Investigation and analysis of a human orf outbreak among people living on the same farm

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INTRODUCTION

Human orf is a viral zoonotic infection caused by Parapoxvirus. The skin lesions of human orf can be misdiagnosed as cutaneous anthrax leading to overtreatment and also fear: This study was conducted to analyze an outbreak which led to deaths among kids and lambs in the same flock, and skin lesions in some persons who were living on the same farm that were initially diagnosed as cutaneous anthrax by a practitioner. Eight patients with skin lesions and eleven persons who had no skin lesion were considered as patients and control groups, respectively. The cultures obtained from the lesions of all patients were negative for Bacillus anthracis. The diagnosis of skin lesions was done by clinical findings, histopathological examination and PCR as human orf. To be under 20 years of age, direct contact with the animals, and contact with flayed skin of sick animals were the risk factors for human orf (Odds Ratio 7.5; 95% Confidence Interval 1.02-54.54, OR 12.25; 95% CI:1.3-100.9, OR 16.67; 95% CI:1.65-148.20, respectively). Orf should be kept in mind in the differential diagnosis of skin lesions resembling anthrax. For control and prevention of orf, transmission routes should be known; good hand hygiene and other personal protective measures have to be implemented.

KEY WORDS: Human orf, Outbreak, Zoonoses

SUMMARY

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INTRODUCTION

Human orf is a self-limiting viral zoonotic infection that usually involves the hands of people handling infected small ruminants such as sheep and goats, and/or newly contaminated equipment (Lederman et al., 2007; Tom et al., 2008; Unal et al., 2002). The aetiology of human orf is a member of the genus Parapoxvirus of the family Poxviridae. Orf virus infection is not usually seen in routine clinical practice, but it is common in persons engaged in sheep and/or goat husbandry as an occupational infection (Lederman et al., 2007). Skin trauma either overt or incidental may cause virus transmission from animals to humans (Lederman et al., 2007; Uzel et al., 2005). Contagious pustular dermatosis, contagious eczema, scabby mouth disease, sore mouth disease, and infectious pustular dermatosis are also used as synonyms of human orf (Tom et al., 2008). Skin lesions due to orf are dramatic but benign and resolve spontaneously, except in immunocompromized conditions (Steinhart, 2005; Geerinck et al., 2001).

When orf is not kept in mind, the disease can be misdiagnosed as more serious conditions such as cutaneous anthrax, leading to overtreatment and also unnecessary medical procedures (Steinhart, 2005). The incubation period is generally 3-7 days. The lesions slowly progress from papule, vesicle, shallow annular ulcer, scab, and to healed...
skin with little or no scarring. Six stages of orf, each of which approximately lasts for one week, are named as maculopapular, target, acute, regenerative, papillomatous and regressive (Tom et al., 2008).

This study was conducted to analyze the orf outbreak which led to deaths among kids and lambs in the same flock, and skin lesions in some persons who were living on the same farm.

**MATERIALS AND METHODS**

Eight patients with initially diagnosed cutaneous anthrax were referred to our emergency department by a practitioner in the middle of summer. They were admitted to our clinic with skin lesions resembling cutaneous anthrax. The patients were relatives from three families living on the same farm.

Then, our investigation team went to their farm for data and sample collection. The farm is in a village 26 km away from the city of Malatya located in the East Anatolian region of Turkey. Eleven members of three relative families who did not have skin lesions and were living together with the patients and had history of dealing or contact with animals, were included in the control group.

A questionnaire was administered to patients and control groups which consisted of age, sex, direct contact with the animals, contact with puddle of water and flayed skin of sick animals, milking, cutting meat of sick animals, and eating meat of sick animals for considering the risk factors.

Because of an initial anthrax outbreak suspicion, in all thirty samples of soil, goat hair, wool and stored meat of the slaughtered animals were collected for culture of *Bacillus anthracis* according to the WHO Guidelines (Turnbull, 1998) to rule out anthrax.

Samples were obtained from lesions of the patients for Gram staining and routine bacterial culture. Biopsies were performed from three lesions of one patient who had five lesions for histopathological examination and polymerase chain reaction (PCR) testing.

The remaining patients refused biopsy from lesions. The tissue samples were stored until in-house PCR testing. Viral DNA was extracted from tissue samples by the QIAcube automated extraction system with the Qiaamp DNA Tissue Kit (Qiagen, Hilden, Germany). Amplification of the viral DNA was done according to the protocol of Torfason et al. (Torfason and Gunadóttir, 2002), with minor modifications. A 5261 bp sequence dominates as ORF-RPA was selected as a suitable target according to Orf sequence information in GenBank. That gene encodes RPO132, a major component of the viral RNA polymerase. The following 5' and 3' primers flanking a 140 bp sequence at position 985-1125.

