

# Biology of Eggs

## Objectives

After completing this module, participants will be able to do the following:

1. Describe the parts of an egg and their biological importance.
2. List defense mechanisms in eggs.
3. Classify the types of shell eggs used in the processing of egg products.
4. Identify the pathogens of concern in eggs and egg products.
5. List the factors that can inactivate or control the microbial pathogens in the final egg products.

## References

1. USDA/AMS, Egg-Grading Manual, Agricultural Handbook Number 75, Revised July 2000.
2. Federal Register [Docket No. 97N-0322]: *Salmonella* Enteritidis in Eggs. Vol. 63, No. 96, Tuesday, May 19, 1998.
3. Federal Register [Docket No. 04-034N]: *Salmonella* Enteritidis in Shell Eggs and *Salmonella* spp. in Liquid Eggs Products Risk Assessments Technical Meetings. Vol. 69, No. 192, Tuesday, October 5, 2004.
4. USDA/FSIS, 2005 – Regulations and Policies, Federal Register Publications & Documents: Related Documents for Docket 04-034N – Draft Risk Assessments of *Salmonella* Enteritidis in Shell Eggs and *Salmonella* spp. in Egg Products
5. Egg Science and Technology, Fourth Edition, W.J. Stadelman and O.J. Cotterill, editors. Haworth Food & Agricultural Products Press, 1995.
6. Virtual Chicken video – the URL to view the Virtual Chicken video is: <http://www.aufsi.auburn.edu/virtual-chicken/>
7. 9 CFR 590

## Egg Composition

The chicken egg is a complex biological structure. It is one of the most nutritious and versatile of human foods. It consists of approximately 10% shell, 58% white, and 32% yolk. Neither the color of the shell nor that of the yolk affects the egg's nutritive value. An average egg weighs approximately 57 grams (2 ounces) and the nutritive content of a large egg (~50 g of edible egg) includes:

- 6.3 g of protein
- 0.6 g of carbohydrates
- 5.0 g of fat (including 0.21 g of cholesterol)

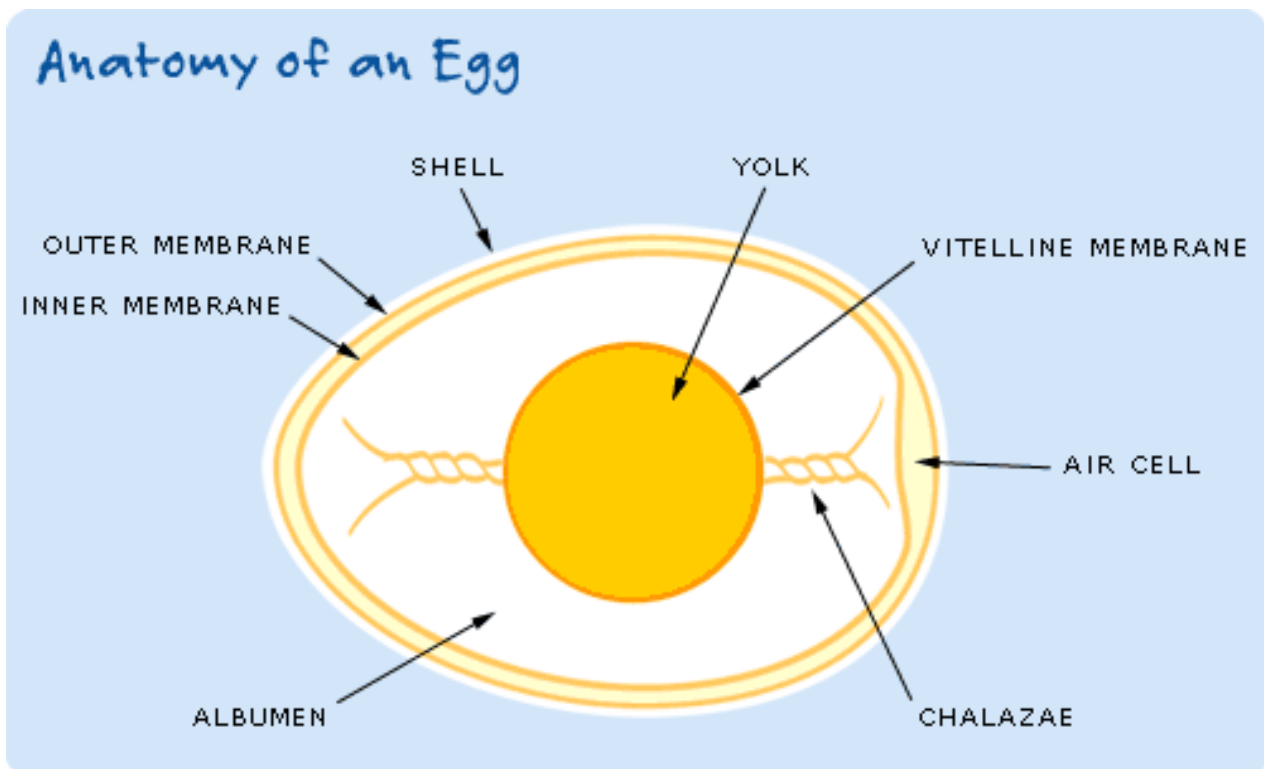
Egg protein is of high quality and easily digestible. Almost all of the fat in the egg is found in the yolk and is easily digested.

