Axone (Akhuni), the miracle of North East

BR Singh

What is it?

- Akhuni (Axone or Aghuni) is the Naga's special food additive, a probiotics, fermented Soy bean product with high culinary and health values, none of the Naga food and feast is complete without Akhuni. Akhuni is prepared in lots sufficient for house hold for a period of 30-45 days. It is available in all markets in Nagaland in case you don't want to prepare yourself. There are some variations in taste in Akhuni from different place due to variation in preparation methods.

How to prepare?

- Take clean and dry Soybean Dal (Naga Dal)
- Boil for about an hour to cook it
- Remove water through straining on thin sieves for about an hour.
- Lay bamboo leaves (fresh and soft) in a suitable bamboo basket and over there lay the strained cooked Soybean Dal, cover from upper side also with the same bamboo leaves (The fast growing bamboos which bent on top are suitable for collecting leaves).
- Cover with a thick cloth and hang over or keep over fire place made in most of the Naga houses) for 3-7 days (depend on season, longer in winter, by the time it starts to give typical smell).
- Pour the fermented product in an aluminum pot and pound it with a thick blunt bamboo stem or a wooden pestle (it can be used without pounding). It is ready to eat Akhuni.
- Divide in parts of about 100 gm and wrap in local wild banana leaves properly in 3 rolling.
- Make bundle and store away from flies, it can also be stored in fridge but taste gradually changes.

What is needed to be done? Objectives

- Establishing Microbiological standards for Akhuni.
- Evaluation and documentation of its probiotic value.
- Characterization of the Bacteria responsible for probiotic value.

Reduction of smelling character with quality preserved.i.e., to evolve **Sweat Smelling Akhuni.**

What we have done? Achievements

- Microbiological analysis of Akhuni from different markets of Nagaland (120 samples).
- Effect of Akhuni on growth (weight gain in pigs, in growers and piglets).
- Effect of Akhuni on health of piglets.
- Effect of Akhuni on immune response.
- Nutritional analysis.

Microbiological analysis

- On testing 120 samples of Akhuni for total bacterial count, coliform count, aerobic spore count, anaerobic spore count and yeast and mold counts we conclude:
- Anaerobic spores could be detected in 87 samples but could not be counted because their numbers might be below 100 cfu per gram.
- Coliform count varied from 0.0 to Log_{10} 10.67cfu/ gram (Avg. Log_{10} 4.24cfu). 63 samples were positive for coliforms.
- Total plate count varied from 7.78 to 12.98 Log_{10} cfu/ gram (Avg. Log_{10} 9.68cfu).
- Y&M count varied from 0.0 to Log_{10} 5.44cfu/ gram (Avg. Log_{10} 4.06cfu). All but 18 samples were positive for yeast and molds. Some were having exclusively molds (12) and some had exclusively yeast (23) colonies.
- Aerobic spore count varied from 5.76 to 10.55 Log_{10cfu} / gram (Avg. Log_{10} 7.80cfu).

Enterobacter spp.	29.5%
Citrobacter spp.	8%
Pseudomonas spp.	15%
Proteus spp.	52%
Klebsiella spp.	8%
Morgenella spp.	6%
Aeromonas spp.	1%
Bacillus spp	From all, 100%

Effect on Growth of grower pigs

- 12 grower pigs (Large Black) of 78 days of age were divided in to two homogenous groups, one group was given Akhuni in feed @ 50gm in 5kg of ration while control group was given equal amount of grounded Soybean meal for 45 days.
- **Weight gain**

	Control	**Akhuni**
Per day/ pig	378g	451g
Total wt gain/pig	17.01Kg	20.3kg

Effect on health and growth of piglets

- Two Ghunghru sows having 16 piglets were fed with Akhuni @ 50g/ sow daily while two sows with 16 piglets were kept control and given grounded soybean instead of Akhuni. Experiment started after 7 days of farrowing till 35 days (still ongoing). In Akhuni group only one piglet had diarrhea and one died (crushed by sow) while in control group a total of 7 piglets suffered diarrhoea at one or more occasions and 4 died.

