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Detection of Beta-lactam and Tetracycline Residues in Raw Dairy Milk in Biskra Region

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Dedication

I dedicate this thesis to my beloved grandmother who believed in me when nobody did, may you rest in peace.

Table of contents

Chapter 6. Results and discussion

List of tables

List of figures

List of abbreviations

ADI: Acceptable Daily Intake

AMR : Antimicrobial Resistance

ASF : Animal Source Foods

AST : Antimicrobial Susceptibility Testing

Ca/P : Calcium/ Phosphorus ratio

CNIEL : Centre National Interprofessionnel de l'Economie Laitière.

CNS : Central Nervous System

DSA : Direction des Services Agricoles.

ELISA: Enzyme Linked Immuno Sorbent Assay

EU: European Unit

FARAD: Food Animal Residues Avoidance Databank

HCL: Hydrogen Chloride

HPLC: High Performance Liquid Chromatography

IM : Intramuscular

IU : International Union

IV : Intravenous

JORA : Journal Official de la République Algérienne.

Lc/Ms: Liquid chromatography/ Mass spectrometry

MRL: Maximum Residues Limit

mRNA: messenger Ribonucleic Acid

ONIL : Organisation National Interprofessionnel de lait et de produits Laitier

ONS: Office National de Statistiques

OTC: Over-The- Counter

PBPs: Penicillin- Binding Proteins

RL: Residues Limit

ROSA: Rapid One Step assay.

tRNA: transfer ribonucleic acid

UV: Ultraviolet

WHO: World Health Organisation

WP: Withdrawal Period

Introduction

Introduction

Antibiotics are widely used in veterinary medicine for therapeutic, metaphylactic, or prophylactic treatment of bacterial infections in farm animals intended for production of human food (meat, milk or eggs). The rationale for veterinary use of antibiotics is to protect animal welfare, to prevent epidemic spread of infectious animal diseases, to provide high efficiency, to warrant safety of (Animal source foods (ASF) and to prevent food-borne diseases (Ungemach *et al.,* 2006).

In Algeria, antibiotics remain among the most used molecules in cattle breeding. Their use, as a therapeutic, prophylactic treatments or as a supplement in animal feed, inevitably leads to the presence of residues in the food products of these animals (Boultif, 2015).

At the same time, milk is considered a complete food whose nutritional value is indisputable in growing youths and adults. In Algeria, this product, widely consumed, occupies a predominate position in the food ratio. As the subsidized price is at the reach of the consumer (Boultif, 2015).

The consumption of a contaminated milk with antibiotic residues leads to a multitude of health risk consequences, such as the disruption of the intestinal flora, toxic and allergic reactions, also some long-term consequences consisting of an increase of antimicrobial resistance, carcinogenicity and mutagenicity.

Aside from the risks of consuming antibiotic residues in milk, the irrational or extensive use of antibiotics in cattle breeding leads to the emergence and selection of antimicrobial resistant bacteria in these animals, which poses a relevant risk for public health by spreading the resistance from farm animals to the human population via different patterns (Ungemach *et al*., 2006).

On a technological point of view, the presence of antibiotic residues in raw milk present a huge problem for the fermentation processes of milk for the production of Yogurt, cheese and other dairy products, causing financial loss for milk processors and for the dairy industry in general.

Currently, the problem caused by antibiotic residues in milk is to be feared because the quantities of raw milk reserved for processing are still insufficient to allow the rejection of milk containing antibiotics (Boultif, 2015).

Today, we are witnessing a total abusive and anarchic use of antibiotics in rural practice in Algeria. The control of antibiotic residues is not being regulated. Even now, our country has

no maximum residues limit (MRL) for milk and other animal product, suitable for the used antibiotics. As a result, no screening is made either at the farm level or on the tanks after milk collection (Boultif, 2015).

In order to evaluate the contamination of milk with veterinary drug residues, the main objective of the study is the screening for antimicrobial residues in locally produced raw milk collected from different region of Biskra. A qualitative method consisting of an immunochromatographic assay will be followed for the screening of antibiotic residues, the screening for residues will focus on the two most used antibiotics in veterinary medicine: penicillin and tetracycline.

A descriptive survey will be carried out, to report the situation of antibiotic therapy in Biskra and to find out how veterinarians operate with antibiotic treatments and which antibiotics are the most commonly used in cattle, the results from this survey will confirm the choice of antibiotics chosen for screening.

Another survey will be carried out, to report the situation of dairy production and processing in Biskra. In order to assess the situation of the dairy sector.

In addition, a literature review will be drawn up on the inhibitors and the means of their detection in the broad sense of the term. The first part includes an overview on milk, the second chapter focuses on antibiotics and their properties and activity as well as their use in veterinary medicine. The third chapter will be devoted to the issue of residues; starting with a background on the concepts of residues, withdrawal period as well as the exposure of problems which these can cause to the consumer and on the dairy industry. Finally, a final chapter will be devoted to the methods applied for the screening and identification of antibiotic residues in milk.

Literature Review

Chapter 1.
General overview on milk

1.1. Milk

1.1.1. Definition

Milk was defined during the 1908 International Congress for the Suppression of Fraud in Geneva as: The full product of total and uninterrupted milking of a healthy female, well fed and not overworked. Milk should be collected properly and should not contain any colostrum (Poughen, 2001).

1.2. Composition and properties of dairy milk

1.2.1. Physical properties

Dairy milk has an average density of 1.032. It's a very complex and unstable mixture, it contains a high proportion of water 87%. It is both a solution (lactose, mineral salts), a suspension (nitrogenous matter) and an emulsion (fatty matter). Its PH is slightly acidic (6.4 to 6.8), the acidity increases over time because the lactose will be broken down into lactic acid (FAO, 1998; Courtet Leymarios, 2010). (tab. 01; Appendix 01) summarizes the main physicochemical properties.

1.2.2. Chemical composition

the composition of milk continuously undergoes changes depending on e.g. breeding, feeding strategies, management of the cow, lactation stage and season (Månsson, 2008).

1.2.2.1. Proteins

A litre of cow's milk, whether whole or skimmed, provides 32 g of proteins. Which consist mainly of casein, lactalbumin and lactoglobulin. They have a high biological value due to the good balance of their essential amino acids; these proteins are very well assimilated by the body (Courtet Leymarios, 2010).

1.2.2.2. Fats

The fat amount of milk is standardized at a minimum rate of 36 g/L of whole milk (can vary from 35 to 45 g/). This lipid amount gives the whole milk a significant energy value (700 Kcal/L). Semi Skimmed and skimmed milk provide respectively 15 to 18 g and 1 g of lipids per litre (Pujol-Dupuy,2004).

1.2.2.3. Carbohydrates

Lactose, an essential carbohydrate in milk, present in large quantities, promotes the absorption of the calcium contained in this food. One litre of milk, whether whole or skimmed,

provides a highly stable amount of lactose which vary from 48 to 50 g (Courtet Leymarios, 2010).

1.2.2.4. Minerals and trace elements

Milk is considered an important source of calcium: 1 200 mg/L. The calcium contained in milk is better absorbed than that of any other source because it provides phosphorus at the same time (Ca/P = 1.4 ratio) and vitamin D. Milk also provides sodium chloride, potassium chloride, Zink and small amounts of sulfur, magnesium and copper (Boultif, 2015). It contains a very low amount of iron (0.6 mg/kg) (Courtet Leymarios,2010).

1.2.2.5. Vitamins

Milk is a considerable source of several water-soluble vitamins (almost all the vitamins of group B), also milk is an excellent source of B12 and contains a significant number of fat-soluble vitamins including vitamins A and D (Lapointe-Vignola et Québec, 2002).

1.3. Milk quality

1.3.1. Organoleptic properties

Milk is a non-transparent white liquid with a golden yellowish coloration due to the presence of carotene pigment. It is slightly viscous liquid which possesses a faint odour nevertheless identifiable. It has a balanced sweet/salty taste mainly related to its lactose/chloride ratio, and both are somewhat moderated with proteins (Chandan *et al*., 2015).

The taste and odor of milk are an important indicator of its quality. The presence of a bad odour and an unpleasant taste with rancidity, reflects a problem in the management and storage of milk (Amiot *et al.,* 2002).

1.3.2. Hygienic quality

Milk of sufficient sanitary quality present according to Renard (2014):

- ✓ A low rate of total germs: less than 100 000 germs/ml;
- ✓ An acceptable level of somatic cells: less than 400 00 cells/ml;
- ✓ An absence of drug residues.

The presence of inhibitory residues in milk can lead to several risks for consumers mainly: changes in intestinal flora, toxic or allergic effects, selection of pathogenic bacteria resistant to antibiotics (Chatigny et Stevens, 2005).

1.3.3. Technological quality

Technological quality of milk depends on its chemical composition (protein and butyric rates), the bacteriological quality and the aptitude for transformation (Cauty et Perreau, 2005).

The presence of an inhibitor in milk blocks or slows down the microbial fermentation and lead to inadequate or no coagulation. Lactic acid bacteria are sensitive to very low doses of antibiotics which partially or completely inhibit the growth of these ferments, and results in numerous accidents in the fermentation process (Broutin, 2005; Zine dine *et al*., 2007).

