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Zoonoses and Food Hygiene News, published four times a year, provides a medium for disseminating technical information on matters related to zoonoses and food hygiene generated in the world, particularly in Nepal. The editors welcome submissions on these topics with appropriate illustrations and references. The views and opinions expressed in the News are those of the authors.

CONTENTS:

Serological Evidence of Brucellosis in Different Species of Meat Animals of Nepal

Birochan Shrestha, D. D. Joshi, A. Aryal, K. Shahi

Background Information

Brucellosis is an acute or chronic contagious disease of domestic animals, that causes placentitis and abortion. It is caused by various species of *Brucella* belonging to family Brucellaceae and order Eubacterials. In human it is characterized by continued, intermittent, or irregular, fever of variable duration, headache, weakness, chills, profuse sweating, arthralgia, constipation, depression, weight loss and general aching after passing an incubation period of 3-16 days. Hence it is termed as" Undulant fever". The disease now called brucellosis, under the name "Mediterranean fever", first came to the attention of British medical officers in Malta during the Crimean War in the 1850s. The causal relationship between organism and disease was first established by Dr. David Bruce in 1887. In 1897 Danish veterinarian Bernhard Bang isolated *Brucella abortus* as the agent and the additional name **Bang's disease** was assigned. Maltese doctor and archaeologist Sir Temi Zammit identified unpasteurized milk as the major source of the pathogen in 1905, and it has since become known as Malta Fever, or *deni rqiq* locally. In cattle this disease is also known as **contagious abortion** and **infectious abortion**. In the 20th Century, this name, along with "brucellosis" (after *Brucella*, named for Dr Bruce), gradually replaced the 19th Century names "Mediterranean fever" and "Malta fever".

Review of the literature

Evidence of brucellosis in animals and man was seen for the first time in Saudi Arabia in 1974 (FAO/ WHO / OIE, 1975). Radwan et al., (1983) reported the disease in Saudi Arabia for the first time and found the incidence of the diseases in small ruminants kept on farms as 11.6% and commercial dairy cattle herds as 6.2%.

Pyakural and Mishra (1977) studied on sero-epidemiological evidence of animal brucellosis in Nepal by tube and plate agglutination tests from different geographical areas from Sept. 1975 to Feb. 1977. The prevalence was found to be the highest in buffaloes from Pokhara (22.64%) followed by cattle from Kathmandu (17.47%) and the lowest to those coming from Biratnagar (1.38%).

Pyakural (1980) studied on Brucella agglutinins in human serum in Nepal. The seropositivity rates of human brucellosis were reported as 5.26% in Solukhumbu 5.36% in Langtang, 2.56% in Malunga, 1.49% in Pokhara, 2.32% in Biratnagar, 3.63% in Bhairahawa, 1.65% in Kathmandu by plate test and 5.26%, 7.14%, 3.84%, 2.23%, 2.79%, 4.54%, 2.47% by tube agglutination test respectively.

Joshi (1983) studied the sero-epidemiological prevalence of human and animal brucellosis in Kathmandu. Total sera samples of 1430 human, 1069 cattle and buffalo and 247 sheep and goat were examined. The incidence rate was found 6.08% (87/1430) in human, 8.7% (93/1069) in cattle and buffalo, and 3.64% (9/247) in sheep and goat.

Mishra and Shah (1990/1991) detected brucellosis serologically in cow, buffalo, goat, sheep and pig in Nepal. They reported that the disease in Nepal is regarded as one of the occupational and public health hazards for veterinarians, animal attendants, dairy man, slaughter-house workers, butchers and meat sellers.

Joshi (1992) conducted serological study on human and animal brucellosis in Nepal. He collected total sera of 558 humans and 120 animals and tested by Brucella Plate agglutination test. The prevalence of disease was reported 4.48% (25/558) in humans and 12.5% (15/120) in animals. He reported that the persons with above 30 years had higher prevalence rate (2.7%). Out of 25 positive samples, 14 (2.5%) were male and 11 (1.9%) were female. But in animal cases higher incidence of diseases was found in females (9.2%) than in males (3.3%).

Pyakural and Mishra (1997) reported 16.66 % and 8.33 % of brucellosis in yak, nak and chauri in Jumla by plate and tube tests respectively.

Pyakural et al., (1997) reported that the incidence of disease was observed 22.64% in water buffaloes, 17.4% in cattle, and 1.54% in sheep.

