An outbreak of peste des petits ruminants in migratory flocks of sheep and goats in Egypt in 2006

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An outbreak of peste des petits ruminants in migratory flocks of sheep and goats in Egypt in 2006

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Summary

An outbreak of peste des petits ruminants (PPR) was recorded in Kalubia province, Egypt in 2006, affecting a large population of migratory goats and sheep over a huge geographical area. Epidemiological, clinical and laboratory investigations were performed. Diseased animals showed pyrexia, erosive stomatitis, enteritis and bronchopneumonia. Clinical manifestations were more severe in goats. The overall morbidity, cumulative mortality and case fatality rates were 26.1%, 10.5% and 40.2%, respectively, and were significantly higher in young animals. Post-mortem examination showed emaciation, congested mucous membranes, lymphadenopathy, hepatosplenomegaly, haemorrhagic necrosis of the abomasal and intestinal mucosa, pleurisy and lung consolidation. Forty oculonasal swabs and 243 serum samples from diseased animals were tested for PPR antigen and antibodies using immunocapture and competitive enzyme-linked immunosorbent assays (ELISA), respectively. PPR antigen was detected in 30/40 (75%) of the swabs. PPR virus was identified in inoculated Vero cells using immunocapture ELISA and fluorescent antibody technique (FAT); 33/40 (82.5%) and 36/40 (90%) samples were positive, respectively. Of 243 sera, 154 (63.4%) contained PPR antibodies. Circulation of PPR among the migratory sheep and goat flocks was demonstrated. Strict serosurveillance and monitoring of PPR with vaccination of migratory flocks at borders is required for effective control of the disease.

Keywords

Cell culture – Clinical manifestation – Egypt – Enzyme-linked immunosorbent assay – Epizootiology – Fluorescent antibody technique – Migratory sheep and goats – Outbreak – Peste des petits ruminants – Post-mortem findings.

Introduction

Peste des petits ruminants (PPR) is an acute febrile viral disease of sheep and goats characterised by mucopurulent nasal and ocular discharge, necrotising and erosive stomatitis, enteritis and pneumonia (16). It affects small ruminants, especially goats, which are highly susceptible, and occasionally wild animals (30).

The virus that causes PPR belongs to the morbillivirus group of the *Paramyxoviridae* family (16). It has created

tremendous problems because of its apparent similarity to rinderpest (26).

Peste des petits ruminants was first described in West Africa (Côte d'Ivoire) in the 1940s by Gargadennec & Lalanne (15). It is an important viral disease of goats and sheep and is prevalent in Africa and the Middle East. In the 1990s, PPR emerged in India, first in South India and later in North India. Outbreaks of PPR have been frequent in recent years in India (25, 36, 42). At least four distinct genetic lineages (lineages I, II, III and IV) of PPR virus with different geographical distributions circulate among small ruminants in endemic regions (6). The fourth lineage is confined exclusively to the Middle East, Arabia and the Indian sub-continent (36).

In Egypt, PPR is one of the major threats to small ruminant populations (1). The current study was planned to investigate the epidemiology, clinical manifestations, post-mortem findings and laboratory diagnosis of a PPR outbreak that occurred during the winter of 2006. The outbreak affected a large population of migratory goats and sheep over a huge geographical area (Banha, Kafr-Shouker, Touhk and Moshtoher districts) in Kalubia province. A further objective of the study was to estimate the seroprevalence of PPR virus in migratory flocks of sheep and goats in the affected areas.

Materials and methods

Epidemiology and clinical examinations

Four migratory flocks of sheep and goats in Banha, Kafr-Shouker, Touhk and Moshtoher in Kalubia province were visited to investigate a complaint of diarrhoea and cough as well as mortality, especially among newborn and young animals. The total number of small ruminants in each flock was recorded. The first flock, in Banha, consisted of 200 sheep and 50 goats; the second, in Kafr-Shouker, consisted of 100 sheep and 70 goats; the third, in Touhk, consisted of 150 sheep and 83 goats; and the fourth flock, in Moshtoher, consisted of 170 sheep and 140 goats.

These animals are managed under a free-range grazing system throughout the day. They usually suffer from the stress of migration and nutritional deficiencies. Most of the migratory small ruminants are tired and they sometimes suffer from thirst and hunger. There is no control over the free movement of such migratory sheep and goats for grazing and/or marketing throughout the country.

Samples

A total of 40 oculonasal swabs and 243 random serum samples were collected from migratory flocks of sheep and goats suspected to be infected with PPR virus in Kalubia province during winter 2006. The sera and swabs were prepared in accordance with the methods of Burleson *et al.* (4) and stored at –20°C until used.

