White Paper

[Microbiological control in Beef Supply Chain]

The beef supply chain is a complex and dynamic system that involves multiple stakeholders, from primary production to retail and final consumption. Microbiological contamination is a key concern, as it impacts food safety, shelf life, and consumer health. Many different contamination sources exist, including animal-borne pathogens, cross contamination during slaughter and processing, and improper handling in distribution and retail. A frequently overlooked factor is water quality, as water used throughout the production process can carry harmful microorganisms and chemical residues. Consuming contaminated beef can lead to serious health issues, including gastrointestinal infections and foodborne illnesses. Ensuring meat safety requires a thorough understanding of contamination mechanisms and the implementation of effective prevention strategies. In industrialized countries, approximately 30% of the population contracts foodborne diseases annually. In the United States, an estimated 76 million people are affected annually, leading to over 325,000 hospitalizations and more than 5,000 deaths. Contaminated water poses significant risks not only to human health but also to animal welfare and productivity. In livestock farming, water is essential for growth, milk production, and reproduction. The presence of microbiological contaminants such as *Escherichia coli*, fecal streptococci, and coliform bacteria, as well as chemical pollutants like nitrates, nitrites, and heavy metals, can negatively impact animal health, reduce productivity, and compromise equipment functionality. Unfortunately, water quality is often overlooked or tested only at the entry point of the distribution system, without considering factors such as piping networks, environmental contamination, and biofilm formation.

Biofilms are complex microbial communities embedded in an extracellular polymeric matrix, that provide a protective environment for bacteria, increasing their resistance to antimicrobials and disinfectants. Studies have shown that biofilms can rapidly develop in drinking troughs even at low temperatures, serving as reservoirs for pathogens capable of causing infections when detached and ingested. The National Institutes of Health (NIH) estimates that approximately 80% of human infections are associated with biofilms. In farms, biofilms can colonize walls, floors, drinking troughs, feeders, and even the animals themselves. In recent years, concerns over microbiological safety in the meat supply chain have led to stricter regulations on water quality control in livestock farming.

Figure 1: Water quality is frequently overlooked, in beef farming

The beef processing chain

Beef production is a complex process that involves multiple stages, each of which is essential to ensuring food safety, product quality, and animal welfare, and each one presents risks of bacterial contamination. Breeding represents the first phase of the beef supply chain and focuses on the growth and development of the animals. Cattle are fed balanced diets that may include forage, grains, and supplements to ensure optimal growth. The primary objective is to help the animal reach an appropriate weight before slaughter, while simultaneously ensuring its health and welfare. Factors such as genetics, diet composition, and environmental conditions play a crucial role in the final quality of the meat. Cattle can harbor pathogenic bacteria in their gastrointestinal tract without showing symptoms. Poor hygiene in barns, contaminated water, and improper handling of feed can contribute to bacterial proliferation. To mitigate these risks, it is essential to maintain clean facilities, provide high-quality feed, and implement vaccination programs against common bacterial infections. During transport, animals are exposed to stress, which can lead to immunosuppression and increased bacterial shedding. Contamination may occur through fecal matter, especially if transport vehicles are not properly sanitized between trips. Ensuring adequate ventilation, hygiene, and minimal transport time can reduce bacterial risk. Upon arrival at the slaughterhouse, the animals are placed in holding pens for a resting period, where they may come into contact with contaminated surfaces or other infected animals. If not managed properly these areas can become reservoirs for bacteria. Veterinary inspections and strict hygiene protocols, including the use of antimicrobial washes, are necessary to prevent bacterial spread. After slaughter, carcasses are sectioned and rapidly cooled to temperatures between 0 °C and 4 °C, to prevent bacterial growth. However, cross-contamination can occur through equipment surfaces and handlers, if hygiene protocols are not strictly followed. Cold temperatures slow bacterial proliferation but do not eliminate existing bacteria, making it crucial to maintain stringent sanitary measures during processing. During packaging, contamination can result from handling or contact with unclean packaging materials. Maintaining the cold chain during transportation and storage is vital to prevent temperature fluctuations that could favor bacterial multiplication.

