Comparison of different diagnostic techniques against Fasciolosis in Buffaloes

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Abstract

The present study was conducted to compare different diagnostic tests viz., Direct Smear (DS), Agar gel precipitation (AGP), Sedimentation (Sd), and Zinc Sulfate ($ZnSO_4$) flotation for fasciolosis in dairy buffaloes as well as the economic losses due to fasciolosis. A total of 200 faecal samples were examined and DS, AGP, Sd, and $ZnSO_4$ flotation techniques showed an overall prevalence of 2, 8, 5 and 4%, respectively. The highest agreement was observed between Sd and $ZnSO_4$ (0.88, 95%CI; 0.74-1.02) followed by AGP and Sd (0.75, 95%CI; 0.62-0.88), $ZnSO_4$ and DS (0.65, 95%CI; 0.52-0.78), AGP and $ZnSO_4$ (0.648, 95%CI; 0.51-0.77), DS and Sd (0.55, 95%CI; 0.43-0.68), and DSM and AGP (0.38, 95%CI; 0.27-0.48). By taking DS as a gold standard, all tests showed 100 percent sensitivity results and same trend was observed in case of negative predictive values (NPV). Highest specificity was shown by ZnSO4, followed by Sd and AGP. Similar trends were observed in the positive predictive values (PPV). Then, by taking AGP as gold standard, all tests showed 100 percent specificity and positive predictive values (PPV) while, Sd showed highest sensitivity followed by ZnSO4 and AGP and similar trend was observed regarding NPV of tests. To this end, most effective test is AGP followed by Sd, $ZnSO_4$ and DS method. Moreover, AGP is the most suitable method for diagnosing the fasciolosis in early stages. Total economic losses due to fasciolosis during three months (Oct. – Dec. 2004) were found very high i.e Rs. 1016400.

Keywords: *Fasciola hepatica,* comparison, diagnostic tests, buffaloes

Introduction

Domestic buffaloes (*Bubalus bubalis*) are the premier dairy and meat producing animals of Asia and Africa and Fasciolosis is undoubtedly the most important helminth affecting buffaloes in these continents due to presence of low-lying swampy areas infested with snails (intermediate host).

These parasites enter into the liver parenchyma, cause hemorrhages and damage the tissue that lead to cirrhosis in chronic cases (Irfan, 1968; Urquhart *et al*., 2000). F. hepatica is found in temperate region and in cooler areas of high altitude whereas F. gigantica dominates the tropical region. The principal pathogenic effects of flukes are anaemia and hyper-albuminaemia. More than 0.5 ml blood per fluke per day can be lost within the bile duct (Urquhart *et al*., 2000). In acute form, there is massive invasion due to immature flukes into the liver which cause sudden death while in chronic from, there is liver cirrhosis caused by the wandering flukes which when mature, lodge into the bile ducts. Calcification of bile ducts and enlargement of gall bladder has been noticed in chronic cases. Sub mandibular edema frequently occurs. The parasites may cause loss of production during winter in milking cows. Its life cycle and prevalence is dependant on climate, which estimate the severity of disease. The factors influencing the population of large number of metacercariae, necessary for outbreaks of fasciolosis depend upon the availability of suitable snail habitat, temperature and moisture (*Urquhart et al*., 2000). *Fasciola* remains endemic throughout the year, with a higher percentage of infection in buffaloes as compared to cattle (Jithendran and Bhat, 1999). In fluke infested areas, this disease entity causes losses in terms of high morbidity and mortality (Bhattacharya and Laha, 1995) leading to heavy economic losses (Togerson, 1999).

The major consequence of infection is hepatic fibrosis. There is traumatic destruction of liver parenchyma, hemorrhages and necrosis. Migration of flukes also results in thrombus formation in hepatic veins that leads to ischemia. Healing leads of fibrosis.

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Subsequent contraction of scar tissues result in considerable distortion of the hepatic architecture (Rhuston and Murray, 1977).

For diagnosis of fasciolosis, carpological technique is considered to be the parasitological method usually employed. The only disadvantage of this technique is its disease occurs as early as 3 weeks after infestation, but parasitological diagnosis possible at 10th week when eggs begin to appear in faeces (Fagbemi and Oberisiagban, 1990). The immuno-diagnostic approach for detection of fasciolosis, has been used for years (Zimmerman *et al*., 1982; Swarp *et al*., 1987; Santiago and Hillyer, 1988; Fagbemi and Oberisiagban, 1990) which makes early diagnosis possible.

Due to low fecundity of trematodes or presence of immature stages, it becomes difficult to diagnose through faecal examination. Recently sero-diagnostic techniques are being recommended for the diagnosis of different parasites. The present study includes comparison of carpological and different sero-diagnostic methods in practice. It also includes the estimation of economic losses in terms of liver condemnation.

