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Acute outbreak of glanders in horses: clinical, serological and pathological studies in Iraq

KEYWORDS

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ABSTRACT *An acute outbreak of glanders occurred in thirteen horses in a stable farm at Diyala province, Iraq. Three horses were died and the rest ten showed different clinical signs of glanders including fever, anorexia, emaciation, mucopurulent, nasal discharge and one to two ulcerative nodules located in the nasal mucosa, submaxillary lymph node enlargement and swelling and enlargement of both testes. Both horses showed multiple ulcerative nodules on the hind limbs and abdomen. From these ulcerative nodules of the skin and nasal cavity, Burkholderia (Pseudomonas) mallei were isolated. Both horses give positive mallein test and variable levels of antibodies titers arranged between 80 – 640 with a mean of (224 ± 66.58). Three horses were died and showed extensive acute bronchopneumonia composed of purulent exudates in the bronchi, bronchioles and in alveoli. In other sections purulent exudates admixed with edema and fibrin filled the alveolar lumina. No granulomatous reactions were seen in the pulmonary tissue examined except that, in certain sections focal necrotizing pulmonary tissue with purulent exudates may be become pyogranuloma in chronic stage of the disease. Upper respiratory tract lesions include mucopurulent tracheitis rhinitis together with ulcerative nodules located in the nasal mucosa. Also enlargement of the bronchial and submixallary lymph nodes. Cutaneous lesions consisted of ulcerative nodules filled with pus distributed on the hind limbs and abdomen with suppurative lymphangitis giving farcy pipes form of cutaneous glanders lesions.*

Introduction
Glanders is an important zoonotic disease occurred when there were large concentration of horses in cities and armies, but now has a sporadic occurrence even in infected area (1). The disease is more wide spread but has been iradicated from most countries (2).Horses, mules, donkeys are the species usually affected. Humans are susceptible to the disease and the infection is usually fatal. Carnivores, Lions, may be infected by eating infected meat and the infections have been observed in the sheep, Goats and laboratory animals(3).Glanders is caused by Burkholderia (Pseudomonas) mallei, these microbial agents induce a contagious disease of solipeds these bacteria is unlikely to survive in a contaminated environment for more than 6 weeks (4). The infected animals or carriers that have made on apparent recovery from the disease are the important source of infection. The infection spreading on fodders, utensils from infected droplets of the nasal and oral secretions, so spread of infection by ingestion and inhalation of infected droplets. Rarely the cutaneous form appears to arise through contamination of skin abrasions by direct contact(5). The disease has two forms acute or chronic and characterized by pneumonia, upper respiratory tract infection and skin form of the disease (farcy) effecting the superficial lymphatic vessels and lymph nodes(6). This study aimed to identify and record the all clinical signs, serological parameters and pathological changes associated with this an acute outbreak of glanders in horses.

Materials and Methods
This study was conducted on theirteen Arabian-race horses, 2-9 years old, they were kept in a part of a stable farm, at Diyala province, Iraq. Three horses were died from acute glanders. The rest live (ten)horses showed different clinical signs of glanders. Burkholderia mallei were isolated from the ulcerative nodules of the nose and cutaneous nodules exudate(7). Few amount of the exudates of the ulcerative nodules of skin and the nose were injected 1/p in male guinea pigs for doing the strauss reaction. Mallein test was also done in the ten horses by injection of 0.1 ml of mallein Ag Intradermopalpebrally in the eye lid. Blood samples were taken from the ten horses for the determination the levels of antibodies using indirect hemaagglutination test (8). For pathological findings, pieces of pneumonic lesions, ulcerative nodular lesions of the upper respiratory tract and skin nodules were taken from the three necropsied horses, the tissue specimen were fixed in 10% neutral buffered formalin processed routinely for histopathological study (9).

The Results
Clinical findings :
All the live(10) horses showed signs of acute glanders composed of high fever, anorexia, loss of apetite and weakness and emaciation. There is coughing and mucopurulent nasal discharge together with 1-2 ulcerative nodules on the nasal mucosa and multiple nodular swellings distributed on the skin of hind limbs and abdomen along the course of lymphatic(Fig-1, Fig-2). Submaxillarly lymphnode was enlarged and swollen(Fig-3). Burkholderia mallei were isolated from the purulent exudate of ulcerative nodules of nose and skin. Strauss reaction was positive in male guinea pigs following 1/p injection of exudates of ulcerative nodules of the nose and skin(Fig-4). Mallein test was positive in all live(10) horses after 48 hrs of injection of mallein Ag. Characterized by edema and swelling of the eye lid(Fig-3) and microscopically, there is extensive congestion, edema and suppurative conjunctivitis(Fig-5).