Weight gain of piglets	**Control**	**Akhuni**
Per day/piglet	90.0g	**151g**
Total/ piglet	3.04kg	**5.3kg**
Average Wt of piglet on start	3.91kg	3.92 kg
Average wt after 35 days	6.95kg	9.22kg

Akhuni piglets

Control piglets

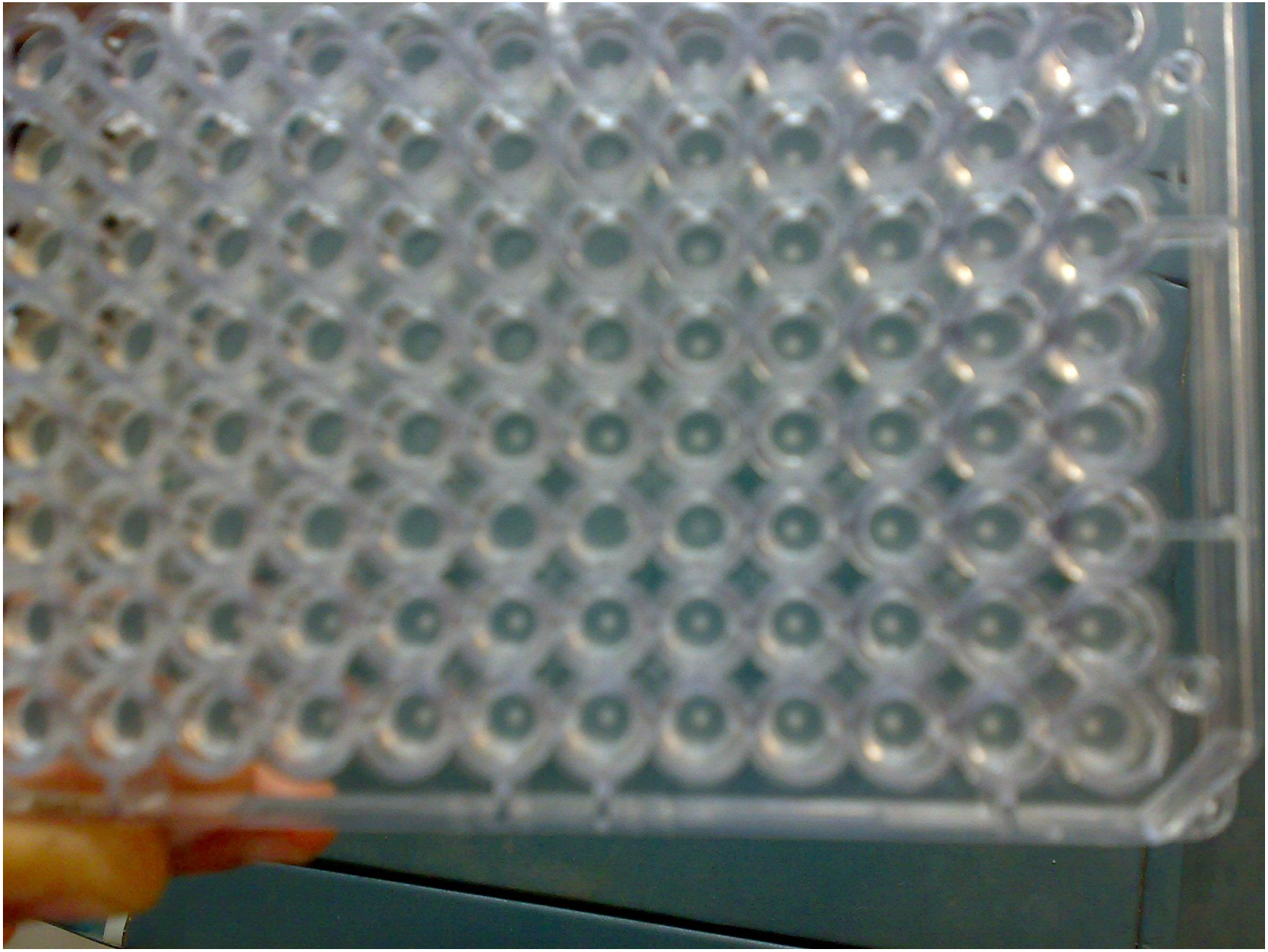
Effect of Akhuni on immune response to *Salmonella enterica* serovar Typhimurium

- Adult (2 month old) Swiss albino mice, 12 in each of the two group.
- In feed of A group akhuni was added @1% (dry Weight basis, the Akhuni sample used had 50% (47-53%) DM, while the control group (B) feed was added with equal amount of grounded Soya bean.
- On Day seven each of the mice of the two groups was inoculated ip with 0.1ml SET antigen, and again on day 12 of the experiment. Blood was collected on day 21st of the first injection and serum was extracted for antibody titration.

