ABSTRACT: Bacillus anthracis is the causative agent of Anthrax. The aim was to detect the presence of Bacillus anthracis in a case of suspected Cutaneous Anthrax in a 30 year old male who had history of handling a sick cow and noticed a painless ulcer on his palm 4 days later. Microbiological investigations revealed the presence of Bacillus anthracis. A diagnosis of Cutaneous Anthrax was made and the concerned authority was immediately notified.

CASE REPORT: A 30 yrs old Muslim male from Laxmikantapur, Karimpur (a district town in West Bengal), gave history of handling a sick cow which eventually died. He consumed its meat on 18.04.2013. He noticed a painless ulcer developing on his right palm 4 days later. The lesion started discharging pus, gradually hardened and turned black (Eschar). No other symptom was reported by the patient and he had no significant medical or surgical history, including Diabetes and Hypertension. The swabs and smears from the lesion were sent to us by the CMOH of District hospital of Nadia, WB for the possible presence of Bacillus anthracis in them. As a precautionary measure all the procedures were performed in Bio safety Cabinet type 2 B with proper personal protection.

The direct smears were stained with Gram stain, Polychrome Methylene blue stain, India Ink and Ziehl-Neelsen stain. The swabs were inoculated in 5% Sheep Blood Agar, Nutrient Agar and Peptone water and incubated at 37°C in O₂ for 24 hrs. Colonies obtained were stained and hanging drop preparation for motility was done. Relevant biochemical tests and antibiotic sensitivity was also done. The results obtained were as per table 1.

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Gram stain</td>
<td>Thick Gram positive bacilli in long chains, few pus cells.</td>
</tr>
<tr>
<td>Polychrome methylene blue stain</td>
<td>M'Fadyean reaction positive</td>
</tr>
<tr>
<td>India Ink preparation</td>
<td>Distinct halo around bacilli(Fig. 2)</td>
</tr>
<tr>
<td>Ziehl -Neelsen stain</td>
<td>Non acid fast bacilli</td>
</tr>
</tbody>
</table>
CASE REPORT

Culture

1. Sheep blood agar - Small, grey white, rough, convex, non-hemolytic colonies with irregular edges-MEDUSA HEAD COLONIES (Fig. 1); a few colonies resembling that of Staphylococcus aureus
2. Nutrient agar - similar non pigmented colonies.

Hanging Drop preparation

Non motile bacilli

Gram stain

Gram positive bacilli with central and sub terminal spores

Biochemical tests

1. Catalase - positive
2. Nitrate reduction - positive
3. Indole - positive
4. Glucose - fermented with acid production.
5. Salicin fermentation - negative.

Antibiotic sensitivity

Sensitive to Penicillin, Tetracycline, Levofloxacin and Erythromycin

Few colonies of S aureus were identified by Gram stain, Catalase positivity, slide and tube Coagulase positivity and biochemical tests. They were sensitive to- Ceftriaxone, Amikacin, Vancomycin and Linezolid; resistant to- Amoxicillin, Piperacillin-Tazobactum and Co-amoxiclav.

Following properties helped us to confirm that it was indeed Bacillus anthracis and not Anthracoid bacilli (Table 2.)

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT (our organism)</th>
<th>RESULT (Anthracoid bacilli)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capsule</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Motility</td>
<td>Non motile</td>
<td>Motile</td>
</tr>
<tr>
<td>Medusa-head colonies</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Hemolysis on Blood agar</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Turbidity in liquid media</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Salicin fermentation</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Penicillin sensitivity</td>
<td>Sensitive</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

Table-2

Thus, in the light of the laboratory results and appropriate clinical history it was concluded that the organism isolated was Bacillus anthracis and it was a case of Cutaneous Anthrax.

The report along with the antibiogram was immediately sent to CMOH of Nadia. He was asked to enquire if similar cases had occurred in the neighbourhood and any untimely cattle death had occurred. The contaminated materials from our lab were autoclaved and properly discarded as per recommendation.5

DISCUSSION: Anthrax, the classical disease caused by endospores of Bacillus anthracis, is primarily a disease of herbivorous animals 1. Bacillus anthracis is Gram positive, aerobic, spore forming bacilli. It is endemic in Asia, Africa, Central and Southern Europe2. Infection is acquired through inhalation, ingestion or contact with contaminated animal or animal products. Although uptake of vegetative forms when, for e.g. meat of an infected animal is eaten by human can cause infection5. Anthrax in
humans can be divided into 4 clinical forms – Cutaneous, Inhalational, Oro-pharyngeal and Gastro-intestinal anthrax based on the mode of acquisition of infection. The main virulence factor is a toxin. Man is moderately resistant to anthrax.

