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Peste des Petits Ruminants (PPR) Outbreak in Tajikistan

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Summary

The occurrence of outbreaks of peste des petits ruminants (PPR) in three districts of Tajikistan is described. The causal strain (PPR Tajikistan) was characterized and the sequence of its N gene was compared with that of 43 other strains isolated since 1968 in Africa, the Middle East and Asia. The study demonstrated (1) the value of the N gene as a target in comparing isolates obtained over an extended period of evolution, and (2) that clustering was related to the geographical origin of strains.

Keywords: goat; peste des petits ruminants; rinderpest; sheep; Tajikistan; viral infection

Introduction

Peste des petits ruminants virus (PPRV) causes a severe disease in sheep, goats and small wildlife ruminants. The virus belongs to the genus *Morbillivirus*, family *Paramyxoviridae*, together with rinderpest virus (RPV), canine distemper virus (CDV), measles virus (MV) and phocine distemper virus (PDV). Peste des petits ruminants (PPR), also known as goat plague, kata and pseudo-enteritis complex (Otte, 1960; Rowland *et al.*, 1969; Rowland and Bourdin, 1970; Hamdy *et al.*, 1976) is similar clinically to rinderpest. The disease is characterized by high fever resulting in depression, anorexia, ocular and nasal discharge, pneumonia, necrosis and ulceration of mucous membranes and inflammation of the gastrointestinal tract leading to severe diarrhoea. Morbidity and mortality rates vary but may reach 90–100% (Lefèvre and Diallo, 1990; Taylor *et al.*, 1990). These rates are usually lower in endemic areas, where mortality may be 20% or less, and serosurveillance is sometimes the only indicator of infection. The seroprevalence rate in sheep and goats rises with age, the symptoms increasing in severity when associated with infections such as sheep and goat pox, and being rapidly fatal in young animals. Morbilliviruses are rapidly inactivated at environmental temperature by solar radiation and desiccation. This means that transmission must occur by direct contact with infected animals or their excretions or secretions. Transmission of PPRV occurs primarily by droplet infection but may also occur by ingestion of contaminated feed or water. PPR was first described (Gargadennec and Lalanne, 1942) in West Africa and for 30 years was thought to be confined to this area. The disease has since been recognized as endemic in West and Central Africa (Scott, 1981) and in the north-east of the continent, Sudan (El Hag Ali and Taylor, 1984; Taylor *et al.*, 1990; Haroun *et al.*, 2002), Kenya and Uganda (Wamwayi *et al.*, 1995) and Ethiopia (Roeder *et al.*, 1994; Abraham *et al.*, 2005). In 1987 it appeared in the Middle East and has since then been confirmed in Jordan (Lefèvre *et al.*, 1991), Pakistan (Amjad *et al.*, 1996),

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southern India (Shaila *et al.*, 1989; Nanda *et al.*, 1996), Turkey (Özkul *et al.*, 2002; Toplu, 2004; Yesilbag *et al.*, 2005) and Israel (Abraham, Libeau *et al.*, unpublished). Recent evidence, however, suggests that the disease appeared in India in the early 1940s, from where it spread to West Africa (Taylor and Barrett, in press). PPR 75-1 vaccine (Diallo *et al.*, 1989), used with some success in infected countries, fails to contain the disease, which causes severe economic losses, especially in goats. In 2004, an outbreak of PPR was detected in Thrace, i.e., the European part of Turkey (Anderson and Sammin, 2005).

Laboratory confirmation of suspected cases is necessitated by the clinical similarity of rinderpest (RP). Enzyme-linked-immunoassay (ELISA) is now routinely used (Libeau *et al.*, 1992, 1995). Virus isolation and differential neutralization in cell culture are slow, tedious and of low efficiency. Immunocapture (Libeau *et al.*, 1994) and reverse-transcription polymerase chain reaction (RT-PCR) followed by nucleotide sequencing (Forsyth and Barrett, 1995; Shaila *et al.*, 1996; Couacy-Hymann *et al.*, 2002) are the current diagnostic methods for all morbillivirus infections.

This report describes outbreaks of PPR in three provinces of Tajikistan in 2004, with characterization of the strain of virus responsible.

Materials and Methods

Samples

These were collected from outbreaks of disease associated with high mortality in sheep and goats in three separate districts of Tajikistan (Fig. 1), for serology or virus detection and identification. From the town of Rasht in the Gharm district, 30 serum samples were collected from 145 surviving goats out of 270 sick animals as reported by the local authorities. In the Farkhror district, sera were collected from nine sheep and one goat out of 30 animals in poor health. In the Tavildara district, investigation of an outbreak in five "households" revealed 10 sick animals. These were examined clinically and one, a young goat aged 8 months, was killed and subjected to necropsy. Twenty-one samples (nasal, oral or rectal swabs, or cardiac fluid) were taken from these animals for virus identification.

