

Unexpected Presence of Diatom in Pathological Specimens of Fetal Membrane of Aborted Cattle Case Report

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THE OBJECTIVE of this article was to report diatom in aborted fetal membrane of cattle. Tissue samples of aborted fetal membrane were fixed in 10% neutral buffered formalin and submitted for pathological evaluation. The tissue samples were prepared for examination and stained with Hematoxylin and eosin, Gram, Gomori's methenamine-silver and Periodic Acid Schiff, and examined using research and phase - contrast microscope. Histopathological examination showed different shapes of diatoms. The most common type of diatoms in the examined tissues was reported. In conclusions, this report is considered the first detection of diatoms in a tissue of aborted fetal membrane of a dairy cows. The source of infection may be from drinking contaminated water, however no pathological changes could be detected around diatoms and the relationship between abortion and algae infection was not detected and further studies were carried out regarding this point.

Keywords: Diatom, Abortion, Cattle, Pathological changes.

Diatoms are considered a major group of algae; they are the most common type of phytoplankton. Diatoms are microscopic, unicellular algae that have intricate glass-like outer cell walls partially composed of silicon. They are abundant in nearly every habitat where water is found in oceans, lakes, streams, mosses, soils, even the bark of trees. Diatoms were detected in cytological samples of aquatic animals (Stacy *et al.*, 2014). Also used in forensic medicine for diagnosis of death by drowning (Hürlimann *et al.*, 2000 and Piette & De Letter, 2006). The aim of this article is to report diatom in aborted fetal membrane of cattle using different stains and novel techniques for detection in fixed tissue sections.

Material and Methods

Tissue samples of two cases of aborted fetus and fetal membrane were submitted from a private dairy farm used desalinated water mixed with water wells located near Giza Governorate, Egypt. The aborted cows did not show specific clinical signs before abortion; the annual rate of abortion in the farm is about 10 %. All cases of abortion were sporadic and the farm is free from *Brucella* infection. Tissue samples of fetal membrane were fixed in 10% neutral buffered formalin and

submitted for pathological examination. The samples were embedded in paraffin, sectioned at 5.0 um thickness, and stained with hematoxylin and eosin (HE), Gram, Gomori's methenamine-silver (GMS) and Periodic Acid Schiff (PAS). The tissue sections were examined using research and phase - contrast microscope. A new novel method for detection of diatoms in tissue by staining with Gram iodine then examined with phase - contrast microscope which give specific reaction with diatoms. The identification of diatom was carried out according to the morphological changes according to Streble and Kraute (1978).

Results and Discussion

The different shape of diatoms were seen and recorded in 2 plates. The most common type of diatoms in the examined tissues were *Melosira granulate* (Fig., 1, 5, 7, 8, 11 & 12), *Cyclotella comta* (Figs. 2, 6, 8 & 9), *Cocconies placentula* (Fig. 3), *Surirella ovalis* (Fig.4), *Nitzschia paradoxa* (Fig.6) *Asterionella gracillima* (Fig. 8) and *Gyrosigma attenuatum* (Fig. 9).

