

## BRIEF REPORT

## Tanapox Infection in a College Student

A. Damian Dhar, M.D., Andrew E. Werchniak, M.D., Yu Li, Ph.D.,  
 Jeoffrey B. Brennick, M.D., Cynthia S. Goldsmith, M.S., Richard Kline, M.S.,  
 Inger Damon, M.D., Ph.D., and Sidney N. Klaus, M.D.

**T**ANAPOX INFECTION IS A POXVIRUS INFECTION THAT IS ENDEMIC TO equatorial Africa. It begins with a febrile prodrome that is soon followed by the eruption of one or more large, superficial nodules, typically on the extremities. Complete recovery after several weeks is the rule, and there are no known permanent sequelae other than a scar at the site of infection. Although the disease is not uncommon in certain parts of Africa, only four previous cases have been reported in the United States. We describe a college student who acquired the disease while working with orphaned chimpanzees in Africa. We also review the important clinical, epidemiologic, diagnostic, and histopathological features to consider in the diagnosis of tanapox infection.

Because of concern about biologic warfare in general and about poxviruses in particular, the prompt identification of tanapox and its discrimination from more worrisome entities such as smallpox, monkeypox, tularemia, and anthrax have important public health implications.

From the Department of Medicine, Section of Dermatology (A.D.D., A.E.W., S.N.K.), and the Department of Pathology (J.B.B.), Dartmouth Medical School, Hanover, N.H., and Dartmouth–Hitchcock Medical Center, Lebanon, N.H.; and the Centers for Disease Control and Prevention, Atlanta (Y.L., C.S.G., R.K., I.D.). Address reprint requests to Dr. Klaus at the Department of Medicine, Section of Dermatology, Dartmouth–Hitchcock Medical Center, 1 Medical Center Dr., Lebanon, NH 03756.

N Engl J Med 2004;350:361-6.

Copyright © 2004 Massachusetts Medical Society.

## CASE REPORT

A 21-year-old female college student from New Hampshire had spent eight weeks observing and caring for orphaned chimpanzees in the Republic of Congo. Two weeks before her departure from Africa, severe headaches, backache, and fever developed, with a temperature reaching 39°C. The student sought medical attention at the local clinic, and empirical treatment with an antimalarial medication was begun. One day later, the fever abated, and a tender papule with an erythematous base erupted from her right elbow. Treatment with the antimalarial medication was discontinued, and the headaches and backache persisted for several days before resolving.

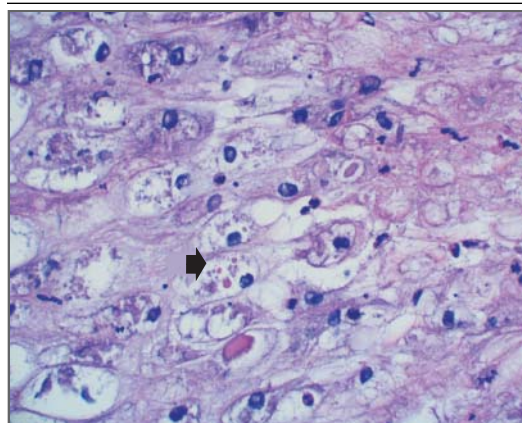
Eight days after the first papule erupted, a similar papule developed on the right lateral calf. The lesions grew steadily, eventually reaching a size of about 2 cm. The student was referred to another local practitioner, who applied liquid nitrogen to the nodule on the right elbow. The practitioner then incised the lesion with the intention of decompressing it; however, it was found to be solid rather than cystic. Since the patient had reported being bitten by several ticks, empirical therapy with dexchlorpheniramine and doxycycline was begun, in case her symptoms resulted from a tick-borne illness. No symptoms developed in any of the student's colleagues, even those who had handled the same animals she had, and none of the animals she had handled had obvious skin or mouth lesions.

On her return to the United States, the student presented for evaluation, two weeks after the eruption of her first papule. She reported having no fever, headache, myalgias, or arthralgias; her only symptoms were tenderness of the nodules and mild local adenopathy.

On examination, the student appeared to be healthy and was afebrile. She had two well-circumscribed, firm, slightly umbilicated nodules located on the right elbow and right lateral calf (Fig. 1), each of which was approximately 2 cm in diameter and sur-



**Figure 1.** Tanapox Nodule on the Right Lateral Calf of the Patient, Approximately Two Weeks after First Appearance.



**Figure 2.** Biopsy Specimen Showing Characteristic Eosinophilic Inclusions Indicative of Poxvirus Infection (Arrow) (Hematoxylin and Eosin,  $\times 400$ ).

rounded by a light red halo. The nodules were slightly tender to palpation; no fluctuance was noted. Mild adenopathy in the right axillary and right popliteal fossa was noted.