Laboratory Investigations

Competitive ELISA (C-ELISA) tests were performed to detect antibodies to PPRV and RPV, as described in the Manual of Standards for Diagnostic Tests and Vaccines of the Office International des Epizooties (edition 2004). The PPR C-ELISA antibodies for detecting the haemagglutinin (H) and the nucleoprotein (N) and the RP C-ELISA anti-H antibodies were those described previously (Anderson and McKay, 1994; Libeau *et al.*, 1995). Reading was carried out with a Multiskan (Titertek, Helsinki, Finland) ELISA reader at 492 nm. Optical density values were converted to percentage inhibition values (PI) by the following formula: PI = 100−(OD test/OD 0 per cent control) × 100.

Detection of PPRV RNA by RT-PCR was performed as described previously (Couacy-Hymann *et al.*, 2002). In brief, viral RNA was extracted from 100 µl of sample suspension mixed with the lysis solution of the RNeasy Mini kit (Qiagen, Courtaboeuf,

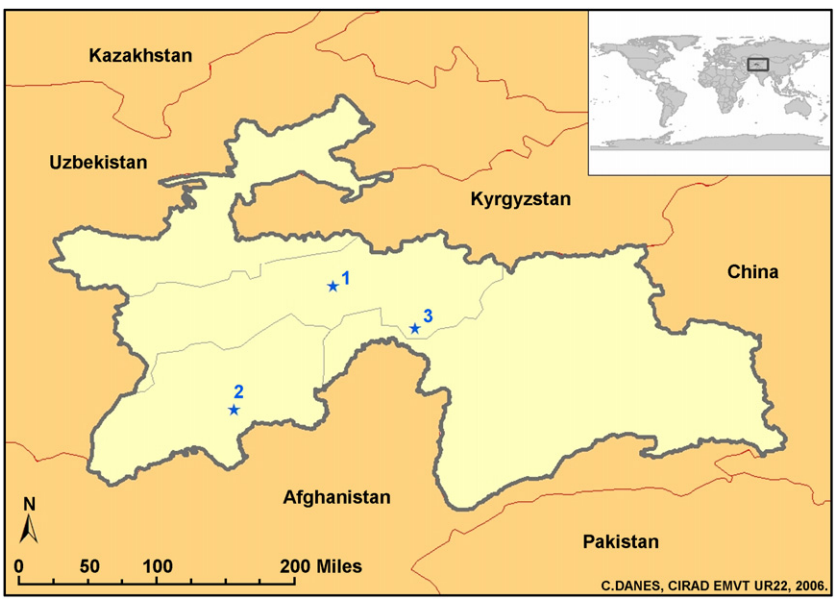

Fig. 1. Map of Tajikistan showing the three districts (Gharm 1, Farkhor 2 and Tavildara 3) in which the samples were collected.

Table 1
List of the oligonucleotides (primers) used for differential diagnosis

Specificity	*Name*	*Purpose*	*Target*	*Location*	*Sequence (5′-3′)*
PM	Nad1	Forward	N gene	790 < 812	5′CAAGCCAAGGATTGCAGAAATGA 3′
PM	Nad2	Reverse	N gene	1012 > 988	5′AATTGAGTTCTCTAGAATCACCAT 3′
PPRV	NP3	Forward	N gene	1232 < 1255	5′GTCTCGGAAATCGCCTCACAGACT 3′
PPRV	NP4	Reverse	N gene	1583 > 1560	5′CCTCCTCCTGGTCCTCCAGAATCT 3′
RPV	Bl2	Reverse	N gene	1270 > 1292	5′CAAGGGGGTGAGATCCAGCACAA 3′
RPV	B2	Forward	N gene	1543 < 1566	5′ATCCTTGTCGTTGTATGTTCTCGG 3′

PM, pan-morbillivirus; PPRV, peste des petits ruminants virus; RPV, rinderpest virus.

France) according to the manufacturer's instructions. First strand cDNA was reverse transcribed with Moloney murine leukaemia virus reverse transcriptase by means of the First-strand cDNA Synthesis Kit (Amersham Pharmacia Biosciences, Amersham, UK). For differential diagnosis of PPRV and RPV, three sets of primers selected on the N protein gene sequence were used (Table 1). Pan-morbillivirus primers located in the middle of the gene (Nad1 and Nad2) allow the amplification of the N gene of all morbilliviruses and give a product of 222 bases. The RP (Bl2, B2) and PPR (NP3–NP4) specific primers situated at the 3′ end of N gene give amplification products of 296 and 351, respectively. The amplification cycle consisted of an initial denaturation at 94 °C, for 2 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. The final cycle had a prolonged extension time (72 °C for 5 min). The amplified products were analysed by electrophoresis in 1.0% agarose gel. PCR products were purified from gel with QIAquick Gel Extraction Kit (Qiagen) and directly used in the sequencing reaction or amplified with a Blunt End Cloning Kit (Roche). In the sequencing reaction, the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Courtaboeuf, France) was used and products were run on an automated DNA sequencer (Prism 377; Applied Biosystems, Courtaboeuf, France,).