An epidemiological survey was conducted by Joshi et al., (1998) among the farmers; who usually sell milk in six different milk producers cooperative society (MPCS) viz; Gaurigunj, Narayani, Navadurga, Krishnapur, Paropakar and Triveni. Out of total 500 milk sample collected, 70 (23.17% of 302) from single, 46(30.06% of 153) from pooled and 13(28.88of 45) from mixed samples were found MRT positive. Similarly out of 500 human sera 7 i.e 1.4% were card test positive. Regarding the prevalence of brucellosis with sex, the disease was found 1.79 % in male and 1.08% in female.

Joshi (2000) studied animal brucellosis in Kathmandu and around the Valley. He collected total 660 milk samples (pooled) of buffaloes and cows from private DDC of Kathmandu and tested by Brucella Ring Test (BRT). The positive reaction was found in 4.69% (31/660). Similarly he examined 4229 milk samples (single) of buffaloes and cows from DDC centers, milk vendors and villages in Kathmandu Valley and around the Valley viz, Panchkhalk, Bhaktapur and

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Banepa and observed 1.25 % positive by MRT. He also collected 53 sera of cows and buffaloes that had aborted in advance pregnancy and tested by Brucella plate agglutination test. The incidence was found to be 3.8% (2/53).

In a epidemiological surveillance conducted by Joshi (2000) of human and animal brucellosis in milk collection area of DDC 4.5% (25 out of 558) goat and 4.5% (25 out of 558) human sera collected from different hospitals showed to be sero-positive.

DHS (2003) indicated that in Nepal around 2-3% of the cattle and buffaloes are seropositive.

CVL (2005) performed RBPT of various livestock species in the year 2005. Out of 210 samples of cattle, buffalo, sheep, goat and pig were collected from 16 districts, 2 were

Aryal (2007) studied seroprevalence of human brucellosis in Kathmandu. Total of 1006 serum samples were collected randomly from human patients among visiting to Siddhi Polyclinic, Charkhal from March, 2007 to June, 2007. Out of 1006 sera samples examined by Brewer's Diagnostic Card, 120 samples showed up positive reaction with the prevalence of 11.93%. The male showed slightly higher prevalence 12.68% (77/607) than that of female of 10.78% (43/399).

Objectives

General objective

- The general objective of the study is to assess the prevalence of brucellosis in different species of animal of the study area.

Methodology

Total 376 blood samples were collected randomly from buffaloes, goats and pigs from different part of Nepal. Blood samples (5ml from each) of 153 buffaloes from Thankot Slaughter House, 70 goats from IAAS farm and 153 pigs from Itahari were collected. They were kept in cold box and brought in laboratory of NZFHRC to centrifugation for collecting serum. Thus obtained serum samples were tested by Brewer Diagnostic Card. 0.03 ml of serum sample were added to every ' tear drop' of card by the help of capillary tube (up to black mark of the tube) and 0.03 ml BBA (Buffered Brucella Antigen) were mixed and stirred with clean sterilized stirrer (each of different tear drop). Positive reactors showed up characteristic agglutination after wet incubation for 4 minutes.

Result

Out of 153 sera-samples of buffalo subjected to card test, all showed up to be negative reactors, while out of 70 sera-samples of goat and 153 sera-samples of pig subjected to card test, 12 (i.e. 17.14%) and 11 (i.e. 7.18%) showed up to be positive reactors respectively. The females showed high prevalence of 18.6% (11/59) than that of males of 9.1% (1/11) in goat. Similarly the females of pigs showed high prevalence of 9.23% (6/65) than that of males of 5.7% (5/88). The result obtained of sero-prevalence of brucellosis of different meat animals of Nepal showed that the prevalence rate is higher in goats than that of pigs and buffaloes.

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Kathmandu Drinking Water Quality Tested for Bacterial and Chemical Analysis

D.D. Joshi, Minu Sharma, Meena Dahal, Arjun Aryal and Kabita Shahi

Safe and Wholesome Water

Water intended for human consumption should be not only "safe", but also "wholesome". Safe water is water that does not harm the consumer, even ingested over prolonged periods. Water may be safe, but if it has an unpleasant taste or appearance, it may drive the consumer to other, less safe, sources. Drinking water therefore should be not only completely safe, but also agreeable to use or wholesome: such a supply may be termed "acceptable" or "potable".

Safe and wholesome water is defined as water that is:

1. free from pathogenic agents;
2. free from harmful chemical substances;
3. pleasant to taste;
4. usable for domestic purposes.

Water is said to be contaminated when it contains infective or parasitic agents, poisonous chemical substances, industrial or other wastes or sewage. The term *polluted water* is synonymous with *contaminated water*. Pollution and contamination are the result of a human activity.