1.4. Importance of milk in Algeria

Milk and its derivatives represent 14% of food expenditure per household. This demand is driven by the demographic growth estimated at 1.6% per year, urbanization estimated at more than 5% per year as well as the improvement in the purchasing capacity (4% to 7% per year). A survey conducted on the Algerian households reveals that "milk and dairy products" category occupies the fourth position with (7.5%) of the total expenditure of these households, after cereals (24.6%), red meat (18.4%) and fresh vegetables and fruits (13.7%) (Bouazouni,2008). In fact, Algeria is the leading dairy consumer in the Maghreb and the second country in the world for importing milk and its derivatives with an annual market estimated, in 2007, at more than three billion litres (Griffoul, 2007; Boultif, 2015) and an average consumption of 115L per capita and per year in 2010 (Ghazi et Niar,2011).

Local production is estimated at just under tow billion litres (Boultif,2015), Algeria is therefore forced to import massive quantities of milk, most of which is in the form of powdered milk which costs more expensive (Griffoul,2007). The lack is therefore enormous; thus, the country has adopted a policy of importing dairy cattle, but they fail to produce the expected results (Ghazi et Niar,2011).

Chapter 2.
Antibiotics in veterinary medicine

2.1. Definition

According to Guardabassi *et al.* (2009), antimicrobial agents are chemical compounds that kill or inhibit the growth of microorganisms but cause little or no damage to the host. They are naturally produced by microorganisms such as fungi and bacteria or can be semi-synthetically produced (e.g. amoxicillin) or totally synthetically produced.

2.2. Classification

Antimicrobials can be classified based on: the spectrum of activity (broad or narrow spectrum antibiotics), their effects on micro-organisms (bactericidal or bacteriostatic antibiotics), mode of action and the chemical structure (Wang, 2012).

The main families of antibiotics are: sulphonamides, tetracycline, quinolones, macrolides and beta-lactams. (see Tab. 02, appendix 02).

2.3. Common antibiotics used in veterinary medicine

2.3.1. Beta-Lactam antibiotics

2.3.1.1. Composition and mechanism of action

Beta-Lactams are a class of broad -spectrum antibiotics characterized by the possession of the four membered beta lactam rings, they include: penicillin, cephalosporin, monobactam, carbapenem and cephamycin (Page, 2012)**.**

All are bactericidal and act by disrupting peptidoglycan synthesis in actively multiplying bacteria, beta-lactams bind to their primary targets, proteins in the cell membrane [Penicillin-Binding Proteins (PBPs)] involved in late stages of bacterial cell wall peptidoglycan synthesis. The peptidoglycan is responsible for maintaining the integrity of bacterial cell walls, disruption of its structure in cells leads to lysis and cell death (Tipper, 1985).

2.3.1.2. Pharmacokinetic of beta-lactam

The digestive absorption of penicillin G is mediocre, (less than 30%) because they are broken dawn by the gastric HCL. Penicillin defuse easily in the extracellular spaces but do not accumulate in the tissues (Milhaud *et al*., 1982; Allain, 2006).

In the earliest two to three hours after an intramuscular injection of penicillin, the concentration in milk is about 100 times lower than the concentration in blood, afterwards the concentration in milk increases slightly while the concentration in blood decreases. At the 6^{th} or 12^{th} hour, the ratio between the two concentration is around 10 (Milhaud *et al*., 1982).

Penicillin undergo little biotransformation and are eliminated in an active form (Hennel,2006). More than 90% of an administered dose is excreted unchanged in the urine by glomerular filtration and active tubular secretion (Hsu, 2008).

The administration through intramammary route, penicillin residues in milk exist not only in the treated area, but also in that of close parts for several days (Milhaud *et al*.,1982).

2.3.2. Tetracycline

2.3.2.1. Composition and mechanism of action

Tetracycline are a broad-spectrum antibiotics, which exhibit their activity against Gram-positive and Gram-negative bacteria., they are bacteriostatic and have bactericidal activity against sensitive organisms at high concentrations (Wang *et al*., 2011).

Tetracycline consist of a common four-ring structure to which a variety of side chains are attached (Chopra et Roberts, 2001). Chlortetracycline and oxytetracycline are natural members of the tetracycline group, while doxycycline and minocycline are semi-synthetic antibiotics (Michalova *et al*., 2004).

Tetracycline inhibit protein synthesis, a mechanism that involves reversible binding of the drug to receptors of the 30S ribosomal sub-unit of susceptible organisms. This, in turn, blocks binding of the aminoacyl-tRNA to the acceptor site on the mRNA ribosomal complex and prevents the addition of new amino acids to the peptide chain. Thus, protein synthesis is blocked (Wang *et al*., 2011;Hauser, 2012).

2.3.2.2. Pharmacokinetic of tetracycline

Tetracycline are administered orally, IM injections or IV every 8–12 hours. Oral absorption of tetracycline ranges from 60–90% of the administered dose except for chlortetracycline, which is only 35% absorbed (Hsu, 2008).

Oral bioavailability varies greatly depending on the dosage and the type of molecule, their high lipid solubility allows them to defuse well through biological barriers and reach effectives concentrations, distribution is wide and includes all tissues except those of the CNS (Hsu, 2008).

Renal excretion by glomerular filtration is the major route of elimination for most tetracycline, but small amounts are excreted into faces via bile and/or diffusion from the blood into the intestine, the enterohepatic cycle prolongs the elimination half-life, it can cause intestinal flora drift (Hsu, 2008).

The Food Animal Residue Avoidance Databank (FARAD) recommends, in cattle, an extra label withdrawal of 28 days for intrauterine treatment. It also recommends testing milk

after intrauterine treatment, as there is inter-cow variability in the residue elimination profiles in milk (Hsu, 2008).

2.4. Antibiotics usage in veterinary medicine

Antimicrobial agents can be individually administered for several reasons:

Therapeutic treatment: involves treatment of individual animals over a short period with doses of antimicrobials exceeding the minimal inhibitory concentration of the known or suspected pathogen. Sometimes, therapeutic treatment is delivered through feed or drinking water (Barton, 2000).

Prophylactic purposes: treatment prevent diseases and involves moderate to high doses of antimicrobials, often given in feed or water for a defined period to a group of animals (Barton, 2000).

Metaphylactic: typically used during disease outbreaks to prevent Infections, thus are treated before their clinical appearance and the treatment period is usually shorter than for therapeutic treatment. administered to clinically healthy animals belonging to the same flock or pen as animals with clinical signs (Guardabassi *et al*., 2008).

As growth promotant: antimicrobial drugs are used as a feed supplement and are continuously administered at sub-therapeutic doses. They exert their effects on feed efficiency, weight gain and are available for purchase over the counter by feed manufacturers and farmers (Wallace *et al*., 1995; Guardabassi *et al.,* 2008).

There are Concerns about the use of antibiotics in animals and the possible impact on human health covers two major issues: the antibiotic agents that are used; the way in which they are used. There is a view that antibiotics that are important in human medicine should not be used therapeutically in food-producing animals, particularly for mass medication. Prophylactic and Growth-promotant use is probably the area of highest concern, as some of the antibiotics used are now regarded as compromising the efficacy of some key human antibiotics (Barton, 2000).

Chapter 3.
Antibiotic residues in milk and dairy industry

3.1. Definition of residues

The term residues describe all active principles and their metabolites which persist in meat or other food products from animals that have been treated with the drug in question. The term metabolite has not been defined. It is generally accepted that it applies to "any by-product of biotransformation of the initial active principle" (Burgat-Sacaze *et al*., 1981).

3.2. Maximum Residue Limit (MRL)

3.2.1. Definition

Maximum residue limit means the maximum concentration of residue resulting from the use of a veterinary medicinal product, which may be legally permitted or recognized as acceptable in or on a food, allocated to individual food commodities. It is based on the type and amount of residue considered to be without any toxicological hazard for human health as expressed by the Allowed Daily Intake (ADI) (Myllyniemi, 2004).

3.2.2. Acceptable Daily Intake (ADI)

The ADI represent a level of daily intake of a chemical that, during an entire lifetime, appears to be without appreciable risk to the health of the consumer. The ADI is used to determine the maximum concentration of a marker residue in edible tissues, honey, milk or eggs that is legally permitted or recognized as acceptable (Giguére *et al.*, 2013). It is expressed in milligrams of the chemical per kilogram of body weight per day (Boultif, 2015).

3.2.3. Algerian legislation

Articale 6 of the interdepartmental decree (the Ministry of Economy, the Ministry of Agriculture and the ministry of Health and Population) of August 18, 1993, relating to the specifications and presentation of certain consumed milk, mentions the fact that milk suitable for human consumption should not contain antibiotic residues but does not specify explicitly a maximum residues limits (Boultif, 2015).

According to the legislation of milk quality, the screening for antibiotic residues is mandatory in raw milk, dehydrated packaged milk, and dehydrated milk intended for food industries (JORA, 1998).

3.3. Withdrawal Period (WP)

This term refers to the interval between the time of the last administration of a drug and the time when the animal can be safely slaughtered for food, milk or eggs can be safely consumed. The withdrawal period provides a high degree of assurance to both producers and consumers that concentration of residues in foods derived from treated animals will not exceed

the MRLs (Vranic *et al*.,2003). Each antimicrobial has a WP which depends on drug type, drug concentration, route of administration, animal kind and the animal product (Riviere, 2009).