Sequence Data, Alignment and Phylogenetic Analysis

The nucleic acid sequences obtained from PCR products obtained with NP3-NP4 primer from this study were aligned with sequences from PPRV strains that were maintained in the database (Table 2). Phylogenetic analysis was performed on the 255 nucleotides located on the 3′ end of the nucleoprotein gene of the virus. The known PPRV sequences were derived from strains isolated over a period of 30 years. They consisted of "historical" strains, such as the Nigeria 75/1, from which the PPR vaccine was developed, and also the Dorcas strain isolated from a Dorcas gazelle in a zoological collection in the United Arab Emirates (Furley *et al.*, 1987). Strains isolated between 1988 and 1996 originated from Burkina Faso, India and Turkey, and more recent strains principally from western Asia, the Middle East (Iran, Israel, Saudi Arabia) and Africa (Mali).

The alignment of nucleotide sequences was created by means of the Clustal W program (Vector NTI, Informax, Inc., Rockville, Maryland, USA). Phylogenetic analysis was carried out by means of the "criterion of neighbourhood based on the principle of parsimony" (Saitou and Nei, 1987). Dissimilarities and edge length of dissimilarities between the sequences were first determined with Darwin software (Perrier *et al.*, 2003). Tree construction was based on the unweighted "Neighbor-Joining" method proposed by Gascuel (1997). Trees were generated with the TreeCon MATRIXW program of Darwin (Van de peer and De Watchter, 1993). "Bootstrap" confidence values were calculated on 1000 replicates. The RBOK vaccine strain of RPV was considered as an out-group.

Results

History of the Outbreaks and Status of Sampled Animals

From April to November 2004, high mortality occurred in sheep and goats from three districts in Tajikistan (Fig. 1). In early November, samples were collected for serology or virus identification. PPRV-specific antibody prevalence was detected in animals from all three districts (Table 3). In Gharm district, according to the veterinarian, the outbreak occurred in May, affecting 270 goats imported from eastern districts of Tajikistan bordering Afghanistan and China. The sick animals had shown fever, coughing, diarrhoea and inappetence, but for the last 2 months no mortality had been recorded among the 145 survivors. Most of the 30 serum samples from this group showed high PI values in the C-ELISA against the N and H protein of PPRV, but two females aged 5 years gave negative results. In the Farkhror district, rumours of high mortality in

Table 2
Selected strains of peste des petits ruminants virus from the database ("historical" and recent isolates) of the FAO/OIE Reference Laboratory for PPR in CIRAD, Département EMVT, France

Lineage	*Year**	*Country*	*Name of the strain*	*Reference or source*	*Genbank Accession No*
I	1968	Senegal	SENEGAL68	Laurent, 1968	DQ840165
III	1972	Sudan	MIELIK72	El Hag Ali and Taylor, 1984	DQ840159
III	1972	Sudan	SINAR72	El Hag Ali and Taylor, 1984	DQ840158
II	1975	Nigeria	NIGERIA75 2	Taylor and Abegunde, 1979	DQ840161
II	1975	Nigeria	NIGERIA75 3	Taylor and Abegunde, 1979	DQ840162
II	1975	Nigeria	VAC_NIGERIA75 1	Diallo *et al.*, 1989	DQ840160
II	1976	Nigeria	ACCRA76	Taylor, Pers com.	DQ840163
II	1976	Nigeria	NIGERIA76 1	Taylor and Abegunde 1979	DQ840164
II	1978	Ghana	ACCRA78	Taylor, Pers com.	DQ840167
II	1978	Ghana	GHANA78	Taylor, Pers com.	DQ840166
III	1983	Oman	IBRI83	Taylor *et al.*, 1990	DQ840168
III	1986	United Arab Emirates	DORCAS86	Furley *et al.*, 1987	DQ840169
I	1988	Burkina Faso	BURKINA88	A.Diallo, Pers com.	DQ840172
I	1988	Guinea	GUINEA88	A.Diallo, Pers com.	DQ840170
I	1989	Côte d'Ivoire	CI89	A.Diallo, Pers com.	DQ840199
I	1989	Guinea Bissau	BISSAU89	A.Diallo, Pers com.	DQ840171
IV	1993	Israel	ISRAEL ARBELLA	KimronVet. Institute	DQ840173
III	1994	Ethiopia	ETHIOP94	Diallo, Pers com.	DQ840175
IV	1994	India	INDIA94	Nanda *et al.*, 1996	DQ840176
IV	1994	India	SASSAYAN94 2	A.Diallo, Pers com.	DQ840179
IV	1994	India	THATAGULLAN 94 1	A.Diallo, Pers com.	DQ840180
I	1994	Senegal	SENEGAL94	M.Diop *et al.*, 2005	DQ840174
IV	1995	India	CALCUTTA95	A.Diallo, Pers com.	DQ840177
IV	1995	India	PRADESH95 1	A.Diallo, Pers com.	DQ840178
IV	1995	India-Tamilnadu	TAMILNADU95 2	A.Diallo, Pers com.	DQ840182
IV	1995	Israel	ISRAEL95 3	KimronVet. Institute	DQ840181
III	1996	Ethiopia	ETHIOPIA96	A.Diallo, Pers com.	DQ840183
IV	1996	Turkey	TURKEY96	A.Diallo, Pers com.	DQ840184
IV	1998	Iran	IRAN98	A.Diallo, Pers com.	DQ840185
IV	1998	Iran	IRAN98 3	A.Diallo, Pers com.	DQ840186
IV	1998	Iran	IRAN98 4	A.Diallo, Pers com.	DQ840187
IV	1998	Israel	ISRAEL98 10	KimronVet. Institute	DQ840191
IV	1998	Israel	ISRAEL98 2	KimronVet. Institute	DQ840188
IV	1998	Israel	ISRAEL98 6	KimronVet. Institute	DQ840189
IV	1998	Israel	ISRAEL98 7	KimronVet. Institute	DQ840190
II	1999	Mali	MALI 99 373	K. Tounkara, Pers com.	DQ840194
II	1999	Mali	MALI99 1	K. Tounkara, Pers com.	DQ840192
II	1999	Mali	MALI99 366	K. Tounkara, Pers com.	DQ840193
IV	1999	Saudi Arabia	SAUDI99 7	A.Diallo, Pers com.	DQ840195
IV	1999	Saudi Arabia	SAUDI 99 9	A.Diallo, Pers com.	DQ840196
IV	1999	Saudi Arabia	SAUDI 99 8	A.Diallo, Pers com.	DQ840197
IV	2004	Tajikistan	TAJIKISTAN/04	This publication	DQ840198
II	2005	Unknown	VAC.RUSSIA05	This publication	DQ837640

