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An Approach for Detection of *Neospora Caninum* Infection in Aborted Bovine Fetuses in Egypt

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Abstract

The present study aimed to detect *Neospora caninum* infection in abortion occurred in aborted bovine fetuses in Egypt as some of foreign breeds cattle aborted without an obvious definite cause. For this purpose 64 pregnant cows in Kalubya and Monofya Governorates were inspected at different time of gestation. The study revealed that among 64 pregnant cows of 2.6 - 6 years old, 9(14.06%) cows were aborted at 6- 7th month pregnancy. Repeated abortion occurred in 1(11.11%) cow. Necropsies and histological examination were performed for all aborted fetuses. Infected tissues specially brain and placenta was used to infect laboratory reared puppies and kitten. Microscopical examination of dog feces fed on infected materials indicated that 5 (55.56%) aborted fetuses; 3 (60 %) and 2 (50%) in Menoufia and Kalubya Governorates respectively were positive for *Neospora caninum* oocysts. The overall infection was 7.81%. The prepatent period, patent period and the morphology of the detected *Neospora caninum* oocyst in puppies feces were described. Kitten feces were microscopically negative for any coccidian oocyst. From faecal, histological examinations, signs of birth of weak calves and repeated abortion, it was concluded that *Neospora caninum* was incriminated in some bovine abortions in Egyptian cattle.

Introduction

Neospora caninum is an apicomlexan intracellular protozoan which is one of the most important infectious causes of abortion in cattle worldwide (15). It was first reported in dogs (9). Dogs are both intermediate and definitive hosts for *N. caninum* (29). It has been found to have a wide host range including cattle. Recently neosporosis has emerged as an important reproductive disease causing bovine abortion in California and many European countries (2). Naturally infected cows can exhibit a rate of

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endogenous transplacental transmission as high as 95% (6) and this may occur during successive gestations (11). Unlike toxoplasmosis, neosporosis can cause repeated abortion in cattle (40). Tachyzoites, tissue cysts and oocysts of this parasite are considered to be the three infectious stages in its life cycle (10). Oocysts of the parasite are shed in the faeces of dogs (29 and 13). The pathogenesis of neosporosis in cows is complex (8). Abortion or fetal infections have been induced in cattle by using a variety of isolates in different laboratories (14). Outbreaks of Neospora-associated abortion in herds can be caused by point source infection by the parasite or by reactivation of the parasite in chronically infected cows (30). Cows and heifers were considered at risk if they had been pregnant for at least 58 to 260 days when the abortion storm started (32). Subsequent abortions can be expected in congenitally infected cows with *Neospora caninum* that have aborted previously (38). It was suggested that abortion occurs when primary parasite-induced placental damage or causes release of maternal prostaglandins that in turn cause autolysis and abortion. Also fetal damage may also occur due to primary tissue damage caused by the multiplication of *N. caninum* in the fetus or due to insufficient oxygen and nutrition secondary to placental damage (8). However, there is no information on Neospora-associated abortion in Egyptian cattle till now, so this study is the first approach for detection of *N. caninum* in aborted bovine fetuses in Egypt.

Material and methods

Aborted cattle: Thirty cows in kalubya and 34 cows in Menoufya Governorates aged 2.6 – 6 years old previously vaccinated against bacterial infection causing bovine abortion like brucellosis, leptosirosis. Cambylobacter fetus was also avoided due to earlier vaccination of the pregnant cattle. Nine cows showed signs of *Neospora caninum* abortion and Oxytetracyclin HCL 15mg / kg BW for 5 successive days administration failed to prevent it. Some calves in Menoufya Governorates showed stunted growth although they were free from any parasites and fed on balanced ration All aborted fetuses were from 6-7th month of gestation.

Necropsy: Complete necropsies were performed on all aborted fetuses and the fetal crown, rump length was recorded and complete abortion screen was performed on fetuses to rule out other causes of infection bovine abortion according to (3). All animals 64 were inspected till parturition to detect abnormalities in newborns.

Experimental animals : for every aborted fetus four puppies and two kitten of four weeks old were obtained from litters of dogs and cats and reared conventionally in individual steel cages and fed on boiled milk and bred to prevent infection with coccidian parasites .The steel boxes were cleaned daily. Fecal examination of these animals was carried out daily from the 4th week of age after birth to the beginning of the experiment according to (18). Infected materials (brain, lung, liver, spleen) beside infected materials from placenta and placentomes were taken from every aborted fetus (approximately 1750gm), then minced and used for feeding 3 puppies (500 gm/puppy) and one kitten (250gm/kitten) on the same day of collection. One puppy and one kitten were not fed on infected materials and kept as non infected controls (13).

