

# The epidemiology of sheep pox in Greece from 1987 to 2007

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## Summary

The authors review the epidemiology of sheep pox outbreaks in Greece between 1987 and 2007. It is believed that sheep pox is introduced into Greece principally from neighbouring countries to the east, and is associated with the movements of infected sheep flocks close to the border and contacts between humans and animals. Disease foci have appeared in several central and north-eastern areas of the country. Between 1982 and 1986, Greece remained free of sheep pox but, in 1987, the disease appeared on the island of Lesbos and, in 1988, outbreaks were seen in the prefecture of Evros. In 1994, a further outbreak occurred in Evros. Over the next four years, more outbreaks occurred in Evros and Thessaloniki (1995); Larissa, Xanthi, Rhodopi, Kavala, Magnissia, Evros and the island of Lesbos (1996); Kavala, Magnissia, Halkidiki, Evros and Rhodopi (1997). In 1998, there were fewer cases of sheep pox, with outbreaks only in the prefecture of Evros. Two years later, a further outbreak was reported in Evros (2000), while the most recent outbreak occurred on the island of Lesbos in January 2007.

## Keywords

Epidemiology – Greece – Sheep – Sheep pox.

## Introduction

Sheep pox is a highly transmissible disease, caused by a large deoxyribonucleic acid (DNA) virus of the *Capripoxvirus* genus, one of eight genera within the *Chordopoxvirinae* sub-family of the *Poxviridae* family (8, 16, 17). The *Capripoxvirus* genus currently comprises: sheep pox virus, goat pox virus, and lumpy skin disease virus, causing disease in sheep, goats and cattle, respectively. Different strains of the virus appear to have different degrees of virulence in different species (13). The virus is transmitted by the aerosol route through close contact and mechanically by biting flies (12, 21). In infected pens, the virus can remain viable in scabs for as long as six months, while affected sheep shed infective viral particles at every stage of the disease and even up to eight weeks after the lesions have resolved (21).

Sheep pox is one of the major viral diseases of sheep, as it causes high morbidity and mortality, has the potential for rapid spread, may cause serious socio-economic or public health consequences, and is of major importance in the international trade of animals and animal products. Sheep represent the livelihoods of shepherds, and provide significant income from their meat, wool and skin. Sheep pox causes losses through:

- reductions in milk yield and meat production
- abortions
- skin damage and depreciation of wool quality
- slaughter of animals in the infected flock.

The loss of export trade in live sheep and their products adds to the economic consequences of this disease (6, 9, 21).

The incubation period for sheep pox is up to 21 days. Following contact with infected animals, incubation usually takes about 12 days, but is more rapid after intradermal inoculation by insects (12). Although clinical signs are indicative of the disease, a laboratory confirmation is necessary. Sheep pox diagnosis is based on virological and serological methods, most of which are time-consuming and laborious. One major obstacle is cross-reaction in the agar gel immuno-precipitation test with the orf virus, a common virus of the *Parapoxvirus* genus (13), which causes contagious ecthyma of sheep and goats and is widely spread in Greece and all over the world.

The tests that are internationally used for virus detection are:

- electron microscopy
- virus isolation in lamb testis or kidney primary or secondary cell cultures
- animal inoculation
- direct immunofluorescent assay
- histopathological tissue sections stained with haematoxylin and eosin
- immunological methods, such as agar gel immuno-precipitation and antigen trapping enzyme-linked immunosorbent assay (ELISA) (4, 23).

The serological diagnostic assays are:

- the virus neutralisation test
- the immuno-precipitation test
- the indirect fluorescent antibody test
- Western blot analysis
- the ELISA test (23).

The use of the polymerase chain reaction (PCR) procedure for sheep pox virus identification in cell cultures, skin biopsies and cell culture supernatants infected with reference viruses is well established (10, 11, 14, 15). This procedure has proved to be simple, rapid and provides a specific diagnosis, reliably differentiating between sheep pox and orf virus DNA in a suspicious sample (25).

In this brief retrospective communication, the authors review the available data on morbidity and mortality rates and surveillance associated with the sheep pox outbreaks that occurred in Greece between 1987 and January 2007.

## Materials and methods

Virus isolation was conducted on all skin biopsy samples collected from sheep suspected of infection with pox virus. These virus isolates were tested by:

- virus neutralisation test
- direct immunofluorescent assay
- agar gel immuno-precipitation test
- PCR (14, 15).

The clinical specimens were processed by grinding the skin and crusted scabs and adding Eagle's medium with antibiotics, making up a 50% (volume/volume) solution. The suspension was frozen at  $-70^{\circ}\text{C}$  and thawed three times. After centrifugation at  $2,000 \times$  gravity for 30 min, 2 ml of the supernatant was collected and stored at  $-70^{\circ}\text{C}$ , until it was used to inoculate cell cultures for virus isolation or DNA extraction.