*Year of isolation or reception in the laboratory.

sheep and goats came to the knowledge of the local FAO office at the end of October. A field visit on November 1 failed to confim this report clinically, but PPR was confirmed by the C-ELISA in 10 animals in poor health, nine giving a positive reaction in the N-based test and eight in the H-based test. The mean PI value in both tests for this group was high (63% and 77.5%, respectively). In this group and the previous group, the H-ELISA gave negative results for RP. In early November 2004 an outbreak was reported in the Tavildara district, affecting five households. Investigations revealed 10 sick animals (nine goats and one sheep) aged 8–18 months. These 10 animals were numbered according to household (1–5) and, when more than one animal per household was examined, also by letter (e.g., 3a, 3b, 3c); in all, 21 samples were taken from this group for serology and virus identification, and results are given in Table 4. The mean PI values were comparatively low (59% and 69% for the N-and H-PPR C-ELISA, respectively), which accords

Table 3
PPRV serological status of animals sampled

District	*Number of animals*	*Age (years)*	*Number of positive animals*	*PPR ELISA (mean positive PI value in %)**		*RT-PCR result*
				N	*H*	
Gharn	30[†]	2–6	28	72.3	79	NA
Farkhror	10[‡]	–	8	63	77.5	NA
Tavildara	10[§]	0.5–1.5	10	59	69	+

NA, tissue samples were not collected.
*Positive threshold, ⩾ 50%.
[†]All goats;
[‡]all sheep except for one goat;
[§]all goats except for one sheep.

Table 4
Laboratory results of PPR cases submitted from Tavildara district, Tajikistan

Animal				*ELISA (PI value in %)**			*RT-PCR*			
								Prime set		
no.	*Age (months)*	*Species*	*Sex*	*N-PPR*	*H-PPR*	*H-RP*	*Sample*	*PM*	*PPRV*	*RPV*
1	NR	Goat	F	48	58	30	NA	…	…	…
2	12	Goat	–	53	72	32	Tears+nasal	+	+	–
3a	12	Goat	–	74	85	31	NA	…	…	…
3b	NR	Goat	F	70	69	19	NA	…	…	…
3c	18	Goat	M	58	71	34	Nasal+rectal	+	+	–
4	9	Sheep	F	83	83	32	Nasal+rectal	+	–	–
5a	18	Goat	M	50	56	20	Nasal	+	+	–
5b	NR	Goat	F	51	54	25	Nasal	+	+	–
5c	8	Goat	–	50	73	27	Cardiac fluid	+	+/–	–
5d	7	Goat	–	52	74	23	NA	…	…	…

NR, age not recorded. NA, tissue samples were not collected.
*Positive threshold, ⩾ 50%. PM, pan-morbillivirus; PPRV, peste des petits ruminants virus; RPV, rinderpest virus.

with the fact that animals were in the erosive phase of the disease and just developing an antibody response. PPRV was detected in a number of the tissue samples, (tear [1], nasal mucus [5], rectal mucus [2] and cardiac fluid [1]), with RPV/PPRV specific primers and also with the pan-morbillivirus primer set.