Fecal examination of infected puppies and kittens:

Fecal examination was carried out daily for a period of 2 months after feeding infected materials according to (18). A quantitative salt centrifugal flotation was performed to detect and count oocysts in puppies and kitten fecal samples according to (31 and 26).

Identification of oocysts of *Neospora caninum*:

The detected oocysts were identified morphologically according to (22 and 4). The sporulation of oocysts was carried out in 2.5% potassium dichromate and calibration of the size of oocysts was carried out by using eye piece micrometer according to (34).

Histopathology: Samples from brain, skeletal muscle, lungs, livers, kidneys, spleens, placenta and placentomes were taken from aborted fetuses and fixed in 10 % natural buffered formalin according to Zhang et al., 2007. Fixed tissues were embedded in paraffin, sectioned, stained with Haematoxylin and eosin and examined by light microscope for the presence of lesions according to Pabon et al 2007.

Results

Incidences of oocysts in aborted cows: Among 64 pregnant cows 9 (14.06%) cows were aborted at 6-7th month of pregnancy and repeated subsequent abortion was recorded in one cow of them (11.11%). Microscopical examination of dog feces experimentally infected with infected aborted fetuses tissues indicated that 5 (55.56%) out of 9 aborted fetuses were positive for *Neospora caninum* oocysts. Three out of 5 cases (60%) were recorded in Menoufia and 2 out of 4 cases (50%) were in Kalubya governorates. The overall percentage of infection was 7.81 (5 out of 64 cows) as recorded in table (1). The faeces of the experimentally infected kitten were free from any coccidian oocysts. Some new born calves from the apparently healthy dams showed signs of delayed maturity (Fig 1: E and I), and stunted growth (Fig 1: D).

Results in table (2) showed that the detected sporulated and nonsporulated oocysts of *N. caninum* (Fig 1: G and H, respectively) ranged from 10.6-11.11 x 9.6-11.2 μm with a mean 10.86 X 10.4 μm . Both, the prepatent and patent periods extended from 5-10 days. The sporulation time was 48-72 hrs.

PM examination

The crown vertebral rump length (CVRL) of the aborted fetuses (Fig1: C) were of 55-57 cm length and showed evidence of enteritis where meconium was smearing the anal region. With cut section in the high limb a blackish colouration and serosanguinous infiltration was detected in the subcutaneous tissues (Fig1: F) was detected in subcutaneous tissues. Slightly bloody stained serosanguinous fluid in the chest cavity and severe bloody stained fluid in abdominal cavity. Liver was severely congested with rare dark uncertain area and spots of hemorrhage with certain dark focal hemorrhage (Fig 1: B). Severe hemorrhage is seen in the serosa of the colon and serosa of the rumen Hemorrhage in the splenic capsule. Prominent enlargement and congestion of prescapular lymph nodes .Kidney was very soft and mashy with preirenal subcapsular hemorrhage and congestion. Heart had irregular gryish white patches (Fig1: A) with congestion in the coronary vessels and subendocardial hemorrhage was seen in the mural and

vulvular portion of right ventricle. Small intestine was severely congested with enlarged edematous and hemorrhagic lymph node.

Histopathology

The histopathological examination of the aborted fetuses revealed disseminated inflammatory lesions which were limited largely to brain, lung, heart and liver. In the brain there was Focal gliosis, congested blood capillaries and hemorrhage (Fig2: C). Liver showed hydropic degeneration and necrosis of hepatocytes (Fig2: D). Lung had pulmonary oedema mixed with mononuclear inflammatory cells (Fig2: A), focal aggregation of mononuclear cells (Fig2: B) and multiple bluish calcified mass (Fig2: E). Heart: showed hemorrhage and hyalinization as well as pericarditis with hyalinization of some cardiac muscles (Fig2: F)

Gynecological examination: All infected cows (N = 5) were suffering from repeat breeder and resist treatment by Oxytetracycline Hcl 15mg / kg BW for 5 successive days .