Laboratory diagnosis

Cell culture and virus isolation

The cell line of African green monkey kidney (Vero) cells was obtained from the Department of Pet Animal Vaccine Research at the Veterinary Serum and Vaccine Research Institute in Abbasia, Cairo. Cell culture inoculation was carried out according to the method of Burleson *et al.* (4).

Immunocapture enzyme-linked immunosorbent assay

A commercial immunocapture enzyme-linked immunosorbent assay (ELISA) (27) was used to detect PPR viral antigen in both clinical samples and inoculated Vero cells. It was applied according to the manufacturer's instructions (Biological Diagnostic Supplies Ltd [BDSL®]).

Competitive enzyme-linked immunosorbent assay

A commercial competitive ELISA kit (28) was used to detect seropositive animals. It was applied according to the manufacturer's instructions (BDSL®).

Fluorescent antibody technique

Anti-PPR and anti-ovine antibodies conjugated with fluorescence isothiocyanate (Sigma Chemicals®) were used to demonstrate PPR virus in the inoculated fixed Vero cells in accordance with the method of Durojaiye (11).

Results

Epidemiology and clinical examinations

Clinical examinations of the diseased animals revealed high fever, ocular and nasal discharge, erosive and necrotic stomatitis, diarrhoea and cough. The clinical signs were more severe in goats than in sheep. Newborn and young animals were more severely affected than adults. Table I shows the morbidity, mortality and case fatality rates in sheep and goats in the four flocks. Overall, the morbidity, mortality and case fatality rates were 26.1%, 10.5% and 40.2%, respectively. No mortality was observed among sheep in the migratory flock in Banha. The mortality and case fatality rates were higher in goats than in sheep in the four flocks investigated.

Table I
Morbidity, mortality and case fatality rates of peste des petits ruminants during the 2006 outbreak in Kalubia province, Egypt

Location	Total number in flock		Morbidity rate (diseased/total)		Mortality rate (dead/total)		Case fatality rate (dead/diseased)	
	Sheep	Goats	Sheep	Goats	Sheep	Goats	Sheep	Goats
Banha	200	50	6% (12/200)	40% (20/50)	0% (0/200)	10% (5/50)	0% (0/12)	25% (5/20)
Kafr-Shouker	100	70	25% (25/100)	64.3% (45/70)	13% (13/100)	27.1% (19/70)	52% (13/25)	42.2% (19/45)
Touhk	150	83	33.3% (50/150)	22.9% (19/83)	2% (3/150)	14.5% (12/83)	6% (3/50)	63.2% (12/19)
Moshtoher	170	140	18.8% (32/170)	34.3% (48/140)	5.9% (10/170)	27.9% (39/140)	31.3% (10/32)	81.3% (39/48)
Total	**620**	**343**	**19.2% (119/620)**	**38.5% (132/343)**	**4.2% (26/620)**	**21.9% (75/343)**	**21.8% (26/119)**	**56.8% (75/132)**
	963		**26.1% (251/963)**		**10.5% (101/963)**		**40.2% (101/251)**	

Laboratory diagnosis

Detection of viral antigen

Eight (53%) out of 15 oculonasal swabs from sheep and 22 (88%) out of 25 swabs from goats were positive for PPR viral antigen using the capture ELISA. In total 30 (75%) out of 40 oculonasal swabs examined were positive (Table II).

Table II
Detection of peste des petits ruminants antigen in oculonasal swabs by immunocapture enzyme-linked immunosorbent assay

Animal species	Number of examined oculonasal swabs	Positive samples	
		Number	Percentage
Sheep	15	8	53
Goats	25	22	88
Total	**40**	**30**	**75**

Identification of virus

Table III shows the results of the identification of PPR virus in the inoculated Vero cell cultures using immunocapture ELISA and fluorescent antibody technique (FAT). The results showed that 33/40 samples (82.5%) were positive using the immunocapture ELISA while 36 (90%) were positive using FAT.

Detection of antibodies against peste des petits ruminants virus

Table IV reveals that the seroprevalence of PPR in ovine sera was 80/150 (53.3%) and it was 74/93 (79.6%) in caprine sera. The total number of serum samples positive for PPR antibodies was 154/243 (63.4%).