Most of the clinically affected animals from the Tavildara district showed severe lachrymation, nasal discharge, coughing, signs of bronchopneumonia, and blood-stained diarrhoea. All animals had a rectal temperature of 39.5–41.5 °C. Some had isolated dry crust formations at the corners of the lips and a white-yellow crustation on the surface of the tongue and inside the lips. Lesions seen at necropsy (animal 5c, Table 4) included focal haemorrhages in the mucous membrane of the trachea, focal haemorrhagic inflammation with isolated necrotic foci of various sizes in the right apical lobe of the lung, bronchopneumonia with fibrinous pleural effusion and pleural adhesions, dot-shaped necrotic foci in the rumen, and petechiae in the small intestine. Large intestine contents were liquid and contained sexually mature *Strongloides* sp. larvae.

Characterization of the Strain and Determination of its Geographical Lineage

To obtain epidemiological information and analyse the genetic relation of the Tajikistan strain 2004 (from Tavildara district) with strains of different geographical origin, the sequence of this isolate was included in a large analysis of PPRV genotypes from strains collected in various parts of the world. These strains, representative of the geographical diversity of the virus, were analysed on the 255 nucleotides that are located on the C terminus end of the nucleoprotein of the virus (84 aa). The method applied ("Neighbor-joining") to compare the strains on this sequence resulted in a phylogenetic tree bearing four different lineages which were supported by the "bootstrap values", suggesting a co-evolution of the lineages in relation to their geographical

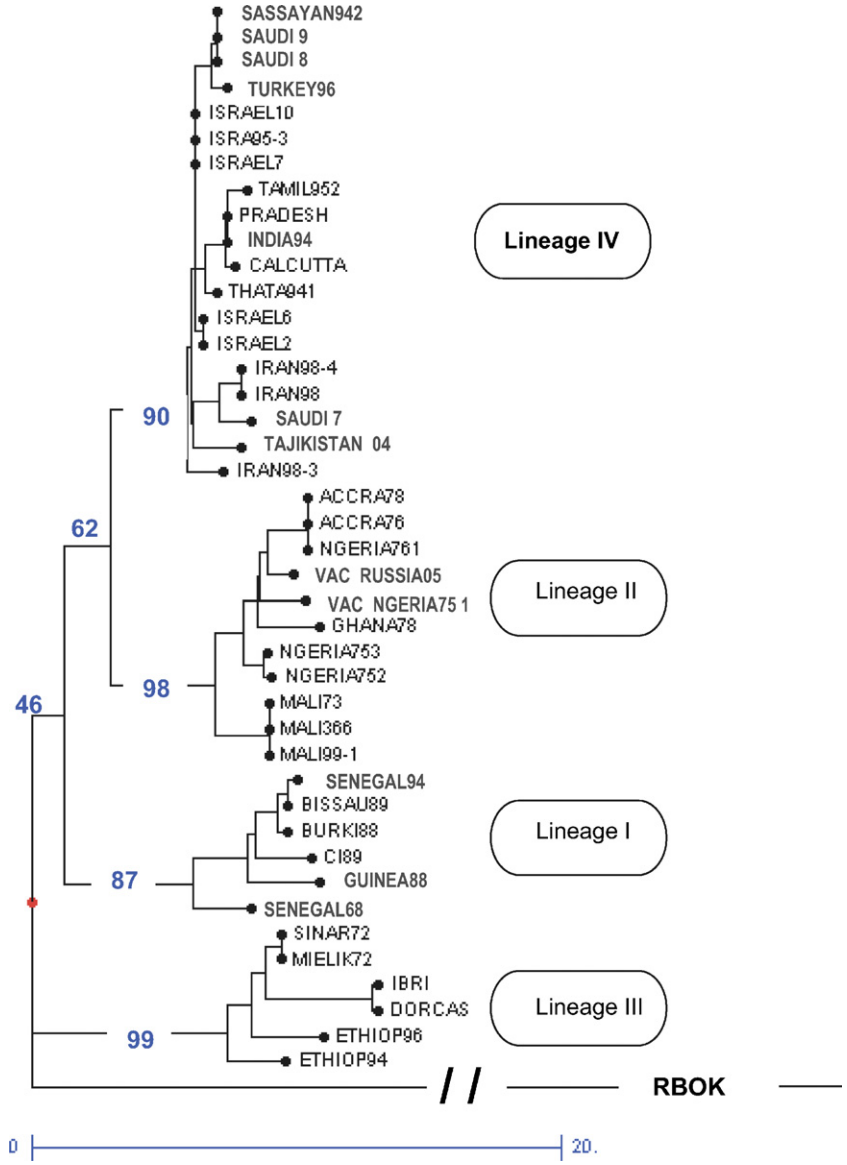

Fig. 2. Majority rule consensus tree of PPRV based on the alignment of the nucleotide sequences (1232–1560) of the N protein gene ("Neighbor-joining method"). Distances were generated with TreeCon MATRIXW program. "Bootstrap values" on 1000 tree replicates are shown on each edge of the majority rule consensus tree. The scale represents the edge length of dissimilarities between the sequences, thus illustrating the relationship between the RBOK vaccine strain of rinderpest virus collected from Genbank (Z30697) and all other PPRV strains.

