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CASE REPORT

Severe Ketoalkalosis as Initial Presentation of Imported Human Rabies in France

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We report a patient with an unusual initial metabolic presentation of imported human rabies who became symptomatic within 2 weeks of returning from Mali to France. This is the single case of imported human rabies identified in France within the past 11 years and the first report of viral RNA in bronchial secretions.

CASE REPORT

A 57-year-old male without past medical history presented to an emergency room in March 2014 because of fever and generalized pain. He had been a resident of France for the previous 15 years and had returned 2 weeks earlier following a 6-month stay in Mali. Approximately 1 month before his return to France, he underwent a right foot injury from a tree branch which eventually healed with local care.

On admission, he was conscious and cooperative but appeared anxious; he had fever (38.2°C) and abundant sweating and complained of generalized pain, mostly in the lower limbs. A clean, noninflammatory wound scar was noted on the right foot. He had marked tachypnea (rate, 40 inspirations/minute) and periodic deeper inspirations. Chest examination results were unremarkable. Arterial blood gas measurements while the patient was breathing room air were as follows: pH 7.79; partial pressure of carbon dioxide ($PaCO_2$), 11 mm Hg; PaO_2, 136 mm Hg; bicarbonates, 16 mmol/liter; and lactate, 3.7 mmol/liter. The capillary blood glucose was measured at 12 mmol/liter, and a urine dipstick revealed glycosuria (3+) and ketonuria (3+). Serum creatinine was at 94 μmol/liter and sodium at 136 mmol/liter. Thick and thin blood smears were negative for malaria. Chest X-ray results were normal. The patient was transferred to our intensive care unit (ICU) with a presumptive diagnosis of uncontrolled diabetes and unexplained severe respiratory alkalosis. He received intravenous fluids, continuous insulin infusion, and tetanus prevention with vaccination and serotherapy. Hyperventilation, ketonuria, and metabolic abnormalities resolved within 2 days, with normalization of pH and $PaCO_2$. Glycated hemoglobin was 6.5%, and the patient required no further insulin administration during his ICU stay.

Two days after ICU admission, he developed bouts of hyperactivity, disorientation, and delirium with thoughts of impending death associated with persecution ideas, alternating with periods of drowsiness and returns to normal behavior when he seemed aware of his disorder and criticized it. Hypersalivation was remarkable, and the patient occasionally spat on ICU personnel. Motor weakness, deep tendon reflexes, and limb sensory perceptions were normal. The results of a computed tomography scan and magnetic resonance imaging (MRI) of the brain were unremarkable. Electroencephalogram results showed no epileptic activity. The cerebrospinal fluid (CSF) test result was normal, with a negative PCR test for herpes simplex virus 1 (HSV-1) and HSV-2. Serological tests for human immunodeficiency virus type 1 (HIV-1) and HIV-2 and HIV-1 p24 antigen were negative. Thyroid-stimulating hormone and ammonia levels were in the normal range. Syphilis serologic tests, including Venereal Disease Research Laboratory (VDRL) and Treponema Pallidum Hem Agglutination (TPHA) tests, were negative.

On day 8 of ICU admission, the patient developed a rapidly extensive flaccid and areflectic tetraparesis without involvement of cranial nerves. His condition deteriorated, with altered consciousness and hypercapnic acidosis, and the patient required mechanical ventilation on day 9. Results of a repeated CSF analysis were normal. Results of a second cerebral and cervical MRI test were normal. Electroneuromyography showed an acute motor axonal neuropathy of both upper and lower limbs without sensory impairment. The results of a search for antineuron antibodies were negative, and the urinary porphobilinogen level was normal. The patient received methylprednisolone (1 g/day from day 8 to day 10) and intravenous immunoglobulin (2 g/kg of body weight from day 8 to day 12) for suspected paraneoplastic limbic encephalitis or Guillain-Barré syndrome. A search for rabies was performed on a skin biopsy specimen and salivary swabs obtained on day 13. A profound coma with periodic inspiratory spasms persisted, and the patient died on day 19 after ICU admission.

