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Survey Microbiological Analyses of Freshly Laid and Stored Domestic Poultry Eggs in Selected Poultry Farms in Sari, Mazandaran State, Iran

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Abstract: Freshly laid and stored domestic fowl eggs of 7, 14 and 21 days old, respectively were microbiologically analyzed for organisms on their shells in the chalazae, albumen and yolks. All the egg parts were analyzed by plating on nutrient, mcconkey and sabourands dextrose media, respectively. Nine bacterial isolates which include *Salmonella* sp., *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter* sp., *Bacillus subtilis*, *Alcaligene* sp., *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Serratia marcescenes* and five fungal genera namely; Aspergillus, Penicillium, Cladosporium, Monilia and Rhizopus were isolated and identified in all the samples. The bacterial load was in the range of 3.3×106-1.5×107 cfu mL⁻¹ with the shell having the highest and the albumen the lowest load, respectively. Result showed presence of pathogenic microbes in the samples analyzed and it was concluded that domestic fowl eggs should not be consumed raw.

Key words: Domestic poultry eggs, organisms, shels, yolk, albumen, samples

INTRODUCTION

Eggs are complex biological systems produced primarily for reproduction in animals. The yolks containing the female germ cells are produced in the chicken's ovaries and are dropped into the mouth of the oviduct. As this happens, the egg is covered with layers egg white from albumen secreting cells with membranous tissue from other protein secreting cells and then finally with calcium and other minerals from mineral secreting cells near the bottom of the oviduct. This results in eggshell. egg yolks produce embryos (Adams and Moss, 1995). Fully mixed egg contains about 65% water, 12% proteins and 11% fat. Fresh egg has three structures which are an outer waxy shell membrane, the shell and the inner shell membrane and each is effective to some degree of retarding the entry of microorganisms. Internally, lysozyme is present and this is quite effective against gram-positive bacteria. Egg white also contains avidin which forms a complex with biotin, thus making the vitamin unavailable to microbes.

Although, considered as proteinous food eggs contain every vitamin and mineral needed by human beings except vitamin C (Mehas and Rodgers, 1994). Proper storage of eggs maintains the quality. However, both physical and chemical changes occur as eggs deteriorate. Physically, egg white becomes less viscous and more watery. Water from the egg white moves into

the yolk thereby making it thinner. Evaporation of water takes place through the shell and carbon oxide CO2 escapes causing an increase in the pH of the content. Due to this, the protein begins to breakdown and other changes occur too. Owing to poor storage conditions of freshly laid poultry eggs, more complex spoilages are usually associated with freshly laid and poorly stored eggs. Poor treatment of freshly laid eggs results in the movement of bacteria into the shell leading to the rotting of eggs when the bacteria are in sufficient numbers. This is the commonest form of bacterial spoilage of eggs. Several kinds of bacterial rotting of eggs include green rots (Pseudomonas and Acinetobacter sp.), black rot (Proteus, Salmonella and Aeromonas sp.), pin rots (Pseudomonas sp.), red spot (Serratia sp.) and custard rots (Proteus vulgaris and Pseudomonas intermedium). Several spoilage rots have been associated also with moulds like Penicillium and Cladosporium sp. Apart from the spoilage organisms, several pathogens have been isolated from domestic fowl eggs. These include Salmonella and Escherichia sp. (Jones et al., 1991). Domestic fowl eggs form a basic food for the masses, especially in the aspect of protein and vitamins. Thus in the local setting in Nigeria, eggs are treasured so much due to their use in feeding the infants, malnourished and the aged. This has led to the development of various egg recipes among the poor and the rich. Such include mayonnaise, eggnog, baked egg custard, stuffed egg,

fried, boiled and pouched eggs (Smittle, 1977). The consumption of raw domestic fowl eggs is a common habit among many people and professions. Such habit is highly recommended and practiced by many musical artists who claim that drinking of raw eggs improves the quality of their voice thereby improving their performance. Some people have at one time or the other taken raw eggs or mix the same with other drinks like malts as away of enhancing blood-building process. This is very common among malnourished and/or anaemic patents. Previous studies revealed the presence of pathogens like Salmonella in raw eggs with high incidence in duck eggs (Jones et al., 1991). Apart from commercial poultry farms, many individuals keep birds in free-range style which lay eggs for use as food. Consequently, this research carried out in 2005 aimed at the microbiological examination of freshly laid and stored commercially produced fowl eggs in selected commercial poultry farms and retailers in Sari, Mazandaran state, Iran.