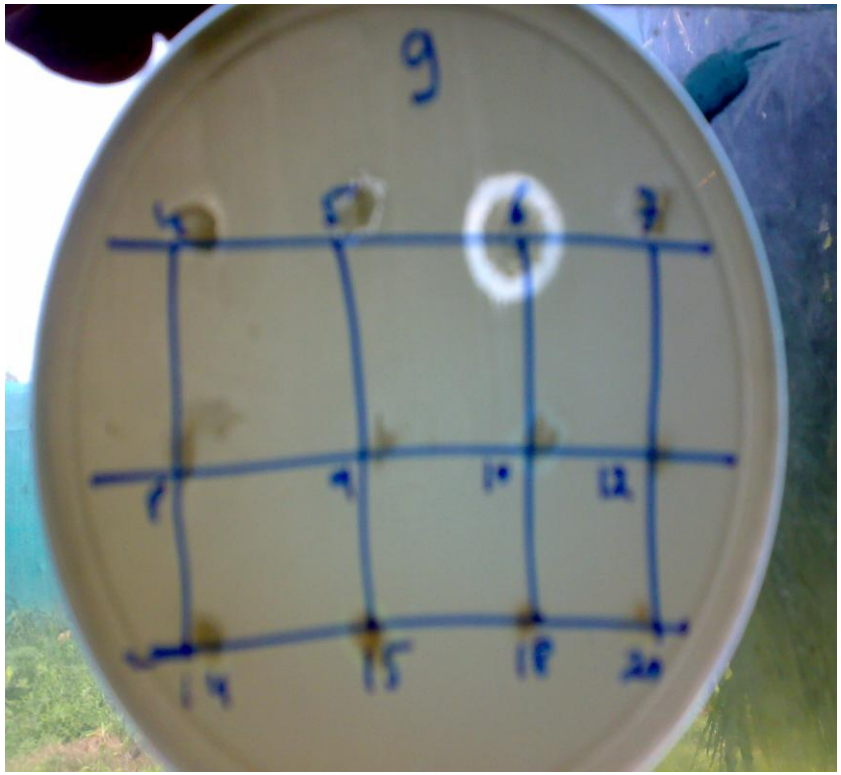
Observations	**Control**	**Akhuni**
Average titre (Log_2)	**7.0**	**9.0**
Range of titres	**6-9**	**8-12**

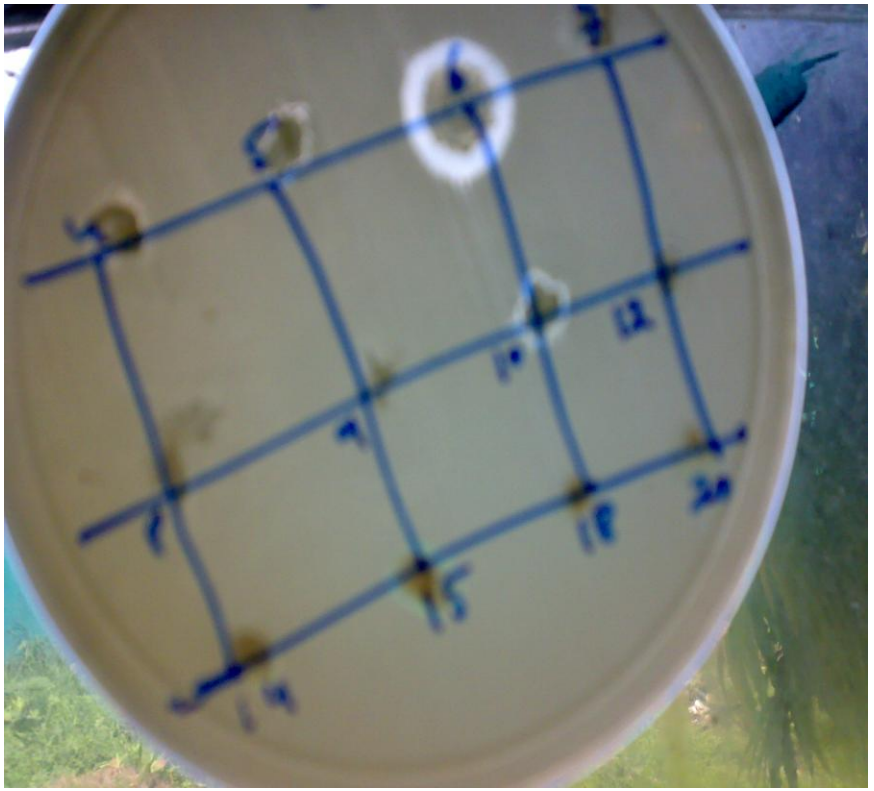
- T-test, Z-test and F-test Significant difference at level of <5%