Discussion

Neospora caninum has two host life cycle including intermediate and definitive hosts. Dogs are proven to be the only definitive host of the parasite and can shed oocysts in their feces after ingestion on infected bovine tissues (29). Several animal species including cattle serve as intermediate hosts. Cattle can be infected by ingestion of oocysts-contaminated water and food (41)

Neosporosis is recognized as a major cause of bovine abortion in many parts of the world (37, 42, 41 and 2). Non pregnant *Neospora* – infected adult cattle show no clinical signs of infection. A majority of pregnant infected cows transmit the infection to their fetuses and this result in abortion, birth of weekly calves, or birth of clinically healthy but persistently infected calves (41). Both acutely or persistently *N. caninum* infected cows are more prone to abort than non infected cows. Infection

increases the risk of abortion with 2-7 times and congenitally infected heifers seem to be most at risk to abort during their initial pregnancy (38). Congenital infection in cattle is considered the dominating route of infection this assumption is supported by investigations undertaken in Europe and USA showing that up to 78-95 % of calves born to infected cows are themselves infected (27 and 6).

Since the first isolation of *N. caninum* oocysts shed by dogs in natural conditions (4), only rare reports of naturally infected dogs shedding oocysts are available (35; 31 and 33). Thus the prevalence of *N. caninum* oocyst in surveys depend on fecal examination is predicated to be very low.

In fact, mainly farm dogs might be considered a high risk population because the opportunity to eat bovine infected tissues especially bovine placenta (7).

Our preliminary diagnosis of neosporosis depended on history of giving birth to weak calves, abortion which are the major signs of neosporosis in bovine (38) and time of abortion which was 5-7th month of gestation according to (24 and 1). Further confirmatory diagnosis was carried out by isolation of *N. caninum* oocysts by feeding naïve puppies free from parasites and reared in the laboratory on infected bovine aborted tissues from brains, placenta and other infected tissues. Several authors confirmed shedding of *N. caninum* oocysts by feces of dogs after ingestion of infected bovine tissues (7 and 26).

The morphology of the detected *N. caninum* oocysts was similar to that described by (29 and 22). Such identification was accompanied by histological investigation of aborted bovine tissues that agreed with (43).

Our differential diagnosis was carried out between *N. caninum*, *Toxoplasma gondii* and *Hammondia hammondia*. In the present study, *Toxoplasma gondii* and *Hammondia hammondi* were excluded because none of the experimentally infected kitten shed any oocysts although cats act a definitive hosts for these two parasites. This means that the reported abortion not happened neither due to *T. gondii* infection nor *Hammondia*

hammondia infections. In addition, the oocysts of *Hammondia heydorni* has the same morphological character of that of *Neospora caninum* and this also was excluded till now as there is no evidence mentioned that *Hammondia heydorni* cause bovine abortion (34). This was also confirmed by the large size of its oocysts compared to that of *Neospora caninum* as they measures up to 14 um in diameter (4 and 10). Yet, we did not observe oocysts larger than 11.5 um. Bacterial infection causing bovine abortion like brucellosis, leptosirosis and cambylobacter fetus were also avoided due to earlier vaccination of the pregnant cattle beside the unbenefit uses of antibiotics. On the other hand, *N. caninum* had been confirmed and isolated from aborted bovine fetuses and calves. Such results were also recorded by (36), (6), (20); (16), (28), (5), (39), (21), (43), (25) and (13) in, UK, Korea, Malaysia, Australia, New Zealand, Brazil, Japan China, Spain and USA respectively.

In the present study neosporosis- induced abortion occurred at 6-7 months of gestation. This result was in accordance with (43) in China who recorded that neosporosis- induced abortion occurred at 5-7 months of gestation. Fetuses were died in utero and some of them autolysed. Such results agreed with (10) who mentioned that fetuses may die in utero, resorbed, mummified autolysed or stillborn.

In the present study, repeated abortion due to neosporosis occurred in 1 (11.11%) out of 9 cows. This result was lower than that recorded by (25) in Spain who recorded 26.7% repeat abortion as a result of neosporosis. Such differences may be due to the change of the scheme of work. (25) mentioned that, repeated abortion due to neosporosis indicated that *N. caninum* can be very stable through time and the infected cows can show a high rate of repeat abortions

The effect of congenital infection with *Neospora caninum* on neonatal calves maturity agreed with that of Hoar et al., 2007 who reported that

weight and average daily gain of calves that were seropositive for *N. caninum* was less than that of seronegative steers in one pen.

