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# COMPARATIVE STUDY FOR DIAGNOSIS OF FASCIOLIASIS IN CATTLE AND BUFFALOES BY ANTIBODY AND ANTIGEN DETECTION

(With 5 Tables and One Figure)

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## دراسة تشخيصية مقارنة لمرض الفاشيولا في الابقار والجاموس بالكشف عن مولدات الضد والاجسام المضادة

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يعد مرض الفاشيولا من أهم الأمراض التى تصيب المجترات وتسبب خسائر إقتصادية هامة. وتتبنى الدراسة الحالية أسلوبين مختلفين فى تشخيص هذا المرض فى الأبقار والجاموس المصاب طبيعياً بالفاشيولا. يعتمد الأسلوب الأول على الكشف عن الأجسام المضادة التى يفرزها الجهاز المناعى للحيوانات فى السيرم ضد أنتيجينات الطفيل أما الأسلوب الثانى فيعتمد على الكشف عن الأنتجيينات نفسها. ويتم تقييم كفاءة هذين الأسلوبين مقارنة بالإختبارات الطفيلية التقليدية. تم فى هذه الدراسة فحص ١٣٩ عينة من الجاموس المصرى وكذلك ٨٠ من الأبقار المحلية للبحث عن بويضات طفيل الفاشيولا وكذلك تم فحص عينات السيرم الخاصة بالحيوانات المذكورة للبحث عن الأجسام المضادة الموجهة ضد إفرازات وإخراجات الطفيل وكذلك ضد أنتيجينات الديدان البالغة والبويضات وذلك باستخدام إختبار الإليزا الغير مباشر ثم تم الكشف عن الأنتيجينات ذاتها فى عينات السيرم (الأنتيجينات السارية) وكذلك فى عينات البراز (الأنتيجين البرازى أو الكوبرو أنتيجين) وذلك باستخدام اختبار السندوتش إليزا ... وقد أسفرت النتائج عن تفوق اختبارى الكشف عن الأجسام المضادة والانتيجينات على الإختبارات الطفيلية التقليدية كما أظهرت النتائج تفوق إختبار الكشف عن الأجسام المضادة الخاصة بإفرازات وإخراجات الطفيل (٦٨,٩%) على تلك الخاصة بالديدان البالغة (٦٤,٤%) والبويضات (٥٧,٩%). بينما أثبت إختبار الكشف عن الانتيجينات السارية كفاءة فى تشخيص مرض الفاشيولا (٦٦,٧%) على إختبار الكشف على الأجسام المضادة ضد كل من أنتجينات الديدان البالغة والبويضات وذلك فى جميع الحيوانات التى تم اختبارها. ولكن أثبت إختبار تحديد الأجسام المضادة ضد إفرازات وإخراجات الطفيل تفوق (٦٨,٩%) على إختبار الكشف عن الانتجينات السارية فى عينات السيرم الخاصة بالجاموس والأبقار معا. وقد لوحظ فى عينات الأبقار أن إختبار تحديد الانتيجينات السارية سجل نسبة إصابة لمرض الفاشيولا أعلى من تلك التى سجلت بواسطة اختبار الكشف عن الأجسام المضادة الموجهة ضد إفرازات وإخراجات الطفيل وضد أنيتجينات الديدان البالغة وكذلك البويضات. ومن ناحية أخرى أثبت الكشف عن الانتيجين البرازى تفوقه على كل

الإختبارات المقدمة فى هذا البحث وسجل أعلى نسبة تواجد لمرض الفاشيولا بين الجاموس والأبقار (٨١%) وهكذا يقدم البحث الحالى إقتراح باستخدام كل من اختبار الكشف عن الانتجين البرازى وكذلك إختبار الكشف عن الأجسام المضادة الخاصة بإفرازات وإخراجات الطفيل معاً فى تشخيص مرض الفاشيولا فى الجاموس أما فى الأبقار فيوصى باستخدام كل من اختبار الكشف عن الانتجين البرازى وكذلك الانتيجين السارى معاً لتشخيص مرض الفاشيولا.