Indirect hemaagglutination test : All the live(10) horses showed variable levels of antibodies against the mallein Ag, the level of the Ab were arranged between 80 – 640 with the mean of(224 ± 66.58).

Gross pathological findings :
There is peticheal hemorrhage throughout the body, congestion of the all organs mucopurulent nasal discharge, mucopurulent, tracheitis, purulent bronchopneumonia and enlargement of the bronchial and submaxillary lymph nodes(Fig-3). There is 1 – 2 ulcerative nodules on the nasal mucosa from which mucopurulent exudates released. Cutaneous lesions composed of multiple ulcerative nodules, edematous and purulent, distributed along the lymphatic vessels(Farcy pipes) of the hind limbs and abdomen(Fig-1, Fig-2).Both testes showed enlargement, edematous and swollen giving a feature of acute orchitis.

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Microscopic pathological findings :
Pulmonary lesions were composed of suppurative bronchopneumonia characterized by filling of bronchi, bronchioles and alveoli with the neutrophils (Fig-6). In certain section alveoli filled with fibrin, edema and neutrophils(Fig-7).The neutrophils infiltrate the mucosa of bronchi, and bronchioles, extensive congestion of alveolar capillaries together with neutrophils infiltration causing thickening of alveolar walls. The neutrophils cellular exudates and adjaeent pulmonary tissue showing focal necrotizing lesions may be develop in to the early pyogranuloma. Bronchial and submaxillary lymph nodes showed congestion, edema and filled with neutrophils cellular exudates giving the feature of acute purulent and edematous lymphadenitis(Fig-8). Upper respiratory tract showed mucopurulent tracheitis and rhinitis with ulceration of the nasal mucosa from which tenacious pus exudates released. Cutaneous lesions were composed of ulcerative nodules, necrotic from which releasing thick tenacious pus composed of neutrophils and necrotic tissue debris(Fig-9). attached to the wall of the ulcers.These ulcerative nodulesconnected by thick walled, tortuous, indurated lymphatic vessels filled with suppurative exudates i.e suppurative lymphangitis (Fig-10)

Fig .1 : Ulcerative nodules distributed on the skin of hind limbs (farcy form of glanders)

Fig. 2 : Ulcerative nodules distributed on the skin of hind limbs and abdomen (farcy form of glanders)

Fig .3 :mallein test was positive and enlargement of submaxillary lymph node

Fig .4 : Strauss reaction was positive in male guinea pig .

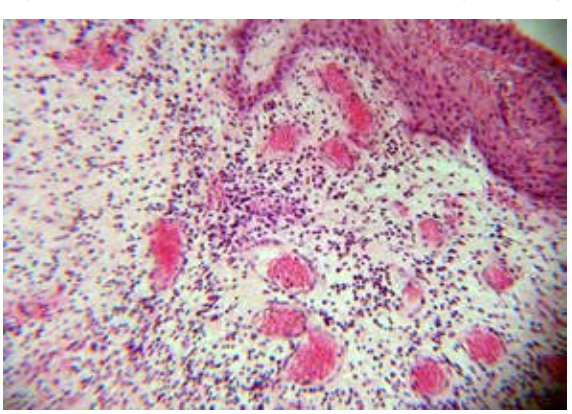

Fig .5 : microscopic picture of mallein test positive in the eye lid showed congestion , edema and suppurative conjunctivitis (H & E) X200

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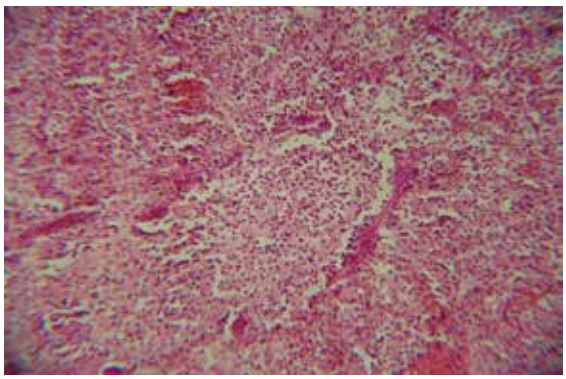

Fig .6 :suppurative bronchopneumonia characterized by filling of bronchi , bronchioles and alveoli with neutrophils (H&E) X100

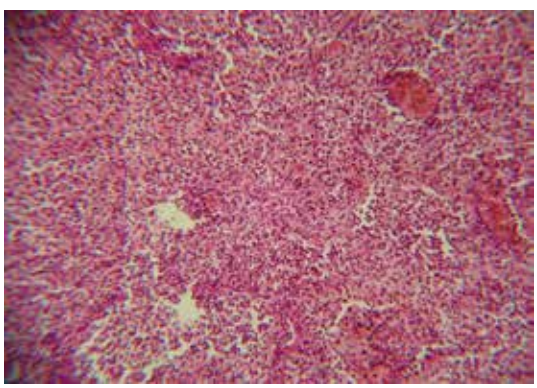

Fig .7 :suppurative bronchopneumonia characterized by filling of alveoli with neutrophils edema , fibrin and necrosis (H&E) X100