## Anatomy of an Egg

The egg is composed of many parts, namely:

- Yolk
- Albumen or egg white
- Shell membranes
- Shell

The following figure provides a better understanding of its physical structure.



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## The Shell

The shell is the egg's outer covering, accounting for about 9 to 12% of its total weight depending on egg size. The shell is the egg's first line of defense against bacterial contamination. This bumpy and grainy outer covering has approximately 17,000 tiny pores and is made almost entirely of calcium carbonate. It is semi-permeable, which means that air and moisture can pass through its pores.

However, the shell also has a thin outermost protective coating called a cuticle. By blocking the pores, the cuticle helps to preserve freshness and prevent microbial contamination of the contents.

## The Shell Membranes

Lying between the eggshell and albumen, these two transparent protein membranes provide efficient defense against bacterial invasion. These tough membranes are made partly of keratin, a protein also found in human hair.

## Air Cell

An air space forms when the contents of the egg cool and contract after the egg is laid. The air cell usually rests between the outer and inner membranes at the egg's larger end, and it accounts for the crater you often see at the end of a hard-cooked egg. The air cell grows larger as an egg ages.

## Albumen

The egg white is known as the albumen, which comes from *albus*, the Latin word for "white." Albumen accounts for most of an egg's liquid weight, about 67%. It contains more than half the egg's total protein, niacin, riboflavin, chlorine, magnesium, potassium, sodium and sulfur. Four alternating layers of thick and thin albumen contain approximately 40 different proteins, the main components of the egg white in addition to water. The pH of the albumen as the egg ages increases from a neutral pH, which support bacterial growth, to a pH of ~10 that cannot support bacterial growth.

However, there are very functional proteins in the albumen that are important to food quality and food safety. From a food safety aspect, the albumen provides both a chemical and physical defense against microbial infection of the yolk. Very little can grow in the albumen; the albumen has both bacteriostatic and bacteriocidal proteins. There are proteins with bacteriostatic (prevents bacterial growth) properties, such as ovotransferrin, avidin, and others. In addition, lysozyme has proven to have bacteriocidal (destroy bacterial cells) properties. All of these things combined make the albumen a great antimicrobial defense mechanism.

When referring to functionality, ovalbumin (foam formation) and ovomucin (foam stability – ability to retain shape and hold air during heating) are the two most important proteins.

### **Chalazae**

Opaque ropes of egg white, the chalazae hold the yolk in the center of the egg. Like little anchors, they attach the yolk's casing to the membrane lining the eggshell. The more prominent they are, the fresher the egg.