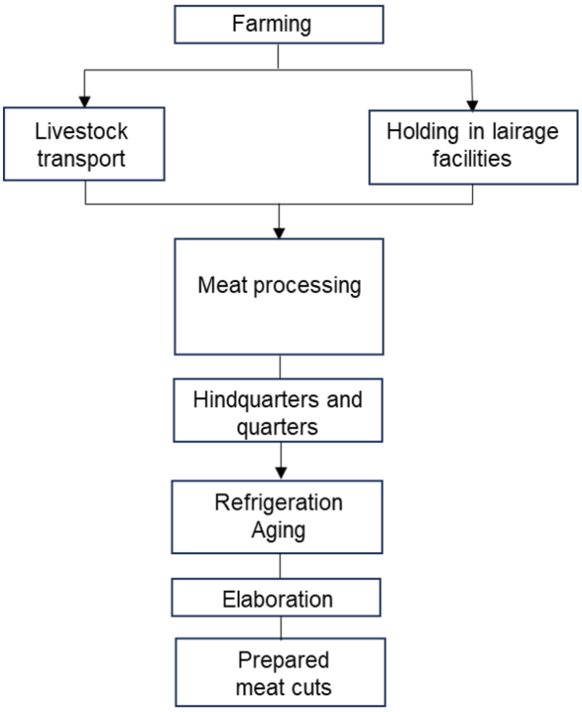

Figure 2: Steps of beef supply chain

Water quality in cattle farming

Cattle farming is a globally widespread agricultural sector, with significant differences in management practices depending on the geographic region. In Europe and the United States, cattle farming has evolved toward intensive farming, where animals live year-round in barns and are fed a variety of feeds. The water supply is ensured by automatic drinking troughs to ensure continuous access to potable water, efficiently meeting the animals' hydration needs. In cattle farming, water is sourced from various locations, each with specific characteristics that influence its quality and availability. The main water sources include surface water such as rivers, streams, lakes and artificial reservoirs used mainly in farms located near watercourses, but subject to seasonal flow variations and quality fluctuations. They can be contaminated by industrial or agricultural runoff, as well as waste from wild animals. Some farms build storage reservoirs to collect rainwater or divert water from rivers, particularly in arid regions. Another source is underground water such as artesian wells, one of the most reliable sources, as deep groundwater tends to have better microbiological quality. However, it may contain high levels of minerals (iron, manganese, nitrates), which can affect livestock health and productivity. Some livestock farms, especially in regions with regular rainfall, install rainwater collection and storage systems to reduce reliance on other sources. However, proper treatment is necessary to remove potential contaminants. Some farms, particularly large-scale operations, source their water from municipal water systems or private consortia. This is often the safest option in terms of quality, but can be expensive and subject to usage restrictions.

Figure 3: Providing fresh and clean water in proportion to animal needs is essential

Ensuring safe drinking water requires continuous monitoring and management of contamination risks. Water quality is defined by its microbiological, chemical, and physical properties. Regulatory agencies such as the United States Environmental Protection Agency (EPA) and the German Federal Ministry of Food and Agriculture have established threshold values for livestock drinking water. However, these standards often focus on water quality at the point of entry into the farm's distribution system, rather than the actual water consumed by animals. Drinking water can be provided to livestock through direct troughs, feeders, or automatic drinking systems. Regardless of the method, the system must meet essential criteria: providing fresh and clean water in proportion to animal needs, minimizing waste, and requiring minimal maintenance. However, factors such as biofilm formation, environmental contamination and water stagnation can degrade water quality before it reaches the animals.

In animal farming, drinking water is also used to provide drugs and supplements, ensuring homogeneous treatment for all livestock. If biofilm forms in water distribution system, it can absorb or degrade the active ingredients of drugs administered in the water, altering their concentration and reducing the therapeutic efficacy, leading to unpredictable underdosing or overdosing in animals. Furthermore, if the biofilm accumulates in the tanks, it can retain part of the drug, releasing it irregularly and altering the dosage administered to the animals. Antibiotics are among the treatments administered to sick animals, and one of the main threats to public health is precisely antibiotic resistance. Biofilm in livestock water infrastructures contributes to the spread of antibiotic resistance through several mechanisms. Bacteria within the biofilm can survive much higher concentrations of antibiotics than free bacteria in the liquid, favoring the selection of resistant strains. Biofilm creates an environment favorable to horizontal gene transfer (HGT), facilitating the spread of resistance genes between different bacterial species. Even after antibiotic treatment, resistant bacteria can remain embedded in the biofilm and re-emerge when the system is exposed to environmental stress again.

