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PARASITOLOGICAL AND SEROLOGICAL STUDY ON FASCIOLA DIAGNOSIS IN CATTLE AND BUFFALOES IN ASSIUT GOVERNORATE

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ABSTRACT

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The present study was carried out to investigate the prevalence of fascioliasis among 100 cattle and buffaloes of different age and sex in Assiut Governorate. Samples were examined by microscopic examination, Agar Gel Diffusion Test AGDT and ELISA techniques to detect *Fasciola species* eggs and antibodies, respectively. Prevalence of Fasciola was 8% by microscopic examination, 32% by AGDT and 60.86% by ELISA test. Cattle showed higher prevalence of infection 61.4% than buffaloes 29.4%. Female cattle and buffaloes were more susceptible to the infection 64.55% than male 23.8%. Fasciola was more frequently recorded in 2-4 years old cattle and buffaloes 76% followed by more than 4 years 46.7% then less than 2 years of age 20%. All positive results with fecal egg examination and AGDT were also positive with ELISA. Serological examination by AGDT and ELISA confirms the microscopical examination. The results of this study revealed that the agar gel diffusion test and ELISA could become a useful tools to diagnose fascioliasis in cattle and buffaloes.

Key words: *Fascioliasis, AGDT, ELISA, Assiut, prevalence.*

INTRODUCTION

Haridy *et al.* (2002); Mas-Coma, *et al.* (2005) and Soliman (2008) mentioned that fascioliasis is a serious infectious parasitic disease infecting domestic ruminants and humans, tops all the zoonotic helminthes worldwide. In Egypt, animal and human fascioliasis is an endemic clinical and epidemiological health problem.

Lotfy and Hillyer (2003); Dar *et al.* (2005); El-Shazly *et al.* (2006) and Periago *et al.* (2008) mentioned that losses due to animal fascioliasis in Egypt, were estimated at 190 million Livre Egyptienne (LE) annually according to the Egyptian Academy of Scientific Research and Technology Report. Both acute and chronic fascioliasis have been found in almost all governorates and in the reclaimed desert land. Fasciolosis is a widespread parasitosis responsible for immense economic losses in cattle and buffaloes in terms of condemnation of livers, decreased milk and meat production, loss of weight and poor carcass quality. So it was considered an important limiting factor for livestock productivity. It is characterized by sudden death with blood stained froth at the natural orifices in acute cases. Diarrhea, jaundice and bottle jaw are predominant features in chronic cases Solusby (1982); Meaney *et al.* (2002); Khan *et al.* (2009) and Kuchai *et al.* (2011).

Urquhart *et al.* (1996); Urquhart *et al.* (2000) and Yildirim *et al.* (2007) found that detection of Fasciola eggs is simple and confirmatory but it is 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 feces when the flukes are already matured (usually between 10 and 14 weeks of infection). By this time, major damages to the liver may have already occurred due to Fasciola enter into the liver parenchyma, cause hemorrhages and damage the tissue that lead to cirrhosis in chronic cases. So, Serological diagnoses have been developed as an alternative approach to fecal egg detection. Serological methods can test a large number of sera at a time and also detect infection earlier than fecal egg examination. There are evidences to show that serodiagnosis can detect the presence of infection as early as 2 weeks after infection. Enzyme Linked Immunosorbent Assay (ELISA) can detect serum antibodies to specific antigens of *Fasciola sp.* using adult fluke extracts. Santiago and Hillyer, (1988); Fagbemi and Guobadia, (1995); Sampaio-Silva *et al.* (1996) and Ghosh *et al.* (2005).

Linh *et al.* (2003) and Adedokun *et al.* (2008) revealed that the Agar Gel Diffusion Test (AGDT) could become a useful tool to diagnose fasciolosis in cattle and buffaloes. The test was specific for Fasciola infection because the Paramphistomum antigen did not react with sera from the cattle infected with

Fasciola sp. They detected that AGDT is also a simple and low cost technique. So, it considered a better test for the herd diagnosis of bovine fasciolosis for veterinarians and other investigators that lack suitable equipment for fecal examination and also have no access to an expensive serological ELISA kit.

Allam, (1992) found relatively high prevalence of Fasciola have been reported along the Nile valley, especially in Lower Egypt. Indeed, in the Northern part of the country, in Abis II village, 44% and 27%, were detected in cattle and buffaloes, respectively. While, Abdel-Aziz, (1993) showed that the prevalence of fascioliasis was 5.3% and 3.5% in cattle and buffaloes, respectively in Assiut. Also, Haridy *et al.* (1999) found that the prevalence of fascioliasis was 3.5% and 1.5% for cattle and buffaloes, respectively in Egypt.