The differential diagnosis included tropical ulcer, poxvirus infection, atypical mycobacterial infection, fungal infection, and cutaneous anthrax. Tissue samples were submitted for Gram's staining, culturing, and histopathological examination. Microscopic examination of tissue stained with hematoxylin and eosin revealed acanthosis, reticular degeneration of the epidermis, eosinophilic intracytoplasmic inclusions in keratinocytes, and papillary dermal edema (Fig. 2). In the context of African travel, these findings raised the suspicion of a poxvirus infection, specifically the orthopoxvirus monkeypox; after preliminary discussions with the Centers for Disease Control and Prevention (CDC), the diagnosis of tanapox was suggested. Fresh tissue was obtained from the patient and forwarded to the CDC, where the diagnosis of poxvirus infection was confirmed with the use of electron-microscopical analysis, and the diagnosis of tanapox was made with the use of polymerase-chain-reaction (PCR) testing. No further treatment was initiated; the lesions resolved after several weeks, and the patient remained otherwise healthy.

#### METHODS

##### DNA EXTRACTION

Material from a frozen biopsy specimen from the patient was homogenized, subjected to freeze-thaw

cycles, and sonicated after resuspension in 500  $\mu$ l of sterile water. This suspension was used for the extraction of DNA and for the preparation of electron-microscope grids to be used for negative staining.

DNA was extracted with the use of an isolation kit (AquaPure Genomic DNA Isolation kit, Bio-Rad) with minor modifications. In brief, 100  $\mu$ l of the suspension was added to 500  $\mu$ l of the lysis buffer and then incubated at 55°C for 60 minutes. This procedure has been shown to inactivate a suspension containing 10 million infectious monkeypox virions, leaving a suspension containing fewer than 10 infectious virions. After the suspension was cooled to room temperature, 5  $\mu$ l of commercially prepared RNase solution was added and incubated at 37°C for five minutes. The suspension was again cooled to room temperature; 200  $\mu$ l of the protein-precipitation solution was added; and the mixture was vortexed for 30 seconds and centrifuged at 13,000 $\times$ g in a microfuge for 20 minutes. The supernatant was removed from the pellet, added to 600  $\mu$ l of isopropanol, mixed through 20 inversions of the tube, and centrifuged at 13,000 $\times$ g in a microfuge for five minutes. After the removal of isopropanol, the pellet was washed with 70 percent ethanol and recentrifuged, and all traces of the ethanol were removed. Next, the pellet was air-dried for five minutes and reconstituted to form an aqueous solution with the use of the supplied buffer.

##### PCR

DNA samples from the patient and controls were amplified in a thermocycler (model 9700, Applied

Biosystems), with the use of specific primers for tanapox and orthopoxviruses. The primers for each reaction are listed in Table 1.

#### *Yatapoxvirus–Tanapox 2L Gene*

Primers (Table 1) were designed with homology to the published yabapox-virus–like open reading frame 2L. During each reaction, PCR assays were performed in thin-walled tubes with the use of 0.25 µg of each primer, 0.5 µl of 10 mM deoxyribonucleoside triphosphate, and 1 µl of Expand polymerase (Roche). PCR cycling conditions consisted of an initial incubation at 92°C for 2 minutes, followed by 30 cycles at 92°C for 10 seconds, 55°C for 20 seconds, and 70°C for 30 seconds, with a final hold at 4°C.

#### *Yatapoxvirus–Tanapox 332-bp Amplicon from Pst 1 L Fragment*

Primers TP-1 and TP-1a were used as described by Stich et al.<sup>1</sup> to amplify 50 ng of input-template nucleic acid.

#### *Multiplex PCR Orthopoxvirus Assay (E9L)*

The primers listed in Table 1 were also used in the multiplex PCR assay. For this assay, we used 0.1 µg of each primer, 0.5 µl of 10 mM deoxyribonucleoside triphosphate, and Roche Expand kit number 2 buffer and polymerase mixture. We added 1 µl of template per 50 ng of total input nucleic acid. The PCR cycling conditions were 92°C for 2 minutes, followed by 10 cycles at 92°C for 10 seconds, 61°C for 30 seconds, and 70°C for 30 seconds, and then 20 cycles of 92°C for 10 seconds, 61°C for 30 seconds, and 70°C for 30 seconds plus an additional 2 seconds for each successive cycle. The cycling was held at 4°C until a 1 percent agarose electrophoretic gel was prepared and used to size the amplicons.