## SUMMARY

As a disease of domestic ruminants, fascioliasis is of considerable economic importance. The current research adopted two main methods for diagnosis of fascioliasis in naturally infected cattle and buffaloes. Antibody and antigen detection were compared with the conventional parasitological examination. A total of 139 Egyptian buffaloes and 80 native breed cattle were examined parasitologically for *Fasciola* eggs detection in faeces. The serum samples of animals were examined for antibodies detection to excretory/secretory products, adult worm extract and egg antigen by indirect ELISA. These samples together with faeces of the same animals were investigated by sandwich ELISA for circulating and coproantigen detection respectively. The results proved that antibody and antigen detection by far better than parasitological examination. Moreover, antibody detection to excretory/secretory (ES) products is more sensitive (68.9%) in the diagnosis of fascioliasis than to adult worm extract (64.8%) and to egg antigen (57.9%) in the examined animals. Furthermore, circulating antigen detection assay is preferable (66.7%) than antibody detection assays to both adult worm and egg antigen in the total examined bovines. While antibody detection assay to ES products recorded higher infection rate (68.9%) than circulating antigen detection one. These comparative diagnostic potentials are also observed in buffaloes but with different infection percentages. It is worthy to note that in cattle, detection of circulating antigen recorded infection percentage higher than that recorded using antibody detection assays to ES products, adult worm and egg antigens. Coproantigen detection assay possesses the highest immunodiagnostic potential for fascioliasis either in cattle or in buffaloes than any other assays introduced in the present study and recorded infection percentage reached to 81% in the total bovines. In conclusion, the current research recommended a combination of both coproantigen detection assay and antibody detection test to ES products for fascioliasis diagnosis in

buffaloes, while in cattle, circulating and coproantigen detection assays are recommended.

**Key words:** *Fascioliasis, diagnosis, antibody, antigen, ELISA.*

## INTRODUCTION

Fascioliasis is caused by infection with *Fasciola hepatica* and *Fasciola gigantica* parasites. It is not only an important human disease but also affects buffaloes, cattle and sheep, causing worldwide economic losses of approximately two billion dollors per year (Torgerson and Claxton, 1999). Diagnosis of fascioliasis is usually accomplished by conventional parasitological techniques based on coprology. However, this approach lacks accuracy and sensitivity. While pathology and disease occur as early as 3 weeks post infection, parasitological diagnosis is only possible at about 10-14 weeks after infection when eggs begin to appear in faeces. At that time, damage of the liver parenchyma may be severe (Anderson *et al.*, 1999). Serological diagnosis is an alternative approach to overcome the deficiencies of fascioliasis diagnosis by parasitological means (Chen and Mott, 1990; Hillyer *et al.*, 1992 and Hillyer, 1993). Serodiagnosis of *F.hepatica* infection in cattle and sheep is repeatedly reported (Anderson *et al.*, 1999; Ortiz *et al.*, 2000; Abdel-Aziz *et al.*, 2001; Cornelissen *et al.*, 2001 ; Mezo *et al.*, 2003 and Concelcao *et al.*, 2004). While few trials have been done on serodiagnosis of *F.gigantica* infection in ruminants (Swarup *et al.*, 1987; Fagbemi and Obarisiagbon, 1990 and Guobadia and Fagbemi, 1995). Serodiagnosis of fascioliasis is generally performed by ELISA using different antigen preparations (Chen and Mott, 1990; Hillyer *et al.*, 1992; Fagbemi and Guobadia, 1995; Clery *et al.*, 1996 ; Sampaio-Silva *et al.*, 1996 ; Intapan *et al.*,2003 and Dalimi *et al.*,2004). Alternatively, direct measurement of *Fasciola* antigens shed into sera (Sanchez- Andrade *et al.*, 2000 and 2002; Paz-Silva *et al.*, 2003 and Velusamy *et al.*, 2004) or faeces (Dumenigo *et al.*, 1996; Abdel-Rahman *et al.*, 1998; Dumenigo and Mezo, 1999; Almazan *et al.*,2001 and Mezo *et al.*,2004) of infected animals is described. These antigen detection tests are preferable than antibody detection ones in that antigenaemia implies current rather past infections. Moreover, antigen detection minimizes false diagnosis due to parasites cross-reactivity. As treatment in the initial stages of the infection considerably reduce liver injury, it is therefore desirable to have a simple, sensitive and specific test for the early diagnosis of fascioliasis. Consequently, the current

research adopts two approaches to develop more sensitive diagnostic methods for diagnosis of *F.gigantica* infection in cattle and buffaloes. One approach focused on antibodies detection in sera of infected animals by indirect ELISA using excretory / secretory products, adult worm crude extract and egg antigen. The second approach relied on detection of circulating *F.gigantica* antigen in bovines sera and coproantigen in fecal samples of infected animals using sandwich ELISA.

