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Studies on Histoplasmosis Farciminosii (Epizootic Lymphangitis) in Egypt

III. Application of a skin test ('Histofarcin') in the diagnosis of epizootic lymphangitis in horses.

Untersuchungen über Histoplasmosis Farciminosii (Lymphangitis Epizootica) in Ägypten

III. Anwendung eines Hauttests („Histofarcin") in der Diagnostik der Lymphangitis Epizootica bei Pferden.

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Key words: Epizootic lymphangitis - Histoplasma farciminosum - 'Histofarcin' antigen - 'Histofarcin' skin test

Schlüsselwörter: Lymphangitis Epizootica - Histoplasma farciminosum - „Histofarcin"-Antigen - „Histofarcin"-Hauttest

Summary: Two hundred horses in a horse farm with a history of infection with epizootic lymphangitis were examined clinically, mycologically, serologically and by skin test using soluble antigen ('Histofarcin') prepared from H. farciminosum. 3 % of the animals showed clinical manifestations of the disease and 26.5 % of the horses had antibodies demonstrated by Immuno Diffusion (ID) and Complement Fixation Test (CFT). The 'Histofarcin' skin test was applied to 40 horses representing serologically negative and positive cases, either apparently healthy, recovered from previous infection or with clinical manifestations of the disease. All serologically positive cases showed significant increase in skin thickness (8–20 mm) 24 h after injection of the antigen, and a maximum increase in skin thickness (50 mm) was recorded after 48–72 hours. The 'Histofarcin' skin test proved to be a reliable and easy test.

Zusammenfassung: 200 Pferde einer Pferdefarm, bei denen eine Lymphangitis epizootica vorlag, wurden klinisch mykologisch, serologisch und mit einem Hauttest untersucht. Hierfür wurde ein lösliches Antigen („Histofarcin") benutzt, das aus H. farciminosum gewonnen worden war. 3 % der Tiere zeigten klinische Manifestationen der Erkrankung und 26 % hatten mit Hilfe der Immunodiffusion oder der Komplementbindungsreaktion nachweisbare Antikörper. Der „Histofarcin"-Hauttest wurde bei 40 Pferden durchgeführt. Diese Pferde waren zum Teil serologisch negativ, z. T. positiv. In der Gruppe fanden sich offensichtlich gesunde Tiere, solche, die von früheren Infektionen genesen waren und Tiere mit klinisch manifester Erkrankung. Bei allen Pferden mit positiver Serologie fand sich im Hauttest eine Verdickung der Haut (8–20 mm) 24

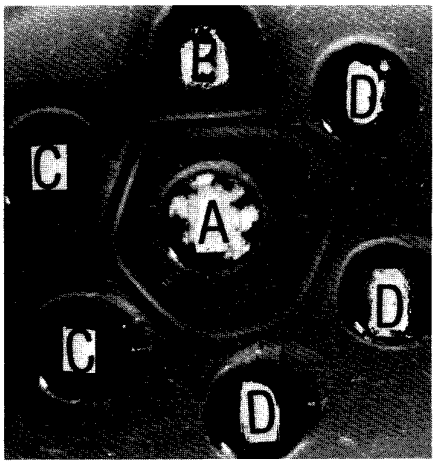

Fig. 1: Interaction between histofarcin antigen and serum from horses infected or recovered from H. farciminosum infection as well as rabbit anti-H.-farciminosum serum using agar gel immunodiffusion test.
Well A: Histofarcin antigen.
Well B: Rabbit H. farciminosum antiserum (positive control).
Well C: Serum from horse actively infected with H. farciminosum.
Well D: Serum from horse recovered from H. farciminosum infection.

Stunden nach der Injektion des Antigens und ein Maximum der Hautverdickung (50 mm) nach 48–72 Stunden. Der „Histofarcin"-Hauttest erwies sich als verläßliche und leicht durchzuführende diagnostische Methode.

Introduction

Epizootic lymphangitis is an endemic disease of equines in Egypt, particularly in the Delta region (Refai and Loot, 1970). Although the policy of condemnation of infected animals is applied since more than 20 years, the disease still causes serious economic losses among equines.

The routine diagnosis depends on direct microscopic examination of stained smears prepared from the discharge or purulent content of unopened nodules in cases with lymphangitis. In view of the concept of systemic infection of equines by Histoplasma farciminosum (Bennet, 1931; Marcato, 1947; Artioli, 1948 and Singh, 1966), other diagnostic methods should be added in order to be able to trace cases with internal infection without manifestation of skin lesions. Soliman et al. (1984) demonstrated the efficiency of the agar gel immunodiffusion test as a rapid test for the diagnosis of the disease.

The aim of the present work was to evaluate the efficiency of an antigen prepared from H. farciminosum ('Histofarcin') in evoking hypersensitivity reactions among infected horses for the purpose of using the 'Histofarcin' skin test as a routine diagnostic test.