The most common form of anthrax is Cutaneous – around 90% of the cases worldwide\(^3\). It is more common in butchers, veterinarians and professionals who come in contact with animals often. Common sites are hands, neck and face (exposed sites)\(^4\). After incubation period of 2-5 days a painless papule develops at the site of contact surrounded by erythema and edema. The lesion evolves into a vesicle and fluid becomes black due to haemorrhage\(^6\). It ulcerates and develops an eschar. Localized or generalized lymphadenopathy and constitutional symptoms may occur. Compartment syndrome of hand requiring extensive plastic surgery has been reported\(^7\). Mortality rate is 10-20% if untreated\(^1\). It is an agent of bio-terrorism and in 2001 bio-terrorism activities involving U.S. Postal Service affected 22 people with anthrax\(^12\). Treatment is with Ciprofloxacin or Doxycycline as per CDC recommendation. Penicillin or Erythromycin can also be used.

In India anthrax is not common as majority of the population does not consume beef, though sporadic cases do occur\(^8\). Actual scenario in India is largely unknown due to under diagnosis and under reporting\(^9\). Anthrax is one of the 11 major zoonotic diseases accorded a priority status in India by the expert group of RZCI\(^11\). In Murshidabad West Bengal Anthrax outbreak was associated with slaughter of sick cows in 2007 and numerous cases of Cutaneous Anthrax was reported\(^8\). A significant number of Cutaneous Anthrax cases have been reported from Vellore\(^13\) and JIPMER, Pondicherry\(^14\). 5 cases of Cutaneous Anthrax have been reported from Vishakhapatnam, Andhra Pradesh\(^10\). A study on outbreak of Cutaneous Anthrax in a village of West Bengal also reported use of contact history, staining and culture to identify the organism as in our study\(^9\).

In our case the source of infection was the cow which had probably died of Anthrax. The spores might have entered through breach on the skin of the patient and led to the characteristic lesion. Discharge of pus can be attributed to secondary infection by S. aureus which is quite common. A striking feature is that most of the previously reported cases were from tribal villages in West Bengal whereas, this case occurred in a district town. Secondly, we received samples and not the patient himself which is very common in Microbiology Laboratory of referral hospitals. Thus multiple samples and blood samples could not be collected which made it challenging for us to conduct our investigation. Finally, identification of Bacillus anthracis was easily done by simple and routine tests, which is heartening as most of the peripheral centres lack advanced facilities.

**CONCLUSION:** Although very common, Cutaneous Anthrax is highly under reported. As Anthrax can cause epidemics and be devastating for human and livestock, clinicians and microbiologists must be alert in diagnosing and reporting any such case. With the threat of Bio-terrorism looming in the background, we must remember – ignorance is not bliss!

**REFERENCE:**

11. http://zoonoses.phfi.org/Anthrax.html. RCZI.

Fig. 01, Medusa head colonies
Fig. 02, India Ink preparation showing capsule.

AUTHORS:
1. Gargi Bhattacharya
2. Indrani Bhattacharyya
3. Pratip Kumar Kundu
4. Md Samidul Hoque

PARTICULARS OF CONTRIBUTORS:
1. Post Graduate Trainee, Department of Microbiology, Calcutta School of Tropical Medicine.
2. Assistant Professor, Department of Microbiology, Calcutta School of Tropical Medicine.
3. Professor, Department of Microbiology, Calcutta School of Tropical Medicine.
4. Laboratory Technician, Department of Microbiology, Calcutta School of Tropical Medicine.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Indrani Bhattacharyya,
37/1D, Raja Manindra Road,
Kolkata – 700037, West Bengal.
Email – indranichaudhuri@yahoo.com

Date of Submission: 11/11/2013.
Date of Peer Review: 13/11/2013.
Date of Acceptance: 23/11/2013.
Date of Publishing: 04/12/2013