origin (Fig. 2). As expected, the Tajikistan strain of PPRV fell into lineage IV and was closely related to strains isolated since 1998 in Asia, principally in Iran and in Saudi Arabia. Viruses from Israel and Turkey formed a cluster which was distinct—albeit slightly—from the India, Calcutta, Pradesh and Tamilnadu strains. Three lineages present in Africa, were numbered I–III, in accordance with the apparent progression of the disease from west to east. Viruses isolated from West Africa (Senegal, Guinea, Guinea-Bissau, Côte d'Ivoire and Burkina Faso) formed lineage I and those from Ghana, Nigeria and Mali formed lineage II. The African viruses isolated in the East of the country (Ethiopia and Sudan) were genetically close to those found in the southern part of the Arabian Peninsula (e.g., Oman and the United Arab Emirates) which were grouped in lineage III. This nomenclature differs from that of the publication of Shaila *et al.* (1996), based on sequence data derived from part of the fusion protein. The present paper refers to the original lineage I as lineage II and *vice versa*.

Discussion

Over a period of several months, outbreaks of respiratory disease with high mortality in sheep and

goats were reported to FAO in Tajikistan. The local veterinarians described the outbreaks as pasteurellosis, and indeed the national laboratories also frequently isolated *Pasteurella* spp. from lung tissue of these cases. Facilities identifying *Pasteurella* spp. are not available in Tajikistan, but it is believed that *Pasteurella multocida* and *Pasteurella hamolytica* are the species most commonly present. As a result, sheep and goats in Tajikistan are generally vaccinated against pasteurellosis but not against PPR, which has not been diagnosed previously.

The clinicopathology of the outbreaks in Tajikistan, as well as the serological and virological findings in this study, confirmed that the disease was PPR. Clinical and post-mortem findings may be sufficient for the diagnosis of PPR in endemic areas, but molecular methods of characterization are indispensable in areas where the disease is introduced for the first time or in which outbreaks occur sporadically (Scott, 1990). PPR has also been confused with rinderpest and pasteurellosis. Clinical rinderpest in sheep and goats has been reported in the past in Kenya (Libeau and Scott, 1960; Rossiter and Jessett, 1982), Nigeria (Beaton, 1955; Johnson, 1958), Uganda (Scott and Brown, 1961) and India (Taylor *et al.*, 2002). However, some of these infections were later confirmed as PPR. In India, PPR was reported for the first time in 1987, having previously been misdiagnosed as rinderpest.

Characteristic erosive-ulcerative lesions of PPR associated with high body temperature were observed in the sick goats in the present study. PCR analysis of samples confirmed the diagnosis of PPR and led to genetic characterization of the strain by sequencing of a limited area of the viral genome. Genotypic classification based on the N-protein gene appeared to be an efficient method for discriminating between geographically separated lineages. Phylogenetic analysis of the PPR Tajikistan strain showed that it belonged to lineage IV, which is known to occur in countries bordering Tajikistan. The Arabian Peninsula, the Middle East and parts of the Indian sub-continent were swept by an epidemic of PPR in 1993–1995, caused by a virus now known to be of lineage IV (Nanda *et al.*, 1996). PPR is believed to be widespread in Afghanistan, where the disease was reported in association with a rinderpest outbreak in 1995 (Geiger and Amir, personal communication). Since then, the disease has remained endemic in most of these regions and in much of the Indian sub-continent (Nanda *et al.*, 1996; Shaila *et al.*, 1996). The data presented here improve our understanding of the molecular epidemiology of PPRV. The geographical distribution of lineage IV covers a large area, probably because of the importance of trade in small ruminants in Asia. In contrast, viruses of lineage I and II, distributed in the west and central part of Africa, seem to be indigenous to that continent and have certainly evolved independently due to the north–south orientation of livestock transhumance. Lineage III is, however, shared by the eastern part of Africa and the southern part of the Middle-East on both sides of the Red Sea. Similarly, the isolates covering most of the major geographical areas of Asia, from Turkey to Tamilnadu, were found to belong to lineage IV. Thus, the areas covered by strains belonging to lineages III and IV reflect the traditional routes of exchange and trade of small ruminants. The genetic relationship between viruses of distinct geographical origin based on the N gene supports the study of Shaila *et al.* (1996), which was based on sequence data derived from the fusion protein gene. However, the N gene appears to be more powerful in clustering strains in accordance with their geographical origin. For example, two viruses (Sinar72 and Mielik72), both isolated in 1972 from the Sudan, were previously grouped in two separate lineages (I and III), whereas the present study placed them both in lineage III with the Oman (Ibri) and United Arab Emirates (Dorcas) isolates. The presence of four distinct genogroups suggests that the disease may have settled in Asia, the Middle-East and Africa long before the initial isolation and characterization of the causative agent. These four genogroups probably originated from a common ancestor, which may or may not have been genetically distinct. In any event, the distribution of strains into these different genogroups argues against the hypothesis of the spread of infection in recent decades from West to East. Indeed, it suggests that lineages have evolved independently over a prolonged period within large, mainly unconnected areas.