The formulae that were used to analyse the epidemiological data were:

a) incidence rate = (number of cases per annum/average sheep population during this year)  $\times 10^5$

b) mortality rate = (number of deaths/average sheep population during this year)  $\times 10^5$

c) case fatality rate = (number of deaths/number of cases)  $\times 100$ .

## Results

In 1987, four outbreaks of sheep pox were recorded in the Lesvos prefecture and in August 1988, seven further outbreaks occurred in the Evros prefecture. According to the World Organisation for Animal Health (OIE), sheep pox outbreaks were also reported in neighbouring Turkey, in January and February of the same year. The disease was quickly controlled using eradication measures such as the slaughter of affected animals and vaccination of the neighbouring flocks in the protection zone. The vaccine was a live, attenuated Algerian strain of sheep pox virus, passaged in lamb testis primary and secondary cell cultures, produced in the Athens Institute of Biological Products. However, vaccination has not been used as a control measure since 1992, because of European Union (EU) controls on the presence of seropositive animals in Member Countries.

In 1994, a single sheep pox outbreak occurred in the Evros prefecture and was quickly brought under control. A year later, in 1995, a new epizootic started, spreading in

mainland Greece and persisting for the following four years. At the same time, sheep pox was recorded in Bulgaria and Turkey (22). The close proximity of infected flocks to the Evros River and Turkish border and the practice of common grazing and watering provided the opportunity and the means of spreading the disease. More specifically, the Evros River is very shallow in some parts and scabs from dead animals probably came into close contact with flocks that were watering in nearby areas. Another possible mode of spread is the employment in Greece of workers and farmers from neighbouring countries, where sheep pox outbreaks have been occurring. Between December 1998 and October 2000, no outbreaks of sheep pox were recorded in Greece; however, in November 2000, a single outbreak was found in the Evros prefecture. A provisional diagnosis was made on the basis of clinical signs and this was confirmed in the laboratory, using blood and tissue samples collected from clinically infected animals. The last outbreak in Greece was reported on Lesbos Island in January 2007.

A total of 202 outbreaks occurred in 9 of Greece's 51 prefectures between 1987 and 2007 (Fig. 1) and 40,519 animals were slaughtered (Table I). Following each epizootic, and between the years 1994 and

2007, 14,752 sheep and goat sera were tested, using agar gel immuno-precipitation and virus neutralisation tests (Table I).

## Discussion

Sheep pox is considered exotic to the EU and is classified in the list of notifiable diseases (formerly List A) by the OIE. The disease is endemic in Asia and Africa (e.g. in Turkey, the Middle East, India, Kenya) (1, 3, 5, 13, 18, 19, 20, 24). In endemic areas, the morbidity rate is 70% to 90% and the mortality rate is 5% to 10%, although it can approach 100% in imported animals (22). Sheep pox has been absent from the countries of Central and Western Europe for many years. Table II presents the last recorded sheep pox outbreaks in European countries. The numbers of outbreaks in Mediterranean countries where sheep pox outbreaks have been reported in the last three years are shown in Table III.

In Greece, epizootics of sheep pox, rather than goat pox, are recorded. The transmission of sheep pox virus to goats has not been observed, but in mixed flocks with both



**Fig. 1**  
**Map of Greece showing the areas infected with sheep pox**

Years when outbreaks occurred:

- |   |                                     |
|---|-------------------------------------|
| 1. Evros prefecture: 1988, 1994, 1995, 1996, 1997, 2000 | 6. Halkidiki prefecture: 1997       |
| 2. Rhodopi prefecture: 1996, 1997                       | 7. Island of Lesbos: 1987, 2007     |
| 3. Xanthi prefecture: 1996                              | 8. Larissa prefecture: 1996         |
| 4. Kavala prefecture: 1996, 1997                        | 9. Magnissia prefecture: 1996, 1997 |
| 5. Thessaloniki prefecture: 1995                        |                                     |

**Table I**  
**Total numbers of outbreaks of sheep pox and animals slaughtered in Greece from 1987 to 2007**

Year	Outbreaks	Prefectures affected	Animals slaughtered	Sera tested
1987	4	1	520	Surveillance
1988	7	1	515	not applied as
1989-1993	0	0	0	a control measure
1994	1	1	86	389
1995	9	2	1,617	948
1996	114	7	26,035	6,812
1997	58	5	9,878	3,332
1998	7	1	1,202	255
2000	1	1	507	2,511
2007	1	1	159	505
<b>Total</b>	<b>202</b>	<b>20</b>	<b>40,519</b>	<b>14,752</b>

