

ALMOND89

PERSISTANCE OF SALMONELLA IN ALMOND ORCHARDS WHERE  
ANIMAL MANURE IS USED AS A FERTILIZER

Project Leaders:

Syed A. Bokhari, Farm Advisor  
University of California Cooperative Extension  
733 County Center 3 Court,  
Modesto, CA. 95355  
209-525-6654

Dr. Constantin Genigeorgis  
Professor of EPM - Food Safety, Microbiology  
Department of Epidemiology & Preventive Medicine  
University of California  
Davis, CA. 95616  
916-752-1555

Wes Asai, Farm Advisor  
University of California Cooperative Extension  
733 County Center 3, Modesto, CA. 95355  
209-525-6654

Objectives:

To determine the prevalence of Salmonella in almond orchards in counties where manure is applied to the soil as a fertilizer, and to study the dynamics of salmonella contamination in almond orchard soils.

Background:

In most almond orchards, the nuts are mechanically knocked to the ground and allowed for several days to dry before being picked up. Growers prefer to knock several acres at one time and the knocking is often done a week or more ahead of picking up. This may introduce other problems in handling. The use of animal manure in almond orchards is growing, and so is the concerns about the potential of almond crop contamination with salmonella or other microorganisms from orchard soils where animal manure is applied as a fertilizer.

The ability of salmonellae to remain viable in the environment may contribute to their persistence in almond orchard soils and may contaminate almonds at harvest time. Literature on the ability of salmonella to live under various conditions is rather limited. Gibson (1967) determined that salmonellae could survive the common slurry system and remain alive when manure containing them was spread upon pastures. Once applied to pastures, salmonella organisms could survive 6 to 12 months. Galton (1963) also estimated that salmonella organisms could survive up to 1 year after pasture application. Rankin & Taylor (1969) noted the

gradual die-off of Salmonella dublin, S. typhimurium, Staph. aureus, and E. coli in one manure slurry. These were still detectable after 12 weeks in slurry.

Large differences in the survival of various serotypes of salmonella in liquid manure were also reported by Hahn (1967). Similarly, Tucker (1967) found that S. thompson survived longer than either of the more delicate host-adopted S. pullorum or S. gallinarium in used poultry litter. In addition, several studies have shown that used poultry litter becomes actively salmonellacidal. For example, Fanelli et al (1970) reported that S. infantis and S. typhimurium did not persist as long in built-up poultry litter as in fresh litter. Bokhari (1989) found that recovery of Salmonella typhimurium in new pinewood shavings was significantly influenced by the moisture contents and holding temperature. S. typhimurium survive better at lower moisture levels and lower holding temperatures when pH remained constant at 4.2.

The colloidal character and microbial population of soil tends to effectively inhibit downward movement of pathogenic organisms. In laboratory and field tests, McCoy (1969) found that the top 14 inches of a silt loam soil retained most of the fecal bacteria from manure applied at the rate of 5 to 80 tons per acre. A sandy soil was slightly less efficient in straining bacteria.

### Experimental Design

1. The first phase of this project will include an extensive survey of almond ranches where animal manure is used as a fertilizer during the crop year. Random samples of field run nonpareil variety of almonds weighing 4 pounds will be taken from a composite sample collected from each individual orchard. These samples will be divided into two parts. One part will be tested as "inshell nuts" and the other as "almond meat" for the presence or absence of salmonella. Soil samples from each site will also be taken for salmonella testing. The data will be summarized and reported in an appropriate fashion.

To study the complete profile of salmonella contamination and its dynamics, there are several variables involved, requiring extensive experimentation and field trials for several years and require financial support. After the completion of phase one, we purpose the phase two for next years' funding.

2. The second phase of this project will start in the spring of 1991 to study the dynamics of salmonellae contamination in almond crop harvested from orchard where animal manure will be applied at a controlled rate. The manure will be experimentally inoculated with salmonellae. The population dynamics will be studied. Soil and manure samples will be collected before the application of manure on the orchard soil. Subsequent soil samples will be taken on a periodic basis until the harvest time when crop samples will also be

taken along with soil samples. All those samples will be tested for the presence or absence of salmonella. The number of salmonellae during the study period in the soil and almond harvest will also be determined. The percentage survival rate of salmonella will be determined.