Both, the prepatent and the patent periods extended from 5-10 days in experimentally infected dogs. The relative low mean number of oocysts /gm of feces may accounted by the gut immunity which develops after primary infection in dogs thus preventing repeated shedding (17) or by the low number of excreted oocysts under experimental and natural conditions. This opinion was previously recorded by (4; 7 and 33)

Conclusion

As a general conclusion, *N. caninum* can be stable over time in infected cattle and cause repeat abortion and congenitally transmit to newborn so we recommended selective culling of infected cows and /or reducing daughters born to *N. caninum* infected cows that remain in the herd. Dogs that reared in bovine herds must not feed infected bovine tissues like aborted fetuses or placenta to minimize the risk of transmission of infection between canine and bovine

To our knowledge, this was the first report dealing with *N. caninum* infection in aborted bovine fetuses in Egypt. Several studies are needed to confirm this report through isolation of *N. caninum* tachyzoites from bovine tissues obtained from dairy farms in Egypt or application of serological tests like ELISA and /or polymerase chain reaction (PCR) for canine and bovine hosts. Epidemiological, ecological, threpeutical and immunological studies are very necessary to prevent the spread of *N. caninum* infection between farm animals in Egypt.

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Table (1): Incidence of neosporosis in faeces of dogs and cats fed minced aborted bovine fetuses in two seasons

Locality	Total No of cows	No. of aborted fetuses	History of Repeated abortion	Positive infection cases	over all percent of infection
Monofya Governorate	34	5 (14.71%)	1 (20 %)	3 (60 %)	8.82
Kalubya Governorate	30	4 (13.33%)	0.00	2 (50%)	6.67
Total	64	9 (14.06%)	1 (11.11%)	5 (55.56%)	7.81

Table (2)The number ,dimensions of oocysts , Prepatent period, Patent period and Sporulation time of recovered coccidian parasites in faeces of dogs fed on minced aborted bovine fetuses in different localities

The infected dogs	Mean number of oocysts '	The prepatent period	The patent period	Sporulation time	Dimensions of oocysts
1	24.300	7 days	9	48-72 hours	10.6-11.12X 9.6-10.8um
2	40.200	5 days	10		
3	12.500	10 days	5		
4	44.100	5 days	10	48-72hours	10.6-11.11X 9.8-11.2 um
5	16.200	8days	7		

Cases 1, 2, 3 were reported in Monoufia Governorate

Cases 4, 5 were reported in Kalubya Governorate



A



B



C



D



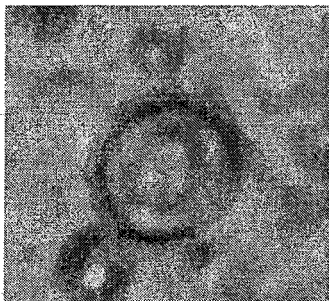
E



F



G

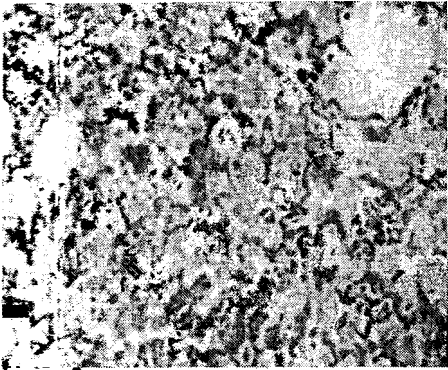


H

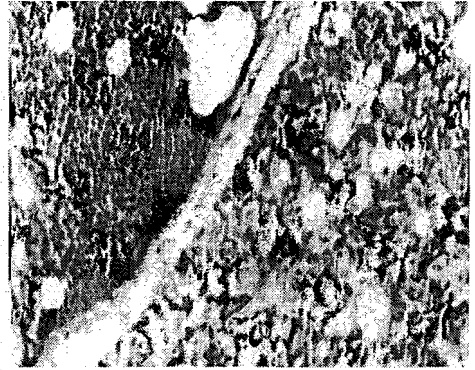


I

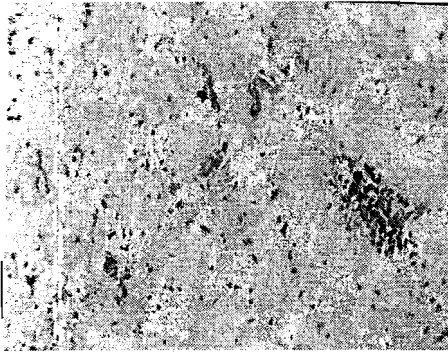
Third Inter. Sci. Conf., 29 Jan.- 1 Feb./ 2009, Benha & Ras Sudr, Egypt Fac. Vet. Med. (Moshohor), Benha Univ