3.4. Reasons for antimicrobial residues in milk

The most likely cause of violated drug residues is the failure to observe withdrawal times. Improper maintenance of treatment records or failure to identify treated animals adequately. Violated drug residues can also occur as a result of improper use of licensed product through the illegal use of unlicensed substances. Extra-label dosages and use of drugs which have not been approved for the species in question (Asredie et Engdaw, 2015).

Antibiotic residues in milk may occur when a treated cow is accidentally routed in to the pipeline, an antibiotic treated dairy cow is unintentionally milked, the milking unit is not cleaned and sanitized between uses, equipment used to milk treated cows is handled carelessly (Asredie et Engdaw, 2015).

3.5. Consequences of the presence of antibiotic residues in milk

3.5.1. Public health aspect

3.5.1.1. Allergic reactions problems

Allergies to antibiotic occur when the body's immune system attacks the antibiotic, which is often a haptenic metabolite of the antibiotic and some carrier tissues. Small levels of antibiotics can be very hazardous to susceptible humans causing acute to severe reactions, that include anaphylaxis, serum sickness, cutaneous reaction delayed, hypersensitivity reactions (Singh *et al*., 2014).

It has been suggested that the present recommended limit of 0.05 IU of penicillin/ml of milk is too high and offers no guarantee of safety (Singh *et al*., 2014).

Moderately high levels of tetracycline residues in milk can provoke allergic reactions in some hypersensitive individuals, the maximum residue level for tetracycline in milk has been established in the EU at 100,000 ng/l (Unusan, 2009).

3.5.1.2. Antimicrobial Resistance (AMR) hazard

The WHO states that AMR "is resistance of a microorganism to an antimicrobial medicine to which it was previously sensitive". Which suggests that disease causing organisms develop a gene being resistant to antimicrobial medicines which were previously used to treat bacterial diseases (Negi, 2014).

Misuse and overuse of antimicrobial may culminate in the development of drug-resistant pathogens resulting in poor response to treatment. Long-term and low-level exposure to

antimicrobials may have greater selective potential of antibiotic resistance strains (Hao *et al*., 2014; Singh *et al*., 2014), the unintentional consumption of small amounts of antibiotics in foods might result in the same problem (Albright *et al*., 1961).

3.5.1.3. Disruption of the normal human intestinal flora

The bacteria that usually live in the intestine act as a barrier to prevent incoming pathogenic bacteria from getting established and causing disease. Antimicrobials might reduce total numbers of these bacteria or selectively kill some important species (Doyle, 2006).

this reduction of the colonization resistance to pathogenic invaders could lead to an overgrowth of these pathogens and might favour the growth of antibiotic resistant bacteria in the gut. The reservoir of resistant genes would thus enlarge, increasing the probability that a resistance gene is transferred to a pathogen (Corpet, 2000).

3.5.1.4. Toxicity

Generally, consists of chronic toxicity which is expressed only after repeated consumption of food containing residues of the same antibiotics (Jeon *et al*.,2008).

Chronic adverse effects of antibiotics residues have been suggested like carcinogenicity (Sulphamethazine), mutagenicity (Gentamicin), hepatotoxicity, reproductive disorders toxicity (Chloramphenicol), chronic exposure to oxytetracycline include blood changes such as leucocytosis, lung congestion and brown discolouration of the teeth (Priyanka *et al*., 2017).

3.5.2. Technological problems

Antibiotic residues in milk are of great concern to dairy farmers and milk processors, since these residues may interfere and adversely affect the manufacture of some dairy products due to the inhibition of the fermentation processes (Cámara *et al*., 2013).

Concentrations of .0017-unit delay starter activity for cheese and yogurt with considerable inhibition occurring at .005 units. Antibiotics also decrease acid and flavour production associated with butter manufacture and reduce curdling of milk and cause improper ripening of cheeses (Jones et Seymour, 1988). This can produce an important loss of monetary income to the milk processors (Cámara *et al*., 2013).

Chapter 4.
Screening methods for the detection of antibiotic residues in milk

4.1. Screening methods used for the detection of antibiotic residues in raw milk

The different methods of screening for antibiotic residues in milk are mentioned in table.03; appendix 03.

4.1.1. Microbiological methods

Microbiological assays are qualitative or semi-quantitative methods, based on a specific reaction between a susceptible organism and the antibiotic present in the sample. Advantages of these assays are their reliability, cost effectiveness and simplicity (Cháfer *et al*., 2010).

4.1.1.1. Delvo test

The DelvoTest is the best-known microbial inhibitor test, designed to detect wide spectrum of antimicrobials (Neaves, 1999). The test is applicable for use on the farm site or in a laboratory (Wang *et al*., 2011).

DelvoTest is based on the inhibition of growth of *Bacillus stearothermophilis*. The test is presented in an ampoule which a standardized number of bacterial endospores are embedded in an agar medium containing selected nutrients and a pH-sensitive dye. After the addition of a fixed volume of a milk sample to the ampoule, the test is incubated. In the absence of an inhibitory substance in the sample, the bacterial spores will germinate and multiply. causing a decrease in pH that can be measured as a purple-yellow colour change. If the sample contains inhibitory substances above the detection concentration of the test, no growth will occur and the agar colour will remain purple (Wang *et al*., 2011).

4.1.2. Enzymatic tests

4.1.2.1. Penzym

Penzym test is a qualitative enzymatic colorimetric method for a rapid determination of beta-lactam antibiotics in milk. The test concept is based on establishing the level of inactivation of the DD-carboxypeptidase enzyme by β-lactam antibiotics. These residues bind specifically with the enzyme and inactivate it.

This enzyme is present in the sealed vials to which a milk sample is added and incubated. During this time any l3-lactams that may be present in the milk bind to the enzyme and inactivate a portion depending on the antibiotic concentration present. A reagent tablet specific for the enzyme is then added to the milk sample, and the sample is incubated again. The end products of the substrate and enzyme reaction are measured. An orange colour indicates a negative result while a yellow colour indicates a positive result (Mitchell *et al*.,1998).

4.1.3. Immune-enzymatic tests

4.1.3.1. BetaStar

The Beta Star Combo is a rapid one-step test of detection of Beta-lactam and tetracyclineas well as the Desfuroyl-Ceftiofur antibiotic residues separately. It can be used for cow, goat or sheep milk.

The test use specific receptors linked to gold particles and an immune-chromatographic support in the form of strip. During the incubation, the strip absorbs the "Milk+ mixture reagents" present in the well. In the presence of antibiotics, receptors bind to residues during the migration phase resulting in change of colour (CNIEL, 2016).

4.1.3.2. ELISA test

ELISA (Enzyme Linked Immuno Sorbent Assay) is a rapid technique, specific for a class of antibiotics and sensitive for the latter (Boultif, 2015).

ELISA technique allows to visualize an antigen-antibody reaction due to a coloured reaction produced by the action on a substrate of an enzyme previously attached to the antibody (Hanzen, 2008). This method is qualitative when the colour reaction occurs once the enzyme catalyses the substrate, while the quantitative analysis is carried out according to (colour intensity) (Ahmed *et al*., 2020).

4.1.4. Physico-chemical methods

physicochemical assays target specific properties of the molecule such as size, charge, binding characteristics or reactive properties (Parthasarathy *et al.*, 2018).

4.1.4.1. High Performance Chromatography on a Liquid Phase (HPLC)

The High-performance liquid chromatography (HPLC) has been widely used for screening and confirming antibiotic residues in milk (Pelvan, 2011). this method allows the detection and quantification of the residues of a wide range of antibiotics (Boultif,2015).

The screening of residues follows a solid-phase extraction clean-up then filtration and finally injection step into reverse-phase HPLC combined with UV diode array detection (Pelvan,2011). This method makes it possible to separate compounds of variable molar mass and different chemical nature (Boultif, 2015).

Experimental part

Chapter 5.
Material and methods

Objectives

The lack of regulation for the control of antibiotic residues in milk, in addition, to the lack of awareness displayed by the citizens concerning the health risks caused by the consumption of a potentially contaminated raw milk with these residues, has strongly motivated us for choosing this theme. Milk, a widely consumed product by the Algerian citizen, therefore represents a potential danger. On the other hand, the dairy industries have a direct impact on the national economy.

With this lack of control, a number of questions arise: is the Algerian citizen, and more particularly in Biskra, safe from indirectly consuming antibiotic residues? Does this contamination come from raw milk produced locally and sold in dairy shops or from uncontrolled manufactured raw milk sold in the markets? On the other hand, is antibiotic therapy in cattle breeding being practiced in a rational way or is it violated by veterinarians and farmers, further complicating the situation? and how aware are the cattle farmers of the risks of self-medicating their herds and to the non-compliance to the withdrawal period of antibiotics that causes this problem in the first place?

To answer these questions, we developed several strategies, the first consists of a questionnaire survey for veterinary practitioners in Biskra, in order to assess the situation of dairy cattle treatments. In the same section, we will also carry out a survey for dairy factories to have an idea about the situation of dairy production and processing in the same region.