Micro-agglutination test for detecting antibody titres

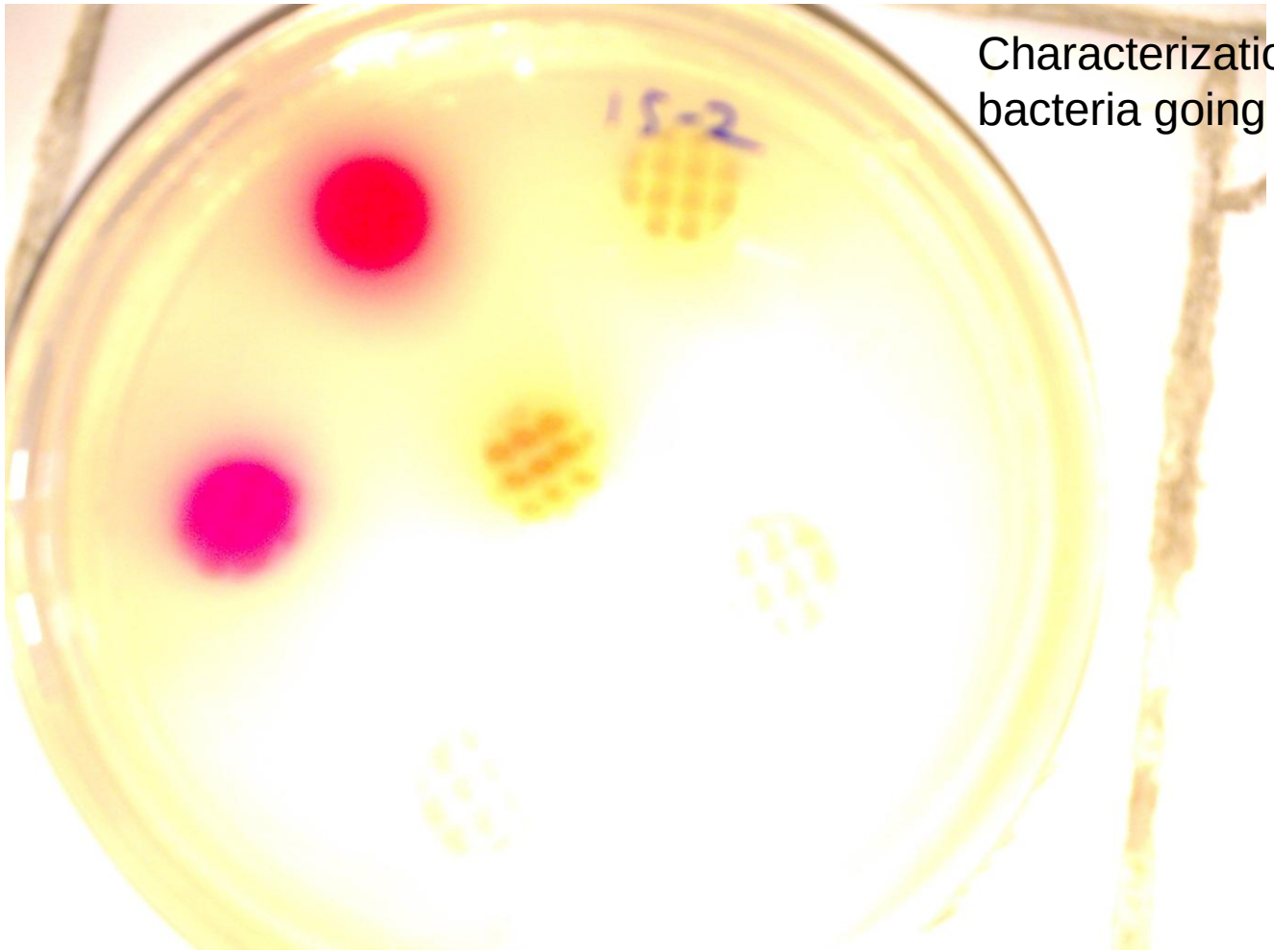


Akhuni Bacteria inhibit growth of other Bacteria





More work



Characterization of bacteria going on

Nutritional Value of Akhuni/ Soybean seed

	Water	CP	Ash
Range of Nutrients in Fresh Akhuni (in Percent)	42.39-57.39	18.4-23.5	2.33-3.03
Range of Nutrients in Soybean seeds (in Percent)	8.50-11.00	36.5-37.9	4.5-4.9
Range of Nutrients in Akhuni on DM basis (in Percent)	0.0	40.7-43.3	5.25-5.50
Range of Nutrients in Soybean seeds on DM Basis (in Percent)	0.0	41.01-43.4	5.05-5.35

What we need to go ahead?

- Facilities to characterize the proper Akhuni starter Bacteria (PCR and Electrophoresis assembly).
- Evaluation of bacteria for its probiotic qualities (more animal experiments).
- Fractionation of the bacterial extract to find out the active ingredient which promote growth, inhibit other bacteria, enhance humoral immune response (needing FPLC/HPLC).
- A proper microbiology laboratory.
- Trained technician and laboratory attendant.
- Funds and support.

Requirements: Equipment

Equipment	**Approximate cost in Rs. In Thousands**
HPLC/FPLC	390
Deep freeze-20^{o}C one	50
Homogenizer- 1	30
Automatic pipettes (two sets)	30
PCR Machine (one)	350
Electrophoresis Assembly (one) with power pack	75
UV spectrophotometer (1)	75
Anaerobic Culture Unit & CO_2 incubator(1)	100
Total	**~Rs. 11.00 lakhs**

Requirements: Consumables

Head of expanses	**First Year**	**2nd Year**	**3rd Year**	**Total**
Consumables (chemicals, glassware, plastic ware etc.)	500000	200000	200000	900000
TA and other contingencies	15000	15000	20000	50000

Requirements: Man Power

- Technician (one)
- Laboratory assistant (one)

Total outlay: About 20 Lakhs for next three years.

Publications this Year

Article

- **MAIL TODAY ePaper** Saturday, July 18, 2009