Material and Methods

Two hundred horses in a horse farm with a history of epizootic lymphangitis were examined clinically, mycologically, serologically and by skin test.

For the serological examination materials obtained aseptically from unopened nodules from cases with skin lesions were examined microscopically after stained with Grams' stain and inoculated onto PPLO agar medium enforced with 2 % glucose and 2.5 % glycerine (Selim et al., 1984). The tubes were incubated at 25 C and examined weekly up to 10 weeks.

For serological examination, 10 ml blood samples were collected from all animals. The separated sera were tested for the presence of antibodies by the agar gel immunodiffusion test and complement fixation test after Palmer et al., 1977.

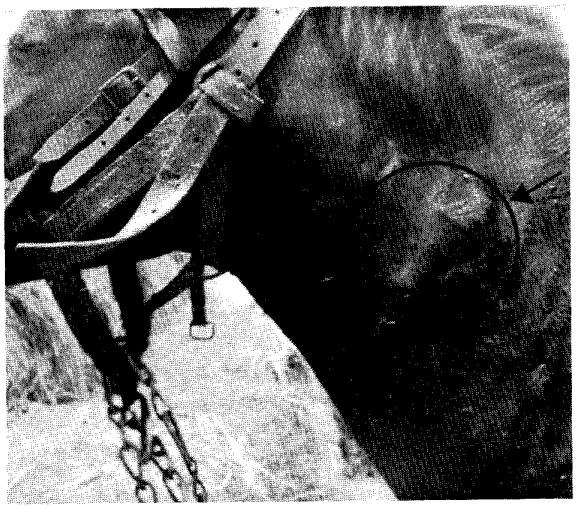

Fig. 2: A horse showing positive skin reaction (delayed type) to histofarcin antigen (see the indicating arrow).

The 'Histofarcin' skin test was applied to 40 horses representing five groups of serologically positive and negative cases, either apparently healthy, recovered from previous infection or with clinical manifestations of disease. Each horse was injected intradermally with 0.1 ml of H. farciminosum antigen ('Histofarcin') at the right side of the neck. On the left side 0.1 ml of the fluid medium used for the preparation of the antigen was injected as control. The size of the skin of the injected area was measured with a tuberculin caliber before injection and 24, 48 and 72 hours after injection.

The antigen used for serological examination and skin test was prepared from the mycelial form of H. farciminosum (Strain 8303) previously isolated from infected horses in the same farm. The fungus was grown on the surface of polysterine discs floating on the surface of 250 ml PPLO fluid medium enforced with 2 % glucose and 2.5 % glycerine in 500 ml flasks. The flasks were incubated in stationary state at 25 C for 4 months. The fungus-free culture filtrate was then mixed with double its volume acetone and kept at 4 C for 48 hours. The supernatant was decanted, the remaining acetone was evapourated and the precipitate was reconstituted to 1/10 of the original volume in destilled water and used as antigen.

Results

1. Clinical examination: clinical symptomes of the disease were observed in 3 % of the cases. 3 horses had nodules along the lymphatics of the forelimbs, some of which were ulcerating and 2 horses had similar lesions on the right side of the neck. One horse suffered from purulent conjunctivitis.

2. Mycological examination: in Gram-stained smears double contoured yeast-like cells were detected in large numbers both free and phagocytized. On PPLO agar medium the fungus appeared as minute grey flakes after 4–6 weeks' incubation at 25 C. On further incubation the colonies became raised, folded, convoluted and white to chocolate brown in colour.

3. Serological examination: The results are summarized in table 1. For further details cp. Soliman et al. (1984).

Table 1
Result of 'Histofarcin' skin test in horses in comparison with clinical and serological examinations

Group No.	Number of examined horses	Clinical picture and history	Serological exam.		Histofarcin skin test		
			ID	CFT	increase in skin thickness in mm ± SD_n		
					24 h	48 h	72 h
I.	8	apparently healthy*	-	-	0.75 ± 0.66	1.25 ± 0.43	1.25 ± 0.83
II.	8	apparently healthy*	-	-	2.75 ± 0.83	4.88 ± 0.17	3.88 ± 0.93
III.	9	recovered from previous infections	+	1/8 : 1/16	8.22 ± 0.76	12.89 ± 4.15	12.56 ± 4.17
IV.	9	apparently healthy	++	1st reading = 1/8** / 2nd reading = 1/32 : 1/64	11.67 ± 5.57	26.00 ± 5.20	26.78 ± 8.31
V.	6	clinical cases (various forms of epizootic lymphangitis)	++	1/16 : 1/64	20.67 ± 3.99	43.00 ± 7.23	49.67 ± 4.64

SD = standard deviation
* = horses recently introduced to this farm (one week)
** = second examination was made 45 days after the first examination
\+ = one precipitation band
++ = two precipitation bands
ID = Immuno Diffusion Test
CFT = Complement Fixation Test

Discussion

The difficulty to isolate the causative fungus of epizootic lymphangitis was the main reason why direct microscopic examination of stained smears prepared from skin lesions is routinely used for the diagnosis of the disease in Egypt. The condemnation of positive cases has been applied in Egypt since 20 years as the only official method of control, however, the disease still represents a threat to equines and causes considerable economic losses.