### **Vitelline Membrane**

This clear casing encloses the yolk. Its strength protects the yolk from breaking. The vitelline membrane tends to become more fragile as the egg ages.

### **Yolk**

Yolk color ranges from just a hint of yellow to a magnificent deep orange, according to the feed and breed of the hen. The yolk has important nutritional and functional qualities. From a nutritional perspective, the yolk contains less water and more fat than the white, a little less than half of the protein of the white, and most of the vitamins and minerals of the egg. These include iron, vitamin A, vitamin D, phosphorus, calcium, thiamine, and riboflavin. The most important functionality of phospholipids (lecithin) in the yolk is the emulsion formation. An emulsion is a stable mixture of two immiscible liquids, like oil and water. An emulsion occurs when one liquid is dispersed in another.

Since the yolk is the major source of nutrients in the egg, it can serve as an ideal culture media for microorganism. Once microbes enter the yolk, they grow and cause spoilage, fermentation, or they may not produce any quality problems (like *Salmonella*), but if contaminated eggs are consumed they will cause illness.

## **Steps in Egg Formation**

A hen requires roughly 24 to 26 hours producing an egg. The hen's reproductive system is divided into two main parts:

- Ovary – is the organ where the egg develops.
- Oviduct – is a long, tube-like organ along the hen's backbone and attached loosely between the ovary and the tail. There are areas along the way that performs certain functions in completing the formation of the egg (white; shell membrane and shell are secreted).

The fully formed egg moves into the vagina, enters the cloaca and the vent where the egg is laid. The time from ovulation to laying is usually 26 hours.

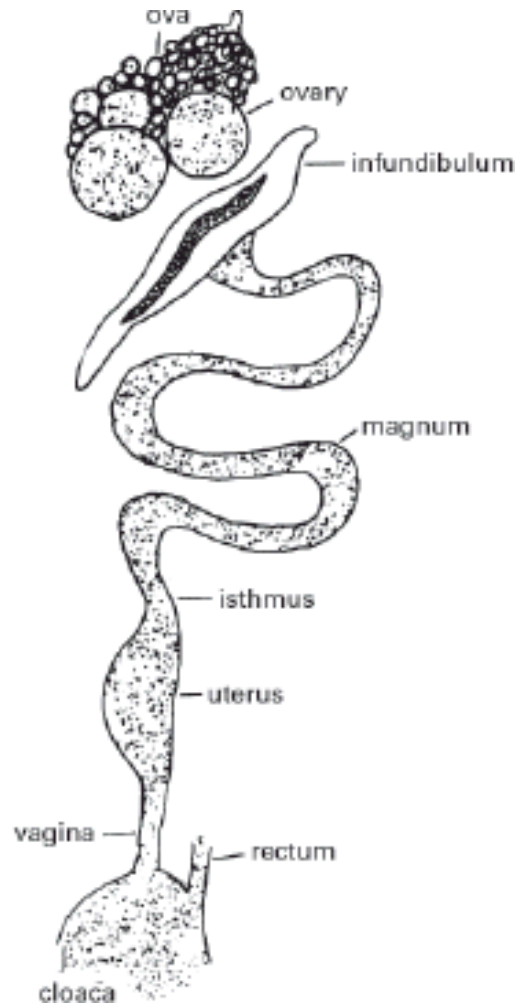
Thereafter, in about half hour after the hen has laid an egg, she releases another yolk (ovulation), where it will travel the length of the oviduct as described above.

The hen, unlike most animals, has only one functional ovary, the left one, situated in the body cavity near the backbone. At the time of hatching, the female chick has up to 4,000 tiny ova (reproductive cells), from some of which full-sized yolks may develop when the hen matures.

Each yolk (ovum) is enclosed in a thin-walled sac or follicle, attached to the ovary (see figure below). The follicle is richly supplied with blood vessels, which carry nourishment to the developing yolk. The vitelline membrane then forms around the yolk prior to ovulation.