Materials and Methods

The study was conducted at the Department of parasitology, University of Veterinary and Animal Sciences, Lahore (Pakistan). To this end, a total of 200 fecal samples from the buffaloes suspected for fasciolosis were collected from the buffaloes attending at slaughterhouse of metropolitan corporation, Lahore (Pakistan). The samples were examined by Direct smear, Zinc sulphate floatation and Sedimentation techniques as described by Foreyt, (2001). Blood samples were also collected from each buffalo. Sera thus collected were stored at 20 ºC. Agar gel precipitation test was used for serodiagnosis (Bui Khanh linh *et al*., 2003).

In order to record the prevalence of fasciolosis in buffaloes, slaughterhouse of Lahore was surveyed at weekly intervals during the period of three months. Liver, bile duct and gall bladder of each of 200 randomly selected buffaloes were examined for the presence of flukes. To this end, each bile duct, gall bladder and liver was cut transversely two to three times and squeezed to expel flukes from the bile ducts. Each liver was cut into 20 small slices, pressed to squees-out flukes from liver. Few flukes were stained in fresh state with semichon's carmione and were identified on the basis of their characteristic features (Soulsby, 1982).

Statistical Analysis: Sensitivities, specificities, accuracy, and predictive values were calculated by standard formulas (Martin, 1977). Kappa statistics were calculated using standard Win Episcope, version 2.0).

Results and Discussion

Results of 4 different diagnostic tests viz., Direct smear method (DSM), Sedimentation test (Sd), Zinc sulfate test ($ZnSO_4$) flotation, and Agar gel precipitation test (AGPT) performed on buffaloes (n = 200) for detection of fasciolosis with respect to age (Young, 60; Adult, 140), sex (Male, 32; Female, 168) and breed (Nili Ravi, 170; Kundi, 30) are shown in the Table I. Among young animals (<2yrs) highest prevalence was shown by Sd (4; 6.6%) and DSM (4; 6.6%), while among adult animals (>2yrs) highest prevalence was detected by AGPT (12; 8.5%). Same trend was observed in sex-wise (both male, 32; female, 168) prevalence among buffaloes. Among different breeds viz., Nili Ravi (170; 85%) and Kundi (30; 15%) of buffaloes observed in this study, highest prevalence was observed by AGPT as 14 (8.2%) and Sd (2; 6.6%) and AGPT (2; 6.6%), respectively.

In the present study DSM was used for the diagnosis of fasciolosis of the 200 fecal samples examined and prevalence was found to be 2 per cent which coincides with the previous studies conducted by Ducommun and Pfister (1991), Maqbool *et al*. (1994), and Aal Abdel *et al*. (1999) which reported 1.6, 1.79 and 3.9 percent prevalence of fasciolosis, respectively. Minor difference may be due to difference in environment and seasonal condition as summer infection of snails in more severe than winter infection (Sousby, 1982). In the present study, a total of 200 fecal samples of buffaloes were examined by $ZnSO_4$ 8 were found positive thus a prevalence of 4%. Mahdi and Al-Badawa (1987) found 4.8 % prevalence of fasciolosis by using this technique. Sd detected 5% prevalence. Lotfy (2003) reported nearly similar results by using this technique, while AGPT detected 8% prevalence that was proved to be highly sensitive and improved test for early diagnosis of fasciolosis. Ahmad *et al.* (2004), reported 15 % prevalence, respectively. To this end, it appears that AGPT proved to be the most effective test followed by Sd, $ZnSO_4$ and DSM.

In the present study prevalence of fasciolosis was higher in older buffaloes (above 2 years) 15.7% than younger (below 2 year) i.e. 10 %. Many workers have reported an increased incidence of fasciolosis in buffaloes and cattle with advancing age. Lin *et al*. (1974), Soecetya (1975), AlBarwari (1977), Mahdi and Al Badawi (1987), Swarp *et al.* (1987), Wee *et al*. (1987), Chowdhury *et al*. (1994), Maqbool *et al.* (1994), Maqbool et al (2002) and Mairia (2003) also reported similar observations. Hence, our observations are inline with those mentioned above. A higher infection

Table-1. Percent prevalence of fascioliasis in buffaloes with respect to age, sex and breed by using different diagnostic tests.

Groups/	No. of buffaloes	DSM*		Sd**		ZnSO4***		AGPT****	
Sub-groups	(n= 200)	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
Age									
Young (<2yrs)	60 (30%)	0(0%)	60(100%)	4 (6.6%)	56(93.3%)	2(3.3%)	58(96.6%)	4(6.6%)	56 (93.3%)
Adult (>2yrs)	140 (70%)	4(2.85%)	136 (97.1%)	6 (4.2%)	134 (95.7%)	6 (4.2%)	134(95.7%)	12 (8.5%)	128(91.4%)
Sex									
Male	32 (16%)	0 (0%)	16 (100%)	2 (6.2%)	14 (43.7%)	0 (0%)	16 (100%)	2 (6.2%)	14(43.7%)
Female	168 (84%)	4 (2.36%)	80 (4.7%)	8 (4.7%)	76 (45.2%)	8 (4.7%)	76 (45.2%)	14 (8.3%)	60 (35.7%)
Breed									
Nili Ravi	170 (85%)	4 (2.3%)	166 (97.6%)	8 (4.7%)	162 (95.2%)	8 (4.7%)	162 (95.2%)	14 (8.2%)	156 (91.7%)
Kundi	30 (15%)	0 (0%)	30 (100%)	2 (6.6%)	28 (93.3%)	0 (0%)	30 (100%)	2 (6.6%)	28 (93.3%)