Water parameters monitored in cattle farms

Drinking water in cattle farms must be of good quality - otherwise, it can lead to reduced production performance, altered product quality, and significant damage to equipment and facilities. According to national and international regulations, water must meet specific parameters:

1. Chemical parameters (nitrates, nitrites, heavy metals, arsenic, chromium, copper, lead, mercury, etc.).
2. Indicator parameters (color, odor, taste, turbidity, hardness, presence of aluminum, ammonium, chloride, iron, manganese, sulphate, sodium, etc.).
3. Microbiological parameters (monitoring of *Escherichia coli,* enterococchi, etc.).

Chemical contaminants are divided into two categories organic and inorganic. Organic contaminants contain carbon and decompose slowly, as they are not easily degradable. Inorganic contaminants include nitrates, nitrites, sulphates, chlorides, hydrogen sulphide, some mineral elements and heavy metals deserve particular attention.

Water hardness depends on calcium and magnesium salts. Hard water contains high levels of these salts, while soft water has low or none. Hard water can hinder digestion, reduce nutrient absorption, and cause limescale buildup in water systems, leading to blockages. High turbidity, due to particles like sand or algae, can cause similar issues.

Drinking water typically has a pH between 6 and 9. A pH outside this range can lead to metabolic and fertility disorders, digestive issues, poor feed conversion, and drug residue accumulation. Acidic water can cause urinary and skeletal problems, while highly alkaline water may affect milk production and growth rates.

Salinity is a crucial parameter for animal drinking water. It is equivalent to total dissolved solids (TDS), which are expressed as milligrams of fixed residue per litter of water after drying at 180 °C. Water with a TDS above 3,000 mg/L causes the first negative effects on the organism.

Figure 4: Microbiological monitoring of drinking water in farms helps to prevent pathological consequences in animals

Untreated drinking water from sources used on livestock farms may contain coliforms, with *E. coli* used as a primary indicator for monitoring this kind of pollution. This bacterium is found in the feces of all mammals and has been chosen as a biological indicator due to its ability to survive in drinking water for 4 to 12 weeks. During rainfall, these coliforms can be carried into streams, rivers, watercourses, lakes, or groundwater. Microbiological monitoring of drinking water in farms helps to prevent pathological consequences in animals, including gastrointestinal, digestive, respiratory, urogenital, and reproductive disorders. A coliform count below 50 per 100 mL is considered safe, for adult cattle. For calves, less than 1 per 100 mL is advisable. One of the major challenges in maintaining microbiological water quality in livestock farms is biofilm formation. As briefly mentioned, biofilm is a complex matrix of microorganisms embedded in an extracellular polymeric substance (EPS), which adheres to the inner surfaces of pipes, storage tanks, and drinking troughs. This protective layer enables bacteria to thrive and resist conventional disinfection treatments, compromising the microbiological safety of water supplied to animals. Biofilm formation starts when bacteria adhere to the internal surfaces of the water system and starts to produce EPS. This layer fosters the growth of a diverse microbial community, including coliform bacteria, *Pseudomonas spp.*, *Legionella spp.*, fungi, and protozoa. Over time, biofilms can harbor pathogens such as *Escherichia coli*, *Salmonella spp.*, and *Campylobacter spp.*, increasing infection risks in livestock. These pathogens may be transferred to the meat supply chain, affecting food safety and posing health risks to consumers.

Commonly used laboratory analyses to assess water quality in cattle farming have several limitations that reduce their representativeness and reliability. Water samples are often collected at the entry point of the farm's water system, without considering variations in quality along the internal distribution network. As mentioned above, the water reaching drinking points may undergo significant changes due to biofilm formation inside pipes and troughs, stagnation and sediment accumulation in storage tanks, and secondary contamination caused by corroded materials or organic residues. Additionally, sampling is conducted at specific times and does not account for daily and seasonal fluctuations in water quality, such as temperature variations that promote microbial growth. Many standard tests focus on basic parameters such as turbidity and pH, electrical conductivity and hardness, total bacterial count (TBC) and detection of fecal indicators such as *Escherichia coli,* as previously explained. While useful, these parameters do not provide a complete picture of microbiological and chemical risks. Fecal indicator bacteria (*E. coli* and total coliforms) do not always correlate with the presence of hazardous pathogens like *Salmonella*, *Campylobacter*, or *Cryptosporidium*. Conventional microbiological tests do not detect enteric viruses or bacterial toxins, which may be more relevant to animal and human health. Total bacterial count does not distinguish between pathogenic and harmless bacteria and does not account for the protective effect of biofilm within distribution systems. Moreover, some microorganisms can enter a "viable but non-culturable" (VBNC) state, in which they remain viable and potentially infectious, without being detectable with traditional culture techniques. Last but not least, traditional microbiological analyses, based on laboratory culture methods, require 24 to 72 hours to deliver results, making timely intervention difficult in case of contamination. Meanwhile, animals continue consuming potentially unsafe water.