The second section will be devoted to a qualitative screening for antibiotic residues (beta lactams and tetracycline antibiotics were chosen for screening) by a rapid immunochromatographic assay (BetaStar® Combo).

5.1. Study area

The study is carried out in Biskra region. It is a province located in the south-east of Algeria (fig. 1). It is delimited to the north by the Wilaya of Banta, to the northeast by the Wilaya of M'sila, to the southwest by the Wilaya of El-eoud, to the northeast by the Wilaya of Khenchela. The province stretches over 20 986 km^2 and is made up of 33 municipalities belonging to 12 Districts (tab. 1). the population was estimated with 721 356 inhabitant since the last census made in 2008 (ONS, 2011).

Tableau 1. Distribution of municipalities per district in the study area (ONS, 2011).

N°	Districts(Daïra)	Municipalities
1	Biskra	Biskra – El Hadjab
2	Djemourah	Djemourah – Branis
3	El Kantara	El Kantara – Ain Zaatout
4	El Outaya	El Outaya
5	Foughala	Foughala – El Ghrous
6	M'chounche	M'chounche
7	Ouled Djellal	Ouled Djellal – Chaiba – Doucen
8	Ourlal	Ourlal – Lioua – M'Lili – Mekhadma - Oumache
9	Sidi Khald	Sidi Khald – Besbes – Ras El Miad
10	Sidi Okba	Sidi Okba – Ain Naga – Chetma – El Haouch
11	Tolga	Tolga – Bordj Ben Azzouz –Bouchagroune – Lichana
12	Zeribet El Oued	Zeribet El Oued – El Feid – Khenguet Sidi Nadji – Meziraa

Figure 1. Geographical situation of the study area (Biskra) (Drouiche *et al.,* 2011).

The number of cattle farms in Biskra region, during the year 2020 is estimated at 176farms with a total of 2069 of identified cattle heads. 867 dairy cows are counted among this herd (Source: DSA Biskra, June 2020).

5.2. Sample collection

For our study, a total of 30 raw milk samples from local dairy shops were collected in an arbitrarily manner. the samples were collected from six districts out of twelve, belonging to Biskra province and at the rate of 5 samples per district.

The milk samples were collected in sterile plastic bottles (capacity 0.5 L), labelled and hermetically sealed closed. The date and origin of each sample Are mentioned on these bottles. All samples are collected from individual shops; the milk vendors were interrogated about the sources of the raw milk (farms) which was also recorded.

The samples are then brought to the AMIRALAIT factory laboratory in bins isotherms, where they were analysed immediately.

This study was carried out over the period extending from February to September 2020. All the information related to the sources of the cow's milk samples, are detailed in table 23; appendix 07.

5.3. Antibiotic selection

The choice of antibiotics for screening is made based on the fact that beta lactams are the most used antibiotics in veterinary medicine for the treatment of diseases that regularly affect livestock animals, also beta lactams are used for prophylactic purposes and as supplements, supplied illegally, in order to promote growth in food-producing animals (Kantiani *et al*., 2009). In addition, Penicillin are known to be the ones that cause the more damage in the dairy industry (Boultif, 2015) due to the fact that starter cultures appear to be more sensitive to the action of penicillin than other antibiotics (Albright *et al*., 1961).

Tetracycline are extensively used in the prophylaxis and treatment and as animal growth promoters (chopra et Roberts, 2001) ,tetracycline residues are known to cause technological problems by possible alteration of the fermentation process by interfering with starter cultures causing a decrease in pH, which may affects various aspects of dairy products manufacture, the residues of tetracycline in milk is known to cause allergic reaction in consumers, as well as disturbance of the intestinal flora and having a potential to stain teeth of young children (Gajda *et al*., 2017).

Antibiotic stability: the stability of penicillin is at its maximum around the normal pH of milk, and the pasteurization has little effect on the alteration of these drugs. Similarly, momentary heating of milk does not affect the biological activity of tetracycline. Also, active antibiotics have been detected in cheese, butter and milk powder (Boultif, 2015).

5.4. Questionnaire surveys on veterinary practice and dairy industry in Biskra

In order to have a better perspective on dairy production in the Biskra, in a broad sense of the term; two survey studies were carried out. the first was administered to veterinary practitioners, to collect as much information as possible about their prescribing practices, the most encountered infectious diseases and their treatment (especially those based on antibiotics). The second was administered to some dairy factories, located in Biskra to assess the situation of dairy production and processing in the same province. Both surveys were conducted simultaneously during the period from February to September 2020.

5.4.1. Questionnaire survey for veterinary practitioners

A descriptive survey was carried out, to report the situation of antibiotic therapy in Biskra.

The aim is to find out how veterinarians operate with antibiotic treatments and whether these treatments are rationally prescribed or are they on the contrary violated.

Another objective of this investigation was, eventually, to correlate risky behaviours such as the non-compliance to the instructions of antibiotic administration (administration routes, drug dose calculation...) and other criteria such as the non-compliance to the withdrawal period with the emergence of Antimicrobial Resistance (AMR).

In order to achieve the main purpose of this investigation, 40 copies of questionnaire survey were distributed arbitrarily throughout all the territory of Biskra, during which personal visits were carried out to interview 19 veterinarians, and due to the current corona virus pandemic and the restricted quarantine, the interrogation of the rest of the 21 veterinary practitioners was carried out through phone calls and emails.

The consent of the selected veterinary practitioners was sought and obtained after explaining the purpose of the study.

5.4.1.1. Questionnaire description

A survey instrument (questionnaire) on antibiotic usage was developed for collecting information on antibiotic usage. A convenient sample of 40 veterinary practitioners variously located was interviewed to capture the diversity of ideas held regarding antibiotic therapy. The questionnaire consisted of 19 questions about:

- Personal and questions about the veterinarian’s professional activities.
- The most encountered infectious diseases in the field.
- The prescribing practices for two specific conditions: mastitis, and respiratory diseases

- Antibiotic administration: intramammary and parenteral routes and their WP in milk.
- The criteria for antibiotic prescription: Spectrum, administration route, dosage …
- prophylactic therapy,
- the withdrawal periods,
- Compliance with the conditions of use.
- Self-medicating animals by breeders.
- Antibiotic resistance: importance and frequency.

The survey instrument is provided in appendix 04. It includes questions and suggested or free answers. Both French and English versions of the questionnaire survey are included.

5.4.1.2. Data analyses

After obtaining the completed questionnaire surveys, we classified them according to the responses obtained for each processed parameter, all the data collected was entered and stored in a Microsoft Excel file.

The treatment of data was restricted to descriptive statistical analysis (percentage and frequencies) without performing statistical tests due to the limited number of the veterinarians interviewed.

5.4.2. Questionnaire survey for dairy factory managers

A descriptive survey was carried out, to report the situation of dairy production and processing in Biskra.

The aim is to identify the procedures and the laboratory methods adopted by the dairy factories to ensure the sanitary security of their final product regarding antibiotic residues, also the aim was to find out the level of awareness concerning the correlation between the consumption of contaminated milk with antibiotic residues with the emergences of antimicrobial resistances for the consumers.

The study covered the two main dairy units in Biskra region; one in Ourlal and the other in Biskra. This research is a preliminary study that describes the working conditions as well as the problems encountered within the dairy manufacturing and processing units, and the strategies adopted by the factories.

The consent of the selected dairy factories managers was sought and obtained after explaining the purpose of the study.

5.4.2.1. Questionnaire description

A survey instrument (questionnaire) was developed to collect as much information as possible, related to essential points, that we considered useful for the assessment of the dairy production in Biskra.

The sample consisted of the two main dairy units located in the Wilaya of Biskra; one in Ourlal and the other in Biskra. We carried out personal visits, within each of the dairy factories, during which we interviewed laboratory technicians, engineers, and heads of production units. To capture the diversity of ideas held regarding dairy processing. The questionnaire consisted of 19 questions related mainly to:

- The capacity of production,
- The types and qualities of productions,
- The supply in raw materials (powder and cow's milk),
- Types of laboratory quality control check,
- Problems of starting culture during transformations,
- The strategies of sanitary and technological control,
- Traceability plans that may have been implemented.

The survey instrument is provided in appendix 05. It includes questions and suggested or free answers. Both French and English versions of the questionnaire survey are included.

5.5. Qualitative analysis of antibiotic residues by BetaStar® S Combo assay for beta-lactam and tetracycline residues

5.5.1. Material

5.5.1.1. BetaStar® S Combo Kit

BetaStar® S Combo is a rapid immunochromatographic assay based on a competitive immunoassay format, used for the detection of beta-lactams, tetracyclines, and desfuroylceftiofur antibiotics. BetaStar® S Combo kit contains:

- 25 plastic vials,
- 1 container with 25 test strips,
- 25 disposable pipettes.

Figure 2. Betastar® kit (CNIEL, 2016).

5.5.1.2. BetaStar® S Combo incubator

It is a device specially designed for the incubation of the BetaStar® S Combo vials, capable of maintaining a temperature of 47.5 ± 1 °C.

Figure 3. BetaStar® S Combo incubator.

5.5.1.3. Sampling

All samples of locally produced raw milk (30 samples) previously cited, will be analysed by BetaStar® S Combo. See aims and objectives part (sampling part).