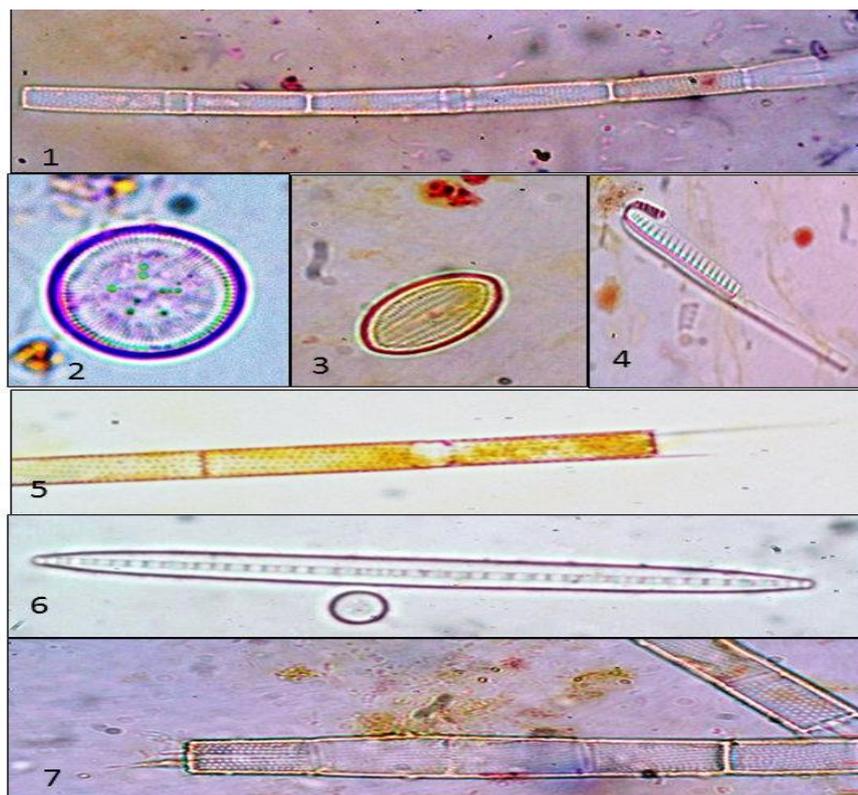


Plate 1. Showing different shapes of diatoms stained with Gram iodine except Fig. 2 with PAS, X 1000). Fig. 1, *Melosira granulate* .Fig. 2, *Cyclotella comta* .Fig. 3, *Cocconies placentula* . Fig. 4, *Surirella ovalis* .Fig. 5, *Melosira granulate* .Fig. 6, *Nitzschia paradoxa* and *Cyclotella comta* .Fig. 7, *Melosira granulate* .