## RESULTS

Negative-stain and thin-section electron microscopy of material prepared from the patient's biopsy specimen revealed the presence of enveloped, brick-shaped virions measuring approximately 295 nm by 160 nm (Fig. 3). PCR reactions targeting three separate loci specific to orthopoxviruses revealed no amplicons consistent with an orthopoxvirus. These loci included both nonessential gene targets (hemagglutinin and A-type inclusion protein, data not shown) and essential gene targets (E9L) (Fig. 4). The novel E9L multiplex assay for orthopoxvirus

**Table 1. Multiplex PCR and Yatapoxvirus–Tanapox PCR Assay Primers.**

Assay	Primer Sequence*
Cowpox virus	
Forward (1531)	TGGAACCTAAAACCATCTTAGCG
Reverse (2021)	CTAAATCCCATCAGTCCATACATC
Monkeypox virus	
Forward (573)	CGATTTAAGTGGTAAACGATTGC
Reverse (1171)	TGTAAAACCTTTGCAAATGTGT
Vaccinia virus	
Forward (2290)	GTCGATAAGTCTATAGAAATAGCGAGA
Reverse (2786)	TAATATCGTTCTCCAAGTTCTATAGCT
Variola virus	
Forward (1964)	CCATGCAGTATACGTACAAGATCA
Reverse (2908)	TTCGTAAAATATTCTTTGATCACC
Tanapox virus 2L	
Yaba161	GCCAAGTAACATAAAATACTTACCCACC
Yaba161 reverse	TGCAGTTTGTAAAAGTTGACGATACC

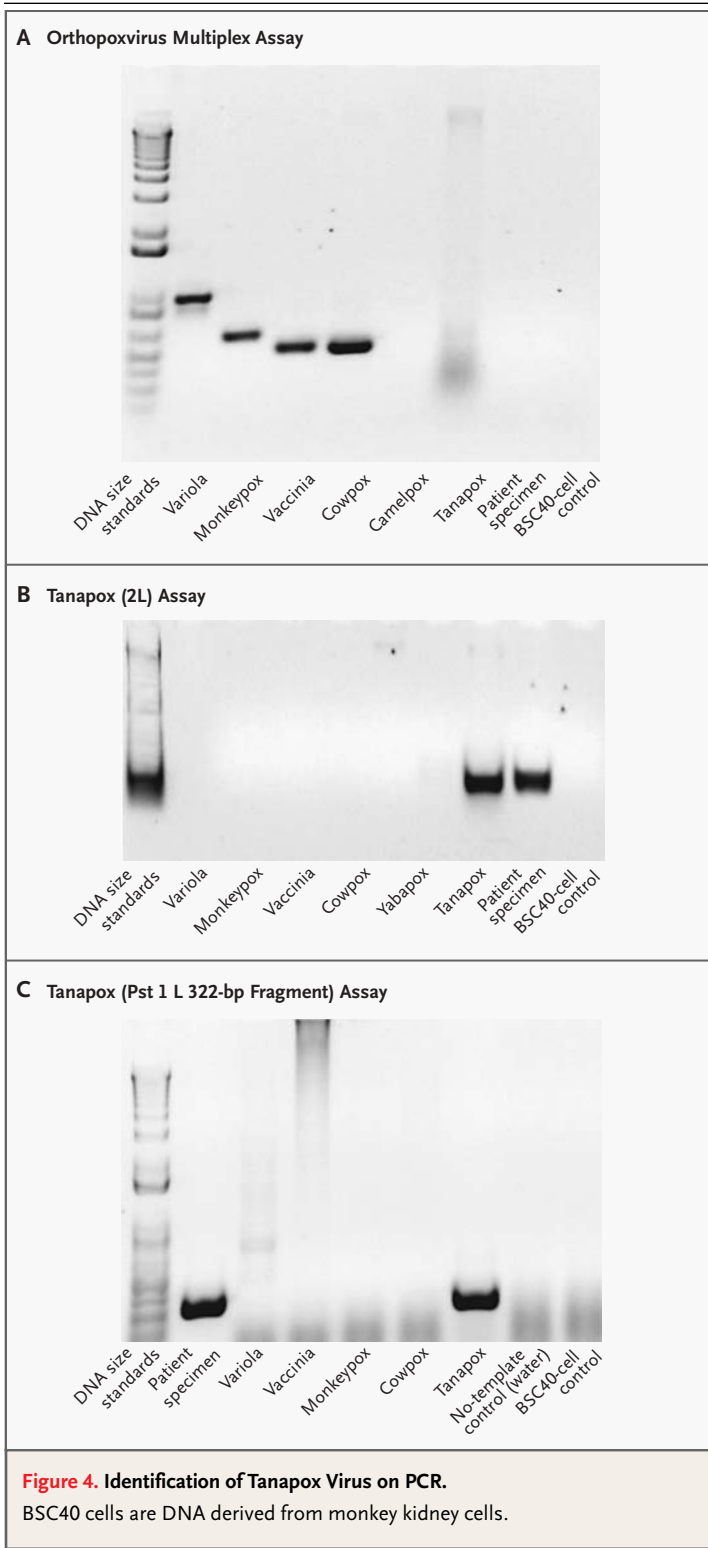
\* Primer sequences are given according to the 5'-to-3' convention.