5.5.2. Method

5.5.2.1. Test preparation

A daily temperature check of the heater block is recommended. We Ensure the heater block has been turned on and preheated and the temperature is maintained at 47.5 ± 1 °C.

BetaStar® S Combo is designed for use under normal ambient environmental conditions (15-30°C). The kit is removed from the refrigerator and the test strip container is kept at room temperature (15-30°C) for 10-15 minutes prior to opening to prevent condensation.

Test strips that have been removed from the test strip container must be kept clean and dry.

5.5.2.2. Test procedure

The bottles containing the samples are removed from the refrigerator, the milk sample is mixed, homogenized, a sufficient quantity of 0.4 ml is taken with a pipette and placed into the bottom of the vial. This was achieved by pressing the pipette tip to the bottom of the vial to release the sample. The vials are identified by a marker for each milk sample to be tested. pipette tips are discarded after each use to avoid contamination between samples.

Afterword, the vials containing the samples are placed into the incubator for a first incubation, the test strips are Placed into the vials that is in the incubator for a second incubation. The arrows on the test strip must be oriented downward in the vial. the test strips are Incubated in the vials for 5 minutes at 47.5 ± 1 °C.

Figure 4. Protocol of screening for antibiotic residues in milk using BetaStar® S Combo

After the incubation period, the test strips were removed from the vials, the Interpretation of the device was done visually by comparing the intensity of the test line with the control line.

5.5.2.3. Result interpretation

After the 5 minutes' incubation, the test strip is removed from the vial. Immediately the intensities of the antibiotic test lines (lines 2, 3, and 4) are compared to the control line (line 1). The intensities are interpreted as follow:

- If the intensity of the test line is greater than or equal to the control line, the milk is negative for the presence of the antibiotic.

- If the intensity of the test line is less than the intensity of the control line, the milk sample is positive for the antibiotic.

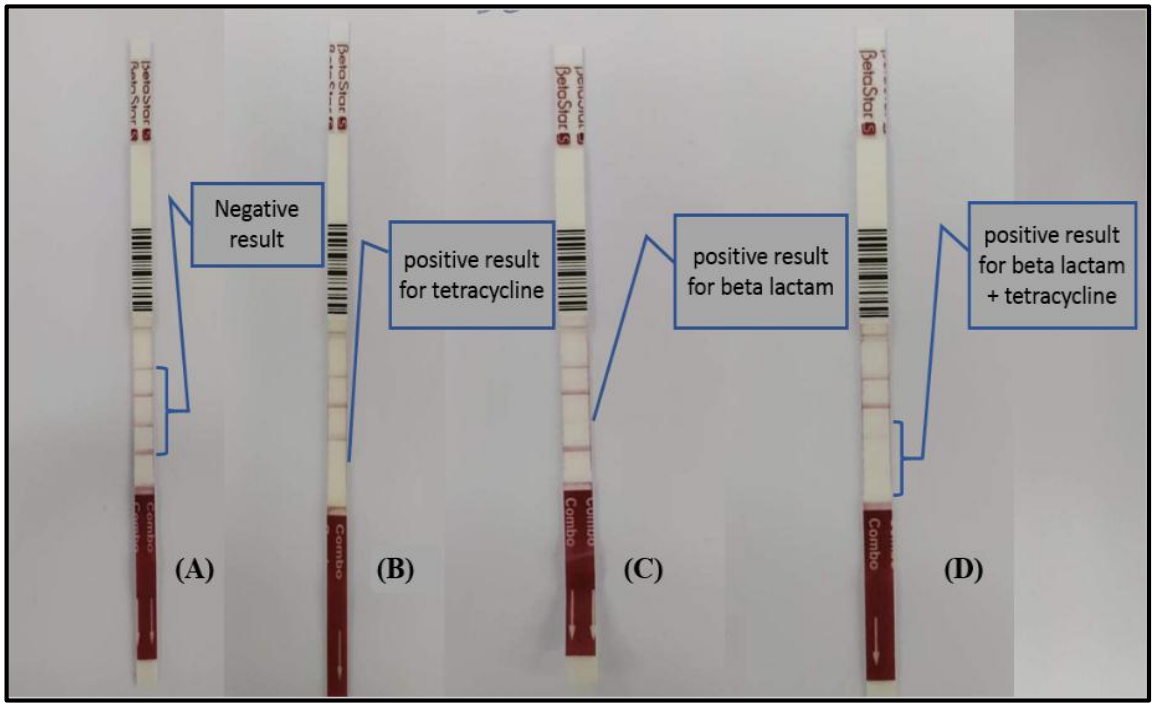

Figure 5. Result interpretation: (A) negative result, (B) positive for tetracycline, (C) positive result for beta-lactam, (D) positive result for beta-lactam + tetracycline.

Chapter 6.
Results and Discussion

6.1. Results of the questionnaire surveys on veterinary practice and dairy industry in Biskra

6.1.1. Results of the questionnaire survey for veterinary practitioners

The results of the questionnaire are organised in tables containing the number and percentage of the given answers which were attached in appendix 04.

6.1.1.1. Distribution of veterinarians according to the study area

A total of forty (40) questionnaire surveys were distributed in different parts of Biskra, 10 out of 12 districts were covered in the investigation (fig. 6). Where the most covered districts are considered the leading suppliers for the dairy industry, Tolga district was represented with 9 questionnaire surveys with a rate of 22.5%, While Biskra and Ouled Djellel were represented with rate of 20% and 22.5% respectively.

The results are detailed in table 04; appendix 04.

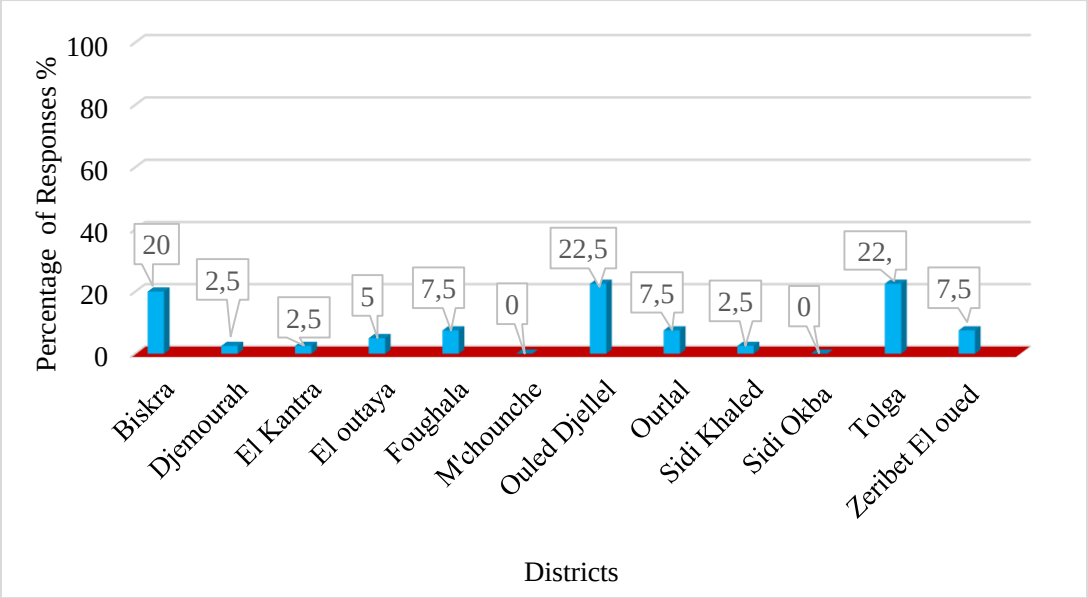

Figure 6. Distribution of the interviewed veterinarians per district according to the study area.

6.1.1.2. Personal and professional information of the interviewed veterinarians

The first section of the questionnaire survey had the main purpose of acquiring certain personal information such as the gender of the interviewed veterinarians, years of practices and experience in the field, in addition to other characteristics of their current practices.

The majority of the interviewed veterinarians were males (fig.7) with a rate of 97,5% while females contributed only by a rate of 2.5%. The results are mentioned in table 05; appendix 04.

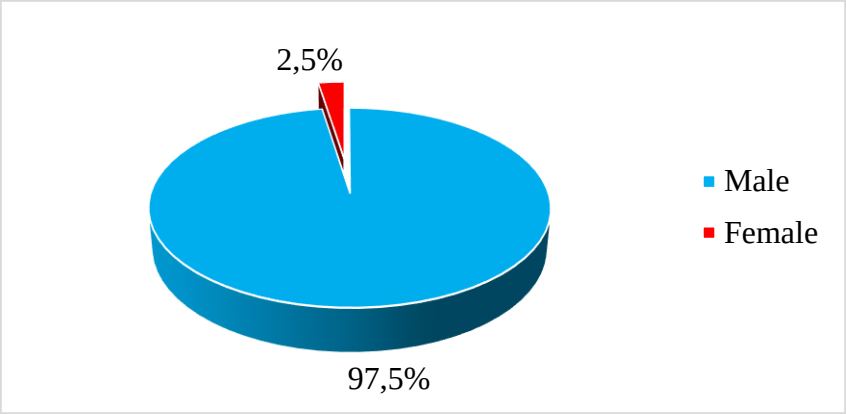

Figure 7. Gender-ratio of the interviewed veterinarians in the study sample (40 responses)

In terms of the experience of the interviewed veterinarians in the field the study showed a diversified result (fig.8) where:

- The category of 1 to 5 years of practice was represented with a rate of 27.5%
- The category of 6 to 10 years of practice was represented with a rate of 30%.
- The category of 11 to 15 years of practice was represented with a rate of 12.5%
- The category of 16 to 20 years of practice was represented with a rate of 15%
- The category of more than 20 years of practice was represented with a rate of 15%

The results are reported in table 06; appendix 04.