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TABLE 1 Lyssavirus diagnostic testing of samples obtained along the disease course in a patient with imported human rabies

Collection day (D) and sample type[a]	Test	Technique[b]	Value or result (value type or no. of samples with indicated result/ total no. of samples)
D1			
Serum	Neutralizing antibody	RFFIT	<0.06 IU/ml
	Specific anti-rabies virus glycoprotein IgG	ELISA	<0.05 EU/ml
D11			
CSF	Neutralizing antibody	RFFIT	<0.06 IU/ml
	Specific anti-rabies virus glycoprotein IgG	ELISA	<0.05 EU/ml
	Viral RNA	RT-hnPCR	Negative
		RT-qPCR	Negative
D12			
Whole blood	Neutralizing antibody	RFFIT	0.5 IU/ml
	Specific anti-rabies virus glycoprotein IgG	ELISA	0.9 EU/ml
D13			
Serum (×3)	Neutralizing antibody	RFFIT	0.8 IU/ml (mean value)
	Specific anti-rabies virus glycoprotein IgG	ELISA	1.2 EU/ml (mean value)
Skin biopsy specimen	Viral RNA	RT-hnPCR	Positive
		RT-qPCR	Positive
Saliva swabs (×6)	Viral RNA	RT-hnPCR	Positive (6/6)
		RT-qPCR	Negative (6/6)
Saliva swabs (×4)	Viral RNA	RT-hnPCR	Positive (2/4)
		RT-qPCR	Negative (4/4)
D16			
Whole blood	Neutralizing antibody	RFFIT	1 IU/ml
	Specific anti-rabies virus glycoprotein IgG	ELISA	2.5 EU/ml
D17			
Serum	Neutralizing antibody	RFFIT	3.3 IU/ml
	Specific anti-rabies virus glycoprotein IgG	ELISA	>3.8 EU/ml
D19			
Skin biopsy specimen	Viral RNA	RT-hnPCR	Positive
		RT-qPCR	Positive
Bronchial secretion aspirate	Viral RNA	RT-hnPCR	Positive
		RT-qPCR	Negative
	Virus isolation	Cell culture/suckling newborn mice	Negative

[a] All times are indicated with reference to the date of hospital admission (day 1 [D1], 16 March 2014).
[b] Rabies laboratory diagnosis was performed according to World Health Organization (WHO) recommendations using reference methods (2, 3). RFFIT, rapid fluorescent focus inhibition test, quantified in international units (IU) (reference value, <0.06 IU/ml); ELISA (enzyme-linked immunosorbent assay), Platelia Rabies II kit (Bio-Rad, Marnes-la-Coquette, France), quantified in equivalent units (EU) (reference value, <0.05 EU/ml); RT-hnPCR, reverse transcription-heminested PCR; RT-qPCR, reverse transcription–real-time quantitative PCR.

Biological diagnosis of rabies was performed at the National Reference Centre for Rabies (Pasteur Institute, Paris), where lyssavirus RNA was detected by heminested PCR after reverse transcription (RT-hnPCR) (1) in the skin biopsy specimen and salivary swabs obtained on day 13 but not in the CSF sample collected on day 11 (Table 1). The presence of viral RNA was confirmed in another skin biopsy specimen and in bronchial secretions collected on the day of the patient's death (day 19). Detection of lyssavirus RNA in both skin biopsy specimens was also obtained with a reverse transcription–real-time quantitative PCR (RT-qPCR) method. Both PCR techniques target the viral polymerase gene and were conducted each time with appropriate internal positive and negative controls. Results of attempts at viral isolation from bronchial secretions on cell cultures and intracranial inoculation into suckling newborn mice were negative. Specific rabies antibodies (both IgG anti-rabies virus glycoprotein and neutralizing antibodies) were undetectable in the CSF but were detected in serum on day 12 by the use of a Platelia Rabies II kit (Bio-Rad, Marnes-la-Coquette, France) (0.9 equivalent units [EU]/ml; reference value, <0.05 EU/ml) and by the seroneutralization test (0.5 IU/ml; reference value, <0.06 IU/ml), and then the levels increased slightly during hospitalization (Table 1). Phylogenetic analysis based on the full-length viral nucleoprotein nucleotide sequence indicated that the isolate belonged to the Africa 2 phyloclade within the *Rabies virus* (RABV) species, which is known to be circulating in dogs in West Africa (4) (Fig. 1). After repeated interviews with the patient's relatives, no history of animal bite or of exposure to animals known to be or suspected of being infected

