An epizootic of equine influenza in Upper Egypt in 2000

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Summary
This study describes an epizootic of respiratory tract disease caused by influenza virus infection in a large population of equines in Luxor and Aswan, Upper Egypt, during the winter of 2000. The epizootic started in January and the infection rate reached its peak in February before gradually decreasing until the end of April, 2000. Horses, donkeys and mules of all ages and both sexes were affected. Free movement of the infected equines and direct contact between the animals at markets facilitated the rapid spread of the disease. The cause of the epizootic was established by use of serological testing and the identification of the influenza virus in nasal secretions. Egg inoculation and the haemagglutination test were used to detect the influenza virus. Both haemagglutination inhibition (HI) and agar gel precipitation tests were performed to identify the isolated influenza virus using reference antisera against A/Equi-1 (H7N7) and A/Equi-2 (H3N8). Antibodies against the equine influenza virus were demonstrated in 416 (95.6%) out of 435 collected sera using the HI test. High rectal temperature, inappetence, conjunctivitis, redness of nasal mucosa, a serous to mucopurulent nasal discharge and a harsh dry cough were the most common clinical manifestations. Stress factors, such as using equines for heavy transportation and drawing, precipitated the onset of the disease, intensified the clinical syndrome, delayed recovery and facilitated secondary bacterial infection. The present study suggested that the absence of a vaccination programme against equine influenza was one of the principal causes of the spread of infection during this outbreak. In conclusion, the implementation of a national equine influenza vaccination programme, using an effective updated vaccine, is essential in Egypt.

Keywords

Introduction
Equine influenza is a highly contagious viral disease that affects equine populations and occurs commonly in epizootic form (4, 17, 25). It is an acute, contagious respiratory disease caused by two distinct subtypes (subtype 1: H7N7 and subtype 2: H3N8) of influenza A viruses within the genus Influenzavirus A, of the family Orthomyxoviridae (26). It was not recognised as a specific disease entity until the mid-1950s, but after that, cases of the disease started to be reported from all over the world (9, 14, 30, 31, 32).

The first equine influenza virus ever isolated was in the Czech Republic in 1956. Later, it became a reference strain for subtype 1 and was designated A/Eq 1/56 (H7N7) (27).
In 1963, major epizootics of respiratory disease, affecting 50%-90% of horses, occurred in the United Kingdom (UK) and the United States of America (USA) (30). One of the outbreaks in the USA that year occurred in Miami, where a new subtype of equine influenza virus emerged and was designated A/Equi/1 (H3N8). The disease spread to horses in various geographic areas in a pattern which appeared to follow the routes taken by racehorses being transported from place to place (15, 33). In the autumn of 1963, an outbreak of equine influenza was reported in horses in England and investigations revealed that the disease was caused by influenza A/Equi/1 (1).

In 1965, serological evidence indicated that an A/Equi/1 or related strain of virus was enzootic in horses in New York (12). In that same year an acute outbreak of equine influenza was recorded in a riding school in Germany, where equine influenza virus (A1/equi/Berlin/65) was isolated for the first time. Some of the horses had secondary infections of bacterial origin (2).

Three epizootics of equine influenza were reported in the USA in the early 1990's, one in 1990, another in 1991 and a third in 1992. The disease was identified in 28% of 390 horses during the 1990 epizootic, 16% of 375 horses during the 1991 epizootic, and 18% of 398 horses during the 1992 epizootic. During these epizootics, young horses had a much greater risk of the disease than older horses. Horses less than 2 years old were approximately five to eight times more likely to develop upper respiratory disease than horses that were more than 5 years old (19).

During November and December 1992, an outbreak of equine-2 influenza virus A (H3N8) occurred among horses in Hong Kong (29). In May 1993, a severe epizootic of equine influenza was reported in Inner Mongolia and China. Serological evidence of the continued circulation of the avian-like H3N8 influenza virus in horses indicates that this virus has probably established itself in horses in Asia (11). Table I shows the previously reported equine influenza epizootics in different parts of the world.