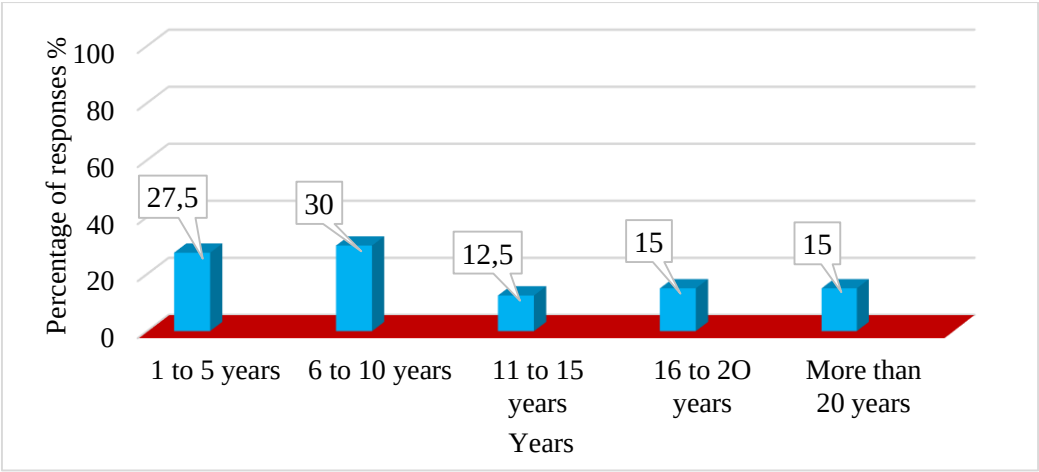

Figure 8. Frequency of the years of practice of the interviewed veterinarians in the sample.

According to the results, the majority of interviewed veterinarians, i.e. a rate of 62.5%, reported that they rarely intervene in dairy cattle treatment (fig. 9), while only 37.5 % said they regularly intervene in the practice.

The obtained responses are detailed in table 07; appendix 04.

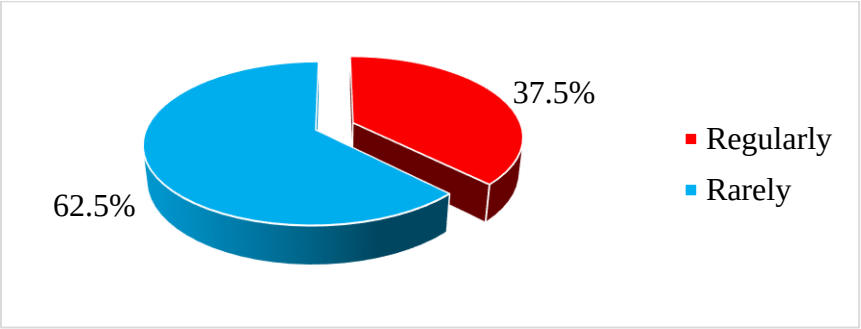

Figure 9. Frequency of veterinarian's intervention in dairy cattle breeding

In terms of dairy cattle farms under the supervision of each veterinarian, the study showed:

- The category of <25 dairy farms was the most represented with a rate of 65%.
- The category of 25-50 dairy farms was represented with a rate of 25%.
- The category of 51-75 dairy farms was represented with a rate of 5%.
- The category of 76-100 dairy farms was represented with a rate of 15%.
- The category more than >100 dairy farms was represented with a rate of 15%.

The results are reported in table 08; appendix 04 and illustrated in the figure 9.

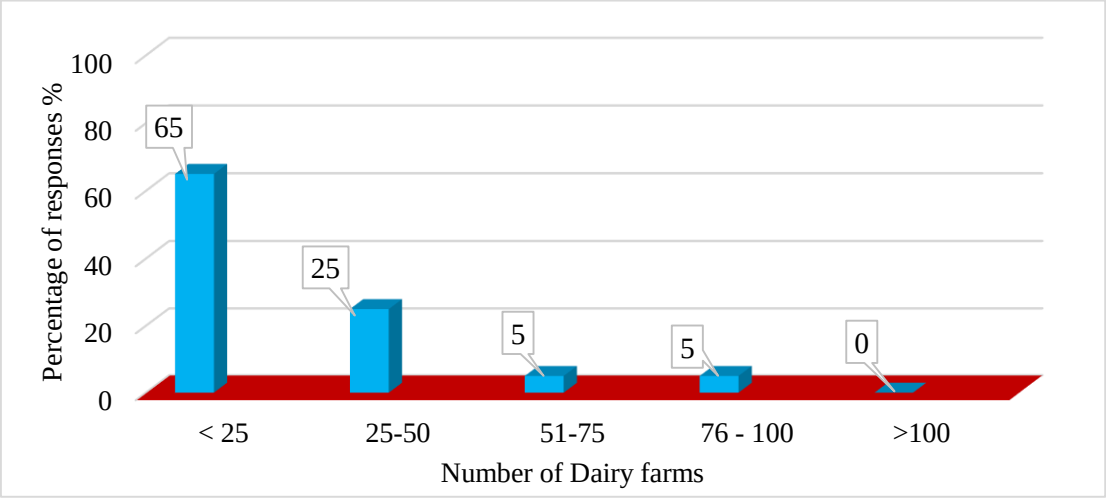

Figure 10. Frequency of dairy farms under the supervision of each interviewed veterinarian.

In terms of the distribution of dairy cattle heads among the dairy farms, the study showed that the category of <5 dairy cattle heads was the most represented (52.5%.) followed

by The categories of 5-10 and 11-20 dairy cattle heads represented with 40% and 7.5% respectively. The categories 21-50 and more than >50 was not represented.

The results are reported in table 09; appendix 04 and illustrated in the figure 11.

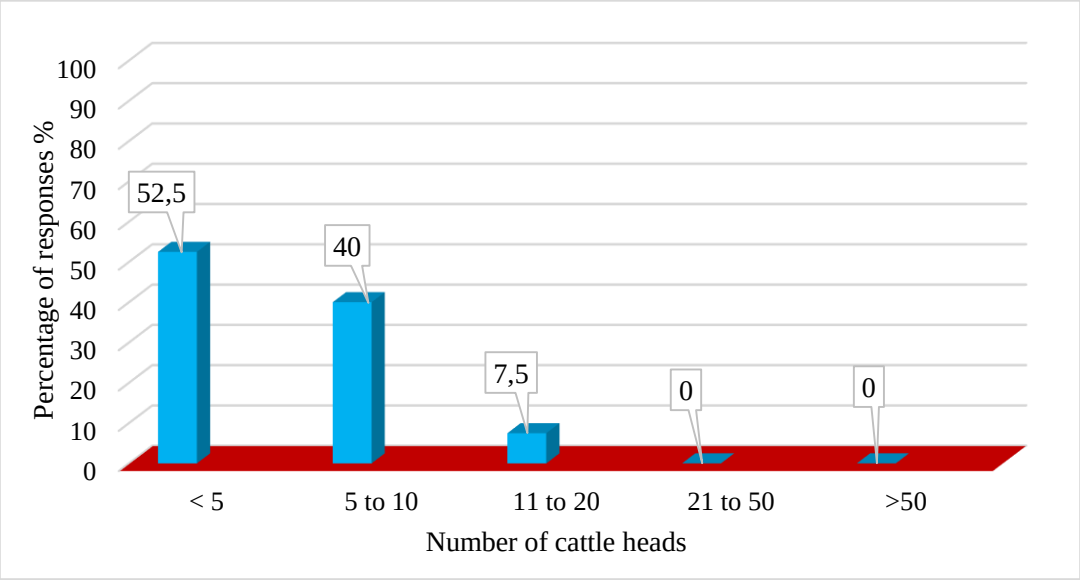

Figure 11. Frequency of dairy cattle heads in each dairy farm under the supervision of the interviewed veterinarians.

6.1.1.3. The most common encountered diseases

According to the obtained results (fig.12), several pathologies exist. Where the most frequent diseases in the field were those with bacterial infections and complications that demands antibiotic treatments such as respiratory problems with a rate of 47.5%, followed by udder problems (mastitis) in 35% of cases and neonatal enteritis in 7.5% of cases. The categories of metritis and digestive diseases represent 5% and 3% of the cases respectively. Other diseases encountered are less common such as locomotion disorders, reproductive and lameness diseases represent only 2.5%. Result are mentioned in the table 10, appendix 04.