Hussein and Khalifa (2010) found that the prevalence of fascioliasis was 28.6% in cows, 33.7% in buffaloes in Qena by coprological examination. Also, Hamed, *et al.* (2013) detected that the prevalence of fascioliasis was 4.4% by microscopic examination of buffaloes in Assiut. While, Abdo (2014) reported that the prevalence of fascioliasis by using fecal examination (sedimentation technique) among cattle and buffaloes was 7.41% and 11%, respectively in Assiut governorate. The present study aimed to spot light on the evaluation of the fecal egg examination, AGDT and ELISA in the determination of the prevalence of bovine fascioliasis in Assiut Governorate.

MATERIALS and METHODS

A survey was conducted during May 2012 to December 2013 to screen the prevalence of fascioliasis in cattle and buffaloes in Assiut. A total of 100 fecal and blood samples were collected from cattle (n =83) and buffaloes (n =17) of different ages groups (Up to 2 years, 2 to 4 years and more 4 years) Bhutto *et al.* (2012).

Preparation of feacal samples:
Fecal samples were collected in a suitable air tight containers such as plastic bags. The collected samples were labeled for breed, sex and age and placed in cool boxes and transported to the laboratory for examination and stored at 5°C. All samples collected were examined within 36h. Fecal samples were examined by direct smear, flotation and sedimentation techniques for the presence of fluke eggs (Urquhart *et al.,* 1996).

Preparation of blood samples:
A 10 ml of whole blood samples were taken from Jugular vein of each cattle and buffaloes to serum tubes and was allowed to clot. Sera were separated by centrifugation at 3000 r/min for 15 minutes after being kept in the refrigerator overnight. Sera were kept at –20 C° until used.

Antigen preparation from adult Fasciola worms according to (Linh *et al.*, 2003):
Adult worms of *Fasciola sp.* (Figure 4) were collected from the bile ducts of infected slaughtered cattle and buffaloes. The worms were washed with physiological saline and stored at –20°C until examination. The antigen was prepared by homogenizing 0.1 g of each adult fluke for 30 minutes in 5 mL of physiological saline (Figure 3). The emulsion was then frozen and thawed twice and centrifuged at 5,000 rpm for 30 min. The supernatant of each emulsion was used as the antigen (Yoshihara *et al.,* 1981).

Agar gel diffusion test:
15 mL of 1% agarose solution (Bioshop ® Canada Inc., Burlington, ON. L7L 6A4) in 8% saline was poured into a Petri dish 9 cm in diameter. Several wells were prepared in the agarose gel at a distance of 3 mm between the wells. Undiluted and untreated serum was used in this study. Each well in the gel was filled with the antigen or serum to be tested (Figure 6). The dish was kept in a moist chamber at room temperature for 4 days and the reaction was observed daily. When a distinct precipitin line was found in the gel, the serum was considered to have the antibody against fasciolosis (Linh *et al.,* 2003).

Enzyme Linked Immunosorbent Assay (ELISA) technique: according to (Voller *et al.*, 1977).

Antigen preparation from dult Fasciola sp worms according to (Moazeni *et al.*, 2005):
Coating of the plate with diluted antigen: Flat bottom 96 well plate of ELISA were sensitized over night with 200 µl of diluting antigen (1: 50) with Phosphate buffer saline (PBS) PH 9.2 in each well [100µl antigen with 100 µl carbonate – bicarbonate buffer 0.1 M of PH 9.6 per well (1 : 1)]. Then washing the plate 3 times with washing buffer. The Plate wells were blocked with 5% bovine serum albumin (Spectrum, Egyptian company for Biotechnology) (200 µl in each well). Blocked plate was incubated 1 hour at 37C^{o}. Then the plate was washed 3 times with washing buffer. Then addition of the serum, 100 µl of diluted serum samples (1 : 50) with Phosphate buffer saline (PBS) PH 7.2 were added in each well and incubated 30 minutes at room temperature. After incubation the plate was washed 5 times with washing buffer. Then Enzyme conjugate (Rabbit Anti–bovine (IgG) (Biomedical lab. Inc.) is diluted to 1 : 500 with diluting solution (P. B. S.). Then 100 µl of diluted enzyme conjugate were transferred to each well and incubated for 1 hour at 37Co. After incubation, plate was washed 5 times with washing buffer. The plate was washed and substrate 3, 3, 5, 5 tetramethyl Benzidine dihydrochloride (TMB) liquid

contain (hydrogen peroxide) (Bioshop ® Canada Inc., Burlington, ON. L7L 6A4) was added in each well and the plate was incubated in the dark for about 30 minutes at room temperature. Then stopping of the reaction, The color reaction was stopped with 50µl 0.1M sulphoric acid per well. Reading of the plate, color changes were measured in ELISA reader at 450nm filter. The optical density in each well of the plate was read with a spectrophotometer and the data were stored on a microcomputer.