Uses of Water:

Water is required for many purposes. These may be stated broadly as follows:

1. domestic uses:
2. public purposes:
3. industrial purposes:
4. agricultural purposes:

Sources of Water Supply in Kathmandu Valley are as follows

In general, water sources must conform to two criteria: (1) the quality of water must be acceptable; (2) the quantity must be sufficient to meet present and future requirements.
There are three main sources of water:

1. RAIN
2. SURFACE WATER
    a. Impounding Reservoirs
    b. Rivers and Streams
    c. Tanks, Ponds and Lakes
3. GROUND WATER
    d. Shallow Wells
    e. Deep Wells
    f. Springs

The basic parameters requirements for drinking water determined by WHO guideline which are as follows:

- Free from pathogenic (disease causing) organisms.
- Containing no compounds that have an adverse effect, acute or

in the long term, on human health.
- Fairly clear and colourless.
- Not saline or salty.
- Containing no compounds that cause an offensive taste or smell.
- Not causing corrosion or encrustation.

WHO Guideline for Drinking Water Quality Standards.
(Results Expressed As mg/L)

S.N.	PARAMETERS	WHO Guideline Value	Acceptable	Allowable
1	pH	6.5-8.5	6.5-8.5	6.5-9.2
2	Turbidity NTU	5	<5.0	10.0
3	Colour TCU	15	<5.0	15.0
4	Taste & Odour	Inoffensive	Palatable	Not offensive
5	Free Residual Chlorine	0.2	0.2	0.5
6	Conductivity µmhos/cm		60.0	250.0
7	Dissolved Oxygen @ 30ºc		7.6	>5.0
8	Iron	0.3	0.3	1.0
9	Manganese	0.1	0.1	0.5
10	Ammonia NH_4-N	1.5	1.5	5.0
11	Orthophosphate PO_4		0.05	1.0
12	Nitrite NO_2-N	< 1.0		3.0
13	Nitrate NO_3-N	50.0	10.0	50.0
14	Chloride	250.0	10.0	50.0
15	Sodium	200.00	50.0	
16	Total Hardness	500.0	100	150
17	Calcium		75.0	
18	Magnesium		30.0	
19	Total Alkalinity		135.0	185.0
20	Suspended Solids		25.0	50.0
21	Total Dissolved Solids	1000.0	600.0	
22	Silica SiO_2		20.0	40.0
23	Hydrogen Sulfide	ND	ND	0.05
24	Sulphate	250.0	20.0	50.0
25	Aluminum	0.2	0.2	0.2
26	Langelier Saturation Index		-1.3	-4.5
27	Water Quality Index		4.6	7.5
28	Arsenic	0.01	0.01	0.05
29	Fluoride	1.5	1.5	1.5
30	Copper	1.0	1.0	
31	Zinc	3.0	0.1	3.0
32	***Lead***	0.01	0.01	
33	Chromium	0.05	0.05	
34	Cyanide	0.07	0.07	
35	Mercury	0.001	0.001	
36	* **Total Coliforms** cfu/100ml	Nil	Nil	Nil
37	**Faecal Coliforms** cfu/100ml ***E. coli*** MPN/100 ml	Nil	Nil	Nil

ND: Not Detected.

A. Bacteriological
*Total Coliforms - 3 MPN/100ml in Untreated Water
10 MPN / 100 ml in Unpiped Water
Not to occur repeatedly in distribution networks

B. Protozoa:
i) *Giardia lamblia* Not Detected (ND)
ii) *Entamoeba histolytica* ND

C. Helminthes:
i) Round Worms, Flat WormsND
ii) Ascaris ND

D. Free Living Organisms:
i) Fungi, Algae ND
ii) Chironomids, Snails ND

E. Biomass:
Chlorophyll a ND

Objective

- To collect water samples from different sources of water supply of Kathmandu and quality testing at NZFHRC lab.

Methodology

Water samples from different wards of Kathmandu Metropolitan City (KMC) and three Village Development Committee (VDC) like Bhimdhunga, Ramkot and Ichangu VDC of Kathmandu district were collected by NZFHRC staff. Altogether 140 water samples brought to our NZFHRC lab for testing. Out of which 5 from Ramkot, 4 from Bhimdhunga, 19 from ward 19 of KMC, 43 from ward 20 of KMC, 29 from 12 of KMC and 19 from ward 15 of KMC. The sample bottles were of 1 liter (1000ml) capacity, clean, of white hard plastic, and free from oil and detergents. From each sector 1000ml water samples was collected. All the water samples were brought the same day to the NZFHRC Lab and test was started for the following water test parameters given in the agreement:

- Physical analysis
- Bio- chemical analysis
- Bacteriological analysis
- Chemical analysis.