MATERIALS AND METHODS

Sources of materials: Freshly laid eggs (a day old) and stored eggs of 7, 14 and 21 days, respectively labeled A-D collected from four different poultry farms in Umuahia metropolis were microbiologically analyzed for bacterial and fungal contaminations.

Sample collection: Twenty eggs placed in groups A-D (five eggs per group) were collected using sterile disposable hand gloves.

Isolation and identification of organisms: From the samples collected, an egg was picked from sample A and washed with 400 mL of with distilled water in a sterile beaker. Six-fold serial dilution of the wash water was made and 0.1 mL of 10⁻³ and 10⁻⁴ dilutions of the wash water was inoculated on Nutrient and MacConkey media using spread plate method. Another egg was broken with sterile blade and using a sterile forceps, the chalaza was transferred into 9 mL of normal saline in a test tube and was shaken for 30 sec. From there, 0.1 mL of the normal saline was inoculated on Nutrient and MacConkey media, respectively. About 1 mL each of the yolk and albumin of the remaining egg samples was add to 9 mL of normal saline in test tubes using sterile pipette from which 0.1 mL was taken from each of the test tubes and inoculated into the media. All inoculations were carried out in duplicates. At the end, the plates were labeled A1-A4 and incubated at 37°C for 24 h. This procedure was carried out on the remaining samples (B-D). The Sabourands Dextrose agar used for the fungal isolation was fortified with 0.005%

chloramphenicol to inhibit bacterial contaminants. The plates were inoculated in duplicates and incubated at 22°C for 2-6 days.

At the end of the incubation period, bacterial colonies were counted using the illuminated colony counter (Gallenkamp, England). The counts for each plate were expressed as colony forming units (cfu mL⁻¹) of the suspension. Total bacterial count was performed on nutrient agar. Discrete colonies on nutrient and MacConkey agars were examined carefully for cultural characteristics such as the bacterial shapes, colour and size.

Gram staining and necessary biochemical tests were carried out to confirm the isolates (Ogbulie *et al.*, 1998). Morphological features, slide culture technique and slide mount in lactophenol-cotton-blue of each fungal isolate was carried out according to Barnett and Hunter (1972). The prevalence of the microbial isolates in the four parts of the eggs analyzed was expressed in percentages.

RESULTS AND DISCUSSION

A total of nine bacteria (Salmonella sp., Escherichia coli, Staphylococcus aureus, Enterobacter, Bacillus subtilis, Alcaligenes sp., Proteus vulgaris, Pseudomonas aeruginosa and Serratia marcescenes) and five fungal genera (Aspergillus, Penicillium, Cladosporium, Monilia and Rhizopus) were isolated from different parts of the egg samples analyzed.

For the 0 day eggs, all the bacterial isolates were present on the shells of the four groups while for the chalaza Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella sp., made 100% appearance in the four groups. Serratia marcescenes and E. coli made the lowest appearance (25%). In the albumin, Bacillus subtilis, Staphylococcus aureus and Salmonella sp., made 100% appearance while E. coli was not isolated from it. In the egg yolk, Salmonella sp., made the highest appearance (100%) while Alcaligenes made the lowest appearance (25%) (Table 1). For 7 days old eggs, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella sp. and Serratia marcescenes made a 100% appearance each on the shells while Enterobacter was not isolated from the shells. Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, E. coli and Salmonella sp., had 100% appearance in the chalaza while Serratia marcescenes had the lowest appearance (50%) in it. In the albumin, all the bacterial isolates were found in the four groups analysed except E. coli and Alcaligenes that made 75% appearance each. In the yolk, Staphylococcus aureus, Pseudomonas