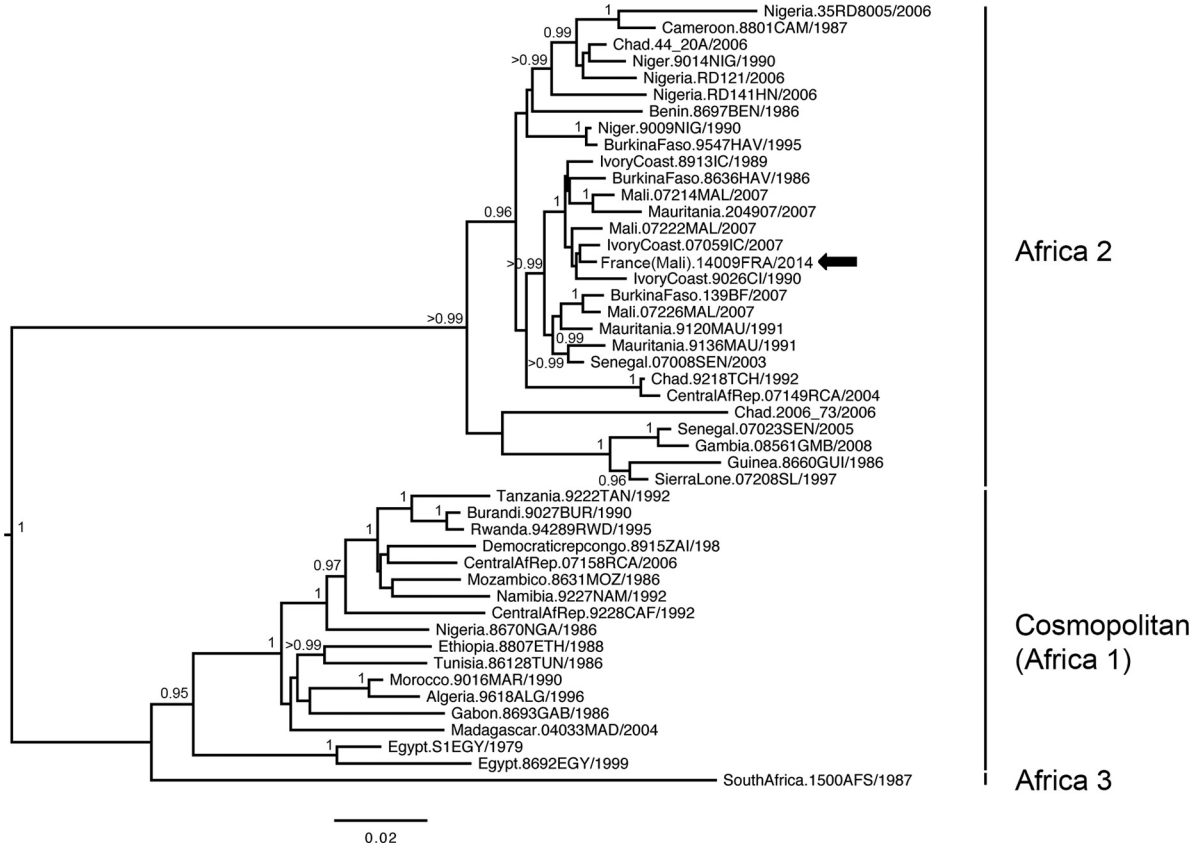

FIG 1 Maximum clade credibility phylogenetic tree based on the full-length nucleoprotein nucleotide sequence of a panel of representative rabies viruses circulating in Africa and the viral isolate obtained from a patient with imported human rabies. Bayesian Markov Chain Monte Carlo (MCMC) phylogenetic analysis was implemented using the BEAST package (version 1.8.1) (15) and a panel of full-length nucleoprotein gene sequences from rabies viruses belonging to the three phyloclades circulating in Africa (i.e., the Cosmopolitan clade with Africa 1 lineage and the Africa 2 and Africa 3 clades, indicated at the right of the tree) (1), in addition to the complete nucleoprotein gene sequence of the viral isolate obtained from the patient. The general time-reversible model with proportion of invariable sites plus gamma-distributed rate heterogeneity (GTR+I+ Γ4) was chosen as the best-fit nucleotide substitution model using jModelTest (version 2.1.7) (16). A relaxed molecular clock (uncorrelated lognormal) was the best supported under Bayes factors, with the BEAST output being summarized using Tracer (version 1.6). The maximum clade credibility (MCC) tree was chosen using TreeAnnotator (version 1.8.1) after the first 10% of trees were discarded, and the resulting tree was visualized using FigTree (version 1.4.2). Posterior probability values of 95% and over are indicated in black at the relevant nodes of the tree. The viral isolate obtained from the patient is indicated with an arrow.

during his stay in Mali was elicited, although stray dogs and bats were reported from his residency area there.

A risk assessment was undertaken among all health care professionals possibly exposed to the patient's body fluids. Of 158 personnel evaluated, 52 were considered to have been possibly exposed to the patient's body fluids and were offered rabies vaccination. Two of them (having been in close contact with the salivary secretions) also received rabies immunoglobulin.