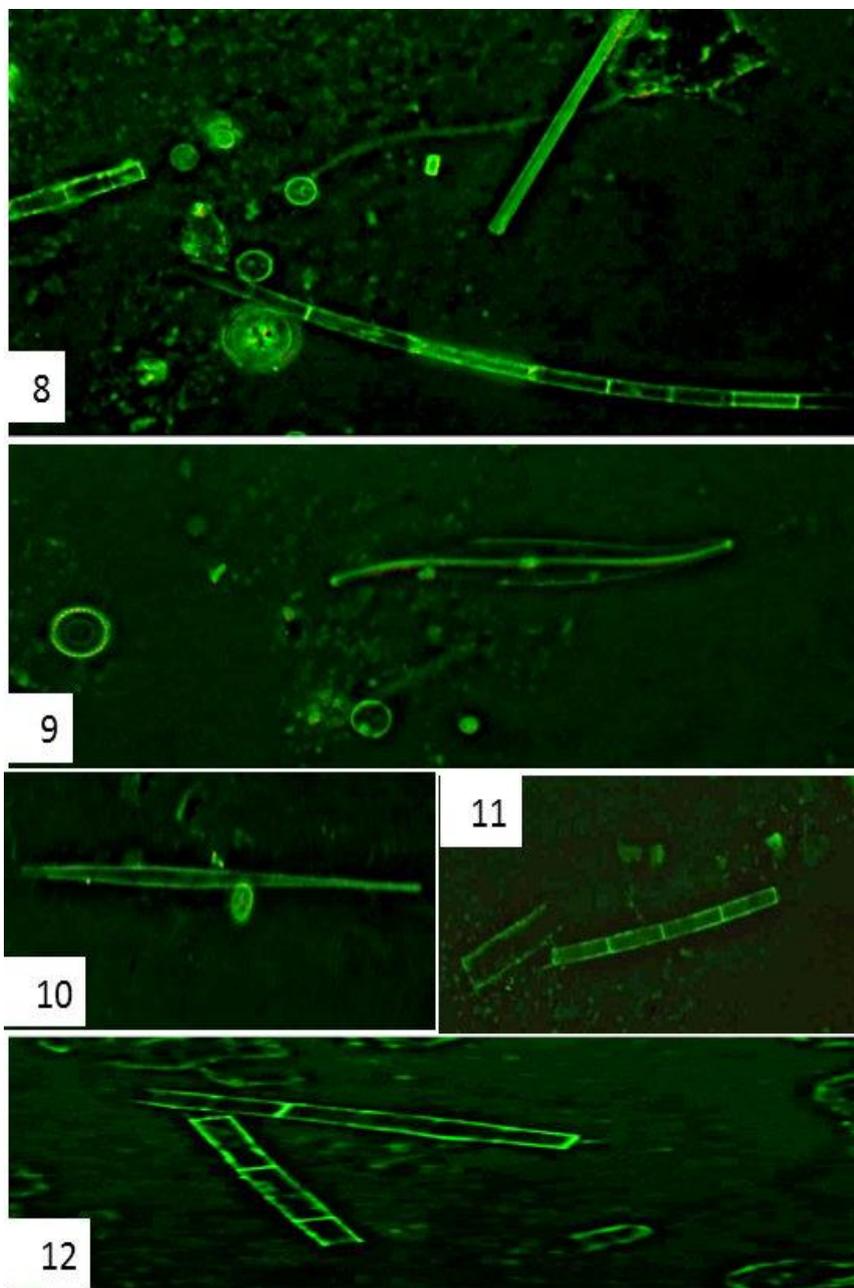


Plate 2. Showing different shapes of diatoms stained with Gram and examined with phase contrast microscope, X 400). Fig. 8, *Melosira granulata*, *Cyclotella comta* and *Asterionella gracillima* .Fig. 9, *Cyclotella comta* .Fig. 10, *Synedra ulna* Fig. 11, *Melosira granulata* .Fig. 12, *Melosira granulata* .

No cellular inflammatory reaction could be seen around the diatoms in tissue may be due to glass-like outer cell walls which partially composed of silicon. The ordinary H&E stain was not recognize diatoms clear in tissue section, and PAS reaction was positive at the outer wall of diatoms and algae (Fig. 2). Diatoms were clear with Gram stain by using research microscope (Fig. 1,3,4-9) however, the best novel method for detection of diatoms in tissue section was by staining the tissue with Gram then examined by phase contrast microscope which gives clear bright green illumination for all types of diatoms in tissue section and dark background for tissues (Fig. 8-12). Detecting methods of diatoms are essential to diagnose drowning and considered one of the hottest topics in forensic medicine. Takeichi and Kitamura (2009) detected diatom in formalin-fixed tissue by proteinase K digestion, and Hu *et al.* (2013) detected diatoms in water and tissues by combination of microwave digestion, vacuum filtration and scanning electron microscopy. The novel method reported in this study is considered beneficial, for the detection and morphological classification of diatoms in formalin fixed tissue without enzymatic digestion microwaves and vacuum filtrations and can easily use for forensic medicine. The use of GMS stain showed positive reaction for all diatoms however, it showed black coloration and the detail structures of some diatoms. Detail identification of diatoms may needed further studies using ultrastructure of the silica cell wall and DNA genome of identification of different species. There are many challenges to pathologist for detection of diatoms and algae in tissue by H&E stain and needed special stains also, tissue response for diatoms and algae is not clear and need further studies. The mechanism of entrance the diatom and algae to placenta and fetal membrane of pregnant cows may be by mouth in drinking contaminated water or feed, and then they invaded the gastrointestinal tract to circulate via blood stream and reach to the fetal tissues. In conclusion the presence of diatom in fetal membrane may be playing a role in abortion in animals.

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Conflict of interest

The author declares that they have no conflict of interest.

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اكتشاف غير متوقع للدياتوم في عينات باثولوجيه لانسجه أغشيه جنينيه نافقه في الابقار

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الهدف من هذه المقالة هو تسجيل وجود الدياتومات فى الاغشيه الجنينيه المجهزه فى الابقار. وهو ما لم يسجل مثل هذا الكشف سابقا واطهر الفحص الميكروسكوبى لانسجه الاغشيه الجنينيه لحالتي اجهاض متأخر وجود اشكال مختلفه من هذه الدياتومات وذلك بعد الفحص باستخدام العديد من الصبغات المختلفه Hematoxylin and eosin, Gram, Gomori's methenamine –silver and periodic Acid Schiff) وتم الفحص باستخدام الميكروسكوب الضوئى كذلك (phase contrast microscope). وتم استخدام تقنية جديدة للكشف عن الدياتومات فى الانسجه المحفوظه فى الفورمالين. تم تسجيل الاشكال والاسماء لهذه الدياتومات. الخلاصه يعتبر هذا التقرير أول اكتشاف من الدياتومات فى أنسجة غشاء الجنين المجهض من مزرعة الألبان، ومصدرا للعدوى قد يكون من شرب المياه الملوثة، وبالرغم من عدم وجود مظاهر التهابات حول الدياتوم او الطحالب وعلاقتها بالاجهاض فى الابقار فسوف تجرى دراسات اخرى لهذا الموضوع.