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References

Abraham, G., Sintayehu, A., Libeau, G., Albina, E., Roger, F., Laekemariam, Y., Abayneh, D. and Awoke, K. M. (2005). Antibody seroprevalences against peste des petits ruminants (PPR) virus in camels, cattle, goats and sheep in Ethiopia. *Preventive Veterinary Medicine*, **70**, 51–57.

Amjad, H., Qamar-ul-Islam Forsyth, M., Barrett, T. and Rossiter, P. B. (1996). Peste des petits ruminants in goats. *Pakistan Veterinary Record*, **139**, 118–119.

Anderson, J. and McKay, J. A. (1994). The detection of antibodies against peste des petits ruminants virus in cattle, sheep and goats and the possible implications to rinderpest control programmes. *Epidemiology and Infection*, **112**, 225–231.

Anderson, J. and Sammin, D. (2005). Peste des petits ruminants in the Thrace region of Turkey. *EMPRES Transboundary Animal Diseases Bulletin*, **27**, 12–15.

Beaton, W. G. (1955). Natural rinderpest in sheep and goats. *Bulletin of Epizootic Diseases of Africa*, **2**, 415.

Couacy-Hymann, E., Roger, F., Hurard, C., Guillou, J. P., Libeau, G. and Diallo, A. (2002). Rapid and sensitive detection of peste des petits ruminants virus by a polymerase chain reaction assay. *Journal of Virological Methods*, **100**, 17–25.

Diallo, A., Taylor, W. P., Lefèvre, P. C. and Provost, A. (1989). Atténuation d'une souche de virus de la peste des petits ruminants: candidat pour un vaccin homologue vivant. *Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux*, **42**, 311–319.

Diop, M., Sarr, J. and Libeau, G. (2005). Evaluation of novel diagnostic tools for peste des petits ruminants virus in naturally infected goat herds. *Epidemiology and Infection*, **133**, 711–717.

El Hag Ali, B. and Taylor, W. P. (1984). Isolation of peste des petits ruminants virus from Sudan. *Research in Veterinary Science*, **36**, 1–4.

Forsyth, M. A. and Barrett, T. (1995). Evaluation of polymerase chain reaction for the detection and characterisation of rinderpest and peste des petits ruminants viruses for epidemiological studies. *Virus Research*, **39**, 151–163.

Furley, C. W., Taylor, W. P. and Obi, T. U. (1987). An outbreak of peste des petits ruminants in a zoological collection. *Veterinary Record*, **121**, 443–447.

Gargadennec, L. and Lalanne, A. (1942). La peste des petits ruminants. *Bulletin des Services Zootechniques et des Epizooties de l'Afrique Occidentale Française*, **5**, 16–21.

Gascuel, O. (1997). BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data. *Molecular Biology and Evolution*, **14**, 685–695.

Hamdy, F. W., Dardiri, A. H. and Nduaka, O. (1976). Etiology of stomatitis-pneumoenteritis complex in Nigerian dwarf goats. *Canadian Journal of Comparative Medicine*, **40**, 276–284.

Haroun, M., Hajer, I., Mukhtar, I. and Ali, B. E. (2002). Detection of antibodies against peste des petits ruminants virus in sera of cattle, camels, sheep and goats in Sudan. *Veterinary Research Communications*, **26**, 537–541.

Johnson, R. H. (1958). An outbreak of rinderpest involving cattle and sheep. *Veterinary Record*, **70**, 457–461.

Laurent, A. (1968). Aspects biologiques de la multiplication du virus de la peste des petits ruminants sur les cultures cellulaires. *Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux*, **21**, 297–308.

Lefèvre, P. C. and Diallo, A. (1990). Peste des petits ruminants. *Revue Scientifique et Technique de l'Office International des Epizooties*, **9**, 935–981.

Lefèvre, P. C., Diallo, A., Schenkel, F., Hussein, S. and Staak, G. (1991). Serological evidence of peste des petits ruminants in Jordan. *Veterinary Record*, **128**, 110.

Libeau, G., Diallo, A., Calvez, D. and Lefèvre, P. C. (1992). A competitive ELISA using anti-N monoclonal antibodies for specific detection of rinderpest in cattle and small ruminants. *Veterinary Microbiology*, **31**, 147–160.

Libeau, G., Diallo, A., Colas, F. and Guerre, L. (1994). Rapid differential diagnosis of rinderpest and peste des petits ruminants using an immunocapture ELISA. *Veterinary Record*, **134**, 300–304.

Libeau, G., Prehaud, C., Lancelot, R., Colas, F., Guerre, L., Bishop, D. H. and Diallo, A. (1995). Development of a competitive ELISA for detecting antibodies to the peste des petits ruminants virus using a recombinant nucleoprotein. *Research in Veterinary Science*, **58**, 50–55.

Libeau, J. and Scott, G. R. (1960). Rinderpest in Eastern Africa today. *Bulletin of Epizootic Diseases of Africa*, **8**, 23–26.