As is often the case in rabies-free countries, the diagnosis of human rabies was considered late in the course of the disease, with several factors contributing to this delay. First, physicians have a very low index of suspicion for rabies in countries such as metropolitan France, where only sporadic imported cases have been identified. Indeed, only 20 cases were reported between 1970 and 2013, with the latest one dating back to 2003 (5). Second, a history of animal bite or exposure to rabies was lacking, whereas most of the recent cases of human rabies imported to western Europe have been associated with a dog bite (6, 7). Third, the initial clinical presentation was disconcerting, with prominent metabolic disturbances followed by pseudopsychiatric symptoms revealing the classical spastic ("furious") form of rabies, which evolved subsequently to a paralytic form (8).

Severe respiratory alkalosis resulting from hyperventilation and spontaneous inspiratory spasms with periodic deeper inspirations may be part of the early autonomic dysfunction associated with rabies encephalitis. Inspiratory spasms, similar to the hydrophobic reaction, may occur in the absence of apparent stimulus (8) and may even result in alveolar barotrauma and spontaneous pneumomediastinum (9). Hyperglycemia and glycosuria have been reported as initial presentations of human rabies (10), possibly associated with stress-induced catecholamine discharge, whereas ketonuria may be attributed to fasting induced by difficulty in swallowing and phobic spasms (8, 11).

Our patient nevertheless exhibited some typical signs of spastic rabies, including fluctuating consciousness and autonomic stimulation signs (inspiratory spasms, hypersalivation, and excessive sweating). While still conscious, he secondarily developed acute flaccid limb weakness consistent with the paralytic form of rabies

(8, 11). Mixed components of furious and paralytic rabies in the same patient have already been reported (12).

Biological confirmation of rabies infection was obtained through detection of viral RNA in saliva and skin biopsy specimens, which remain the specimen categories that provide the most sensitivity in testing (13). As in our patient, a late and moderate specific serum immune response is a common feature in human rabies, occurring 8 days or more after the onset of symptoms (13). Although the geographical origin of the virus was confirmed by the phylogenetic analysis (4), the source of the infection, possibly resulting from exposure of the lower limb wound to rabies virus, could not be confirmed in our patient.

While contamination via aerosolization remains exceptional and has been reported in only two laboratory cases (14, 15), the presence of rabies virus in human bronchial secretions has never been reported. In our patient, the detection of viral RNA in bronchial aspirates, together with unsuccessful attempts to isolate the virus from those specimens, suggests that this potential source remains at very limited risk for rabies transmission. Indeed, the presence of viral RNA in bronchial secretions could still result from contamination with saliva, since the inflated cuff of the endotracheal tube does not guarantee that there cannot be some leakage of oropharyngeal secretions in the lower airways. Nevertheless, endotracheal intubation in rabid patients still represents a procedure that presents a high risk of rabies exposure of personnel through respiratory secretions. A mask and gloves should be routinely worn by personnel when performing endotracheal intubation and suctioning for any patient receiving mechanical ventilation. When performing procedures that present a risk of generating aerosols in a patient suspected of rabies, full personal protective equipment, including gowns and gloves, filtering facepiece level 1 (FFP2) mask, and face shield, should be worn by personnel. Exposure of many intensive care unit workers to aerosols and salivary secretions occurred in this case before a diagnosis of rabies was considered; many had to receive postexposure prophylaxis with vaccination, and some received immunoglobulins. Fortunately, all the health care workers exposed to this case were doing well a year later.

This report serves as a reminder that rabies should be suspected in patients presenting with encephalitis or paralysis of unknown etiology and returning from or living in countries of rabies virus enzooticity, even when a history of animal bite is lacking. Skin biopsy specimens and salivary secretions should be systematically collected and tested for viral RNA detection to rapidly confirm the diagnosis so that appropriate precautions can be taken to avoid the risk of personnel exposure.

Nucleotide sequence accession number. The complete nucleoprotein gene sequence of the viral isolate obtained from the patient has been submitted to GenBank under accession number KP345881.

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C.B.-B. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the report and data analysis. C.B.-B., D.C., H.B., and L.D. were responsible for the study concept and design. Acquisition, analysis, and interpretation of data were performed by all of us. Drafting of the manuscript was performed by D.C., L.D., and C.B.-B. All of us performed critical revision of the manuscript for important intellectual content. Study supervision was by C.B.-B. and L.D.

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