STATISTICAL ANALYSIS

Pearson Chi-square Test was used to compare the effect of breed, sex and age on the prevalence of fascioliasis in cattle and buffaloes.

RESULTS

Out of the 100 cattle and buffaloes examined for Fasciola species 8% (8/100) were positive by microscopic examination (Figure 5), 32% (32/100) by Agar Gel Diffusion Test (AGDT). While antibody detection by ELISA tests resulting in a prevalence of fascioliasis was 60.87% (56/92) by ELISA (Table 1). In some cases, antibody against the *Fasciola sp.* was detected even though eggs were not detected in the feces. Fecal egg examination failed to detect (24) 24% of the positive samples detected by AGDT and failed to detect 52.17% (48/ 92) detected by ELISA. All positive results with fecal egg examination and AGDT were also positive with ELISA.

During this study, 83 cattle and 17 buffaloes were examined of which 51 (61.4%) of cattle and 5 (29.4%) of buffaloes were found to be infected with Fasciola in Assiut. Overall infection rate was 56 (56%). High significant differences were found in infection rate with regard to the host breed, cattle showed higher prevalence of infection (61.4%) than buffaloes (29.4%) (Table 2). Also, High significant differences were recorded in female cattle and buffaloes which were more susceptible to the infection (64.55%) than male (23.8%) (Table 3). High significant difference of fasciolosis was more frequently recorded in 2-4 years old cattle and buffaloes (76%) followed by more than 4 years (46.7%) then less than 2 years of age (20%) (Table 4).

Table 1: Comparison of AGDT (Agar gel diffusion test) and ELISA with microscopic examination for detecting Fasciola in cattle and buffalo.

Detection methods	No. of examined animals	Positive samples	Prevalence (%)
Microscopic examination	100	8	8%
Agar gel diffusion test	100	32	32%
ELISA	92	56	60.87%

Table 2: Prevalence (%) of fasciolosis in cattle and buffaloes according to breed

Breed	No. of examined animals	Positive samples	Prevalence (%)	χ^2	P
Cattle	83	51**	61.4%	7.29	0.01
Buffalo	17	5	29.4%		
Total	100	56	56%		

Table 3: Prevalence (%) of fasciolosis in cattle and buffaloes according to sex

Sex	No. of examined animals	Positive samples	Prevalence (%)	χ^2	P
Male	21	5	23.8%		
Female	79	51**	64.56%	12.04	0.01
Total	100	56	56%		

Table 4: Prevalence (%) of fasciolosis in cattle and buffaloes according to age

Age	No. of examined animals	Positive samples	Prevalence (%)	χ^2	P
Up to 2 years	20	4	20%		
2:4 years	50	38**	76%	19.235	0.01
More than 4 years	30	14	46.67%		
Total	100	56	56%		

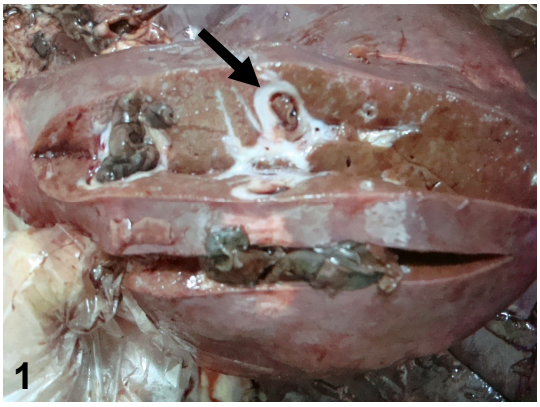

Figure 1: Fasciola infected bovine liver. Note fibrosis and thickening of the wall of bile duct (arrow). Liver flukes also can be seen protruding from cut bile ducts.