## MATERIALS and METHODS

- **Animals:** A total number of 139 Egyptian buffaloes and 80 native breed cattle located in Giza governorate were used to perform this study. Serum and faecal samples were collected from each animal for parasitological and immunological studies.

- **Samples:**

**Serum samples:** used for antibody detection ELISA (indirect ELISA) and for detection of circulating *F.gigantica* antigen in bovine sera.

**Faecal samples:** used for parasitological examination and detection of coproantigen by sandwitch ELISA.

- **Parasites:** *F.gigantica* adult worms were collected from condemned livers of buffaloes slaughtered in Cairo abattoir. Eggs of *F.gigantica* were collected from gall bladder of slaughtered buffaloes.

- **Antigen preparation:**

**A: Egg and whole worm antigen**. *F.gigantica* eggs and adult worms were washed thoroughly with distilled water to remove all traces of host tissues and bile. Antigens were prepared by homogenizing eggs and worms separately in 0.15M phosphate buffer saline (PBS), pH 7.2 supplemented with 2mM phenyl methyl sulphonyl fluoride (PMSF) and 0.02% $NaN_3$ in a ten Broeck tissue grinder. The homogenates were centrifuged at 10.000 rpm at 4°C for 1h. Clear supernatants corresponding to each antigen were collected as egg and worm antigens.

**B: Excretory/secretary antigen (ES).** *F.gigantica* adult worms were washed several times in dechlorinated water and incubated in RPMI 1640 medium pH 7.3, containing 2% glucose, 20 mM Hepes and 25 mg/L gentamicin at 37°C overnight as described by McGonigle and Dalton (1995). The culture medium (ES) was centrifuged and the supernatant was lyophilized then reconstituted in small amounts of physiological saline in use.

- **Faecal specimen preparation.** Faecal supernatants were prepared for coproantigen immunodetection according to Allan *et al.* (1990) by

vigorously shaking the fecal sample in an equal volume of PBS containing 0.3% tween until a slurry formed. The fecal suspensions were centrifuged at 2000x g for 30 min. The supernatants were used in sandwitch ELISA.

- **Rabbit immunization with ES antigen.** Three native breed (1.5kg) rabbits were immunized subcutaneously with *F. gigantica* ES antigen for preparation of hyperimmune serum as described by Fagbemi *et al.* (1995).

- **Antibody detection ELISA.** ELISA for the detection of antibodies to *F.gigantica* whole worm, egg and ES antigens was performed as described by Wijffels *et al.* (1994). In brief, the optimum antigen concentration and sera dilution were determined by checkerboard titration. ELISA plates were coated with 80μg/ml of each antigen. The selected dilution of the examined sera was 1: 100, and of anti-bovine IgG horse-radish peroxidase conjugate was 1: 1000. ELISA OD cut off values were calculated by the method of Abdel-Rahman *et al.* (1998).

- **Antigen capture ELISA.** A sandwich ELISA for detection of circulating ES antigen in serum samples and coproantigen in faecal suspensions was performed as described by Espino *et al.* (1998).

## RESULTS

**I- Parasitological examination.**

A total of 139 buffaloes and 80 native breed cattle located in Giza Governorate were examined parasitologically for detection of *Fasciola* eggs. In buffaloes, the examination revealed 53 animals infected with *Fasciola* (38.1%). While in cattle 21 animals were infected recording a percentage of 26.2%. Collectively, from a total of 219 buffaloes and cattle only 74 animals were infected with *Fasciola* (33.8%) as shown in table 1.

**II- Immunological examination .**

**A. Antibody detection**

**1- Antibodies reacted with ES antigen.**

Detection of antibodies against *F.gigantica* infection using ELISA plates sensitised by ES antigen revealed that 95 from 139 buffaloes serum samples (68.3%) were positive as indicated by the obtained OD values. 56 from a total of 80 cattle serum samples (70%) tested positive for ES antigen. In bovines, 151 from 219 samples (68.9%) reacted positively with ES antigen as observed in table 1 and

figure 1.The positive samples showed different degrees of positivity as depicted in table 1.

**2- Antibodies reacted with adult worm antigen.**

As depicted in table 2 and figure 1, antibodies in 92 buffaloes serum samples reacted positively with adult worm antigen in ELISA with a percentage of 66.2%. In cattle 50 from 80 samples showed positivity with the same antigen recording 62.5%. Consequently, 142 from a total of 219 examined animals (64.8%) tested positive to adult worm antigen (Table 2 and Figure 1).