**Table II**  
**The most recent outbreaks of sheep pox in European countries and territories, by year, according to data from the World Organisation for Animal Health (22)**

Country	Year of last outbreak	Country	Year of last outbreak
Albania	1934	Ireland	1850
Andorra	1952	Israel	2004
Armenia	1987	Italy	1983
Austria	1954	Moldavia	1994
Azerbaijan	1996	Norway	1882
Bosnia and Herzegovina	1955	Poland	1950
Bulgaria	1996	Portugal	1970
Croatia	1955	Romania	1957
Cyprus	1989	Russia	2003
Czech Republic	1950	Slovakia	1950
Denmark	1879	Spain	1968
France	1964	Sweden	1934
Georgia	1997	Turkmenistan	2000
Germany	1920	United Kingdom	1866
Greece	2007	Uzbekistan	1996
Hungary	1957		

**Table III**  
**Mediterranean countries that reported sheep pox outbreaks during the last three years according to data from the World Organisation for Animal Health (22)**

Country	Number of outbreaks
Algeria	9
Israel	30
Tunisia	109
Turkey	399

sheep and goats, where infected sheep were present, the serological examination of goats resulted in the detection of antibodies against capripoxvirus. Although positive serology is present in goats, the absence of clinical signs indicates that the transmission of the virus from goats, or their involvement in the epidemiology of outbreaks, is unlikely. The disease occasionally occurs in Greece and is transmitted from neighbouring Turkey and Bulgaria. The geographical position of Greece between Europe and Asia makes the rapid and accurate diagnosis and control of sheep pox and other exotic diseases very important.

Since 1992 a stamping-out/non-vaccination policy has been applied to control the disease whenever there has been an outbreak in Greece. Routine control measures include the cleaning and disinfection of depopulated premises and establishment of protection and surveillance zones, with a radius of 3 km and 10 km, respectively, around the outbreak, as specified in EU Council Directive 92/119 (7). A census is taken of all susceptible animals inside these zones and a 'standstill' on animal movements, animal products and feedstuffs out of the zones is enforced for 21 days. Serological testing of animals in the protection zone is also undertaken. A serological survey is conducted on a sample of 10% of the total number of sheep and goats in the protection area and, if this survey proves negative, free movement of the animals is allowed. The available data on the number of animals in the protection zone come from the year 1996, when the greatest number of outbreaks was recorded. About 1,650 sera, from 16,220 animals of 158 flocks in the protection area, were tested.

Many different factors have a direct influence on the epizootiology of sheep pox (2, 24), as follows:

- characteristics of the host (age, sex, breed, nutritional status, immunological status)
- characteristics of the agent (strain, virulence, pathogenicity)
- environmental factors
- poor management
- feed scarcity
- inadequate veterinary services.

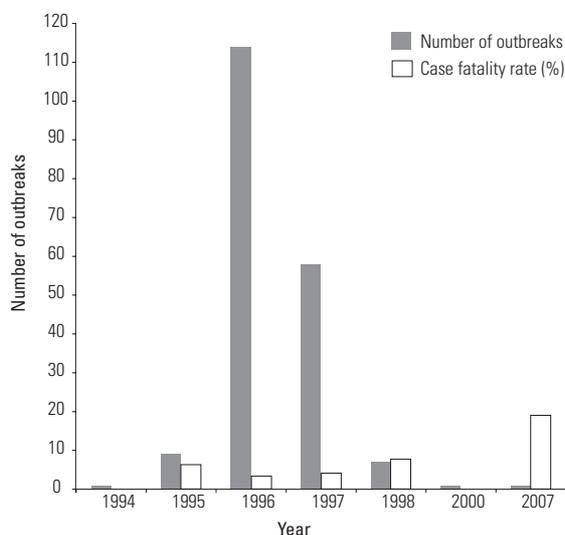
In this survey, the incidence rate ranged from 4.21 per 100,000 to 59.87 per 100,000; the mortality rate from 0 to 2.44 per 100,000; and the case fatality rate from 0% to 19.04% (Table IV, Fig. 2). In the single outbreak of 1994, no deaths were reported and a low incidence was seen. The high incidence rate observed in 1996 may be due to factors such as:

- efficient reporting of outbreaks
- a higher number of susceptible animals
- host and agent factors.