Nanda, Y. P., Chatterjee, A., Purohit, A. K., Diallo, A., Innui, K., Sharma, R. N., Libeau, G., Thevasagayam, J. A., Bruning, A., Kitching, R. P., Anderson, J., Barrett, T. and Taylor, W. P. (1996). The isolation of peste des petits ruminants virus from northern India. *Veterinary Microbiology*, **51**, 207–216.

Otte, E. (1960). Clinical studies on « abu nini » in the Sudan: a contagious disease of goats and sheep possibly caused by pleuropneumonia-like organism. *Veterinary Record*, **72**, 140–145.

Özkul, A., Akca, Y., Alkan, F., Barrett, T., Karaoglu, T., Dagalp, S. B., Anderson, J., Yesilbag, K., Cokcaliskan, C., Gencay, A. and Burgu, I. (2002). Prevalence, distribution, and host range of peste des petits ruminants virus, Turkey. *Emerging Infectious Disease*, **8**, 708–712.

Perrier, X., Flori, A. and Bonnet, F. (2003). Data analysis methods. In: *Genetic Diversity of Cultivated Tropical Plants*, P. Hamos, M. Seguin, X. Perrier and J.C. Glaszmann, Eds, Gifield Science Publishers, Montpellier, pp. 43–76.

Roeder, P. L., Abraham, G., Kenfe, G. and Barrett, T. (1994). Peste des petits ruminants in Ethiopian goats. *Tropical Animal Health and Production*, **26**, 69–73.

Rossiter, P. B. and Jessett, D. M. (1982). Detection of rinderpest virus antigens in vitro and in vivo by direct immunofluorescence. *Research in Veterinary Science*, **33**, 198–204.

Rowland, A. C. and Bourdin, P. (1970). The histological relationship between peste des petits ruminants and kata in West Africa. *Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux*, **23**, 301–307.

Rowland, A. C., Scott, G. R. and Hill, D. H. (1969). The pathology of an erosive stomatitis and enteritis in West African dwarf goats. *Journal of Pathology*, **98**, 83–87.

Saitou, N. and Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology Evolution*, **4**, 406–425.

Scott, G. R. (1981). Rinderpest and peste des petits ruminants. In: *Virus Diseases of Food Animals*, Vol. II, E.P.J. Gibbs, Ed., Academic Press, New York, London, pp. 401–432.

Scott, G. R. (1990). Peste des petits ruminants. In: *Virus Infections of Ruminants*, Vol.3, Z. Dinter and B. Morein, Eds, Elsevier, Amsterdam, pp. 355–361.

Scott, G. R. and Brown, R. D. (1961). Rinderpest diagnosis with special reference to agar double diffusion test. *Bulletin of Epizootic Diseases of Africa*, **9**, 83–125.

Shaila, M. S., Purushothaman, V., Bhavasar, D., Venugopal, K. and Venkatesan, R. A. (1989). Peste des petits ruminants of sheep in India. *Veterinary Record*, **125**, 602.

Shaila, M. S., Shamaki, D., Forsyth, M. A., Diallo, A., Kitching, R. P. and Barrett, T. (1996). Geographic distribution and epidemiology of peste des petits ruminants virus. *Virus Research*, **43**, 149–153.

Taylor, W. P. and Abegunde, A. (1979). The isolation of peste des petits ruminants virus from Nigerian sheep and goats. *Research in Veterinary Science*, **26**, 94–96.

Taylor, W.P. and Barrett, T. (in press). Peste de petits ruminants and rinderpest. In: *Diseases of Sheep*, 4th Edition, I.D. Aitken, Ed, Blackwells Science, Oxford.

Taylor, W. P., al Busaidy, S. and Barrett, T. (1990). The epidemiology of peste des petits ruminants in the Sultanate of Oman. *Veterinary Microbiology*, **22**, 341–352.

Taylor, W. P., Diallo, A., Gopalakrishna, S., Shreeramalu, P., Wilsmore, A. J., Nanda, Y. P., Libeau, G., Rajasekhar, M. and Mukhopadhyay, A. K. (2002). Peste des petits ruminants has been widely present in southern India since, if not before, the late 1980s. *Preventive Veterinary Medicine*, **52**, 305–312.

Toplu, N. (2004). Characteristic and non-characteristic pathological findings in peste des petits ruminants (PPR) of sheep in the Ege district of Turkey. *Journal of Comparative Pathology*, **131**, 135–141.

Van de peer, Y. and De Watchter, R. (1993). Treecon: a software package for the construction and drawing of evolutionary trees. *Computor Applications in the Biosciences*, **9**, 177–182.

Wamwayi, H. M., Rossiter, P. B., Kariuki, D. P., Wafula, J. S., Barrett, T. and Anderson, J. (1995). Peste des petits ruminants antibodies in East Africa. *Veterinary Record*, **136**, 199–200.

Yesilbag, K., Yilmaz, Z., Golcu, E. and Özkul, A. (2005). Peste des petits ruminants outbreak in western Turkey. *Veterinary Record*, **157**, 260–261.

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