The mature yolk is released when the sac ruptures and is received by the funnel of the left oviduct. The left oviduct is a coiled or folded tube about 80 cm (31.5 inches) in length.

**Note:** The rupture of a blood vessel on the yolk surface causes **blood spots** during formation of the egg or by a similar accident in the wall of the oviduct. Either **meat spots** have been demonstrated to be older blood spots, which have changed in color due to chemical action, or tissue sloughed off from the reproductive organs of the hen, although most meat spots are from sources other than blood spots. Less than 1% of all eggs produced have blood spots.



### Reproductive Organ of the Hen

The oviduct is divided into five distinct sections, each with a specific function:

- Infundibulum (funnel) – a funnel-shaped membrane that captures the yolk when it is released. This is where fertilization, if it occurred, would take place (commercially produced eggs are not fertilized).
- Magnum – after about 15 minutes, the yolk passes along to the *magnum*. The albumen (white) proteins are secreted and layered around the yolk (in approximately 3 hours). As the albumen is formed, the yolk rotates, twisting the albuminous fibers to form the chalazae.
- Isthmus – is where the inner and outer shell membranes are added (in 1 – 1.25 hrs), as are some water and mineral salts.
- Uterus (shell gland) – initially some water is added, making the outer white thinner. Then the shell material (mainly calcium carbonate) is added. Depending on the hen breed, pigments may also be added to make the shell brown. The egg stays in the uterus the longest (~21 hours) because of the layering that occurs with the deposition of the shell.

- Vagina/cloaca – after a few minutes pause in the vagina, the uterus inverts through the vagina, the cloaca (the junction of the digestive, urinary and reproductive systems), and the vent to release the egg outside the hen's body. Laying of the egg is known as oviposition.

Worth mentioning is the fact that during the formation of the egg shell in the oviduct, pores are formed through the spongy layer connecting some space between the knob-like mammilla with the surface. When the egg is laid the pores are filled by the matrix material and covered by the cuticle (the chemical composition is similar to the shell membrane) sealing the pores when it dries.

As mentioned previously, the purpose of the cuticle is to keep dust and bacteria out. Bacteria penetrate the shell through the pores (trans-shell contamination).

## Eligibility of Shell Eggs

A freshly laid egg is usually free of bacteria on the inside and is well protected from bacteria by the shell, shell membranes, and several chemical substances in the egg white. However, if the eggs are subjected to warm temperatures or moisture, or both, bacteria are able to penetrate the egg and overcome the egg's defense.

Because of the versatility, usage, and storage convenience of egg products, the market for shell eggs for breaking has increased over the years. Therefore, the egg products industry must meet the 9 CFR 590 regulatory requirements throughout processing.

### Shell eggs eligible for breaking

Shell eggs are to be checked for loss, leakers, dirties, strong odors, and eggs other than those of domesticated chicken, then segregated prior to entering the breaking room in the egg products plant. As per 9 CFR 590.510(c), clean shell eggs, when presented for breaking, shall be of edible interior quality and the shell shall be sound and free of adhering dirt and foreign materials. It is critical that inspectors understand some of the terms applicable to shell eggs as defined in 9 CFR 590.5:

- *Clean and Sound*: a clean and sound egg is defined as any egg whose shell is free of adhering dirt or foreign material and is not cracked or broken. As mentioned above, only clean eggs are satisfactory for breaking.

In addition, eggs with *meat or blood spots* may be used for breaking as long as the spots are removed (590.510(c)(3)). This interior defect is detected during the candling process. Blood spots show up as a cloudy image through candling.

Meat spots are usually from sources other than blood spots, as discussed earlier in this module, and are generally located within the white. They are observed as floating particles of foreign material during candling.

*Hatchery culls or classified* shell eggs from hatcheries are also eligible for breaking (9 CFR 590.510(a)(5)). These are eggs from a commercial hatchery which are removed (culled) before incubating due to defects such as cracked shells, thin-shelled eggs (body checks), and double-yolk eggs (these hatch very poorly).