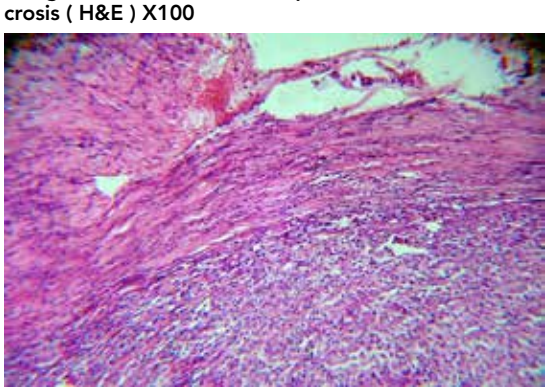

Fig .8 :lymph nodes : congestion . edema and filled with neutrophils cellular exdate (farcy form of glanders) (H&E) X100

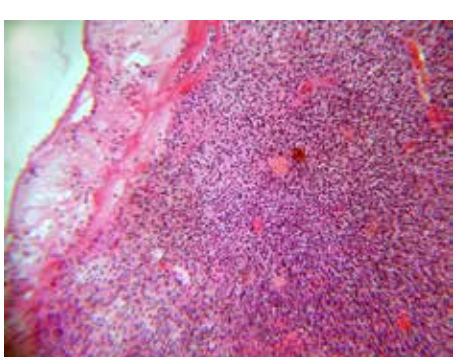

Fig .9 : Cutaneous ulcerative nodules composed of neutrophils , edema , congestion and fibran (H&E) X100

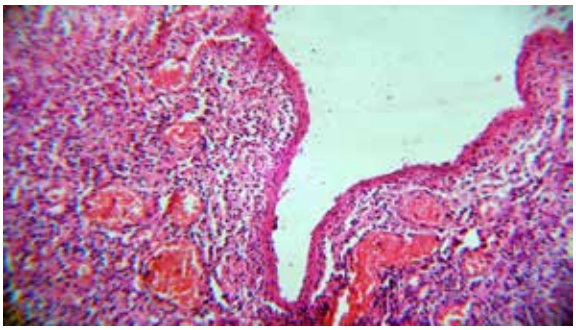

Fig .10 : lymphatic vessels filled with neutrophils , congestion , edema and fibrin I.eSuppurative lymphangitis (H&E) X100

Discussion

Burkholderia(Pseudomonas)mallei is an obligate parasite unlikely to survive more than 6 weeks in the contaminated environments and infect the horses which believed to be the principle resorviour host for this microbial agent(10).However, mules, donkeys, goat, sheep, lions, carnivores and human could be naturally infected(11) and even the laboratory animals could be experimentally infected. The glanders is widely distributed among the horses which is more evident in this outbreak in horses through the clinical signs, positive strauss reaction in male guinea pigs and positive mallein test in horses in addition to the death of three horses in this outbreak, A similar findings were reported by (12) in Iraq. The case fatality is considerably higher in old ages than in young horses(12) due the effect of age, continuous exposure and stress factor of management and the outbreak occurred in these group of horses reared together , for this reason, all the horses showed the typical clinical signs , similarly were recorded by others(13), in addition to the positive strauss reaction in male guinea pigs using infective exudates of the ulcerative nodules of the nose and skin and from this infective exudates B.mallei were identified.Also the positive mallein test in all the live(10)horses, the mallein test remains a valuable screening test for the diagnosis of the glanders. All the horses in this outbreak showed high titers of antibodies arranged between 80-640 with the mean of(224±66.58), all these findings with clinical signs give obsolute indication for the diagnosis of the glanders in this outbreak. Most of authors reported (14) that B.mallei induces both cellular and humoral immune responses which was evident in this outbreak through positive mallein test and high level of antibodies.Some authors considered that the titer 640 and above was positive for the diagnosis of glanders(15) whereas other workers considered 80 and above (16) which is evident in this outbreak is positive for diagnosis of the glanders.To confirm the diagnosis of this outbreak of glanders is the isolation of B.mallei from the ulcerative nodules exudate of the nose and skin together with positive strauss reactionand mallein test and high level of antibodies in addition to the typical clinical signs on horses in this outbreak. Three horses were died in this outbreak, the cause of death were due to septicemia occurred following invasive activity of B.mallei through the pharyngeal or intestinal mucosa into the lymphatic vessels and into the blood reaching the different internal organs such as mainly the lungs(5). Also the infection of the horses may be through inhalation of infective droplets directly into the lungs and cause bronchopneumonia and upper respiratory tract intections(5),which is more evident in this outbreak . Cutaneous lesions may be occurred through either direct contact of the horses with ulcerative nodular lesions exudates (5) or even the infected nasal discharge may be in contact with skin of hind limbs and abdomen and induces the cutaneous nodular lesions which more observed in this outbreak .Different pathological lesions were recorded in this outbreak .The main lesions were located in the lungs following the reaching of the B.mallei