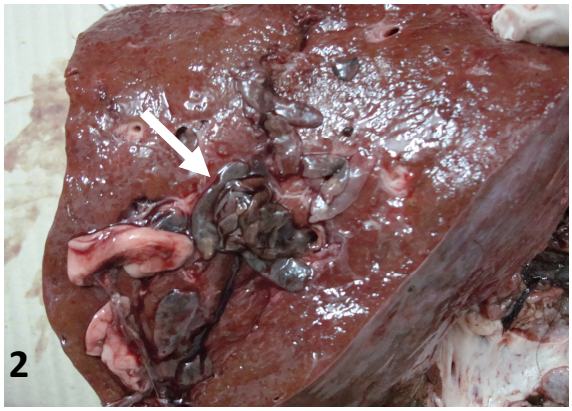

Figure 2: Showed severe infestation of liver with *Fasciola spp.* worms (arrow).

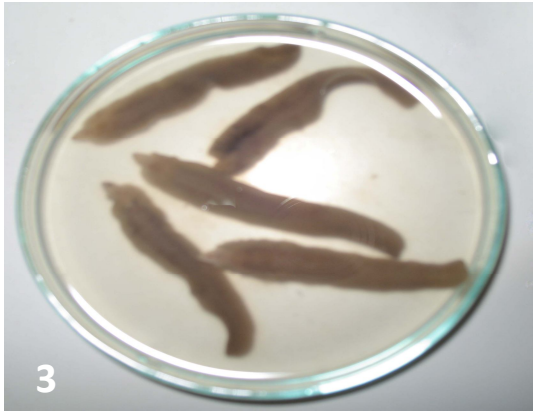

Figure 3: Adult Fasciola worms recovered from an infected bovine liver washed by physiological saline in Petri dish.

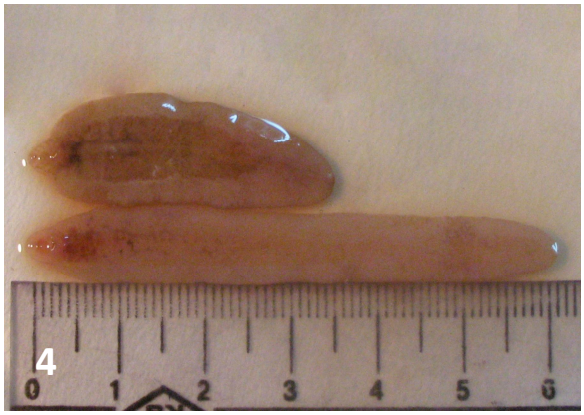

Figure 4: Adult Fasciola worms recovered from an infected bovine liver. Worms are large and leaf shaped (approximately 1 cm × 6 cm).

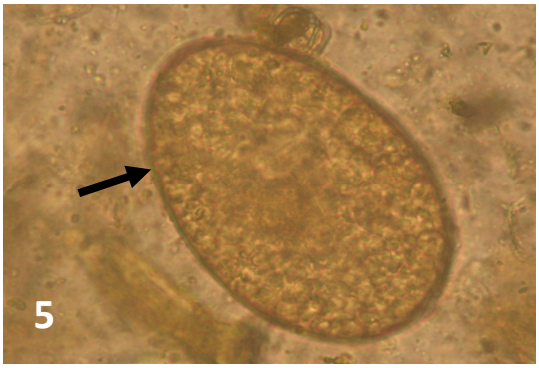

Figure 5: Showed *Fasciola species* egg (40x magnification).

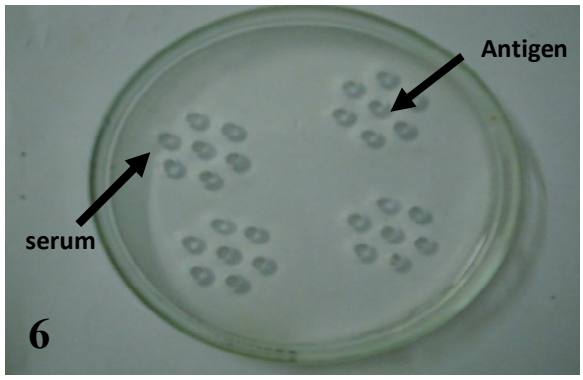

Figure 6: Agarose gel with antigen and test sera in Petri dish

DISCUSSION

Vercruysse *et al.* (1988); Bhattacharya and Laha (1995); Torgerson (1999) and Dar *et al.* (2005) stated that fascioliasis is asymptomatic disease in most animal cases, but it has substantial effects on milk production and a reduction in food conversion efficiency with reduced weight gain leading to high morbidity and mortality with heavy economic losses.