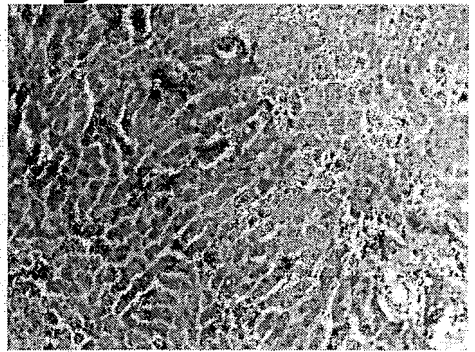
A



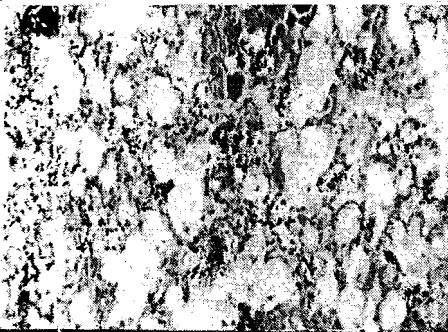
B



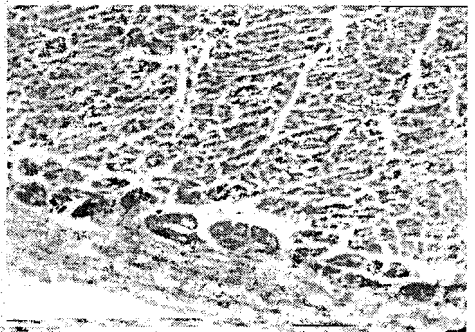
C



D



E



F

All Fig. X 400.

محاولة اقتراب من اكتشاف طفيل نيوسبورا كانينم في أجنة الأبقار المجهضة في مصر

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الملخص العربي

هدفت الدراسة إلى اكتشاف طفيل نيوسبورا كانينم في أجنة الأبقار المجهضة في مصر بعدما ظهرت أعراض تشبه أعراض النيوسبورا مثل تقزم العجول ، إجهاض متكرر عند ٦-٧ شهور حمل ، شبق متكرر ، موت العجول بعد ولادتها مباشرة في بعض مزارع الأبقار في محافظة المنوفية والقليوبية وقد تم فحص ٦٤ بقرة عشار ٣٤ منها في محافظة المنوفية و ٣٠ في محافظة القليوبية وظهرت حالات إجهاض علي ٩ بقرات خمسة منها في محافظة المنوفية و ٤ في محافظة القليوبية ولم يجدي معها العلاج بالأوكسي تتراسيكلين ولعزل طفيل نيوسبورا كانينم تغذية ٣ كلاب صغيرة وقطة صغيرة / جنيه مجهض علي بعض الأنسجة المصابة مثل المشيمة والمخ بمعدل ٥٠٠ جم/كلب ، ٢٥٠ جم/قطة. وقد تم فحص براز هذه الحيوانات المعديّة يوميا ولمدة شهرين. وقد تم اكتشاف حويصلات طفيلية تشبه حويصلات النيوسبورا في براز ٥ مجموعات من الكلاب ولم يتم وجود مثل هذه الحويصلات في براز أي من القطط التي تم تغذيتها علي الأنسجة المصابة. استنتجت الدراسة أن خمس بقرات من تسعة (٥٥,٥٦%) كانت مصابة بهذا الطفيل وكانت تشبه الإصابة العامة ٧,٨١% وبقياس أبعاد الحويصلات المكتشفة وعمل تشخيص مقارنة مع هثيلاتنا مثل التوكسوبلازما ، الهومونديا تم الترحيح بأن تكون الحويصلات المكتشفة في براز الكلاب المغذاة علي أنسجة مصابة لأجنة أبقار مجهضة هي لطفيل نيوسبورا كانينم.