Table 1: Percentage occurrence of bacterial isolates in 0 day eggs

Table 1.1 creentage	Bacillus		Pseudomonas		Serratia	Enterobacter	Escherichia		
Bacterial isolates	subtilis	aureus	aeruginosa	vulgaris	marescenes	aerogenes	coli	Alcaligenes sp.	Salmonella sp.
Samples									
Shell									
1	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+
Chalaza									
1	+	+	+	-	-	-	-	-	+
2	+	+	+	+	-	+	-	-	+
3	+	+	+	+	-	+	-	+	+
4	+	+	+	+	+	+	+	+	+
Albumen									
1	+	+	-	-	-	+	-	-	+
2	+	+	+	+	+	+	-	+	+
3	+	+	+	+	+	-	-	+	+
4	+	+	+	+	+	-	-	+	+
Egg yolk									
1	-	-	-	-	-	-	-	-	+
2	-	-	+	+	+	+	+	-	+
3	+	+	+	+	+	+	+	-	+
4	+	+	+	+	+	-	-	+	+
Occunence (%)	87.50	87.50	87.50	81.25	68.75	68.75	43.75	62.50	100

Table 2: Percentage occurrence of bacterial isolates in 7 days old eggs

	Bacillus	Staphylococcus	Pseudomonas	Proteus	Serratia	Enterobacter	Escherichia		
Bacterial isolates	subtilis	aureus	aeruginosa	vulgaris	marescenes	aerogenes	coli	<i>Alcaligenes</i> sp.	Salmonella sp.
Samples									
Shell									
1	+	+	+	+	+	-	-	-	+
2	+	+	+	+	+	-	-	-	+
3	+	+	+	+	+	-	+	-	+
4	+	+	+	+	+	-	-	+	+
Chalaza									
1	+	+	+	-	+	+	+	-	+
2	+	+	+	+	-	+	+	+	+
3	+	+	+	+	-	+	-	+	+
4	+	+	+	+	+	+	+	+	+
Albumen									
1	+	+	+	+	+	+	+	-	+
2	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	-	+	+
Egg yolk									
1	+	+	+	+	+	+	+	+	+
2	-	+	+	+	+	+	+	+	+
3	+	+	+	+	+	-	+	+	+
4	+	+	+	+	+	+	-	+	+
Occurance (%)	93.75	93.75	93.75	93.75	87.50	68.75	68.75	68.75	100

⁺⁼ Presant-= Absent; 1-4 = Poultry farms

Table 3: Percentage occurrence of bacterial isolates in 14 days old eggs

Bacterial isolates	Bacillus subtilis	Staphylococcus aureus	Pseudomonas aeruginosa	Proteus vulgaris	Serratia marescenes	Enterobacter aerogenes	Escherichia coli	Alcaligenes sp.	Salmonella sp.
Samples									
Shell									
1	+	+	+	+	+	-	-	-	+
2	+	+	+	+	+	-	-	-	+
3	+	+	+	+	+	-	+	-	+
4	+	+	+	+	+	-	-	+	+
Chalaza									
1	+	+	+	-	+	+	+	-	+
2	+	+	+	+	-	+	+	+	+
3	+	+	+	+	-	+	+	+	+
4	+	+	+	+	+	+	-	+	+

Table 3: Continue

Bacterial isolates	Bacillus subtilis	Staphylococcus aureus	Pseudomonas aeruginosa	Proteus vulgaris	Serratia marescenes	Enterobacter ærogenes	Escherichia coli	<i>Alcaligenes</i> sp.	Salmonella sp.
Albumen									
1	+	+	+	+	+	+	+	-	+
2	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	-	+	+
Egg yolk									
1	+	+	+	+	+	+	+	+	+
2	-	+	+	+	+	+	+	+	+
3	+	+	+	+	+	-	+	+	+
4	+	+	+	+	+	+	-	+	+
Occurance (%)	93.75	100	100	93.75	87.50	68.75	68.75	68.75	100