The success to isolate the causative fungus on PPLO solid media (Selim et al., 1984) and to culture it by the floating method on PPLO fluid medium (Soliman et al., 1984) has rendered it possible to prepare soluble antigen from H. farciminosum. Such an antigen, when concentrated, proved to be efficient and specific in serological tests, particularly ID and CFT, as well as in a skin test. In the present work the results of ID and CFT were in agreement with the clinical manifestations or history of previous infection and were conforming with results of the skin test. The feasibility of application of the skin test, in addition to its specificity, renders the test suitable as a routine diagnostic method for screening of large number of animals. ID and CFT may be used for confirmation. All the 3 tests are of great importance and very useful, particularly in cases with pneumonia, nasal or eye infection i. e. cases which do not show the classical skin infection with involvement of the lymphatics.

The fact that histoplasmosis caused by H. capsulatum has been reported also in equines (Richmond, 1948; Randall et al., 1951 and Menges et al., 1963) and a histoplasmin skin test using an antigen prepared from H. capsulatum has been used for diagnosis (Marx et al., 1972 and Jones et al., 1972) would render it confusing if the antigen prepared from H. farciminosum was also called histoplasmin.

Therefore, in order to avoid confusion, the authors suggest the application of the term 'Histofarcin' for the antigen prepared from H. farciminosum and 'Histofarcin' skin test for the test method.

References

1. Artioli, D. (1948): Contributo alla conoscenza delle localizzazzioni interne del farcino criptococcico. Alti. Soc. Ital. Sci. Vet, 2, 405-414.
2. Bennett, S. C. J. (1931): Cryptococcus pneumonia in Equidea. J. Comp. Pathol. Therap. 44, 85.
3. Jones, M. B., A. Gonzalez-Ochoa, M. B. Marx & M. L. Furcolow (1972): Survey of histoplasmin skin test reactions among horses in Mexico. Am. J. Vet. Res., 33 (8), 1707-1709.
4. Marcato, A. (1947): Le localizzazzioni interne del farcino criptococcico. Nuova vet. 23, 63-77.
5. Marx, M. B., M. B. Jones, D. S. Kemberlin & M. L. Furcolow (1972): Survey of histoplasmin and blastomycin test reactors among thoroughbred horses in Central Kentucky. Am. J. Vet. Res. 33, 1701-1705.
6. Menges, R. W., R. T. Habrmann, L. A. Selby, H. R. Ellis, R. T. Behlow & C. D. Smith (1963): A review and recent findings on histoplasmosis in animals, techniques of veterinary study, Lexington animal study, 1960. Vet. Med. small Anim. Clin. 58, 331-338.
7. Palmer, D. F., L. Kaufman, W. Kaplan & J. J. Cavallaro (1972): Serodiagnosis of Mycotic Diseases. Charles C. Thomas. Publisher Springfield. Illinois, USA.
8. Randall, C. C., M. F. Orr & F. Schell (1951): Detection of tissue culture of an organism resembling H. capsulatum in apparently healthy horses. Proc. Soc. Expell. Biol. & Med. 78, 447-450.
9. Refai, M. & A. Loot (1970): Incidence of epizootic lymphangitis in Egypt with references to its geographical distribution. mykosen 13 (5), 247-252.
10. Richmond, H. (1948): Studies on epizootic lymphangitis. North Am. Vet. 29, 710.
11. Selim, S. A., R. Soliman, O. Kamillia, A. Padhay & L. Ajello (1984): Studies on Histoplasmosis farciminosii (Epizootic lymphangitis) in Egypt. I. Isolation of H. farciminosum from cases of epizootic lymphangitis in horses and its morphological characteristics. Am. J. Vet. Med. Assoc. (under publication).
11. Singh, T. (1966): Studies on Epizootic Lymphangitis. Study of clinical cases and experimental transmission. Ind. J. Vet. Sci. 36, 45-59.
12. Soliman, R., M. Ismail, E. Hatem, O. Kamillia & S. A. Selim (1984): Studies on Histoplasmosis farciminosii (Epizootic Lymphangitis) in Egypt. II. Serodiagnosis of epizootic lymphangitis using agar gel immunodiffusion test. Vet. Med. J. (Faculty of Veterinary medicine, Cairo University, Giza-Egypt). Under publication.

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