In the present study, the prevalence of fascioliasis in cattle and buffaloes was 8% by microscopic examination, 32% by Agar Gel Diffusion Test (AGDT) and 60.86% by ELISA tests (Table 1). The high prevalence of fascioliasis in this study may be due to insufficient treatment and control measures and irregular usage of anthelmintics might have been responsible for the overall high prevalence of *Fasciola species*.

This result was similar to that reported by, Mahmoud (1984) 68.9% in Behera, Samaha (1989) 68.8% in Alexandria, Keyyn *et al.* (2005) 28.4- 63.8% in Tanzania, Yildirim *et al.* (2007) 65.2% in Turkey and Adedokun *et al.* (2008) 58.5% in Nigeria.

Our study revealed that the prevalence of Fasciola infestation in the examined animals was generally high in comparison with that recorded by Ayoub (1983) 30.7% in Gharbia, Salem *et al.* (1990) 29.7% in Beni- Suef, Mansour (1994) 8.44 % in Cairo abattoir, Abdel-Rahman, (2002) 14% in Egypt, El-Shazly *et al.* (2002) 12.31% in Dakahlia Governorate Bhutto *et al.* (2002) 4% in Sindh, Waruru *et al.* (2004) 31.5% in Kenya, Morsy *et al.* (2005) 20% in AL-Fayoum and Nossair and Abdella (2014) 9.8% in Behera Province. This variation may be attributed to the difference in the study locality and the technique used for detection of the fasciola infestation.

In this study, the results revealed that serodiagnosis by AGDT and ELISA were more sensitive than fecal examination for detection of fascioliasis which may be due to low numbers of egg in the fecal samples, possibly as a result of low worm burden or occlusion of the gastrointestinal tracts by debris (Urquhart *et al.,* 1996). Low sensitivity of microscopic examination could also be as a result of inability of the fecal examination to detect of *Fasciola sp.* eggs in animals at the early stages of infection. It could possibly be due to intermittent emptying of the gall bladder into the digestive tract. Eggs would be detected only when the fluke is present and the infection is patent.

Adedokun *et al.* (2008) and Salam *et al.* (2009) detected that fecal examination was not enough for diagnosis of fascioliasis while AGPT detected more animals with *Fasciola sp.* infection than the microscopical examination. So, AGPT is the suitable method for diagnosing the fasciolosis in early stages, based on the detection of antibodies. Although, AGPT is less sensitive than other serological tests like ELISA, 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 Linh *et al.* (2003). Serological techniques are that they can be easily applied to a large number of samples at once using AGDT or ELISA.

Guobadia and Fagbeni (1996) stated that serodiagnosis of fascioliasis was the good diagnostic assay for detection of early prepatent and active infections. Whereas diagnosis by fecal examination was not possible until 9-10 weeks post infestation, during that time a lot of damage would have been done to the animal. The indirect ELISA is clearly more sensitive than the fecal examination partly because antibodies are present approximately 8 weeks before the infection matures and eggs are shed in the feces, so early detection of infestation is very important because it encourage early chemotherapy.

This study revealed that the prevalence of Fasciola was 61.4% in cattle and 29.4% in buffaloes (Table 2). Cattle showed higher prevalence of infection than buffaloes. Our results are in agreement with previous studies conducted with El-Sherif *et al.* (1959) who revealed 22% and 13% in cattle and buffaloes, respectively in Alexandria, Zaki *et al.* (1965) 15.9% and 10.9% in cattle and buffaloes, respectively in El Gharbia Governorate. Similarly, (Monib, 1977) found that prevalences were 30.3%, and 24.3% in cattle, and buffaloes, respectively in Assiut Governorate, Abdel Aal *et al.* (1999) found that the Fasciola infestation rates was (5.3%) in cattle and (3.7%) in buffaloes in lsmailia province, Raza *et al.* (2007) reported 9% in cattle and 4% in buffaloes in Pakistan and Eslami *et al.* (2009) who recorded that fecal samples of 32.1% of cattle, 17% of buffaloes harbored Fasciola egg. This may be due to differences in the immune response of cattle and buffaloes during infestation with *Fasciola sp.* as well as higher resistance of buffaloes than cattle due to their lower fluke burden or the delayed migration or suppressed development of flukes in buffaloes in compared with cattle (Molina, 2005).