**3- Antibodies reacted with egg antigen.**

Indirect ELISA results revealed that 66 from 139 buffaloes serum samples (47.5%) showed positive reaction with egg antigen. While 61 from 80 cattle samples (76.2%) tested positive. From a total of 219 examined animals, 127 tested positive (57.9%) with egg antigen as shown in table 3 and figure 1.

**B. Antigen detection**

**1- Detection of circulating *F.gigantica* antigen.**

Detection of circulating antigen in the serum samples of buffaloes revealed 77 positive samples with a percentage of 55.4%. In cattle 69 samples carry circulating antigen recording 86.2% . In the total examined bovines, 146 samples were positive (66.7%) as shown in table 4 and figure 1.

**2- Detection of *F.gigantica* coproantigen.**

Detection of coproantigen in the faeces of buffaloes recorded a percentage of positivity reached to 78.4%. In cattle, coproantigen detection assay was able to detect 91.7% of infected animals. Collectively, 81% of total examined bovines tested positive to coproantigen as shown in table 5 and figure 1.

## DISCUSSION

The key to the success of any diagnostic assay which detects antibodies to a given organism is the development of a satisfactory antigen(s). *Fasciola gigantica* is a complex organism that exposes the host to a mosaic of antigens as glandular secretions, surface antigens and digestive excretory products. Each developmental stage of *F.gigantica* may also have unique antigenicity, as well as those common to all stages of the life cycle, thus complicating the isolation of the appropriate antigens. Moreover, not all *F.gigantica* life stages are easily obtained. Adult worms as well as eggs of *F.gigantica* were chosen in the present study as antigen sources because they are readily obtained in substantial

amounts and it was previously suggested that potential antigens in fascioliasis are found in both stages (Reddington *et al.,* 1984).

The immunoenzymatic techniques such as ELISA have been found to be very suitable for the diagnosis of fascioliasis due to their high sensitivity and the possibility of processing many sera samples (Arriaga de Morilla *et al.,* 1989). These techniques which based on detection of antibodies have been successfully utilized to detect early infection (Paz-Silva *et al.,* 1998). In the current research, detection of antibodies reactive to ES products, adult worm and egg antigens in the bovine serum samples by indirect ELISA revealed that 151 from 219 animals (68.9%), 142 form 219 animals (64.8%) and 127 from 219 animals (57.9%) were *F.gigantica* positive seroreactors in the total examined cattle and buffaloes, respectively (Table 1, 2, 3). One of the great advantage of these serological tests is the capability to detect IgG anti *Fasciola* antibodies from the second week post infection, which is remarkably early in the diagnosis of this disease (Fagbemi and Guobadia, 1995 and Ibarra *et al.,* 1998). However, persistence of antibodies for long periods after cure makes it unsuitable for prediction of success of chemotherapy (Rodriguez-Peres and Hillyer, 1995). Comparing these results with that of coprological examination (33.8%), performed in the current research, demonstrated the advantage of antibody detection assay over coprological method. This observation was previously reported by Chen and Mott (1990), Hillyer *et al.* (1992) and Hillyer (1993). Moreover, results also proved the potency of antibody detection to ES products than to adult worm or egg extracts. This notion was expected where Santiago and Hillyer (1988), Sinclair and Wassall (1988), Ferre *et al.* (1995) and Martinez *et al.* (1996) indicated that ELISA with ES products as antigen has been proved to be a sensitive, specific and an early method for diagnosis of *F.hepatica* infection in cattle and sheep. However, disadvantages of immunodiagnostic tests based on antibody detection were that they did not discriminate between recent, past or current infections and also lack specificity due to cross reaction phenomenon (Guobadia and Fagbemi, 1996 and Hassan *et al.,* 2001).