* DSM – Direct smear method, ** AGPT – Agar gel precipitation test, *** Sd – Sedimentation test
**** $ZnSO_4$ – Zinc Sulfate flotation test

rate in females (15.4%) as compared to males (6.4%) may be due to the social practice of keeping female animals under good managemental conditions as compared with the males which are kept for the breeding purpose and let loose freely to graze on pasture (Bedarkar *et al*., 2000; Fuenmayor *et al*., 2000). Agreement (Kappa statistics) between different diagnostic tests was calculated (Table-2). The kappa values vary between 0-1 i.e., good to excellent. The highest agreement was observed between Sd and $ZnSO_4$ (0.88, 95%CI; 0.74-1.02) while evaluating the fasciolosis positive and negative buffaloes followed by AGPT and Sd (0.75, 95%CI; 0.62-0.88), ZnSO4 and DSM (0.65, 95%CI; 0.52-0.78), AGPT and ZnSO4 (0.648, 95%CI; 0.51-0.77), DSM and Sd (0.55, 95%CI; 0.43-0.68), and DSM and AGPT (0.38, 95%CI; 0.27-0.48).

In order to compare different diagnostic tests for detection of fasciolosis in buffaloes, sensitivity was calculated as percentages of fasciola positive buffaloes as positive and specificity was calculated as the percentages of fasciola negative buffaloes as negative in the related test (Table-3). By taking DSM as a gold standard, all tests showed 100 percent sensitivity results and same trend was observed in case of negative predictive values (NPV). Highest specificity was shown by ZnSO4 as 97.95 (95%CI; 95.98-99.93), followed by Sd and AGPT as 96.93 (95%CI; 94.5-99.35) and 93.87 (95%CI; 90.52-97.81), respectively. Similar trends were observed in the positive predictive values (PPV). Then, by taking AGPT as gold standard, all tests showed 100 percent specificity and positive predictive values (PPV) while, Sd showed highest (62.5; 95%CI, 38.7-47.6) sensitivity followed by ZnSO4 (50.0; 95%CI, 25.5-74.5) and AGPT (25.0; 95%CI, 3.7-46.2) and similar trend was observed regarding NPV of tests. Fecal examination was not enough for diagnosis of this disease and the immunological diagnosis (AGPT) of fasiolosis in buffaloes based on the detection of antibodies has proved to be very successful as compared to the usual coprological methods.

It was previously reported that 4% of 92400 lives of buffaloes were condemned during the period of three months study die to fluke infection or lesions attributed to liver in the present study. The total cost/loss of Rs. 1016400/- was calculated during the period of study, this is in line with the previous studies conducted by Miangi and Gichingi (1999), Togerson (1999), Machincka Barbara (2000), and Kithuka *et al.* (2002).

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Table-2. Performance of the four diagnostic tests and 95% confidence interval by taking direct smear method and agar gel precipitation test as gold standards separately.

	Diagnostic tests by taking DSM as gold standard			Diagnostic tests by taking AGPT as gold standard		
	ZnSO4	Sd	AGPT	ZnSO4	Sd	DSM
SN	100 (95%CI; 100.0-100.0)	100 (95%CI; 100.0-100.0)	100 (95%CI; 100.0-100.0)	50.0 (95%CI; 25.5-74.5)	62.5 (95%CI; 38.7-47.6)	25.0 (95%CI; 3.7-46.2)
SP	97.95 (95% CI; 95.98-99.93)	96.93 (95% CI; 94.5-99.35)	93.87 (95% CI; 90.52-97.81)	100 (95%CI; 100.0-100.0)	100 (95%CI; 100.0-100.0)	100 (95%CI; 100.0-100.0)
PPV	50.0 (95% CI; 15.35-84.64)	40.0 (95% CI; 9.63-70.36)	25.0 (95% CI; 3.78-46.2)	100 (95%CI; 100.0-100.0)	100 (95%CI; 100.0-100.0)	100 (95%CI; 100.0-100.0)
NPV	100 (95%CI; 100.0-100.0)	100 (95%CI; 100.0-100.0)	100 (95%CI; 100.0-100.0)	95.83 (95%CI; 93.0-98.6)	96.8 (95%CI; 94.3-99.3)	93.8 (95%CI; 90.5-97.2)

DSM – Direct smear method, ZnSO4 – Zinc sulfate flotation test, Sd –Sedimentation test
AGPT – Agar gel precipitation test, SN - Sensitivity, SP - Specificity, PPV – Positive predictive value, NPV – Negative predictive value

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