The primers were as follows; p1: 5’-cgcagacgtgcgtgagtcgat-3’ and p2: 5'-tgagctggttggcgctgtcct-3’ (Torfason and Gunadóttir, 2002). PCR amplification was performed in 50 μl master mix containing 1X amplification buffer, 0.2 mM of dNTP mix, 1.5 mM MgCl₂ and 2.5 U HotStart Taq DNA polymerase (Qiagen, Hilden, Germany).

**Statistical analysis**

Statistical analysis was performed with SPSS for Windows version 15.0 program. Continuous variables were reported as means ± standard deviation (SD). Categorical variables were reported as number and percent. Normality for continuous variable (age) in groups was determined by the Shapiro Wilk test. The variable did not show normal distribution (p<0.05). So, Mann-Whitney U test was used for comparison of variables between the studied groups. Fisher’s exact test and Odds Ratio (OR) risk factor were used for categorical variables. A value of p<0.05 was considered significant.

**RESULTS**

The patients had reported that they purchased 111 goats and 54 sheep, a total of 165 animals, one month before to graze together at the same place. They had planned to sell these animals in the feast of sacrifice (Eid-al-Adha) after gaining enough weight for several months.

Five lambs and eight kids which were 3-4 months old had become sick in the last fifteen days. According to the history, the animals initially presented blistering lesions on their lips, nostrils and toes, and they also had starvation. The owners of the flock were afraid of anthrax, although a veterinarian had not confirmed anthrax. And also, he diagnosed orf clinically in the sick
animals, not any other disease. Five sick animals died and were buried. The remaining sick three lambs and five kids were slaughtered for meat consumption by the owners. Most of the families’ members had eaten meat and pluck of these animals or stored them for later consumption. All living animals were asymptomatic and additional animal death had not been seen since the last episode.

In the patients group, there were five males and three females with a mean age of 17.4±7.8 SD (year). Five males and six females with a mean age of 32.9±18.4 SD were included in the control group.
Younger age was related to the morbidity for human orf (p=0.028), but sex was not (p=0.65). To be under 20 years of age, have direct contact with the animals and contact with flayed skin of sick

<table>
<thead>
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<th>TABLE 1 - Risk analysis of various factors in human orf.</th>
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<td><strong>Risk factor</strong></td>
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<td>Age &lt; 20 years</td>
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<td>Direct contact with the animals</td>
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<td>Contact with puddle of water</td>
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<td>Milking</td>
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<td>Cutting meat of sick animals</td>
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<td>Eating meat of sick animals</td>
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<td>Contact with flayed skin of sick animals</td>
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OR= odds ratio; CI= confidence interval; NC= not calculated because of a null cell.

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<th>TABLE 2 - The features of the patients and their lesions.</th>
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<td><strong>Patient no</strong></td>
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M: Male, F: Female
animals were determined as risk factors for human orf in the patient group (Table 1). The patients reported no constitutional symptoms. Seven patients presented only one lesion. Six of them were seen as targetoid nodules with elevated border and ulcer on the centre, and one of them was seen as weeping nodule. One patient had five similar lesions located on different sites of the body. Lesion locations, incubation period and healing time of the lesions are summarized in Table 2.

Samples obtained from the lesions of all patients did not show polymorphonuclear leukocytes on the Gram stain, and the cultures were negative for *B. anthracis*. Two cultures yielding coagulase negative staphylococci were considered as contamination. Likely, cultures of thirty samples from soil, goat hair and wool, and also stored meat of sick animals did not yield *B. anthracis*. There were axillary lymphadenopathies in two patients and the remainder of the patients’ physical examinations were unremarkable except the lesions. One patient had been administered oral penicillin for anthrax by the practitioner before referring to our hospital for three days. We did not initiate any antimicrobial treatment to any patients. In all patients, complete blood counts and C-reactive protein levels were normal. The lesions healed spontaneously within 36.6 days (range 28-45 days) on average by wound care with povidone-iodine and keeping the lesion dry.
Histopathological examination of skin punch biopsies from hand and flank area of the patient showed the same pictures which were epidermal ulceration, vesiculation, acanthosis, occasional intracytoplasmic eosinophilic inclusions and mixed inflammatory infiltration and edema in the dermis (Figures 1 and 2). The 140kb amplicon which was previously reported as suitable for diagnosis of human orf was detected in the tissue by in-house PCR (Figure 3).

**DISCUSSION**

Orf virus is hardy and persists on farm material and the ground for months to years (Steinhart, 2005). Humans who handle infected animals, carcasses or contaminated equipment can contract human orf by direct inoculation through cuts or abrasions in their skin.