Table 4: Percentage occurrence of bacterial isolates in 21 days old eggs

Bacterial isolates	Bacillus subtilis	Staphylococcus aureus	Pseudomonas aeruginosa	Proteus vulgaris	Serratia marescenes	Enterobacter aerogenes	Escherichia coli	<i>Alcaligenes</i> sp.	Salmonella sp.
Samples									
Shell									
1	+	+	+	+	+	-	-	-	+
2	+	+	+	+	+	-	-	-	+
3	+	+	+	+	+	-	+	-	+
4	+	+	+	+	+	-	-	+	+
Chalaza									
1	+	+	+	+	-	+	+	-	+
2	+	+	+	+	-	+	+	+	+
3	+	+	+	+	-	+	+	+	+
4	+	+	+	+	+	+	+	+	+
Albumen									
1	+	+	+	+	+	+	+	-	+
2	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	-	+	+
Egg yollk									
1	+	+	+	+	+	+	+	+	+
2	-	+	+	+	+	+	+	+	+
3	+	+	+	+	+	-	+	+	+
4	+	+	+	+	+	+	-	+	+
Occurance (%)	93.75	100	100	93.75	87.50	68.75	68.75	68.75	100

⁺⁼ Present; -= Absent; 1-4 = Poultry farms

Table 5: Percentage occurrence of fungal isolates in all the egg samples

Fangel isolates	<i>Penicillium</i> sp.	Aspergillus sp.	Cladosporium sp.	<i>Rhiozpus</i> sp.	<i>Monilia</i> sp
Egg shell					
A	+	+	-	+	-
В	+	+	+	+	-
C	+	+	-	+	-
D	+	+	+	+	+
Chalaza					
A	+	+	-	+	+
В	+	+	+	-	-
C	-	+	-	-	-
D	+	+	+	+	+
Albumln					
A	-	+	+	-	+
В	+	+	+	+	-
C	+	+	+	+	+
D	+	+	+	-	-
Egg yolk					
A	+	+	+	+	+
В	+	+	+	+	+
C	+	+	+	+	+
D	+	+	+	-	+
Occurrence (%)	87.50	100	75	68.75	56.25

 $A1 = Shell \ of \ 0 \ day; \ B1 = Shell \ of \ 7 \ days; \ C1 = Shell \ of \ 14 \ days; \ D1 = Shell \ of \ 21 \ days; \ A2 = Chalaza \ of \ 0 \ day; \ B2 = Chalaza \ of \ 7 \ days; \ C2 = Chalaza \ of \ 14 \ days; \ D3 = Albumen \ of \ 21 \ days; \ A3 = Albumen \ of \ 0 \ day; \ B3 = Albumen \ of \ 7 \ days; \ C3 = Albumen \ of \ 14 \ days; \ D3 = Albumen \ of \ 21 \ days; \ A4 = Egg \ yolk \ of \ 0 \ day; \ B4 = Egg \ yolk \ of \ 7 \ days; \ C4 = Egg \ yolk \ of \ 14 \ days; \ D4 = Egg \ yolk \ of \ 21 \ days; \ + = Present; \ - = Absent$

aeruginosa, Proteus vulgaris, Alcaligenes, Serratia marcescenes and Salmonella sp. were isolated from the four groups while Bacillus subtilis, Enterobacter and E. coli made a 75% appearance each (Table 2).

From Table 3, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Serratia marcescenes, Proteus vulgaris and Salmonella sp., were found on the shells of the four groups (A-D) while Enterobacter sp. was not isolated from the shells of the 14 days old eggs. However, in the chalaza, Bacillus subtlis, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter, E. coli and Salmonella sp., made 100% appearance each while Serratia marcescenes made the least appearance (50%). In the albumin, E. coli and Alcaligenes made a 75% appearance each while other bacterial isolates made 100% appearance each.

Staphylococcus aureus, Pseudomonas aeruginosa, Serratia marcescenes, Proteus vulgaris, Alcaligenes and Salmonella sp. were isolated from the yolks of all the groups while Bacillus subtilis, Enterobacter and E. coli made a 75% appearance each. From Table 4, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Serratia marcescenes, Proteus vulgaris and Salmonella sp. were isolated from the shells of all the eggs of the four groups while Enterobacter sp. was not isolated from it.