- **PASTEURISATION- RESISTANT BACTERIA STRAINS IDENTIFIED**
- AN INDIAN veterinary scientist has discovered a source of pasteurisation- resistant Escherichia coli and Enterobacter. Both these bacteria are known to cause millions of infections every year and pasteurisation is considered an effective means to kill them. But there have been several instances of these bacteria causing widespread food- borne infection on consumption of pasteurized products like milk as well. This could not be explained and it was believed that such cases could be due to post pasteurisation contamination. But the finding by Dr Bhoj Raj Singh, working at the Indian Council of Agriculture Research (ICAR) centre at Jharnapani, has solved this mystery. He has found that highly drug resistant and pasteurisation resistant E. coli and Enterobacter occur in nature as well. He has isolated several of them from faeces of horses using a novel technique. Singh says these strains can explain the presence of contamination in pasteurised products as these bacteria can withstand high temperatures. The new knowledge can be used to make pasteurized products safer. The finding has been published in The Veterinary Record , a journal of the British Veterinary Association. The pasteurisation resistant bacteria could also be used as vector for vaccines which could be pasteurized without having any adverse effect on their life. But the presence of drug resistance in these bacteria isolated from horses and their stable soil might become a source from which drug resistance could spread to other bacteria.
- *By: dineshc.sharma@mailtoday.in*

The News is based on Research Articles Published in “Veterinary Record”

B.R. Singh. 2009. Thermo-tolerance and multi-drug resistance in bacterial isolates from equids and their environment: source of pasteurization resistant bacteria. **Veterinary Record. 164**: 746-750.