### **Restricted eggs**

As per 9 CFR 590.5, *restricted egg* means any check, dirty egg, incubator reject, inedible, leaker or loss. Some of these eggs can be presented for breaking, such as checks, dirty eggs (dirties), and leakers, provided that the requirements of §590.510(c)(1) and (2) are met. 9 CFR 590.5 defines these defects as well:

- *Checks*: an egg that has a broken shell or crack in the shell but has its shell membranes intact and contents not leaking.
- *Dirty egg or dirties*: an egg(s) that has a shell that is unbroken and has adhering dirt, foreign material, or prominent stains. The eggs must be washed and sanitized (§590.510, §590.515, and §590.516). There is no threshold for “a little dirt or foreign material”; this is a zero tolerance rule.
- *Leaker*: an egg that has a crack or breaks in the shell and shell membranes to the extent that the egg contents are exposed or extruding or free to exude through the shell.

The eggs classified as incubator reject and loss are inedible (§590.510).

### **Inedible Eggs**

When bacteria grow inside the egg, they may form byproducts or cause the contents of the egg to decompose, or both, resulting in the characteristic colors, appearance, or odors that make the egg unfit for human consumption, also called inedible. The most common microbial contaminants of the inedible or rotten eggs are the genera *Alcaligenes*, *Acinetobacter*, *Pseudomonas*, *Serratia*, *Cloacae*, *Hafnia*, *Citrobacter*, *Proteus*, and *Aeromonas*. It is important to emphasize that these organisms are associated with shell eggs and egg products.

As per 9 CFR 590.510 and 590.504(c), filthy and decomposed eggs, including inedible and loss eggs, are classified as inedible. Regulation §590.5 defines the terms inedible and loss.

- *Loss* means an egg that is unfit for human food because it is smashed or



broken so that its contents are leaking; or overheated, frozen, or contaminated; or an incubator reject; or because it contains a bloody white, large meat spots, a large quantity of blood, or other foreign material.

- *Incubator reject* means an egg that has been subjected to incubation and has been removed from incubation during the hatching operations as infertile or otherwise unhatchable (§590.5).
  - *Frozen eggs* – the egg whites are crystallized and the yolk may or may not be hard and almost perfectly round, the texture is pasty or hard – if the yolk is frozen, it will not look flattened out on top like a normal egg yolk.
  - *Bloody White* is an egg that has blood diffused (partially or completely bloody) through the white; usually no blood spot appears on the yolk. Such a condition may be present in freshly laid eggs and this condition is classified as loss.
  - *Large Blood Spots or Meat Spots* in an egg require that they be classified as loss (§590.510). Blood spots, a spot of blood in the egg yolk, shall not be due to germ development; usually no blood appears in the egg whites – not diffused. Meat spots may be blood spots that have lost their characteristic red color or tissue (a reddish-brown chunk in the whites) from the reproductive organs.
- *Inedible* means eggs of the following descriptions: Black rots, mixed rots, sour eggs, eggs with green whites, eggs with stuck yolks, moldy eggs, musty eggs, eggs showing blood rings, and eggs containing embryo chicks (at or beyond the blood ring stage).

As per 9 CFR 590.510(d), *inedible* and *loss* eggs are defined to include:

- *Black Rots* are caused by bacteria from the *Proteus* and *Pseudomonas* groups. Black rots are generally opaque (grey or black color) when viewed before the candling light and are characterized by the presence of gas; since the shell membranes apparently become impermeable, the pressure created within the egg is often sufficient to burst the shell and scatter the egg's contents. When broken, the contents have a muddy brown appearance and give off a repulsive, putrid odor.
- *Mixed Rots* (addled eggs) occur when the vitelline membrane of the yolk breaks and the yolk mixes with the white (usually brown in appearance), resulting in murkiness throughout the interior of the egg when viewed before the candling light.
- *Green Whites or Green Rots* are usually caused by members of the *Pseudomonas* group of organisms, which are commonly found on the surface of the egg shell. Certain species of *Pseudomonas* multiply in the

albumen, from which they synthesize a characteristic fluorescent green pigment. The white of the egg is pale green in appearance and has a musty smell to it. These were very common types of "inedible" eggs 30 years ago; many egg products processing facilities installed black lights on the breaking equipment so that they could readily detect these inedible eggs.