Concerning antigen detection approach adopted in the current research which included detection of circulating *F.gigantica* antigen in the serum samples and coproantigen in the faeces by sandwich ELISA, results revealed that 66.7% and 81.0% of the total examined animals were infected with *F. gigantica,* respectively (Table 4 and 5). Matching these percentages with that of antibody detection ones revealed that

coproantigen detection recorded the highest percentage (81.0%) while circulating antigen detection assay recorded 66.7% which is higher than antibody detection to both adult worm (64.8%) and egg (57.9%) antigens. Meanwhile, detection of antibodies to ES products recorded higher percentage in diagnosis (68.9%) than circulating antigen detection (66.7%). In buffaloes, this observation is also true where the infection percentage using antibody detection assay to ES products is higher (68.3%) than circulating antigen detection one (55.4%). These results clarified two main points; first, it confirmed again the potency of ELISA with ES products as antigen in the diagnosis of fascioliasis in bovines. The second it did not exclude the hypothesis that few numbers of examined animals possibly exposed to chemotherapy. Where negative absorbance values were obtained 3 weeks after chemotherapy using circulating antigen detection assay, while positive absorbance values were still recorded 6 weeks post chemotherapy using antibody assay (Fagbemi *et al.*, 1995). On the other hand, circulating antigen detection assay in cattle is better for diagnosis than antibody detection assays to ES products, adult worm and egg antigens as proved in the current research. In contrary, Abdel-Aziz *et al.* (2001) stated that ELISA technique using *F.hepatica* ES antigen is of diagnostic value for cattle fascioliasis rather than for buffaloes. This could be attributed to *Fasciola* species. It is worthy to note here that few studies have been done on the immunodiagnosis of fascioliasis by the detection of circulating antigens. Among them, those of Viyanant *et al.* (1997); Abdel-Rahman and Derbala (1999); Dumenigo *et al.* (1999); Almazan *et al.* (2001); Attallah *et al.* (2002); Paz-Silva *et al.* (2003) and Velusamy *et al.* (2004), who proved its potential in fascioliasis diagnosis.

The current results also proved that coproantigen detection assay possesses potential in the diagnosis of fascioliasis in buffaloes and cattle over other assays introduced in the present study. Detection of coprooantigen in the faeces of cattle was previously studied by Dumenigo *et al.* (1996) using antibodies to ES products, and they proved a direct correlation between antigen concentration in faeces and the number of adult flukes. The correlation was also proved between antigen concentration in the faeces and egg counts (Espino and Finlay, 1994). The high sensitivity of this assay facilitates detection of low fluke burdens in which the antigen is highly diluted in a large amount of ingesta, as in the case of cattle and buffaloes. The proved correlation between the assay and the number of flukes suggest the possibility of estimating the fluke burden. It is also possible to detect early bile duct

infection before adult fluke mature and start to shed eggs. In addition, the capture ELISA for coproantigen has two distinct advantages over the other diagnostic assays; detection of the antigen as long as the parasite is present in the bile duct and simplicity of faecal specimen collection from pasture or yards.

Collectively, the present study concluded that coproantigen detection assay is scientifically preferable than other assays introduced in the current research for fascioliasis diagnosis in naturally infected cattle and buffaloes. Moreover, antibody detection ELISA to ES products also proved potency in the diagnosis of fascioliasis in buffaloes, while in cattle coproantigen and circulating antigen detection assays are recommended. Consequently, the present study strongly recommended the adoption of coproantigen detection assay in combination with antibody detection assay to ES products in fascioliasis diagnosis in buffaloes while in cattle coproantigen and circulating antigen detection assays are recommended for epidemiological evaluation of therapeutic strategies or protocols.

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**Table 1 :** Detection of antibodies against *F. gigantica* infection in buffaloes and cattle by ELISA using ES antigen.

| Parameter | | Parasitological Examination | | | | ELISA using *F.gigantica* ES antigen | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Positive reactors | | | | | | | | | | Negative reactors | |
| Animals | No. of examined animals | +ve | | -ve | | Total positive | | + (Light)* | | ++(Moderate)** | | +++ (Heavy)*** | | | | | |
| | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | | |
| Buffaloes | 139 | 53 | 38.1 | 86 | 61.9 | 95 | 68.3 | 39 | 28.1 | 29 | 20.8 | 27 | 19.4 | 44 | 31.7 | | |
| Cattle | 80 | 21 | 26.2 | 59 | 73.8 | 56 | 70.0 | 35 | 43.8 | 16 | 20.0 | 5 | 6.2 | 24 | 30.0 | | |
| Total examined animals | 219 | 74 | 33.8 | 145 | 66.2 | 151 | 68.9 | 74 | 33.8 | 45 | 20.5 | 32 | 14.6 | 68 | 31.1 | | |

* + (Light infection): 0.500 > Value ≥ 0.369; ** ++ (Moderate infection): 0.700 > Value ≥ 0.500; *** +++ (Heavy infection): Value ≥ 0.700.