**Figure 3. Tanapox Virus, as Seen with Negative-Stain Electron Microscopy of a Biopsy Homogenate.**

The virus has the characteristic brick shape, and an envelope (arrow) surrounds the particle. Bar represents 100 nm.

that we used can differentiate monkeypox, vaccinia, and variola according to the amplicon size with the use of standard 1 percent agarose-gel electrophoresis. The amplicon sizes were 582 nucleotides for monkeypox, 490 nucleotides for cowpox, 496 nucleotides for vaccinia, and 944 nucleotides for vari-



ola. The cowpox and vaccinia amplicons cannot be differentiated by means of electrophoresis but can be differentiated by means of sequence analysis. The presence of amplified control orthopoxvirus DNA and the absence of amplified material from the patient's sample imply that orthopoxvirus nucleic acid was not present in the specimen.

The definitive identification of tanapox through the detection of its DNA in the biopsy specimen was accomplished with the use of two sets of primers designed to detect unique yatapoxvirus nucleic acid regions. The use of a set of primers that was described previously<sup>1</sup> and one that was newly designed to amplify tanapox-specific gene targets (Fig. 4B and 4C) demonstrated the presence of tanapox virus nucleic acid in the specimen.

DISCUSSION

The poxviruses are a family of large, double-stranded-DNA viruses that has recently received increased attention because of concern over a potential resurgence of its most notorious member, smallpox (variola). Although there are eight genera in the poxvirus family, only four contain species that infect humans. Variola, an orthopoxvirus, and *Molluscum contagiosum* virus, the sole species of the molluscipoxvirus genus, are obligate human pathogens; the other poxviruses produce zoonotic infections and thus infect humans only incidentally. Many poxviruses have vectors and reservoirs that are limited to specific geographic areas, although the precise boundaries of these areas may be somewhat labile.

Tanapox was first recognized in September 1957, when an outbreak occurred among Wapakomo schoolchildren in Ngau, Kenya, a village in the Tana River valley of eastern Africa.<sup>2</sup> After a second outbreak along a different section of the river in 1962, the virus was isolated and identified.<sup>2</sup> Subsequent serologic and clinical surveys have demonstrated that the viral infection is a zoonosis harbored by the local population of nonhuman primates.<sup>3</sup> Transmission to humans is thought to occur primarily through transfer from an arthropod intermediary, and the seasonal variation in infections mirrors oscillations in the local arthropod populations.<sup>4</sup> Direct transmissions from nonhuman primates to humans and from humans to other humans have been described, although they appear to be extreme-

ly rare.<sup>3</sup> Although most common in adults, tanapox occurs in people of all ages and is equally prevalent among people of both sexes.

The first sign of infection is a mild fever (temperature, 38 to 39°C) that lasts two to four days.<sup>4</sup> Fever may be accompanied by headache and myalgias, but patients are rarely incapacitated. Toward the end of the febrile phase, a solitary erythematous macule appears on the skin. As the fever wanes, a central papule appears, and over the course of the next few days, it expands to a diameter of approximately 1 cm. The center can become umbilicated, develop a necrotic crust, ulcerate, or (as most commonly occurs) deepen and form a nodule. Erythema and edema typically surround the nodule as it increases in size. Enlarged regional lymph nodes appear four to five days after the appearance of the papule. The lesion can be extremely tender, and it may also be pruritic. The maximal diameter (up to 2 cm) is reached at the end of the second week. From this point, the lesion slowly involutes and typically resolves completely by about six weeks after the onset of symptoms.<sup>5</sup> The patient is left with a small scar at the site of involvement. Although no effective treatment has been identified, complete recovery is the rule.