- Eggs with diffused blood in the albumen or on the yolk (*bloody white* – as described above).
- *Blood Rings and Embryo Chicks* are caused by germ development, occurring in fertile eggs held at incubation temperatures. At an early stage in incubation (after 24 hrs), the embryo develops a circulatory system and if the embryo dies the blood drains to the outer edge of the germ disc, causing the blood disc. Before the candling light, it appears as a brilliant blood-red circle and the circle's diameter depends on the stage of development.
- *Moldy Egg* is an egg, usually found in humid storage conditions, whose shell is covered in mycelium "whiskers" (appears as greyish or black areas). The shell pores are penetrated and growth occurs on the shell membrane. You often see gelling around the albumen, which shows up as dark or colored patches or rings when candled. As the mold progresses, it will further break down the yolk membrane.
- *Sour Eggs* have a peculiarly pungent odor (sharp, sour odor) when opened, and their defects are not readily detected by candling. The albumen may be turbid, and the yolk and albumen may be somewhat mingled. The *Pseudomonas* bacteria cause sour eggs. These organisms produce a material that fluoresces under ultraviolet light, giving off a green sheen. The use of ultraviolet light ("blight light") in candling has made detecting this type of loss easier.
- *White Rots* occur when the white and yolk have liquefied into a single mass when the egg is broken out, and there is no longer any definition to the yolk or albumen. White rots may be detected in the early stages by the presence of threadlike shadows in the thin white layer. In later stages, the yolk appears severely blemished when viewed before the candling light, and, when broken, shows a crusted appearance. The content frequently gives off a fruity odor.
- *Crusted Eggs* are due to bacterial infection through eggs having been laid in damp nests, and is more likely to occur with dirty eggs. A *crusted yolk* is one which is covered with a light color crust, which has a tendency to flake off, and in which the white is watery and frequently yellowish in color and

- possessing a putrid odor; it may show dark spots on its surface before the candling light.
- *Stuck yolk* occurs when the yolk membrane becomes attached to the shell membrane. It generally occurs in older eggs that have been left in a fixed position for a long time. When the thick white becomes thin, the yolk floats close to the shell and becomes attached to the shell membrane.
  - *Musty eggs* frequently appear clear and free from foreign material when viewed before the candling light and can generally be detected only by the characteristic musty odor emanating from the egg.
  - *Mottled Yolks* result from a non-uniform distribution of water in the yolk or from a separation of the vitelline membrane and chalaziferous layer of the albumen (inner and outer membranes). Mottled yolks defects are rare in the egg products industry since movement of the eggs in transit takes ~15 – 30 days, maximum, at cool temperatures. This type of egg is usually diverted for pet food.

For more detail on the regulatory requirements concerning to shell eggs entering the official plant for breaking, refer to the module on “Egg Products Plant Operations.”

## Pathogen of Concern

Although an egg’s biological structure provides good defense against bacterial contamination, it is still possible for bacteria to pass through the shell into the egg’s content (trans-shell contamination or horizontal transmission). The primary food safety concern associated with eggs and egg products is the consumption of those foods when contaminated with the *Salmonella* species (spp.) bacteria.

Many serotypes of *Salmonella*, as well as other bacteria, have been isolated from laying flocks. *Salmonella* can be found in the intestinal tracts of animals, including chickens. Contact with feces, nesting material, dust, feedstuffs, shipping and storage containers, human beings, and so on contribute to the likelihood of shell contamination. The likelihood of spoilage increases with the duration of contact with contaminated material. This is especially true during storage and while in the production environment.

