 5 September 2008

ADEDOKUN, O. A., A. B. AYINMODE, B. O. FAGBEMI: Usporedno istraživanje triju metoda za određivanje invazije metiljem *Fasciola gigantica* u nigerijskih goveda. Vet. arhiv 78, 411-416, 2008.

SAŽETAK

Uspoređeni su rezultati pretrage na prisutnost jajašaca metilja *Fasciola gigantica* u žuči i izmetu s rezultatima pretrage na prisutnost specifičnih protutijela testom gel-difuzijske precipitacije. Istraživanje je provedeno na 1000 goveda zaklanih u gradskoj klaonici Bodija u Ibadanu u Nigeriji. Određivanjem jajašaca u žuči, prisutnost metilja dokazana je u 196 (33,5%), dok su u izmetu jajašca dokazana u 389 (38,9%) životinja. Metodom precipitacije u gelu protutijela su bila dokazana u 474 (47,4%) životinje. Postupak izravnog dokaza jajašaca u izmetu i žuči vrlo je specifičan i ima pozitivnu prediktivnu vrijednost (100%) u usporedbi s precipitacijskim testom. Međutim, slabija osjetljivost (66,5%) i negativna prediktivna vrijednost (67,9%) dobivene su za pretragu jajašaca u izmetu te 81,0% za osjetljivost i 78,9% za negativnu prediktivnu vrijednost za dokaz jajašaca u žuči. Pretragom izmeta na jajašca nije se uspjela otkriti prisutnost metilja u 33,5% životinja, dok pretragom žuči na jajašca metiljavost nije bila dokazana u 19% goveda u odnosu na precipitaciju u gelu. Rezultati pokazuju da je precipitacija u gelu bolja za dokazivanje govede fascioleze u nekom stadu te vrlo prikladan test za veterinare u Nigeriji.

Ključne riječi: fascioleza, govedo, precipitacija u gelu, izmet, žuč