Table III
Identification of peste des petits ruminants virus antigen in inoculated Vero cell cultures by immunocapture enzyme-linked immunosorbent assay and fluorescent antibody technique

Animal species	Number of inoculated samples	Immunocapture ELISA Positive samples		FAT Positive samples	
Sheep	15	10	(66.7%)	13	(86.7%)
Goats	25	23	(92%)	23	(92%)
Total	**40**	**33**	**(82.5%)**	**36**	**(90%)**

ELISA: enzyme-linked immunosorbent assay
FAT: fluorescent antibody technique

Discussion

Peste des petits ruminants is an economically important viral disease of goats and sheep first described in West Africa in the 1940s. The virus has been circulating in parts of sub-Saharan Africa for several decades and in the Middle East and southern Asia since 1993 (6). The huge number of small ruminants that are reared in the enzootic areas makes PPR a serious disease that threatens the livelihood of poor farmers (8). In Egypt, small ruminants are one of the main sources of meat production. Both sheep and goats are raised under traditional extensive systems, although intensive husbandry systems have recently been established. Infection with PPR virus is common in Egypt, causing high morbidity and mortality and leading to severe economic losses (1, 12, 14). The present study reported a severe outbreak of PPR in four migratory flocks of sheep and goats in four districts (Banha, Kafr-Shouker, Touhk

Table IV
Prevalence of peste des petits ruminants antibodies in sera from sheep and goats using competitive enzyme-linked immunosorbent assay

Location	Sheep						Goats						Total prevalence in sheep and goat sera
			Frequency of positive sera in different age groups						Frequency of positive sera in different age groups				
	Sera examined	Positive sera	<6 months	6 to 12 months	1 to 2 years	>2 years	Sera examined	Positive sera	<6 months	6 to 12 months	1-2 years	>2 years	
Banha	30	15 (50%)	9/12 (75%)	4/9 (44.4%)	1/4 (25%)	1/5 (20%)	12	10 (83.3%)	5/7 (71.4%)	2/2 (100%)	2/2 (100 %)	1/1 (100 %)	25/42 (59.5%)
Kafr-Shouker	50	32 (64%)	17/19 (89.5%)	9/13 (69.2%)	3/8 (37.5%)	3/10 (30%)	15	10 (66.7%)	6/8 (75%)	1/4 (25%)	1/1 (100%)	2/2 (100%)	42/65 (64.6%)
Touhk	30	12 (40%)	4/9 (44.4%)	3/15 (20%)	2/3 (66.7%)	3/3 (100%)	21	16 (76.2%)	8/9 (88.9%)	4/5 (80%)	2/3 (66.7%)	2/4 (50%)	28/51 (54.9%)
Moshtoher	40	21 (52.5%)	11/15 (73.3%)	6/8 (75%)	2/8 (25%)	2/9 (22.2%)	45	38 (84.4%)	19/21 (90.5%)	15/18 (83.3%)	2/3 (66.7%)	2/3 (66.7%)	59/85 (69.4%)
Total	**150**	**80 (53.3%)**	**41/55 (74.5%)**	**22/45 (48.9%)**	**8/23 (34.8%)**	**9/27 (33.3%)**	**93**	**74 (79.6%)**	**38/45 (84.4%)**	**22/29 (75.9%)**	**7/9 (77.8%)**	**7/10 (70%)**	**154/243 (63.4%)**

and Moshtoher) in Kalubia province, Egypt during the winter of 2006. Infection with PPR virus was also confirmed in three flocks of sheep in Giza governorate, Egypt in 1995 (31).

Peste des petits ruminants can occur in an epizootic form; it may have dramatic consequences with morbidity of 80% to 90% and mortality between 50% and 80% (26). In the current study, the overall morbidity, cumulative mortality and case fatality rates among sheep and goats during the extensive PPR outbreak in Kalubia province were 26.1%, 10.5% and 40.2%, respectively. The highest mortality rate (27.9%) was reported among goats in Moshtoher, while no mortality was observed among sheep in Banha. An outbreak of PPR in a flock of 70 goats with a mortality rate of 32% was recorded in Embaba, Giza province in Egypt in January 1987 (20).

It has been shown that goats are more susceptible to PPR than sheep, and that young animals of both species are at higher risk than adults (5, 9, 21, 22). The present study also found that goats were more susceptible to PPR virus infection than sheep, and that kids and lambs were at higher risk than adults.

The stress of migration, coupled with low environmental temperatures, and bolstered by humidity and nutritional deficiency may explain the occurrence of the disease (18, 22). In the Kalubia province epidemic, it was suggested that the stress of migration coupled with nutritional deficiencies acted as a predisposing factor for the disease. It was also noticed that the disease was more severe in tired animals, and those suffering from thirst and hunger. In addition, the free movement of migratory sheep and goats for grazing and/or marketing may have been responsible for the spread of PPR virus infection among sheep and goats in the four districts in Kalubia province.