In addition to contamination caused externally, other routes of infection where bacteria can be inside an uncracked whole egg are:

- Transovarian – the yolk is infected while attached to the ovary. If the hen is contaminated with *Salmonella*, it will then deposit the bacteria in the yolk during development. Since the yolk is a nutrient rich environment for

the pathogen, it is able to survive and grow.

- Oviductal or vertical transmission – the vitelline membrane and/or albumen are contaminated as they pass along the oviduct. Even though there are some defense mechanisms that are found in the albumen of the egg, these defense mechanisms are not 100% bacteriocidal or bacteriostatic and *Salmonella* can still grow.

*Salmonella enteritidis* (SE) is one of the few pathogenic *Salmonella* serotypes known to colonize the reproductive tissues of hens and, consequently, the eggs they lay. For an egg to be contaminated with SE, three conditions must exist: SE must be present on the farm, SE must infect one or more hens, and SE-infected hens must be susceptible to producing SE-contaminated eggs. Multiplication and growth of this pathogen inside the egg depends primarily on time and the temperature of the egg during storage. Once inside the egg, SE survives cleaning and disinfecting of the shell surface and can multiply within the egg depending on how the egg is handled during processing.

The USDA periodically samples pasteurized egg products and has occasionally found evidence of *Salmonella* contamination in these samples, which suggests that the processing practices have not been completely effective at eliminating *Salmonella* spp. from all egg products.

The Centers for Disease Control and Prevention (CDC) has found that human salmonellosis is one of the most commonly reported bacterial infections of any kind in the United States and is the second most prevalent food-borne disease.

*Salmonella* usually causes an intestinal infection accompanied by diarrhea, fever, abdominal cramps, vomiting, headache, and nausea, starting 6 to 72 hours after ingesting contaminated food. These symptoms can last up to a week.

Factors that influence the number and severity of salmonellosis cases are the pathogenicity and virulence of the organism, the dose level, and the susceptibility of the people exposed. Generally, the higher the dose of the organism in the food, the greater the chance the person ingesting it will become ill. Infants, young children, older adults, pregnant women, and people with weakened immune systems are particularly vulnerable to SE infections. In severe cases, the infection can spread to the bloodstream and then to other areas of the body, leading to a severe and occasionally fatal illness unless treated promptly with antibiotics. Furthermore, about 2% of affected persons may later develop joint pains and arthritis.

### **Pathogen Controls in Eggs**

An important component of food safety is preventing or eliminating the presence of pathogenic organisms in the final product. Since contamination from

pathogens can come from many sources, it is the plant's responsibility to minimize pathogen contamination and growth in the final egg products.