In 1996, Daly et al. noted that antigenic and genetic variants of equine H3N8 viruses co-circulated, and that variants circulating in Europe and the USA were distinguishable from one another in terms of both the antigenic reactivity and the genetic structure of the HA1 portion of the haemagglutinin (HA) molecule (7). Serological and virological investigations of outbreaks of influenza in the UK during 1998 indicated that ‘American-like’ strains of influenza A/equine-2 virus were the cause of respiratory disease both in vaccinated and unvaccinated horses (24).

Clinically, equine influenza is characterised by pyrexia, mucopurulent nasal discharge and severe persistent cough (26, 30). Mild clinical signs were seen during an outbreak of equine influenza in vaccinated horses in the UK (23).

Liu et al. noted that antibodies against A-equi-1 virus and A-equi-2 virus derived from colostrums in newborn foals were of low titre and persisted only for a short period (16). However, equine influenza can be prevented by vaccinating with a bivalent, inactivated vaccine, which is administered three times: the first two vaccinations should

### Table 1

**Recorded epizootics of equine influenza**

<table>
<thead>
<tr>
<th>Country</th>
<th>Date</th>
<th>Causative virus</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Czech Republic</td>
<td>1956</td>
<td>A/Equi/1/S6 (subtype 1)</td>
<td>Savinova et al. (31)</td>
</tr>
<tr>
<td>United States of America</td>
<td>1963</td>
<td>A/Equi/1/Miami/1/63</td>
<td>Kitchen et al. (15)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>1963</td>
<td>Influenza A/Equi/1</td>
<td>Beveridge et al. (1)</td>
</tr>
<tr>
<td>Switzerland</td>
<td>1965</td>
<td>A/Equi-2</td>
<td>Gerber and Loehrer (9)</td>
</tr>
<tr>
<td>Germany</td>
<td>1965</td>
<td>A/Equi/Miami/65</td>
<td>Biblm and Zeller (2)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>1973</td>
<td>Equine influenza A subtype 1</td>
<td>Powell et al. (28)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>1976</td>
<td>Equine influenza type 2</td>
<td>Thomson et al. (32)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>1979</td>
<td>Influenza A/Equi 2 virus</td>
<td>Burrows et al. (4)</td>
</tr>
<tr>
<td>United States of America</td>
<td>1983</td>
<td>A/Equi-2 influenza virus</td>
<td>Kemen et al. (14)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>1989</td>
<td>Influenza A/Equi-2 (H3N8)</td>
<td>Livesay et al. (17)</td>
</tr>
<tr>
<td>United States of America</td>
<td>1990, 1991 and 1992</td>
<td>A/Equi-2 (H3N8) subtype</td>
<td>Morley et al. (20)</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>1992</td>
<td>Equine-2 influenza virus A (H3N8)</td>
<td>Powell et al. (29)</td>
</tr>
<tr>
<td>Inner Mongolia and the People’s Republic of China</td>
<td>1993</td>
<td>H3N8 influenza virus</td>
<td>Guo et al. (11)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>1995 and 1996</td>
<td>A/equine-2 viruses (H3N8)</td>
<td>Newton et al. (25)</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>1998</td>
<td>‘American-like’ strains of influenza A/equine-2 virus</td>
<td>Newton et al. (24)</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>1995-2000</td>
<td>All strains belonged to A/Equi/1/Miami/63 (subtype 2)</td>
<td>Pospisil et al. (27)</td>
</tr>
</tbody>
</table>
be administered eight to twelve weeks apart, and the third six months later. Revaccination should be carried out at nine-month intervals (8). Mumford et al. reported that the HA content of a vaccine immunostimulating complex was found to be superior to aqueous whole virus vaccines and consequently they offered longer lasting protection (22). In their 2001 study, Cullinane et al. noticed (6) that the monovalent vaccine induced a significantly better response than the immunostimulating complex vaccine or the multivalent vaccine.

