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Brief Communication

ISOLATION OF ASCARIS SUUM EGGS FOR EXPERIMENTAL PURPOSES

Eggs from the pig roundworm Ascaris suum are easily obtainable in large numbers from uterus of adult worms. It is therefore natural that eggs isolated from that organ have been used almost exclusively in experimental ascariasis, both in the natural host (*Kelley & Nayak* 1965, *Gaafar & Keittevuti* 1972, *Andersen et al.* 1973, *Jørgensen et al.* 1975 and others) and in small laboratory animals (*Jeska et al.* 1969, *Berger* 1971 and others). In some cases no details are given on the origin and preparation of the infective eggs or the eggs may originate both from uteri of adult worms and from pig faeces (*Kelley et al.* 1957).

However, eggs isolated directly from the uterus deviate considerably from eggs naturally passed in the faeces of infected pigs. Egg suspensions isolated from uterus appear white and contain eggs of different morphology. Some eggs are fully developed, but non-finished eggs are also found, without the shell or the albuminous layer. The eggs are very sticky and they tend to gather in clusters or they attach to surfaces such as glassware or the uterine tissue present in the suspension. Even the fully developed eggs deviate from naturally passed eggs in their hatching mechanism and in the structure and chemical composition of the albuminous layer (*Enigk & Dey-Hazra* 1976) since they have not been exposed to the tanning process of passing through the host's digestive system. On the other hand, an egg suspension of naturally passed eggs as they may be seen when isolated from faeces by the method described below, appears dark brown. Large numbers of ascaris eggs may be isolated in clear water containing a minimum of debris. All eggs are fully developed, they are less sticky and therefore they are mostly found as single eggs. Upon cultivation, more than 95 % will embryonate. Therefore, with the present technique in hand, the author suggests that naturally passed eggs are used as a source of infection in experimental ascariasis, rather than eggs isolated directly from uterus of adult worms.

Method

Faeces from a naturally infected pig excreting approx. 400 eggs per g of faeces was collected and thoroughly mixed with water. Approx. 20 l of water was used per kg of faeces. The faecal suspension was poured through a sieve with a diameter of 35 cm and a mesh size of 2—3 mm. The sieved solution was left to sediment overnight. The sediment which was approx. 1/20 of the total volume was mixed with twice its volume of saturated salt solution and distributed into beakers to a level of 15 cm from the bottom. The beakers were left for 30 min. and floated material was poured through a tier of sieves with mesh sizes of 200, 100 and 36 μm, respectively. The flotation procedure was repeated after mixing. The contents of the sieves were thoroughly rinsed with water and the material collected on the 36 μm sieve washed into 75 ml centrifuge tubes and subsequently spun down. The supernatant was removed and the sediment suspended in a sucrose solution which contained 1300 g sucrose/l H_2O (s.g. 1.26). After mixing, a 1 cm layer of water was deposited on top of the mixture. The tubes were then centrifuged at 2500 r.p.m. for 10 min. After centrifugation the interface was mixed with the water layer by means of a syringe fitted with a hypodermic needle. The mixture, which should contain a minimum of sucrose, was collected and transferred to an Erlenmeyer flask. The remaining sediment in the centrifuge tube was resuspended, the centrifugation procedure was repeated and a second portion of eggs in water was collected. The collected suspension was diluted with 20 times its own volume of water. The level of the water suspension in the Erlenmeyer flask did not exceed 15 cm. Cleaning of the suspension by removal of debris and sucrose was carried out in the flask by mixing, sedimentation for 1 hr. and careful removal of the supernatant by means of a suction pump. Further purification was carried out by resuspension of the sedimented eggs. At this stage the sedimentation of the dark brown eggs in clear water may be followed macroscopically and the sedimentation time may be reduced to 30 min. The egg suspension was cultured to the infective stage at room temperature in shallow water which was changed at weekly intervals.

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