#### References Cited

1. Bokhari, S. A., 1989. The effect of Moisture and Temperature on the Recovery of Salmonella typhimurium in Pinewood Shavings. Poultry Science 68(Supp. 1): 15.
2. Fanelli, M. J., W. W. Sadler, and J. R. Brownwell, 1970. Preliminary Studies on Persistence of Salmonella in Poultry Litter. Avian Diseases, 14:131-141.
3. Galton, M. M., 1963. Salmonellosis in Livestock. Public Health Reports, 78:1066-71.
4. Gibson, E. A., 1967. Disposal of Farm Effluents - Animal Health. Agriculture., 74:183-188.
5. Hahn, G. 1967. Tenacity of Salmonellae in Liquid Manure of Various Compositions. Inaug. Diss. Vet. Fak. Giesseen.(quoted by Turnbull and Snoeyenbos, 1972).
6. McCoy, J. H., 1969. The Isolation of Salmonellae. Journal of Applied Bacteriology, 25:213-224.
7. Rankin, J. D. and R. J. Taylor, 1969. A Study of Some Disease Hazards Which Could be Associated With the System of Applying Cattle Slurry to Pasture. Veterinary Records, 85:578-581.
8. Thunegard, E. 1975. On the perisitence of Bacteria in manure. Acte Vet. Scand., Suppl. 56:1-19.
9. Tucker, J. F. 1967. Survival of Salmonellae in Builtup Litter for Housing of Rearing and Laying Fowls. British Veterinary Journal, 123:92.

18TH ANNUAL ALMOND RESEARCH CONFERENCE, DECEMBER 4, 1990, FRESNO

Project No. 90-ZE1 - Persistence of Salmonella in Almond Orchards  
Where Animal Manure is Used as a Fertilizer  
New Project

Project Leader: Dr. Syed A. Bokhari, (209) 525-6654  
University of California Cooperative Extension  
733 County Center III Court  
Modesto, CA 95355

Project Personnel: W. Asai and C. Genigeorgis

Objectives: (1) Determine the prevalence of Salmonella in almond orchards in counties where manure is applied to the soil as a fertilizer. (2) Study the dynamics of salmonella contamination in almond orchard soils.

Interpretive Summary:

The use of animal manure as a fertilizer in almond orchards have caused some concerns about contamination of soil and almond crop with salmonella or other microorganisms. The results reported here were obtained by a survey of twenty-five almond orchards in Stanislaus (13), San Joaquin (6) and Merced (6) Counties. Of these orchards, six were control orchards where no manure was applied as a fertilizer and nineteen were test orchards where animal manure has been used as a fertilizer. Random samples of orchard soil and field run almonds were taken from each site to determine the presence or absence of Salmonella in the orchard soils, and its persistence in the almond crop.

The almond samples were sub-divided into three parts and designated as "field-run", "hulled", and "shelled" samples, respectively. All samples were submitted to the Veterinary Service Inc. Modesto, California for testing. Ten grams of each sample were inoculated into 90 ml of selenite cysteine and tetrathionate broths and incubated at 37 degrees C for 24 hours. The broth cultures were subcultured onto plates of brilliant green agar (BGA) or MacConkey's agar and incubated for 24 hours at 37 C. Lactose negative colonies were subcultured to slant tubes of triple sugar iron (TSI) and lysine iron agar (LIA). API test stripes were used for further identification.

No salmonella was isolated from the soil samples and almond samples collected from control orchards and test orchards. Other harmless bacteria were isolated and identified. Six soil samples (four from test orchards and two from control orchards) showed large white spreadings and were identified as Proteus mirabilis. Three field-run samples (one control and two test orchards), five hulled samples (all from test orchards) and none of the shelled samples showed lactose negative colonies. All other samples showed either lactose positive growth or no growth at all. Lactose positive and negative colonies were identified as Entrobactor agglomeratus and E. cloacae, respectively.