**Table 2 :** Detection of antibodies against *F.gigantica* infection in buffaloes and cattle by ELISA using crude adult worm antigen.

| Parameter | | Parasitological Examination | | | | ELISA using *F.gigantica* crude adult worm antigen | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Positive reactors | | | | | | | | Negative reactors | |
| Animals | No. of examined animals | +ve | | -ve | | Total positive | | + (Light)* | | ++(Moderate)** | | +++ (Heavy)*** | | | |
| | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| Buffaloes | 139 | 53 | 38.1 | 86 | 61.9 | 92 | 66.2 | 50 | 36.0 | 30 | 21.6 | 12 | 8.6 | 47 | 33.8 |
| Cattle | 80 | 21 | 26.2 | 59 | 73.8 | 50 | 62.5 | 29 | 36.2 | 16 | 20.0 | 5 | 6.3 | 30 | 37.5 |
| Total examined animals | 219 | 74 | 33.8 | 145 | 66.2 | 142 | 64.8 | 79 | 36.0 | 46 | 21.0 | 17 | 7.8 | 77 | 35.2 |

* + (Light infection) : 0.400 > Value ≥ 0.334; ** ++ (Moderate infection): 0.600 > Value ≥ 0.400; *** +++ (Heavy infection): Value ≥ 0.600.

**Table 3** : Detection of antibodies against *F.gigantica* infection in buffaloes and cattle by ELISA using egg antigen.

| Parameter | | Parasitological Examination | | | | ELISA using *F.gigantica* egg antigen | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Positive reactors | | | | | | | | Negative reactors | |
| Animals | No. of examined animals | +ve | | -ve | | Total positive | | + (Light)* | | ++(Moderate)** | | +++ (Heavy)*** | | | |
| | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| Buffaloes | 139 | 53 | 38.1 | 86 | 61.9 | 66 | 47.5 | 42 | 30.2 | 19 | 13.7 | 5 | 3.6 | 73 | 52.5 |
| Cattle | 80 | 21 | 26.2 | 59 | 73.8 | 61 | 76.2 | 26 | 32.5 | 24 | 30.0 | 11 | 13.8 | 19 | 23.8 |
| Total examined animals | 219 | 74 | 33.8 | 145 | 66.2 | 127 | 57.9 | 68 | 31.1 | 43 | 19.6 | 16 | 7.3 | 92 | 42.0 |

* + (Light infection): 1.150 > Value ≥ 1.056; ** ++ (Moderate infection): 1.200 > Value ≥ 1.150; *** +++ (Heavy infection): Value ≥ 1.200.



**Table 4** : Detection of circulating antigen in serum samples of *F.gigantica* infected buffaloes and cattle by ELISA.

| Parameter | | Parasitological Examination | | | | ELISA using plates sensitized with rabbit polyclonal antibody raised against ES antigen | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Positive reactors | | | | | | | | Negative reactors | |
| Animals | No. of examined animals | +ve | | -ve | | Total positive | | + (Light)* | | ++(Moderate)** | | +++ (Heavy)*** | | | |
| | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| Buffaloes | 139 | 53 | 38.1 | 86 | 61.9 | 77 | 55.4 | 47 | 33.8 | 20 | 14.4 | 10 | 7.2 | 62 | 44.6 |
| Cattle | 80 | 21 | 26.2 | 59 | 73.8 | 69 | 86.2 | 26 | 32.5 | 26 | 32.5 | 17 | 21.2 | 11 | 13.8 |
| Total examined animals | 219 | 74 | 33.8 | 145 | 66.2 | 146 | 66.7 | 73 | 33.4 | 46 | 21.0 | 27 | 12.3 | 73 | 33.3 |

* + (Light infection): 0.820 > Value ≥ 0.752; ** ++ (Moderate infection): 0.880> Value ≥ 0.820; *** +++ (Heavy infection): Value ≥ 0.880.