Clinically, the recorded outbreak was characterised by high fever, anorexia, mucopurulent nasal discharge, conjunctivitis and encrustation in the medial canthus, ulcerative stomatitis, profuse diarrhoea and bronchopneumonia. These clinical signs are in accord with those described by Taylor (41). The same clinical signs were also observed previously during PPR outbreaks in Nigeria (17), Sudan (13, 29), Saudi Arabia (2), India (5, 21, 32, 33, 37, 42), Iran (3), Turkey (24) and Pakistan (23).

During the outbreak studied here, post-mortem examination showed mild to severe lesions. Emaciation of the carcass, congestion of the mucous membranes, erosions of the buccal mucosa, swelling of the mesenteric lymph nodes, moderate enlargement of the liver and spleen, haemorrhage and necrosis in the abomasal and intestinal mucosa, severe congestion of the lungs, and pleurisy, as well as areas of consolidation in the lungs, were observed. These findings are in agreement with those reported previously (2, 9, 24, 37, 43).

To confirm the presumptive diagnosis, immunocapture ELISA was used in this study for the detection of PPR viral antigen in 40 oculonasal swabs. The results showed that 53% and 88% of swabs from sheep and goats were positive, respectively. In total, 75% of the 40 swabs examined were positive. This indicates that PPR virus was the causative agent of the outbreak and that endemic PPR virus is circulating within and between the small ruminant

flocks. During an outbreak of PPR in the central and southern regions of the Kingdom of Saudi Arabia in 2004, it was concluded that the disease was mostly associated with the presence of enzootic virulent strains of PPR virus. These strains could have circulated between the infected and susceptible animals in flocks of sheep and goats in the absence of a national vaccination regime against the disease (2).

Peste des petits ruminants virus should be isolated from field samples in cell culture for further identification, even when the detection of PPR viral antigen has been carried out by rapid immunocapture ELISA (11, 26). The current study revealed that the inoculation, isolation and propagation of PPR virus in Vero cells was successful from the first passage, with the cytopathic effect (CPE) observed in the form of cell granulation and vacuolation of the cytoplasm. The cells became rounded in shape and then clustered to form cell syncytia, followed by plaque formation and complete drop of the cell sheet. This characteristic CPE is in concordance with that described by the World Organisation for Animal Health (OIE) (44). Out of 40 inoculated samples tested for PPR virus by immunocapture ELISA and FAT in Vero cells, 33 (83%) and 36 (90%) were positive, respectively. Durojaiye (10) detected PPR viral antigen in tissue using FAT. Similarly, the virus was isolated in primary lamb kidney cells and identified by agar gel diffusion testing and immunocapture ELISA by Saeed *et al.* (34).

Detection of antibodies against PPR virus is a reliable tool for the diagnosis of the disease. The ELISA has been proven to be the test of choice for the serodiagnosis of PPR (12, 19, 35, 39, 40, 44). The prevalence of antibodies to PPR virus in the present study was 154/243 (63.4%). The prevalence of antibodies to PPR virus in the northern and central parts of India was noted to differ between species (sheep versus goats), age groups and geographical regions. A greater proportion of the sheep (36.3%) in comparison with the goat (32.4%) population was infected with PPR virus (38). In contrast, in the present study, it was found that the seroprevalence in goats (79.6%) was greater than that in sheep (53.3%).

Peste des petits ruminants is a contagious transboundary disease with a significant impact on poor farmers. Its control should therefore be considered in programmes that aim to alleviate poverty in developing countries (7). The results of the current study suggest that the movement of sheep and goat flocks between different localities of Egypt should be restricted, especially during the risk period for PPR, and that an obligatory PPR vaccination programme should be applied for small ruminants all over the country.

Conclusions

The present study demonstrated the circulation of PPR virus among populations of sheep and goats in Egypt. Movements of the infected migratory sheep and goat flocks should be prohibited to prevent the spread of the disease and the transmission of the virus to different localities. The vaccination strategy for the control of the disease should be applied more strictly all over the country using the homologous PPR vaccine that is recommended by the OIE. In addition, strict serosurveillance and monitoring of PPR is recommended, together with uninterrupted vaccination of migratory flocks at the borders between districts or provinces, for effective control of the disease.