In particular, veterinarians, wool shearers, abattoir workers, and also nonprofessional persons such as farmers’ children and housewives, Muslims in the feast of sacrifice, visitors to zoological gardens and persons who slaughtered their animals for traditional activities are at particular risk (Lederman et al., 2007; Unal et al., 2002; Uzel et al., 2005; Steinhart, 2005; Malik et al., 2009). Although most of the articles related to human orf have been reported as case series, this study presented a human orf outbreak and its risk factors.

Although the most common sites of orf are hands and face, other sites of the body may rarely be affected (Lederman et al., 2007; Unal et al., 2002; Uzel et al., 2005; Gill et al., 1990). In our cases, one of 12 lesions in eight patients was located on the left flank area, one on the lip and the others on the upper extremities (Table 2). Only one patient had more than one lesion.

It has been reported that the lesions are most commonly seen on non-dominant hands (Uzel et al., 2005). However, half of ten lesions were seen on dominant hands and the others on non-dominant hands in our study.

Infected animals may transmit the virus to children in an occupational setting or during recreation (Lederman et al., 2007). One of two children in the patients group had a history of torn cuticle and contact with the animals. Both of them had a history of contact with puddles of water prepared for animals. Moreover, two children had eaten well cooked meat of the sick animals. Two children in the control group had only a history of eating the meat. Younger age, direct contact with the animals and contact with the flayed skin of sick animals were the main risk factors in our study.

Orf infection tends to occur in spring and summer months (Leavell et al., 1968). Young animals are more susceptible to orf infection, so that the warm months after the lambing season attract attention due to a high infection rate. Mortality in young animals is also higher due to starvation, immunosuppression and secondary infection up to 93% (Lederman et al., 2007; Steinhart, 2005; Gumbrell and McGregor, 1997). As mentioned above, current outbreak of orf infection had occurred in the summer. Thirteen lambs and kids had suffered.

Five of them died and the remaining eight moribund animals were slaughtered in the flock consisting of 165 animals.

Constitutional symptoms such as fever may accompany orf infection, although this is unusual (Lederman et al., 2007; Leavell et al., 1968). Fever and other symptoms were not noted in any of the patients. Physical examination revealed axillary lymphadenopathies in only two patients.

In clinical practice, diagnosis is usually made by history and by considering a typical lesion in a person who has a history of contact with sheep and goats (Unal et al., 2002; Uzel et al., 2005; Kuhl et al., 2003). Definite diagnosis is also confirmed by pathological examination of a biopsy specimen (Uzel et al., 2005).

Histopathological evaluation revealed the features of a viral infection such as intracytoplasmic inclusion bodies in the specimens of one of our patients. Polymerase chain reaction method is a more reliable method to identify the viral genome in specimens regardless of disease stage (Torfason and Gunadóttir, 2002; Scaglierini et al., 2004). New PCR methods have been developing for the differential diagnosis of orf virus infection (Chan et al., 2009).

We also detected the 140 bp amplicon of orf virus by in-house PCR. Electron microscopy and immunological assays such as immunohistochemistry or immunofluorescence tests have also been used for the diagnosis of parapoxvirus and orf infection (Zhao et al., 2010; Mangana-Vougouka et
al., 2000). But we could not use these tests because the diagnosis was made by history and clinical findings of the patients and also histopathology and PCR.

There is no specific treatment for human orf which is typically a self-limiting disease. For that reason, prevention is the first line of defence (Steinhart, 2005). Unless lesions are huge and progressive, no intervention is recommended. Topical cidofovir shows promise in immune-compromised individuals (Geerinck et al., 2001; Nettleton et al., 2000).

In our patient group, no antibacterial drug was used, because anthrax and bacterial superinfection were not diagnosed.

It is known that cutaneous anthrax, pyoderma gangrenosum, herpetic whitlow, felon, milker's nodule and malignant melanoma are considered in the differential diagnosis for orf lesions (Lederman et al., 2007; Inceoglu, 2000). Among these diseases, anthrax is endemic and is especially considered by clinicians in Turkey (Doganay and Metan, 2009; Ozcan et al., 2008).

Besides, anthrax has recently gained attention around the world because of its potential as a biological weapon.

Cutaneous anthrax begins as a red papule, then progresses to black ulcer. The surrounding tissue becomes edematous and painful regional lymphadenopathy is common. However, in clinical practice, orf lesions may be confused with cutaneous anthrax according to the terms of the disease and may lead to fear.

Orf is a common cause of zoonotic infection. Differential diagnosis is very important for this dramatic but generally benign infection which may usually not require redundant, invasive and expensive therapies in humans.

It should be known that especially contact with sick animal and their flayed skin and to be of a younger age are the main risk factors for human orf. The young population is especially at risk because they usually do not obey hygiene rules. For infection control and prevention, transmission routes should be known; good hand hygiene and the use of other personal protective measures have to be practised.

Moreover, the education of farmers, animal owners, butchers or cutters of animals for religious or cultural traditions, physicians and veterinarians is very important.

REFERENCES