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into the lungs after either ingestion or inhalation of infective droplets ,so more bronchopneumonia observed in this outbreak and in the dead horses(6), accompanied by enlargement of bronchial and submaxillary lymph nodes together-with mucopurulent tracheitis and rhinitis which more evident in the three dead horses. The infection of upper respiratory tract occurred as a result of invasive activity of B.mallei for the upper respiratory tract mucosa and induced typical lesions of glanders , a similar findings reported by (10,11). The B.mallei reached the upper respiratory mucosa through coughing of contaminated bronchial exudates(6). The pyogranulomas which were reported by other workers(17) in the lungs in chronic type of infection in horses , but not seen in this outbreak , because that this outbreak was acute and only suppurative bronchopneumonia were seen . It need time to become chronic and pyogranulomas seen .Glanders lesions were not seen in other internal organs such as spleen and liver in this acute outbreak may be need time to become seen in these internal organs (17). Multiple cutaneous ulcerative nodules with pus exudates connected by thick walled , tortuous lymphatic vessels together with suppurative lymphangitis were observed in the three dead horses were similarly reported by (11). These cutaneous ulcerative nodules in the hind limbs and abdomen occurred following either direct contact of horses or following dropping of contaminated nasal discharge into these location sites .

REFERENCE

1. Derbyshire, J.B.(2002). The eradication of glanders in Canada, can. Vet. J. 43:722-726. | 2. Hagan, E.L.; Bruner, B.G.; Gillespie, I.H. and Timoney, J.F.(1981). Infectious diseases of domestic animals 7th. Ed. Cornell Univ. Press, Ithaca, USA. | 3. Galati, P.; Puccini, V. and Contento, F.(1973). An outbreak of glanders in Lions. Histopathological findings. Acta Med. Vet. 19:261-277. | 4. Pritchard, D.C.(1995). Glanders. Equine Vet. Educ 7:29. | 5. Radostits, O.M.; Gay, C.C.; Hunchcliff, K.W. and Constable, P.D.(2007). Veterinary medicine: A textbook of the diseases of cattle, sheep, pigs, goats and horses. 10th. Ed. Saunders Elsevier, USA. | 6. Carlton, W.W. and McGavin, M.D.(1995). Thomson's Special Veterinary Pathology, 2nd. Ed. Mosby, USA. | 7. Quinn, P.J.; Carter, M.F.; Markey, B. and Carter, G.R.(1994). Clinical Veterinary Microbiology, Wolfe Publishing Company, London. | 8. Zhang, W.D. and Lu, Z.B.(1983). Application of an indirect hemagglutination test for the diagnosis of glanders and melioidosis , Chinese , J. Vet. Med. 9: 8-9. | 9. Luna, L.G. (1968). Manual of histological staining methods of the Armed Forces Institute of pathology, 3rd. ed. McGraw-Hill Book Company, USA. | 10. Jubb, K.V.; Kennedy, P.C. and Palmer, N. (2007). Pathology of domestic animals, 5th. Ed. Saunders Elsevier, USA. | 11. Jones, T.C.; Hunt, R.D. and King, N.W. (1997). Veterinary Pathology, 6th. Ed. Lippincott Williams and Wilkins, Baltimore, Maryland, USA. | 12. Al-Ani,F.K.;Al-Delaimi,A.K. Ali,H.A. (1987). Glanders in horses , clinical and epidemiological studies in Iraq.Pakistan Vet. J.7:126-129. | 13. Verema, R.D. (1981). Glanders in India with special reference of incidence and epidemiology. Indian Vet. J. 58:177-183. | 14. Hagebock, J.M.; Schlater, L.K.; Frerichs, W.M. and Alson, D.P. (1993). Serological responses to the mallein test for glandersin solipeds J. Vet. Diagn. Investig. 49:97-99. | 15. Gangulee, R.C.; Sen, G.P. and Sharma, G.L. (1966). Serological diagnosis of glanders by hemagglutination test, Indian Vet. J. 43:386. | 16. Al-Ani, F.K.; Al-Rawasheda, O.F.; Ali, H.A. and Hassan F.K. (1998). Glanders in horses : clinical, biochemical and serological studies In Iraq, Veterinary Archiv 68(5)155-162. | 17. Zubaidy, A.J. and Al-Ani, F.K. (1978). Pathology of glanders in horses In Iraq. Vet. Pathol. 15:566-568. |