■

Détection d'un foyer de peste des petits ruminants dans des troupeaux itinérants de moutons et de chèvres en Égypte en 2006

I.H.A. Abd El-Rahim, S.S.A. Sharawi, M.R. Barakat & E.M. El-Nahas

Résumé

Un foyer de peste des petits ruminants (PPR) a été enregistré en 2006 dans la Province de Kalubia en Égypte. Il a affecté une importante population de chèvres et de moutons appartenant à des troupeaux itinérants qui se déplacent sur de vastes étendues géographiques. Le foyer a fait l'objet de plusieurs études

épidémiologiques et cliniques : des tests de laboratoire ont également été effectués. Les animaux atteints présentaient une pyrexie et des signes de stomatite érosive, d'entérite et de bronchopneumonie. Les manifestations cliniques étaient plus sévères chez les chèvres. Les taux de morbidité globale, de mortalité cumulée et de létalité s'élevaient respectivement à 26,1 %, 10,5 % et 40,2 %, avec une incidence plus élevée chez les jeunes animaux. Les examens post-mortem ont révélé une émaciation, une congestion des muqueuses, une lymphadénopathie, une hépatosplénomégalie, une nécrose hémorragique des muqueuses de l'abomasum et des intestins, une pleurésie et une consolidation pulmonaire. Au total, 40 écouvillons oculaires et nasaux et 243 échantillons sériques ont été prélevés sur des animaux atteints, et soumis à des tests de détection de la PPR, faisant appel aux techniques immuno-enzymatiques (ELISA) d'immunocapture et de compétition pour détecter respectivement l'antigène viral ou les anticorps spécifiques. L'antigène viral a été décelé dans 30 des 40 écouvillons (75 %). L'identification virale a été réalisée sur cellules Vero au moyen de la technique ELISA d'immunocapture (33 résultats positifs sur 40 échantillons soit 82,5 %) et du test aux anticorps fluorescents (36 résultats positifs sur 40 soit 90 %). La présence d'anticorps dirigés contre le virus de la PPR a été décelée dans 154 des 243 sérums prélevés (63,4 %). L'étude a confirmé la circulation virale dans les troupeaux itinérants de moutons et de chèvres. Cette situation impose d'exercer une surveillance sérologique rigoureuse et de vacciner les troupeaux itinérants lors de leurs passages aux frontières afin de contrôler efficacement la maladie.

Mots-clés
Culture cellulaire – Égypte – Épidémiologie animale – Épreuve immuno-enzymatique – Examen post-mortem – Foyer – Manifestation clinique – Ovins et caprins itinérants – Peste des petits ruminants – Test aux anticorps fluorescents.

Brote de peste de pequeños rumiantes en rebaños migratorios de ovejas y cabras en Egipto en 2006

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Resumen
En 2006 se registró en la provincia de Kalubia (Egipto) un brote de peste de pequeños rumiantes (PPR) que afectó a una enorme población de cabras y ovejas migratorias en una vasta extensión geográfica. Se llevaron a cabo estudios epidemiológicos y clínicos, así como análisis de laboratorio. Los animales enfermos presentaban pirexia, estomatitis erosiva, enteritis y bronconeumonía, manifestaciones clínicas todas ellas que revestían mayor gravedad en las cabras. Los índices globales de morbilidad, mortalidad acumulada y letalidad fueron del 26,1%, el 10,5% y el 40,2% respectivamente, con guarismos sensiblemente superiores en ejemplares jóvenes. En las necropsias se observó emaciación, congestión de las membranas mucosas, linfadenopatía, hepatoesplenomegalia, necrosis hemorrágica de la membrana abomasal e intestinal, pleuresía e infiltración pulmonar. Se sometieron 40 hisopados de secreción oculonasal y 243 muestras séricas de animales enfermos a sendos

ensayos inmunoenzimáticos (ELISA) de captura de antígeno y de competición, con el fin de detectar antígenos de la PPR y anticuerpos contra la enfermedad, respectivamente. Se observó presencia de antígeno en 30 de los 40 hisopados (un 75%). Después se identificó el virus de la PPR en células Vero inoculadas empleando un ELISA de captura de antígeno y una técnica de inmunofluorescencia, que arrojaron resultado positivo en 33 muestras de 40 (82,5%) y 36 de 40 (90%) respectivamente. De los 243 sueros analizados, 154 (un 63,4%) contenían anticuerpos contra la PPR. Quedó así demostrada la circulación de la enfermedad entre rebaños de cabras y ovejas migratorias. Para combatir eficazmente la PPR es preciso instituir estrictas medidas de serovigilancia y seguimiento de la enfermedad y vacunar a los rebaños migratorios en las zonas fronterizas.

Palabras clave
Brote – Cultivo celular – Egipto – Ensayo inmunoenzimático – Epizootiología – Manifestación clínica – Observaciones postmortem – Ovejas y cabras migratorias – Peste de pequeños rumiantes – Técnica de inmunofluorescencia.

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