In the chalaza, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter, E. coli and Salmonella sp., made a 100% appearance each while Serratia marcescenes had the lowest appearance (50%). In the albumin, E. coli and Alcaligenes sp., made the lowest appearance each (75%) while all other bacterial isolates were isolated from it. In the yolk however, Staphylococcus aureus, Pseudomonas aeruginosa, Serratia marcescenes, Proteus vulgaris, Alcaligenes and Salmonella sp., made a 100% appearance each among the four groups while Bacillus subtilis, Enterobacter and E. coli made a 75% appearance each. Only Salmonella sp. was found in the four parts of all the egg samples analyzed while Enterobacter, E. coli and Alcaligenes had the overall lowest appearance of 68.75% each. Only Aspergillus sp. was isolated from the four parts of the eggs analysed in the four groups while Monilia sp., made the lowest appearance of 56.25% (Table 5). The egg shell had the highest bacterial load while the albumin had the lowest load and the bacterial load in all the egg samples analyzed was in the range of 3.3×106 to 1.5×107 (Table 6). All the bacterial isolates are found on the shells of 1 day old eggs from the 4 samples of eggs analyzed.

The microbial isolates from the analyzed egg samples indicated the presence of both spoilage and pathogenic

Table 6: Bacterial loads of different of the eggs	
Bactarial isolates	Bacterial loads
Egg shell	
A_1	1.30×10^{7}
B_1	1.23×10^{7}
C_1	1.50×10^{7}
D_1	1.20×10^7
Chalaze	
A_2	4.67×10^{6}
B_2	5.33×10 ⁶
C_2	4.67×10^{6}
D_2	3.67×10^{6}
Albumen	
A_3	3.67×10^{6}
B_3	4.00×10^{6}
C_3	3.67×10^{6}
D_3	3.33×10^{6}
Yolk	
A_4	3.67×10^{6}
B_4	4.00×10^{6}
C_4	4.00×10^{6}

4.00×106

organisms. The result agrees with the earlier research of Braun and Fehlhaber (1995). The presence of E. coli and Salmonella in the eggs indicates faecal contamination probably from faecally contaminated poultry feeds or drinking water. Comparing Table 1 and 4, results showed an obvious movement of microorganisms from the shells of 1 day old eggs to the yolk of the same eggs. This movement from the shell to the yolk was probably due to a fall in the pressure as air escaped through the shell. There was a gradual decrease in the contamination of the albumin and yolk by all the bacterial isolates except Bacillus subtilis, Staphylococcus aureus and Salmonella sp., that appeared in the albumin of the four, 1 day old egg samples (Table 2). Salmonella sp. was the only organism found in all the analyzed portions of the four, 1 day old eggs. The presence of the bacterial and fungal isolates in the albumen and yolk of 1 day old eggs could be due to contamination in the oviduct of the hen with the chicken droppings or contaminated poultry feeds.

Although, all the four parts of the eggs were contaminated, there was gradual decrease in number from 1.37×107 to 3.67×106 cfu mL $^{-1}$ for the shell, 1.23×107 to 4.00×106 ; 1.5×107 to 4.0×106 and 1.2×107 to 4.00×106 cfu mL $^{-1}$ for the chalazae, albumen and the yolks, respectively (Table 6).

Interestingly from Table 5, more bacteria were found in the yolk than in the albumen which is a correlation with the report of Banwart (1980). Comparing Table 1 and 4, results revealed that by the 21st day of storage, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Serratia and Salmonella were found in the yolks of the four eggs. It is then obvious that given some more days, the last three organisms mentioned above would have found their way into the yolk of the remaining egg samples. It was reported by Braun and

Fehlhaber (1995) that Salmonella enteridis could migrate from the albumen into the yolk within 24 h depending on the storage temperature and level of contamination. This implicitly implies that consumption of raw 21 days old eggs creates direct access of the isolated bacteria into the body, a situation that could lead to several types of bacterial infections. In this instance, various strain of E. coli have been documented to be Enteropathogenic, Enterotoxigenic, Enterohemorrhagic and Enteroinvasive while Salmonella sp., have been implicated in typhoid fever, bacteremia with focal lesions and enterocolitic consequent upon ingestion of food containing these organisms (Jawetz et al., 1995).