Meat microbiology

Meat microbiology is a critical aspect of food safety and the quality of the final product. Microbial contamination can arise from multiple factors, affecting shelf life and the risk of pathogen transmission. Meat serves as an ideal substrate for microbial growth due to its chemical composition and high-water content. While meat is a highly relevant food from a nutritional and dietary standpoint, it can also pose a public health risk due to potential contamination. Such contamination can be endogenous, originating from the animal's life, or exogenous, occurring during the production process. Endogenous contamination may result from substances intentionally administered to the animal, such as drugs and anabolic agents, or from accidental intake of contaminants through water and feed, including pesticides, heavy metals, mycotoxins, and PCBs, as explained in previous

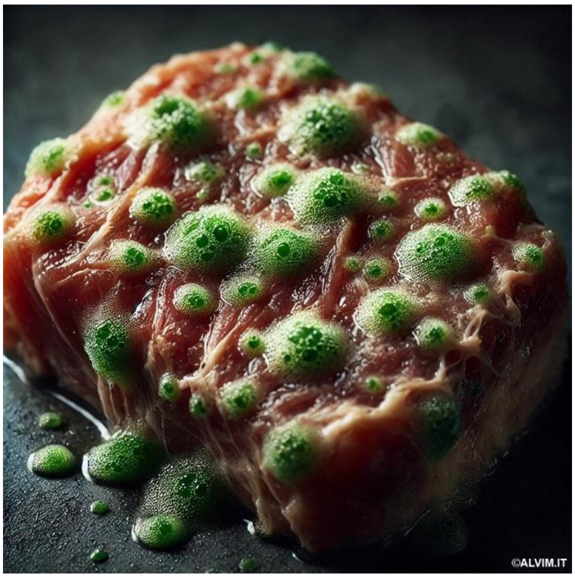

Figure 5: Meat is an ideal substrate for microbial growth

sections. Among these, biological hazards responsible for zoonoses play a key role, including parasites (*Toxoplasma gondii*, *Trichinella spp.*, *Cysticercus bovis*, and *Cysticercus cellulosae*) and bacteria (*Brucella spp.*, *Mycobacterium tuberculosis*, *Burkholderia mallei*, *Bacillus anthracis*). Exogenous contamination, on the other hand, is primarily bacterial in nature and may include pathogens responsible for foodborne infections such as *Salmonella, Campylobacter, Escherichia coli, Yersinia*, and *Aeromonas*. Additionally, spore-forming Gram-positive bacteria like *Clostridium* and *Bacillus*, as well as non-spore-forming bacteria from the *Listeria* and *Staphylococcus* genera, may also be present. During slaughter, meat inevitably comes into contact with environmental contaminants such as air, water, materials, and operators. The number and type of microorganisms present at this stage directly influence the hygienic quality of the final product. Although the introduction of HACCP (Hazard Analysis and Critical Control Points) plans has improved hygiene conditions in slaughterhouses, the risk of contamination cannot be entirely eliminated. For this reason, countries like the United States and Australia have developed carcass decontamination techniques to complement standard hygiene procedures. These methods, which include chemical and physical interventions, have proven effective in reducing bacterial contamination in the final product. However, the effectiveness of microbiological decontamination depends on the combination of chemical and physical methods. The choice of the optimal strategy must balance the reduction of the microbial load with the maintenance of the qualitative and functional characteristics of the final product, ensuring safety without compromising its organoleptic and functional properties. In Europe, the use of chemical substances for carcass disinfection is not permitted.

Disinfection methods in the beef supply chain

The disinfection process involves all stages of production, from farm management to slaughtering and packaging, using physical, chemical, and biological methods. In livestock environments, the proliferation of microbial biofilms and the accumulation of organic material represent a significant challenge. Disinfection strategies based on the mechanical removal of organic residues through high-pressure washing is essential to enhance the effectiveness of subsequent chemical disinfectants. Among these, quaternary ammonium salts, sodium hypochlorite, and aldehydes are used to eliminate pathogens present in barns and contact surfaces. The use of alkaline detergents is particularly effective in degrading proteins and fats present in organic residues, while acidic ones help eliminate limescale and biofilm. The use of bacteriophages, enzymes, and antimicrobial peptides extracted from natural compounds represents an innovative approach to reducing microbial load without resorting to aggressive chemicals. Chlorination is the most commonly used method for water disinfection, but UV rays represent an effective alternative for inactivating bacteria and viruses without altering the organoleptic properties of water. Animal medication tanks and pipelines must be periodically disinfected with appropriate products too, to prevent bacterial growth and the risks discussed above.