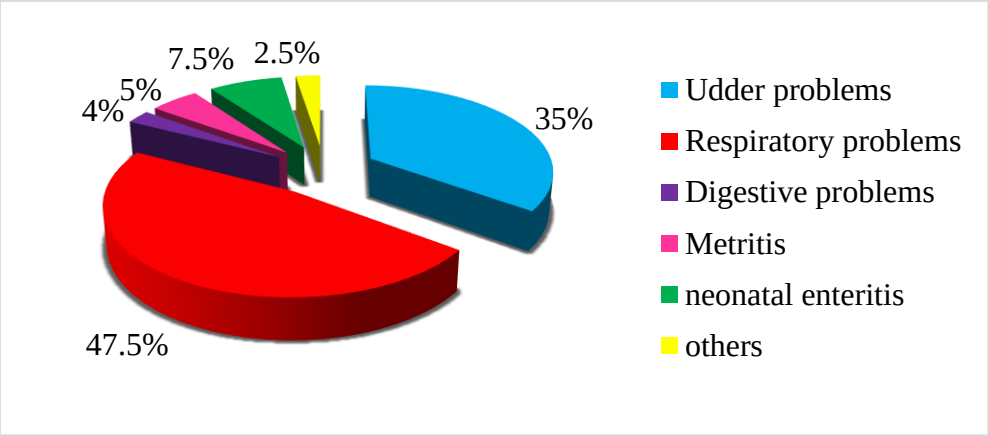

Figure 9. Frequency of the most encountered diseases in dairy breeding treated with antibiotic therapy

This dominance of respiratory diseases even though it's mainly common among young calves, might be explained by the interactions of one or more pathogens with several predisposing factors, such as the stress of moving animals, the environment in which the calves are housed and their nutrition. (Esslemont et Kossaibati,1999). In adult dairy cows, respiratory diseases are less important than mastitis, even though the rate of the later in our study was 35%, still mastitis is the most known disease that negatively affects the animal's condition and decreases productivity and milk quality, in addition to potential public health challenge. Mastitis results in substantial economic loses (Amer *et al*., 2018). Mastitis is mainly caused by inadequate handle of hygienic conditions in dairy farms. A study done by Chatellet, (2007), reported that the most common diseases among dairy cattle were mastitis with a rate of 80% followed by respiratory problems and Foot disease in a rate of 33% and 10% respectively. A study conducted in the central area of Benin by (Mensah *et al.*, 2014), reported that the diseases that led to the use of antibiotics were mastitis (27%), respiratory problems (27%), foot diseases (21%), neonatal enteritis (19%) and umbilical diseases (7%). According to Cazeau *et al.*, (2010) The diseases which motivated the most use of antibiotic treatment mastitis (36%) and locomotion disorders (14%) and respiratory diseases with a rate of 11%.

6.1.1.4. Antibiotic treatments

A. Treatment of mastitis

The results showed (fig. 13) that the most used antibiotics for the treatment of mastitis are: beta-lactam (57.5%), tetracycline (27.5%), macrolide (20%) and Cephalosporin (15%).Results are reported in table 11; appendix 04.

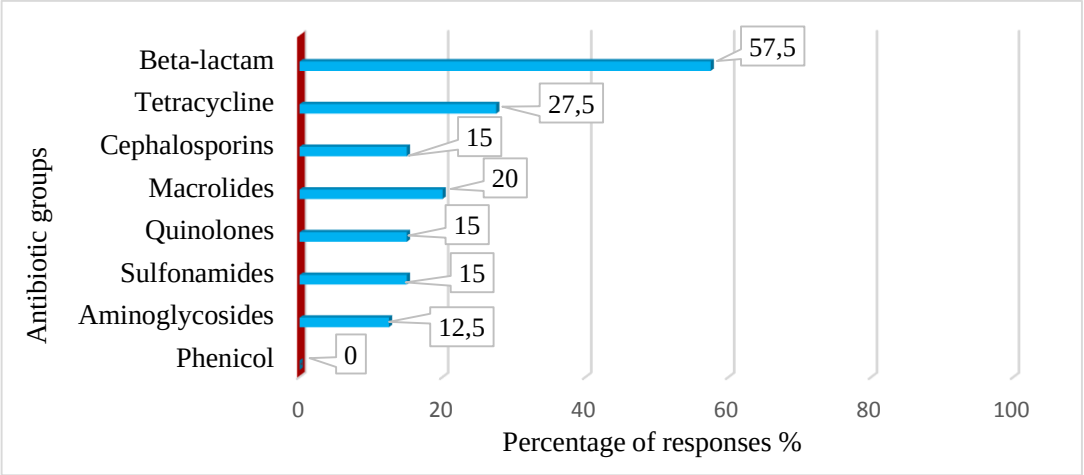

Figure 10. Distribution of the most prescribed antibiotic molecules for the treatment of mastitis

Mastitis might be caused by Gram+ or Gram- bacteria therefore require the use of a broad-spectrum, lipo-solube antibiotics and the combination of both intramammary and general therapy (Boultif,2015).

According to study done by (Boultif, 2015), antibiotics used for the treatment of mastitis and with an intramammary administration are: tetracycline (55%), beta lactam (33%), macrolide (9.67%) and sulphonamides (3.22%).

B. Treatment of respiratory diseases

The results showed (fig. 14) that the most used antibiotics for the treatment of respiratory diseases are: tetracycline (70%), beta-lactam (43%), macrolide (42.5%) and Cephalosporin (20%). Results are detailed in table 12; appendix 04.

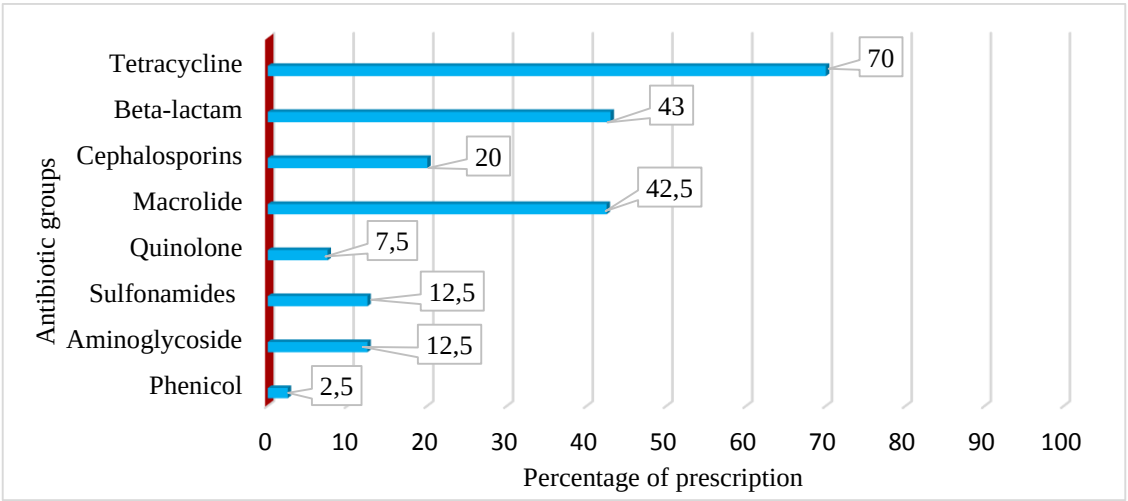

Figure 11. Distribution of the most prescribed antibiotic molecules for the treatment of respiratory diseases

The use of tetracycline especially oxytetracycline in developing countries can be explained by its efficiency in treating respiratory diseases due to their large spectrum of activity, long period of action (one single shot is sufficient), availability as well as low cost.

A study done by Boultif, (2015), reported that the most used antibiotics for the treatment of respiratory diseases are: tetracycline (44%), beta-lactam (43.5%), macrolides (43%) and cephalosporin (22%).

6.1.1.5. Administration route

A. Intramammary route

- **During lactation**

The results showed that the most commonly used antibiotics via intramammary route during lactation are beta-lactam group (fig. 15) with a rate of 72.5% (ampicillin, amoxicillin followed by penicillin and cloxacillin) followed by cephalosporin with a rate of 20% and lincosamides represented by rifamixin with a rate of 7.5%. tetracycline are the least used antibiotic by intramammary rout with a rate of 5%.

Results are detailed in table 13; appendix 04.

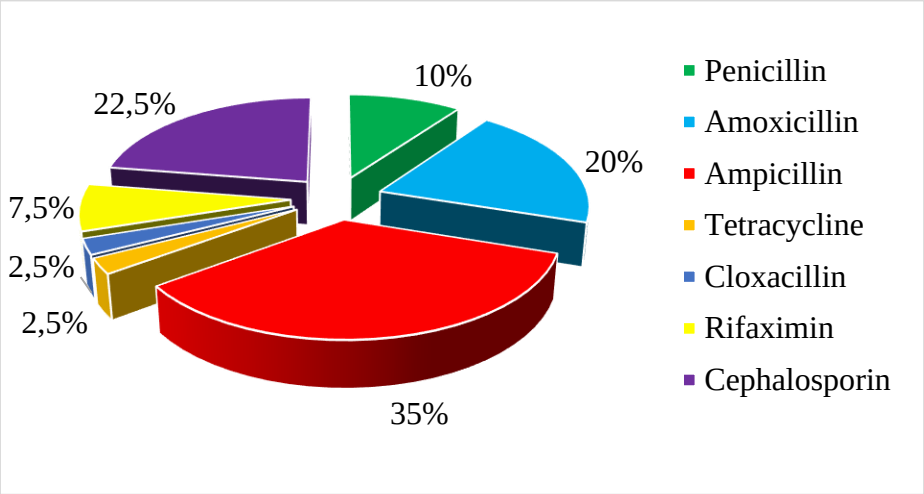

Figure 12. Frequency of the most prescribed antibiotics administrated via intramammary route during lactation.