Thus, the consumption of 7-21 days old eggs without proper cooking increases the probability of occurrence of the before mentioned health problems. From Table 5, Aspergillus sp. was found on the shells, in the chalazae, albumen and yolks of all the eggs of 0-21 days old. This is of great health importance since the presence of Aspergillus in all the egg samples from all the poultry farms analyzed hitherto points to the use of contaminated poultry feeds or poultry feeds raw materials or general low hygienic margins in these farms. Occurrence of aspergillus is a threat to health due to the production of aflatoxins that have been found to be carcinogenic, teratogenic and mutagenic in humans and birds. Aflatoxins have also been found in cow milk following the consumption of contaminated cow feeds. Aflatoxins were discovered in 1960 when 100,000 Turkey poults died from eating Fungus-infected peanut meal.

The toxins are known to cause frame-shift mutation. From Table 5, penicillium has a 82.50% occurrence level in all the egg samples analyzed followed by cladosporium (75%), rhizopus (68.75%) and monilia (56.25%). These mentioned fungi are spoilage in function and their presence in the eggs the insidious initiation and sustenance of various types of spoilages mentioned in the review. This will lead to great food and economic losses to the unsuspecting farm keepers and the consumers. The presence of the spores of these fungi on and in the eggs could lead to several respiratory diseases like coccidioidomycosis, blastomycosis and histoblastomycosis when the fungal spores are inhaled by the humans and the birds. This condition is not less expected in the poultry farms as the eggs are simply kept on wooden shelves and at temperature suitable for fungal growths.

The proliferation of microbes in the inner part of the eggs especially of the 0 and 7 days old eggs was possibly due to high level of contamination coupled with the abundant nutritional materials and growth factors in the egg. However, the incomplete colonization of the yolks by

Bacillus, Enterobacter and *E. coli* after 21 days (Table 4) was obviously due to the bacteriostatic components of the egg such as lysozymes and avidin. The same could apply to penicillium, cladosporium, rhizopus and Monilia growths, respectively.

However under the prevailing poor storage conditions, the organisms will definitely overpower the intrinsic resistance.

CONCLUSION

The foregoing studies have revealed that freshly laid and stored domestic fowl eggs used in the study were contaminated by consortia of microorganisms. They migrated and invaded the inner parts of the eggs due primarily to heavy contamination and then prevailing poor storage conditions.

RECOMMENDATIONS

It is strongly recommended that the government should set quality control standards in the storage conditions of the eggs, especially with respect to the installation of air-conditioning facilities in the ware houses to ensure that the required storage temperature of -1°C is maintained.

This would minimize moisture loss due to evaporation and ensure normal relative humidity. Furthermore, cross-contamination of freshly laid sterile eggs by contaminated poultry feeds and wash water must be checked and stopped entirely in order to prevent microbial migration into the eggs and subsequent spoilages that will ensure.

This will also check aflatoxin production by aspergillus in the eggs from contaminated poultry feeds. Since eggs pick odour and flavour due to the porousity of the shells, freshly laid eggs should be quickly coated with light mineral oil to prevent possible proteolysis and other organoleptical changes as advanced methods of egg preservation are missing in the local setting.

The use of cryogenic gas to effect rapid cooling of eggs should also be introduced in the poultry farms. Necessary microbiological analytical techniques should be incorporated in the poultry farms as part of the good manufacturing practice to ensure the release of wholesome products to the public. The general public should be well informed of the health threats associated with the raw consumption of poultry eggs. Installation of necessary storage facilities would ensure that eggs are in constant supply round the year due to its high nutritional values to the neonates, infants, the sick, the aged and the entire public.

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