**Table IV**  
Annual statistics for sheep pox in Greece from 1995 to 2007

Year	Number of outbreaks	Incidence rate per one hundred thousand sheep	Mortality rate per one hundred thousand sheep	Case fatality rate
1994	1	4.21	0	0
1995	9	28.88	1.81	6.29%
1996	114	59.87	1.91	3.19%
1997	58	58.35	2.36	4.05%
1998	7	31.42	2.44	7.79%
2000	1	6.10	0	0
2007	1	8.45	1.61	19.04%

Source: Veterinary Services of the Greek Ministry of Rural Development and Food



**Fig. 2**  
Distribution of the number of outbreaks of sheep pox in Greece and case fatality rate per year

In 2007, a high case fatality rate possibly indicated an infection with a highly virulent strain or hosts with low immunological status.

An increase in the number of cases is usually observed during summer until January of the following year. There are no reports of sheep pox cases during spring. The most probable reason is that dry scabs separate and fall off in the higher temperatures of the summer months. While flocks

are moving, the sheep pox viral particles present in the dust can easily be carried on cars or clothes and be indirectly transmitted to distant geographical regions. Sometimes sheep pox cases are observed immediately after the end of the surveillance period, indicating slow transmission and suggesting that restrictions should be imposed for a longer duration since, in addition to economic losses, there is always the risk of a new epizootic. To decrease the possibility of a reappearance of the disease, the following measures are recommended:

- continuous awareness of the epizootic situation in neighbouring countries
- strict controls on animal movements near and across borders
- restrictions on the movements of people during epizootics.

In addition, the possibility of pox virus transmission through insects other than *Stomoxys* should be investigated. There is a need for new vaccines which offer protection against the disease without causing diagnostic problems, as well as for the optimisation of associated serological assays which can discriminate between vaccine-derived antibodies and those resulting from natural infection.

This is the first study on sheep pox in Greece during the last 20 years and an analysis of the data leads to the conclusion that sheep pox spreads to Greece from neighbouring countries, in particular, Turkey, where the disease is enzootic.



## L'épidémiologie de la clavelée en Grèce entre 1987 et 2007

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### Résumé

Les auteurs dressent un tableau épidémiologique des foyers de clavelée survenus en Grèce entre 1987 et 2007. La clavelée s'est probablement introduite en Grèce depuis les pays voisins à l'Est ; cette introduction est associée aux déplacements de troupeaux de moutons infectés près de la frontière et aux contacts entre les populations humaines et animales. Les foyers ont fait leur apparition dans plusieurs zones du centre et du nord-est du pays. Entre 1982 et 1986, la Grèce est restée indemne de clavelée ; en 1987, la maladie a fait une première incursion sur l'île de Lesbos puis, en 1988, des foyers ont été observés dans la préfecture d'Évros. Un nouveau foyer est survenu à Évros en 1994. Pendant les quatre années qui ont suivi, des foyers ont été signalés en plusieurs endroits du pays : Évros et Thessalonique (1995) ; Larissa, Xanthi, Rhodope, Kavala, Magnésie, Évros et île de Lesbos (1996) ; Kavala, Magnésie, Chalcidique, Évros et Rhodope (1997). En 1998, le nombre de cas de clavelée a décliné et les seuls foyers enregistrés ont concerné la préfecture d'Évros. Deux ans plus tard, un nouveau foyer a été signalé à Évros (2000). Le dernier foyer de clavelée signalé dans le pays a eu lieu sur l'île de Lesbos, en janvier 2007.

### Mots-clés

Clavelée – Épidémiologie – Grèce – Mouton.



## Epidemiología de la viruela ovina en Grecia desde 1987 hasta 2007

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### Resumen

Los autores repasan la epidemiología en Grecia de los brotes de viruela ovina observados entre 1987 y 2007. Se piensa que la enfermedad penetró en el país básicamente desde países vecinos orientales, a resultas de los movimientos de rebaños infectados en áreas fronterizas y del contacto entre humanos y animales. En varias zonas del centro y el noreste de Grecia han aparecido focos infecciosos. Entre 1982 y 1986, Grecia estuvo libre de viruela ovina, pero en 1987 la enfermedad apareció en la isla de Lesbos y algo más tarde, en 1988, se observaron brotes en la prefectura de Evros. En 1994 se produjo un nuevo brote en Evros. En el cuatrienio siguiente hubo más brotes: en Evros y Tesalónica (1995); en Larisa, Xanthi, Rodopi, Kavala, Magnisia, Evros y la isla de Lesbos (1996); y en Kavala, Magnisia, Halkidiki, Evros y Rodopi (1997). En 1998 se registraron menos casos, y sólo se produjeron brotes en la prefectura de Evros. Dos años más tarde se notificó un nuevo brote en Evros (2000). El más reciente tuvo lugar en la isla de Lesbos en enero de 2007.

### Palabras clave

Epidemiología – Grecia – Oveja – Viruela ovina.



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