The purpose of the present study was to describe the epidemiological pattern, clinical manifestations, virological diagnosis and serological investigations of an epizootic of equine influenza in Luxor and Aswan, Upper Egypt, during the winter of 2000.

Materials and methods

Epidemiology

The authors visited the infected areas at the time of the outbreak to clinically examine diseased equines and collect blood samples and nasal swabs for virological and serological investigations. The infected population had not been previously vaccinated. Epidemiological data about the outbreak and information obtained from the Brooke Animal Hospital in Luxor were used to define the disease status.

Clinical examinations

Thorough clinical examinations were performed during the outbreak, including, mucous membrane examination and percussion and auscultation of the lungs. The pulse rate, respiratory rate and rectal temperature of the diseased equines were also taken.

Virological and serological diagnosis

Laboratory diagnosis of the epizootic was carried out at the Department of Virology, Animal Health Research Institute in Dokki, Cairo.

Serum samples

Ten millilitres of whole blood from clinically-ill animals was collected in sterile test tubes without anticoagulant. A total of 436 blood samples, 274 samples from Luxor and 162 samples from Aswan, were collected. These samples were centrifuged at 1,000 g for 10 min., and the sera were decanted and frozen. Before the serological examinations, all collected sera were heated at 56°C for 30 min. and treated with 25% kaolin solution to eliminate the non-specific inhibitors of HA.

Nasal swabs

Fifty-six nasal swabs (thirty from Luxor and twenty-six from Aswan) were obtained from affected animals and placed in sterile phosphate buffer saline (PBS). The specimens were transported to the laboratory in a chilled state. The swab fluids were clarified at low speed centrifugation, 1,000 g for 5 min. Antibiotics, namely, 100 IU of penicillin and 100 µg of streptomycin per ml, were added to the supernatant, which was left at 4°C for one hour then kept at –70°C until used for egg inoculation. Table II shows the number of nasal swabs and serum samples collected.

<table>
<thead>
<tr>
<th>Location</th>
<th>Nasal swabs</th>
<th>Serum samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Horses (18)</td>
<td>Donkeys (9) Mules (3)</td>
</tr>
<tr>
<td>Luxor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aswan</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>21</td>
</tr>
</tbody>
</table>

Egg inoculation

Doses of 0.2 ml of swab extracts were inoculated into the allantoic cavity of eight-to-ten-day embryonated hen eggs, which were further incubated for three days at 35°C. The allantoic fluids were then harvested and tested for haemagglutinating activity. Harvested fluids which proved negative were kept for a further two blind passages.

Haemagglutination test

The haemagglutination test was used to detect equine influenza virus and was carried out according to the method described by Powell et al. (28). All titrations were carried out in U-shaped microtitre plates. Fifty microlitres of PBS were dispensed into the individual wells of each plate. Fifty microlitres of the suspected virus fluid (allantoic fluid) were added to the wells of the first row and thoroughly mixed. Fifty microlitres were transferred from row 1 to row 2 and so on to make a two-fold dilution. Fifty microlitres of 1% chicken red blood cells (RBCs) suspended in PBS were added to all wells including control wells. The microplate was incubated at room temperature for one hour until the cell control wells showed a button of deposited RBCs. The appearance of a complete red lattice formation at the bottom of the wells was an indication of positive haemagglutination.

Haemagglutination inhibition test

The haemagglutination inhibition (HI) test was used to detect equine influenza antibodies in the collected serum
samples. In addition it was used to identify the recovered equine influenza virus using reference equine influenza antisera against strains H7N7 and H3N8.

The HI test was carried out according to Powell et al. (28). Twenty-five microlitres of PBS were dispensed in the wells of U-shaped microtitre plate. 25 microlitres of each serum sample were added to the wells of the first row. Reference equine influenza antisera were also included as control wells. Both test and control sera were diluted serially to the end of the plate in two-fold dilution steps. Twenty-five microlitres of PBS containing four HA units of the virus (isolated agent) were then added to all wells. Serum/virus mixtures were left at room temperature for one hour before adding 25 microlitres of 1% chicken RBCs. The microplate was covered and incubated at room temperature until an agglutination pattern appeared in the control wells.