Figure 6: If water used for rinsing is contaminated, it can transfer pathogenic microorganisms to the carcasses, instead of eliminating them

To control bacterial cross-contamination during the slaughter phase, disinfection methods include the use of hot water (above 82 °C) or peracetic acid solution to reduce the microbial load on meat surface, as well as the application of high-temperature steam and cycles of disinfectant solutions. The water used for rinsing and disinfecting carcasses and work surfaces can be a vehicle for contamination. if not properly controlled. If the water itself is contaminated with pathogenic bacteria (such as *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*), it can transfer these microorganisms to the carcasses, instead of eliminating them. This risk is particularly high when the water is reused, or when the distribution system is not properly sanitized. It is therefore essential to carry out regular analyses to verify microbiological safety. Enzymatic formulations and quorum sensing inhibitors are increasingly used to prevent the formation of resistant biofilms on meat processing surfaces. After processing, the meat must be kept in sterile conditions to prevent microbial proliferation, through the use of UV rays or antimicrobial gases such as ethylene oxide.

Biofilm monitoring in beef supply chain

As discussed above, the adhesion of bacteria to surfaces, followed by biofilm formation, represents a significant risk to the quality and safety of meat products. For this reason, the implementation of biofilm detection technologies is essential. The use of [ALVIM real-time biofilm monitor] allows for the early identification of biofilm presence and development, enabling the timely adoption of corrective measures to prevent contamination. This is a fundamental step for monitoring bacterial proliferation at critical control points within the facility, such as potable water pipelines, storage tanks, water distribution lines used for carcass washing, Cleaning-In-Place (CIP) systems, and cooling circuits. The integration of ALVIM Biofilm Sensors into quality management protocols optimizes sanitation treatments, enhancing the effectiveness of chemical or physical disinfection strategies. The ability to monitor microbial growth in real-time reduces the risk of failure in decontamination procedures and minimizes the consumption of chemical agents, providing benefits in both food safety and environmental sustainability. The adoption of such technologies aligns with the HACCP approach, based on contamination prevention rather than post-event risk management. Continuous biofilm monitoring not only reduces the likelihood of cross-contamination during various processing stages, but also improves the shelf life of the final product, ensuring high-quality meat.

Figure 7: ALVIM Biofilm Sensors

Conclusions

Meat processing plants play a crucial role in the food supply chain, ensuring product safety, quality, and sustainability. Every stage of the beef production chain, from breeding to distribution, must comply with strict hygiene regulations to guarantee safe, high-quality meat. However, despite preventive measures and best practices, the risk of microbiological contamination remains one of the industry's main challenges. A key factor in food safety and product quality is the microbiological control of water used in farming and processing operations. The presence of biofilm in pipelines and water systems poses a significant contamination risk that is difficult to detect and control with conventional testing methods. In this context, ALVIM sensors provide an advanced solution for real-time biofilm monitoring, enabling early detection and timely, effective intervention. With their high sensitivity, ALVIM probes allow for optimized sanitation treatments, reduced use of chemical agents, and improved operational efficiency in processing facilities. Integrating innovative monitoring systems like ALVIM into water quality management and meat processing operations significantly enhances food safety, reduces waste, and optimizes resource utilization. Investing in these technologies not only ensures greater control over microbiological contamination but also contributes to a more sustainable, efficient, and regulation-compliant production system.

Do you have a similar problem with biofilm? Contact our experts and ask for a free custom-tailored consultancy, you will receive further information about ALVIM products and services.

The ALVIM Biofilm Monitoring System is a reliable tool for the early detection of bacterial growth on surfaces, on-line and in real time, in industrial production lines, cooling water systems, etc.

The ALVIM Technology has been developed in collaboration with the Italian National Research Council, and it is currently used worldwide in many different application fields.

ALVIM Srl | +39 0108566345 | info@alvim.it | www.alvim.it | www.linkedin.com/company/alvimbiofilmsensors