In the present study, female cattle and buffaloes were more susceptible to fascioliasis (64.55%) than male (23.8%) (Table 3). This was in agreement with that described by Phiri *et al.* (2005) who reported significantly higher prevalence rates of fascioliasis in females than males, Yildirim, *et al.* (2007) who found that the fascioliasis was (70.7%) in females and (47.8%) in males, Salam *et al.* (2009) who stated that a higher infection rate in females (15.4%) as compared to males (6.4%), Kuchai *et al.* (2011) who noticed that the prevalence of fascioliasis was higher in females (46.42%) than males (38.46%) and Abdo

(2014) who mentioned that female cattle and buffaloes were more susceptible to fascioliasis (10.95%) and (4%) than male (5.30%) and (0%), respectively in Assiut governorate. These results may be due to the social practice of keeping male animals under good managemental conditions for fattening and breeding purpose as compared with the females which kept for breeding and milk productions.

In the present study, fascioliasis was more frequently recorded in 2-4 years old cattle and buffaloes (76%) followed by more than 4 years (46.7%) then less than 2 years of age (20%) (Table 4). This was in agreement with that described by Kuchai *et al.* (2011) who noticed that a higher infection rate was recorded in younger (>3 years) buffalos (45.83%) than in adult ones (40%).

Animals over two years old were significantly more frequently affected than those under 2 years. Similar findings were reported previously by several researchers Shrestha *et al.* (1992), Ghirmire and Karki (1996), Abdel Aal *et al.* (1999), Maqbool *et al.* (2002), Sanchez-Andrade *et al. (*2002), Marques and Scroferneker (2003), Keyyn *et al.,* (2005), Yildirim *et al.* (2007), Salam *et al.* (2009) and Fatima *et al.,* (2012). Also, Nossair and Abdella (2014) found that the highest prevalence was observed in the age group 2 - 4 years (18.18 %) followed by the age group > 4 years (5.88 %) and lastly the age group (< 2 years) (4.76 %). Many workers have reported an increased incidence of fasciolosis in buffaloes and cattle with advancing age, the higher incidence in older animals might be due to lowering of resistance due to grazing more frequently from pasture which enhance the possibility of infection with Fasciola encysted metacercariae or may be attributed to that the lifespane of fasciola takes longer time to produce the eggs (diagnostic stages) in adult age, or may be due to several times of exposure to the infestation than young.

CONCLUSION

We concluded that serodiagnosis of fascioliasis by agar gel diffusion test and ELISA were more sensitive than fecal examination. AGDT could be a useful, economic technique for herd diagnosis.

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دراسة طفيلية وسيرولوجية عن تشخيص الفاشيولا في الأبقار والجاموس في محافظة أسيوط

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أجريت هذه الدراسة لتحديد نسبة الاصابه بالديدان الكبديه (الفاشيولا) لعدد مائه من الأبقار والجاموس بأعمار وأجناس مختلفة في محافظة أسيوط. وقد تم فحص العينات باستخدام الفحص المجهرى واختبار الانتشار خلال جل الأجار واختبار الإليزا. حيث وجد أن نسبة الاصابه كانت ٨٪ عن طريق الفحص المجهري ، ٣٢ ٪ بإختبار الإنتشار خلال جل الأجار ، ٦٠,٨٦ ٪ باختبار ألإليزا. أظهرت هذه الدراسة أن معدل انتشار العدوى فى الابقار (٦١,٤٪) أعلى من معدل الاصابه فى الجاموس (٢٩,٤٪) . في حين أن نسبة الاصابه في إناث الأبقار والجاموس (٦٤,٥٥٪) أعلى من الذكور (٢٣,٨٪). وعلى الجانب الآخر ازدادت نسبة إصابة الأبقار والجاموس فى عمر ٢-٤ سنوات (٧٦ ٪) يليها (٤٦,٧٪) في عمر أكثر من ٤ سنوات ثم (٢٠٪) في الأعمار أقل من سنتين. وكانت جميع النتائج الإيجابية بالفحص المجهرى وإختبار الإنتشار خلال جل الأجار إيجابية أيضا باستخدام اختبار الإليزا. الفحص السيرولوجى (المصلى) باستخدام اختبار الانتشار خلال جل الأجار واختبار الإليزا أكد نتائج الفحص المجهرى. وكشفت نتائج هذه الدراسة أن الاختبارات السيرولوجيه المستخدمة يمكن الاستفادة منها واستخدامها لتشخيص الاصابه بالديدان الكبديه في الابقار والجاموس.