Results

pH: Most of the water sources had pH within normal range of 6.5 to 8.5. But the pH value of stone tap water from KMC 15 was only 6.07 and highest value was from Ichangu Narayan VDC with 7.73.

Of the total samples examined for clearness, sediment and smell; only 1, 2 and 1 samples were unclear, with sediments and smell respectively. All the water from tap, stone tap, spring and reserve tank were clear, without sediments and smell.

Total of 140 samples so collected were subjected to H_2S test for bacteriological analysis. These samples were compared with positive and negative controls. Positive control was water from Bishnumati River and Negative control was distilled water. Out of which 50 water samples were found positive and rest were negative. Positive samples were identified as the black coloration of the H_2S media in the test tube within three days of the incubation at 37^0C. Out of 50 positive samples 42 were wards 12, 15, 19 and 20 of KMC and 8 from three VDC.

The chemical analysis of water samples were carried out with a standard protocol. These were presented below in VDC and ward wise:

a. Ichangu Narayan VDC: The total hardness and Magnesium were found to be high in sector 1 and 3 of Ichangu Narayan VDC but was within normal range in sector – 2. The chloride content of all sectors were high. The Alkanity of sector – 2 was low but almost normal in sectors 1 and 3. The ammonia content was high in sector 1 and 3 but within normal range in sector 2 as determined by colorimeter.

b. Ramkot VDC: All the 3 samples from Ramkot VDC showed high value of total hardness and Chloride contents. Magnesium value was also high for sample from ward number 5 and 9 but normal for

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ward number 4. Ammonia content was slightly higher in tap water in ward number 5 of Ramkot VDC.

c. Bhimdhunga VDC: Total hardness was high for all samples collected from Bhimdhunga VDC but calcium content was low in samples collected from this area. In contrast to this Magnesium, Ammonia and Chloride contents were higher.

d. Ward number 20 KMC: Water samples collected from ward number 20 exhibited high amount of total hardness, magnesium, ammonia and chloride content. The amount of Iron was also found high in the community well and tap water. In contrast to this, the amount of calcium was low in tap water and stone tap of this area.

e. Ward number 19 KMC: All the samples were high for chloride content. All these samples were low in calcium contents in comparison to magnesium. The Alkanity value was low for tap water from this area but almost normal from stone taps. Ammonia and Iron contents were almost at the normal range as when compared with the standard of WHO.

f. Ward number 12 KMC: All the samples were high for chloride content. All these samples were low in calcium contents in comparison to magnesium. Ammonia and Iron contents were almost at the normal range as when compared with the standard of WHO.

g. Ward number 15, KMC: One of the surface well from this area had water not suitable for drinking purpose. That sample was high in alkanity, hardness, magnesium, chloride and iron. The ammonia content was very high and was turbid too. Lots of mosquitoes egg and larva were obtained during collection time. But the stone tap and tap water from these areas were low in alkanity, high in hardness and low in calcium. The ammonia and Iron contents were within normal from these areas.

Also, all the samples were subjected to Arsenic test for the determination of Arsenic. The commercial arsenic kit as standardized by WHO was used for the determination. No samples were high for Arsenic content. They all were within the normal range.

Acknowledgement

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NEWS:

World Zoonoses Day 2008 Celebrated, Nepal

"Workshop on Status and Challenges of Meat Hygiene and Marketing in Nepal" held on the occasion of World Zoonoses Day July 6, 2008. It was organized jointly by Nepal Veterinary Association (NVA) and National Zoonoses and Food Hygiene Research Centre (NZFHRC). Dr. D. D. Joshi presented a technical paper titled "Epidemiology, Transmission Cycles Main Signs and Symptoms, Prevention and Control of Various Zoonoses in Nepal"

World Rabies Day 2008 Celebrated, Nepal

World Rabies Day September 28, 2008 celebrated jointly by WHO, NVA, NHEICC/MoHP, VPH/DLS/MoAC, NZFHRC, KAT, HICAST, NEVLA, NPA and Heifer. In this occasion NZFHRC has carried out free dog rabies vaccination at two centres. At National Zoonoses and Food Hygiene Research Centre total of dogs 100 vaccinated and at Central Veterinary Hospital Tripureshwor, Kathmandu total of dogs 46 vaccinated.

K.D.M.A. Research Award:

Please kindly submit your research work paper on allergy for trust award consideration by the end of May 2009 to KDMART office Chagal, G.P.O. Box 1885, Kathmandu, Nepal, Phone: 4270667 and Fax 4272694. This award was established by Dr. D.D. Joshi in 2049 B.S. on the memory of his wife, the late Mrs. Kaushilya Devi Joshi. The award includes a grant of NCRs. 10,001 with certificate.

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