Agar gel precipitation test

The agar gel precipitation test (AGPT) was used to identify equine influenza virus using reference antisera against H3N8 and H7N7 (Ames, Iowa), kindly obtained from the Faculty of Veterinary Medicine, Cairo University. The AGPT was carried out according to Boussetta et al. (3). Seven wells (one central well and six peripheral wells) were cut in 1% agarose agar and 50 µl of the suspected virus was dispensed in the central well. Fifty microlitres of reference antisera against the Miami (H3N8) and Prague (H7N7) strains were pipetted into the peripheral wells alternatively. Serum free from equine influenza antibodies was added to two wells as a control negative. After incubation of the plate at room temperature for 24 h to 48 h, a positive result appeared as a precipitin line.

Results

Epidemiology

Table III shows the number of clinical cases in donkeys, mules and horses that were observed in Luxor and Aswan and their surrounding countryside as well as the cases that were submitted to the Brooke Animal Clinic in Luxor. The infection rate reached its peak in February, as shown in Table IV and Figure 1.

Free movement of infected equines and direct contact between animals at market facilitated the rapid and wide spread of the causative virus. The stress of intensive work such as drawing carts and being used for heavy transportation played a large part in worsening the clinical disease and predisposed the diseased equines to secondary bacterial infection.

Clinical symptoms

Thorough clinical examinations of 982 diseased animals revealed high rectal temperature (39°C-41°C), redness of the nasal mucosa, conjunctivitis, a serous to mucopurulent nasal discharge and a harsh dry cough. The lung sounds were characterised by crackles and wheezes and an increase in the intensity of the normal breath sounds. Depression, anorexia and reluctance to move were common signs, especially in the complicated cases.

During this outbreak, animals under stress, as a result of heavy and continued work, suffered from more severe clinical symptoms. Horses and donkeys who were worked particularly hard had a prolonged clinical course of the disease and showed symptoms of secondary bacterial infection with severe purulent nasal discharge, as shown in Figure 2.
Virological and serological diagnosis

Detection of influenza virus

After inoculation into the allantoic cavity of eight-to-ten-day embryonated hen eggs, equine influenza virus was detected in eighteen nasal swabs from animals in Luxor and in twelve nasal swabs from animals in Aswan (Tables V and VI). Three passages were made to increase the virus titres. After the third passage, the isolated virus (allantoic fluid) haemagglutinated chicken RBCs at titres of 1/32 and 1/64.

Identification of the isolated virus

Both the HI test and AGPT were used to identify the isolated influenza virus using reference antisera against A/Equi-1 (H7N7) and A/Equi-2 (H3N8). The results are shown in Tables VII and VIII.

Serodiagnosis

A total of 416 (95.4%) out of 436 collected sera contained antibodies against equine influenza virus as detected by HI test (Table IX). A total of 266 (97.1%) out of 274 sera from Luxor were positive for antibodies against influenza virus, while 150 (92.6%) out of 162 serum samples from Aswan were seropositive.

Discussion

Characteristically, equine influenza virus spreads rapidly in a susceptible population (26) and this was indeed the case with the 2000 outbreak in Upper Egypt where the morbidity rate ranged from 50% to 70% in different locations.

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**Table V**

Detection of equine influenza virus by inoculation into embryonated chicken eggs

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of examined nasal swabs</th>
<th>HA positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st passage</td>
<td>2nd passage</td>
</tr>
<tr>
<td>Luxor</td>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td>Aswan</td>
<td>26</td>
<td>12</td>
</tr>
</tbody>
</table>

HA: haemagglutination

**Table VI**

Number of equine samples positive for isolation of the influenza virus

<table>
<thead>
<tr>
<th>Location</th>
<th>Horses</th>
<th>Donkeys</th>
<th>Mules</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luxor</td>
<td>12/18</td>
<td>5/9</td>
<td>1/3</td>
<td>18/30</td>
</tr>
<tr>
<td>Aswan</td>
<td>7/11</td>
<td>5/12</td>
<td>0/3</td>
<td>12/26</td>
</tr>
<tr>
<td>Total</td>
<td>19/29</td>
<td>10/21</td>
<td>1/6</td>
<td>30/56</td>
</tr>
</tbody>
</table>