**Table 5 :** Detection of coproantigen in faeces of *F.gigantica* infected buffaloes and cattle using ELISA.

| Parameter | | Parasitological Examination | | | | ELISA using plates sensitized with rabbit polyclonal antibody raised against ES antigen | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Positive reactors | | | | | | | | Negative reactors | |
| Animals | No. of examined animals | +ve | | -ve | | Total positive | | + (Light)* | | ++(Moderate)** | | +++ (Heavy)*** | | | |
| | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| Buffaloes | 51 | 36 | 70.6 | 15 | 29.4 | 40 | 78.4 | 22 | 43.1 | 12 | 23.5 | 6 | 11.8 | 11 | 21.6 |
| Cattle | 12 | 9 | 75.0 | 3 | 25.0 | 11 | 91.7 | 5 | 41.7 | 4 | 33.3 | 2 | 16.7 | 1 | 8.3 |
| Total examined animals | 63 | 45 | 71.4 | 18 | 28.6 | 51 | 81.0 | 27 | 42.9 | 16 | 25.4 | 8 | 12.7 | 12 | 19.0 |

* + (Light infection): 1.080 > Value ≥ 1.030; ** ++ (Moderate infection): 1.120> Value ≥ 1.080; *** +++ (Heavy infection): Value ≥ 1.120.

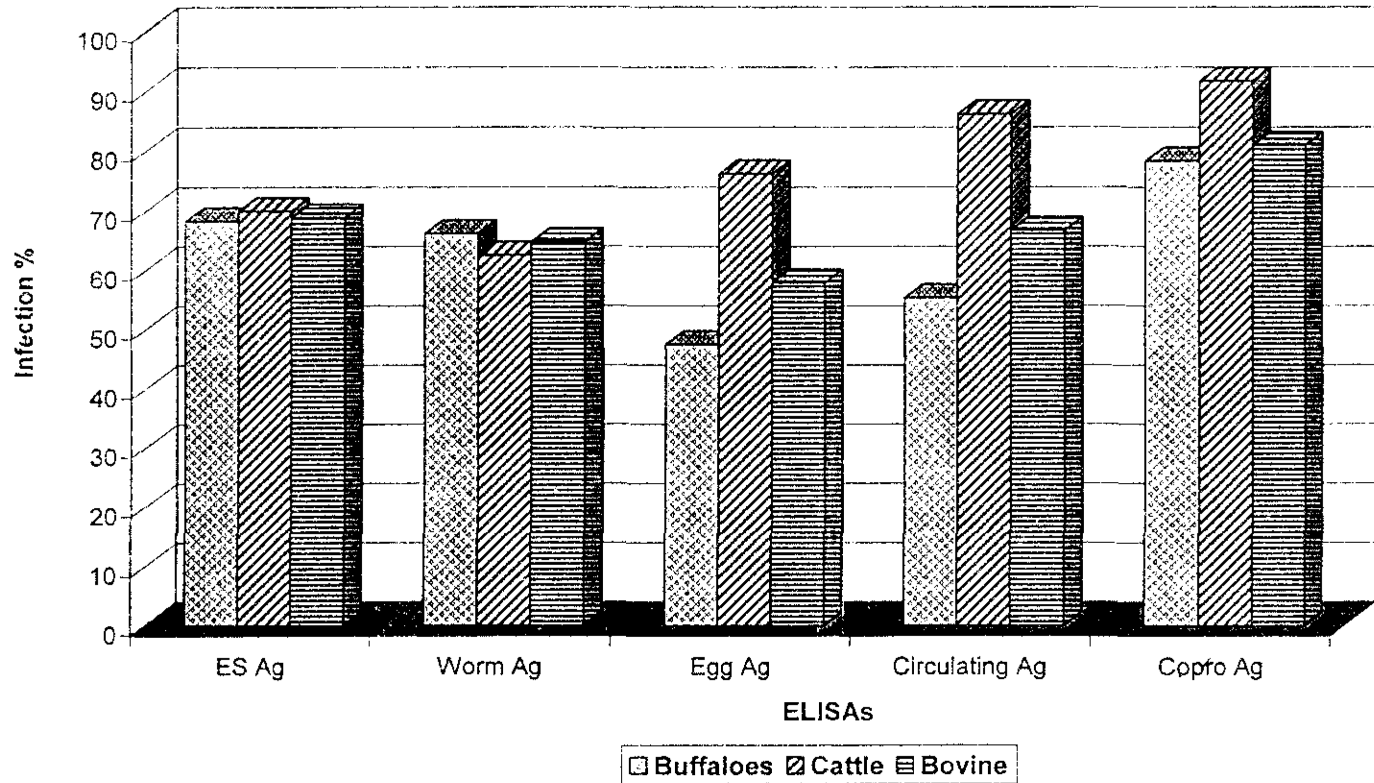


Fig. 1 : Comparative evaluation of five ELISAs techniques for fascioliasis diagnosis