Most cases of tanapox (78 percent in one series)<sup>4</sup> involve a solitary nodule; however, as many as 10 lesions on one person have been described. The most common location for lesions is the legs (in 72 percent of patients), and the least common locations are the face and areas normally covered by clothing.<sup>4</sup> Infection confers lifelong immunity, but immunization with or exposure to other poxviruses (such as vaccinia) does not protect against tanapox. Unique clinical features that allow the differentiation of tanapox from other orthopoxvirus infections are the nodular nature of the lesion, the paucity of lesions, the benign course of disease, and the prolonged process of resolution of the rash.

The disease is extremely rare outside Africa. Only four previous cases have been reported in the United States; three of them involved contact with laboratory animals in research facilities (in 1965 and 1966),<sup>6</sup> and one occurred in a traveler who had recently arrived from Sierra Leone.<sup>7</sup> A case of tanapox in Eu-

rope was recently documented in a traveler who returned to Germany after visiting Africa.<sup>1</sup>

A variety of different laboratory methods, electron microscopy, histopathological analysis, and nucleic acid testing can be used to identify poxviruses. Negative-stain electron microscopy can, as in this case, be used to identify poxvirus in a clinical specimen, but it may not permit the determination of the genus or species. Except for the parapoxviruses, which manifest as ovoid particles, most poxviruses are brick-shaped, which makes it difficult to discriminate one from another. In addition, especially on thin-section electron microscopy, but also, as here, on negative-stain electron microscopy, a high proportion of tanapox particles appear in enveloped forms.<sup>8</sup> On histopathological examination, the poxviruses appear as eosinophilic cytoplasmic inclusions in samples that have been stained with hematoxylin and eosin, and characteristic dermal edema with reticular degeneration may be observed. Currently, methods of nucleic acid detection are the approaches most often used to differentiate the genera and species of this family of viruses. We have described here an additional specific method for confirming the presence of tanapox and a novel multiplex-PCR approach to screening for a variety of orthopoxviruses that infect humans.

The recognition of tanapox is important, given increased international travel and the possibility of biologic warfare and terrorism. Early tanapox infection, particularly if it is atypical, can occasionally resemble more serious conditions such as monkeypox,<sup>4</sup> tularemia, or anthrax. Thus, prompt and accurate diagnosis can have important public health implications. If any of the above conditions is suspected, tissue samples should immediately be sent for Gram's staining, culturing, routine histopathological analysis, electron microscopy, and PCR analysis. In addition, the appropriate public health authorities should be notified, and discussions should be held regarding the appropriate plan of action. Tanapox is most likely transmitted through arthropod vectors or through direct contact with a lesion; lesions should therefore be bandaged to prevent possible spread of the disease.

## REFERENCES

1. Stich A, Meyer H, Kohler B, Fleischer K. Tanapox: first report in a European traveller and identification by PCR. *Trans R Soc Trop Med Hyg* 2002;96:178-9.
2. Downie AW, Taylor-Robinson CH, Caunt AE, Nelson GS, Manson-Bahr PE, Matthews TC. Tanapox: a new disease caused by a pox virus. *Br Med J* 1971;1:363-8.
3. Baxby D, Bennett M. Poxvirus zoonoses. *J Med Microbiol* 1997;46:17-20, 28-33.
4. Jezek Z, Arita I, Szczeniowski M, Paluku KM, Ruti K, Nakano JH. Human tanapox in Zaire: clinical and epidemiological observations on cases confirmed by laboratory studies. *Bull World Health Organ* 1985;63:1027-35.
5. Diven DG. An overview of poxviruses. *J Am Acad Dermatol* 2001;44:1-16.
6. Downie AW, Espana C. Comparison of Tanapox virus and Yaba-like viruses causing epidemic disease in monkeys. *J Hyg (Lond)* 1972;70:23-32.
7. Croitoru AG, Birge MB, Rudikoff D, et al. Tanapox virus infection. *Skinmed Dermatol Clin* 2002;1:156.
8. Knight JC, Novembre FJ, Brown DR, Goldsmith CS, Esposito JJ. Studies on Tanapox virus. *Virology* 1989;172:116-24.

Copyright © 2004 Massachusetts Medical Society.

## IMAGES IN CLINICAL MEDICINE

The *Journal* welcomes consideration of new submissions for Images in Clinical Medicine. Instructions for authors and procedures for submissions can be found on the *Journal's* Web site at [www.nejm.org](http://www.nejm.org). At the discretion of the editor, images that are accepted for publication may appear in the print version of the *Journal*, the electronic version, or both.