**Table VII**

Identification of the isolated influenza virus using haemagglutination inhibition (HI) test

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of virus isolates</th>
<th>Results of HI test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A/Equi-2 (H3N8)</td>
<td>A/Equi-1 (H7N7)</td>
</tr>
<tr>
<td>Luxor</td>
<td>8</td>
<td>1/128</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1/64</td>
</tr>
<tr>
<td>Aswan</td>
<td>5</td>
<td>1/128</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1/32</td>
</tr>
</tbody>
</table>
Secondary bacterial pneumonia almost always accompanies equine influenza (34). Simple influenza infection causes a slight serous nasal discharge which later turns mucoid or muco-purulent, depending on secondary infection (35). In the Luxor and Aswan epizootic, many diseased horses suffered from heavy mucopurulent nasal discharge (Fig. 2), which could be attributed to the secondary bacterial invasion.

Hard work, i.e. use of donkeys and horses for transportation and travelling, may have precipitated the onset of the disease, intensified the clinical syndrome and delayed recovery. Gross et al. (10) have suggested that exercise can affect the course of clinical disease in infected horses (in their study, the exercise group exhibited more severe signs of the clinical disease). The current study suggests that diseased equines should be rested to avoid a severe and prolonged clinical course of the disease.

Age was an important risk factor for disease during three epizootics of equine influenza in the USA in 1990, 1991, and 1992. The risk of disease increased incrementally as age decreased. It is likely that age was a non-specific marker of exposure and immunity, i.e. older horses were more likely to have been exposed to infectious agents through vaccination or natural exposure than young horses (20). In contrast, horses, donkeys and mules of all ages and both sexes were affected during the equine influenza outbreak in Luxor and Aswan.

During the outbreaks in the USA, serological testing was found to be the most sensitive method of detecting influenza virus infections (20). Similarly, during the 2000 epizootic in Egypt, the infectious agent was established by serological investigations and identification of the influenza virus in nasal swabs.

Webster (34) stated that the two biotypes of equine influenza viruses (equine 1 and 2 viruses) co-circulated in horses for many years. Of the two serologically distinct subtypes, all reported outbreaks of equine influenza in the past two decades have been caused by strains of subtype 2 (H3N8) (30), as shown in Table I. In the laboratory investigations of the authors, virus isolates reacted more clearly with antisera against A/Equi-2, where 1/128 and 1/64 HI titre and a faint precipitation band were noted in the AGPT. However, the virus isolates produced 1/32 and 1/16 HI titre and a clear precipitation band were noted in the AGPT. However, the virus isolates produced 1/32 and 1/16 HI titre and a faint precipitation band were noted in the AGPT. The virus isolates produced 1/32 and 1/16 HI titre and a faint precipitation band were noted in the AGPT. The virus isolates produced 1/32 and 1/16 HI titre and a faint precipitation band were noted in the AGPT. 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<table>
<thead>
<tr>
<th>Location</th>
<th>Positive samples/ sera examined</th>
<th>Haemagglutination inhibition titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1/4</td>
<td>1/8</td>
</tr>
<tr>
<td>Luxor</td>
<td>266/274</td>
<td>92</td>
</tr>
<tr>
<td>Aswan</td>
<td>150/162</td>
<td>48</td>
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<tr>
<td>Total</td>
<td>416/436</td>
<td>140</td>
</tr>
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</table>