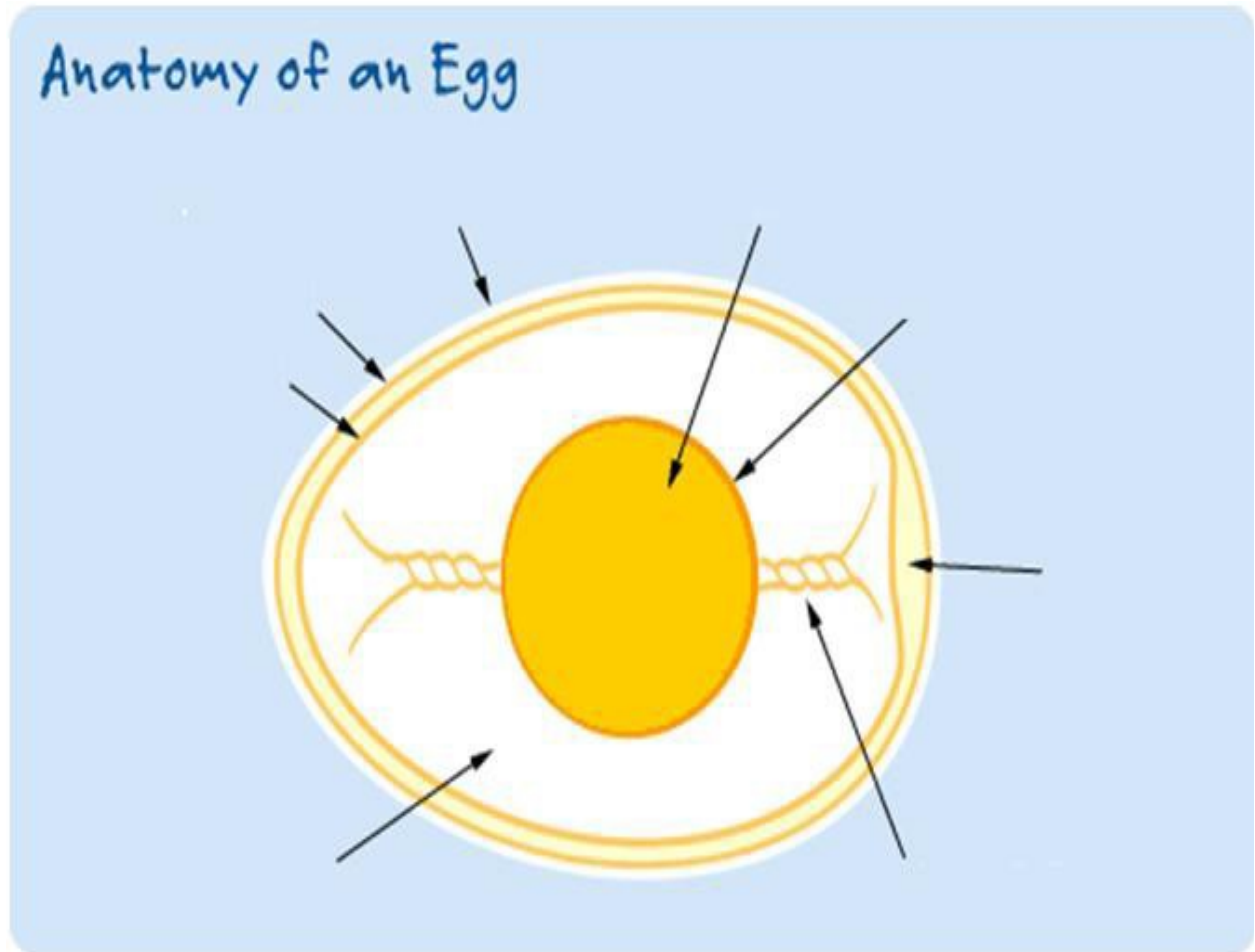
In addition to the shell egg's natural antimicrobial properties, some factors (examples listed below) can control the microbial pathogens that are most likely to cause food safety concerns during egg products processing, egg products handling, and packaging.

- Prevent contamination – production and processing conditions of shell eggs to prevent initial loads of pathogens.
- Temperature control – temperature must be strictly controlled during processing, storage, and heat treatment. Proper pasteurization and heat treatments are great microbial destruction mechanisms; they do not sterilize product, but they can reduce the number of microorganism significantly to ensure a safer product.
- Good sanitation – is extremely important during egg processing and packaging.
- Preventing cross-contamination – this is accomplished through good employee hygiene, strict sanitation, and other good manufacturing practices.

External contamination is more prevalent, and it is important to keep the microorganisms out of the liquid egg during processing. It is important to know that SE growth is inhibited at temperatures below 40°F and that not many bacteria grow at temperatures below 45°F. FDA requires shell eggs to be stored at an ambient temperature of 45°F within 36 hours of lay. In contrast, USDA has requirements for refrigerated egg products at various temperatures depending on the product's formulation and processing operations (9 CFR 590.530). Note that SE is destroyed during pasteurization. We will discuss more about egg products food safety requirements throughout this training.

## Workshop

1. Label the parts of the egg. Attempt to do so without looking back at the diagram on page 2.



2. What might cause an egg to appear fluorescent under a black light?
3. Name two inedible egg types whose defects are difficult to see – even with the candling light – but that give off characteristic odors.