Continue---------

1. **B.R. Singh**. 2009. Thermo-tolerance and multi-drug resistance in bacterial isolates from equids and their environment: source of pasteurization resistant bacteria. **Veterinary Record. 164**: 746-750.
2. **B.R. Singh**, J. Jyoti, M. Chandra, N Babu, G Sharma. 2009. Drug resistance pattern in *Salmonella* isolates of equine origin in India. **Journal of Infections in Developing Countries.** 3(2):141-147.
3. Amit Kumar Verma, **B. R. Singh,** D. K. Sinha, Mudit Chandra, Ravikant Agarwal and Mahima Verma. 2008. Micro-agglutination test (MAT) based sero-epidemiological study of Salmonellosis in dogs. **J. Vet. Pub. Hlth.** 10 (1):pp. 29-35.
4. Amit Kumar Verma, DK Sinha, **BR Singh.** 2008. Micro-Agglutination Test (MAT) based sero-epidemiological study of salmonellosis in dogs. **Journal of Immunology & Immunopathology.** 10, (1)
5. Mahtab Z. Siddiqui, **B.R. Singh,** Mudit Chandra, Ravi Kant Agarwal, R.K. Agarwal and S.K. Srivastava. 2008. Detection and partial purification of *Salmonella* serovar Typhimurium cytotoxic protein affecting seed germination. **J. Appl. Anim. Res.** 33 (2008):77-79.
6. Mudit Chandra, **B. R. Singh**, S. K. Srivastava, P Chadhry, R. K. Agrawal and A. Sharma. 2008. Comparative analysis of protein profiles of wild virulent (E156) and *aro*A-*htr*A double deletion mutant vaccine strain (S30) of *Salmonella enterica* ssp. *Enterica* serovar Abortusequi under *in vivo* and *in vitro* growth conditions. **Indian J. Experiment Biology.** 46:621-626.
7. Mudit Chandra, **B.R. Singh**, B.M. Arora, H. Shankar, R.K. Agarwal and A. Sharma. (2008). Isolation of *Salmonella* from the faeces of captive wolves. **J. Vet. Pub. Hlth**. 6(2): 107-110.
8. N. Babu, **B.R. Singh**, Harishankar, Ravi kant Agrawal, Mudit Chandra, T.V.Vijo, S.K. Srivastava, M.P. Yadav. 2008. Prevalence of *Salmonella* in equids determined by microbiological culture, standard tube agglutination test and PCR. **Haryana Veterinarian** 47:58-63.
9. **BR Singh**. 2009. **Some Naga specials dishes and culinary** on:http://northeasterner.in/index.php?option=com_comprofiler&Itemid=125

Articles accepted for Publication

1. **BR Singh**. Prevalence of vancomycin resistance and multiple drug resistance in enterococci in equids in North India. In the "**Journal of Infections in Developing Countries**" (JIDC 60-09).
2. **BR Singh.** *Salmonella* Vaccines for Animals and Birds and Their Future Perspective**.** In **"**The Open **Vaccine** Journal" (TOVAJ-C).
3. **BR Singh**, BR Gulati, N. Virmani, M Chauhan. Outbreak of abortions and infertility in thoroughbred mares associated with waterborne *Aeromonas hydrophila*. In "**Indian Journal of Microbiology**" (INJM-D-09-00039R1).
4. Javed Alam, **B R Singh**, D Hansda, V P Singh & J C Verma. Evaluation of *aro*A deletion mutant of *Salmonella enterica* subspecies *enterica* serovar Abortusequi for its vaccine candidate potential" Indian Journal of Experimental Biology. (Pub3/4(EB-2908)/09)

Thanking you.