During the 1963 equine influenza epizootic in the USA, horses of all ages were affected and the attack rate was very high. The incubation period was three to five days and the usual clinical signs were a cough, fever, catarrhal rhinitis, and, later in the course of the disease, a mucopurulent nasal discharge. The course of uncomplicated cases was four to seven days. Exercise and stress prolonged the clinical illness. There were no reports of deaths directly related to influenza (15). Similar observations were made in the Upper Egypt equine influenza epizootic, which was characterised by a high morbidity with a high proportion of animals affected at one time. Clinically, the disease was characterised by high fever, rhinitis, conjunctivitis, excessive lacrimation, a serous to mucopurulent nasal discharge and a harsh dry cough. In the non-complicated cases the course of the disease was short and the rate of recovery was high. In the UK, equine influenza-affected horses typically had rapid onset high body temperatures and a hacking dry cough. In addition, there was non-specific malaise with often heavy mucopurulent nasal discharges (36).
seroprevalence of A/Equi-1 and A/Equi-2 subtypes was 9.9% and 12.8% respectively (5). There is a need for this type of seroepidemiological survey in Egypt so that more can be known about the influenza virus infection among equine populations.

During the evaluation of the efficacy of a commercial vaccine against equine influenza, Morley et al. noted that the median duration of clinical disease was three days shorter in vaccinated horses (18). Experimentally, the degree of protection against clinical equine influenza correlated closely with circulating antibody levels. When influenza occurs in vaccinated horses, the duration and severity of clinical signs are significantly reduced, and recovery is usually rapid and uneventful, especially if horses are rested in the acute stage (21, 36). The absence of a vaccination programme against equine influenza in Egypt most probably contributed to the rapid spread of the disease during the outbreak in Luxor and Aswan.

The main cause of the outbreak in Upper Egypt was A/Equi-2 virus. It has been shown that a new modified live equine influenza virus 2 vaccine is safe and does not cause any clinical signs in vaccinated young equids (13). It is advisable to update equine influenza vaccine strains regularly so that they contain similar strains to variants that are circulating in the field (37). An equine influenza vaccination programme, using an effective, updated vaccine, is essential for Egypt.

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Une épizootie de grippe équine en Haute-Égypte en 2000

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Résumé
Epizootia de gripe equina en el Alto Egipto en 2000

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Resumen

Los autores describen una enfermedad epizoótica del tracto respiratorio provocada por la infección por el virus de la gripe que afectó a una inmensa población equina de Luxor y Asuán (Alto Egipto) en invierno de 2000. La epizootia empezó en enero, y la tasa de infección alcanzó su nivel máximo en febrero, para después bajar gradualmente hasta finales de abril de 2000. Resultaron afectados caballos, asnos y mulas de ambos sexos y de todas las edades. La libre circulación de equinos infectados y el contacto directo entre animales en los mercados facilitaron la rápida propagación de la enfermedad. Para determinar la causa de la epizootia se emplearon pruebas serológicas, combinadas con la identificación del virus en las secreciones nasales. La inoculación de huevos y la prueba de hemoaglutinación sirvieron para detectar el virus en cuestión. Después se realizaron ensayos de inhibición de la hemoaglutinación y de precipitación en gel de agar para tipificar el virus aislado mediante antisueros de referencia contra A/Equi-1 (H7N7) y A/Equi-2 (H3N8). Con el primero de estos ensayos se comprobó la presencia de anticuerpos contra el virus de la gripe equina en 416 (95,6%) de los 435 sueros recogidos. Las manifestaciones clínicas más frecuentes de la enfermedad eran: elevada temperatura rectal, inapetencia, conjuntivitis, enrojecimiento de la mucosa nasal, secreción nasal entre serosa y mucopurulenta y tos áspera y seca. La concurrencia de una serie de factores de estrés, por ejemplo el uso de los animales para tareas pesadas de tiro y transporte, precipitó la aparición de la enfermedad, intensificó el síndrome clínico, retrasó la recuperación y facilitó la aparición de infecciones bacterianas secundarias. De este estudio se desprende que la falta de un programa de vacunaciones contra la gripe equina fue una de las principales causas de que la infección se propagara tras el brote inicial. Los autores concluyen que es indispensable aplicar en Egipto un programa nacional de vacunaciones contra la enfermedad utilizando una vacuna eficaz y moderna.

Palabras clave
References