The dominance of beta-lactam is due to their efficiency, large spectrum of activity as well as their briefest withdrawal period in milk (3 days), on the other hand tetracycline has a long withdrawal period in milk (7 days) and has become banned for the treatment of dairy cattle. Therefore, is less used during lactating period.

- **During dry period**

On the other hand, during dry periods, the results showed that beta-lactam group is still the dominant (fig. 16) with a rate of 37.5% followed by tetracycline with a rate of 27.5%, cephalosporin with a rate of 25%% and aminoglycosides represented by streptomycin with a rate of 7.5. Quinolone are the least used antibiotic by intramammary rout with a rate of 2.5%.

The Results are detailed in table 13; appendix 04.

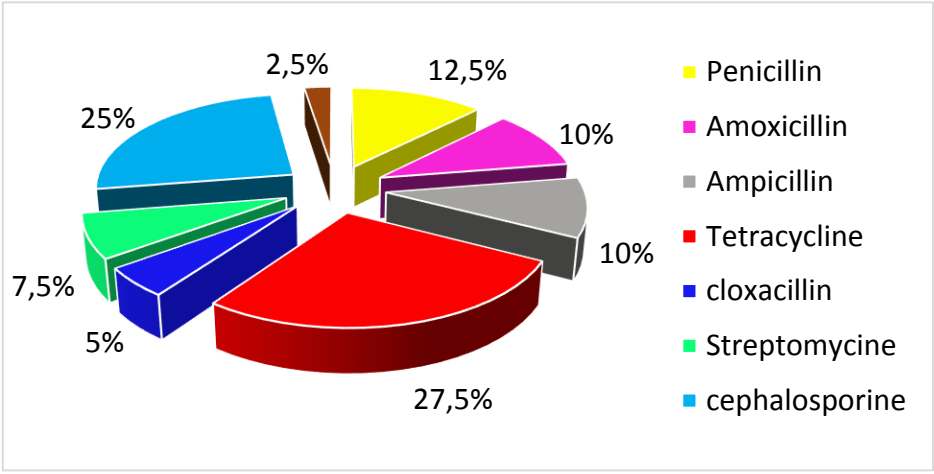

Figure 13. The most prescribed antibiotics administrated via intramammary route during dry period

This diversity in antibiotic use is due to fact that during dry periods of a cow, veterinarians tend to take the liberty in prescribing the most efficient antibiotic without putting into consideration the recommended withdrawal period for milk since the cow is no longer lactating.

B. Parenteral route

The collected data determine seven active molecules of antibiotics administrated parenterally. Where the most frequently used groups of antibiotics were tetracycline (fig. 17) with a rate of 40% (oxytetracycline and tetracycline) followed by beta-lactam with a rate of 35% and Macrolide represented by tylosin with a rate of 12.5%, while cephalosporin and quinolones antibiotics both with a rate of 5%. sulfonamides are the least used antibiotic via parenteral route with a rate of 2.5%. The results are detailed in table 14; appendix 04.

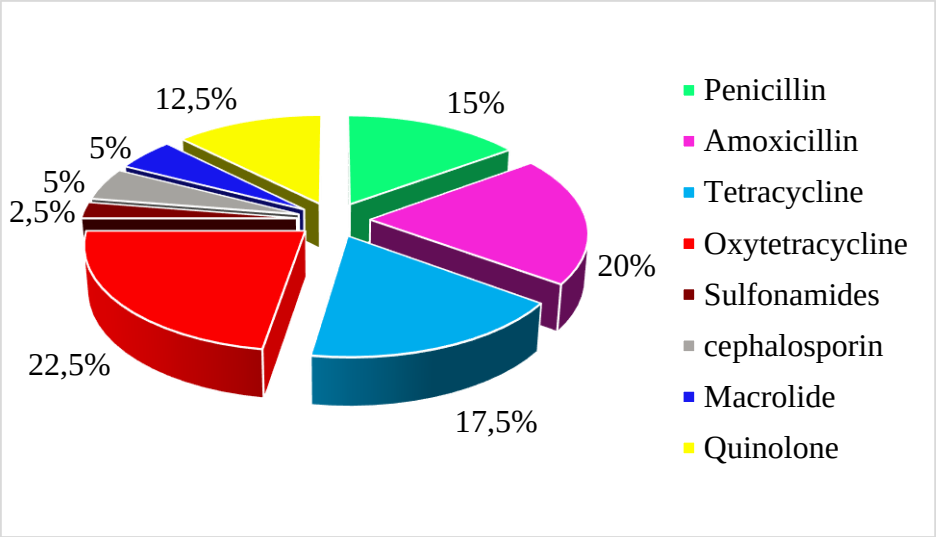

Figure 14. The most prescribed antibiotic administrated parenterally

6.1.1.6. Criteria of antibiotic prescription

According to the results, the criteria of efficiency is the most taken into consideration while prescribing antibiotic treatments (fig. 18) by 97.5% of veterinarians and the withdrawal period by 60%. The criteria of the long period of action and using antibiotics with the least side effects represent 35% and 7.5% of cases respectively. While prescribing antibiotic treatments after operating an antimicrobial susceptibility testing (AST) was not put into consideration by all the interviewed veterinarians, due to the fact that none of them equip themselves with microbiology laboratories nor they send samples to be tested in external laboratories to carry out additional examinations in order to selectively isolate the germs responsible for infections and thus target antibiotic-based treatment. Therefore, veterinarians tend to prescribe a large spectrum antibiotics as empirical therapy based mainly on experience rather than performing a complete laboratory examination for accurate diagnosis of infections, this risky behaviour present a serious problem of selecting drug resistant bacteria.

The results are detailed in table 15; appendix 04.

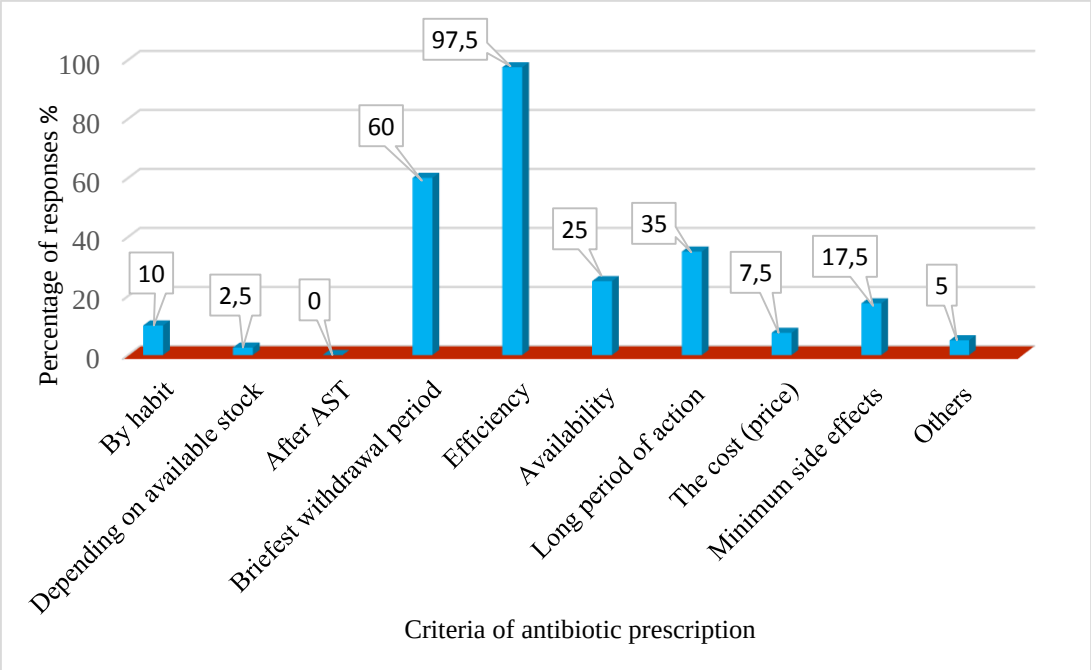

Figure 15. Criteria for prescribing antibiotic treatments.

AST: Antimicrobial susceptibility testing.

6.1.1.7. Compliance with the Withdrawal Periods (WP)

The results showed that out of a total of 40 interviewed veterinarians, a rate of 100% always advise and educate the breeders about the risks engendered by the non-compliance to the withdrawal period.

All the veterinarians interviewed indicate that breeders hardly respect the withdrawal periods, during antibiotic treatments (fig. 19). According to them:

- Only two (2) veterinarians with a rate of 5%, believed that all the breeders respect the withdrawal periods.
- Twenty-seven (27) veterinarian with a rate of 67.5%, confirmed that most of the breeders respect the withdrawal periods.
- Eleven (11) veterinarians with a rate of 27.5%, reported that few of the breeders respect the withdrawal periods.

The obtained responses are detailed in table 16; appendix 04.

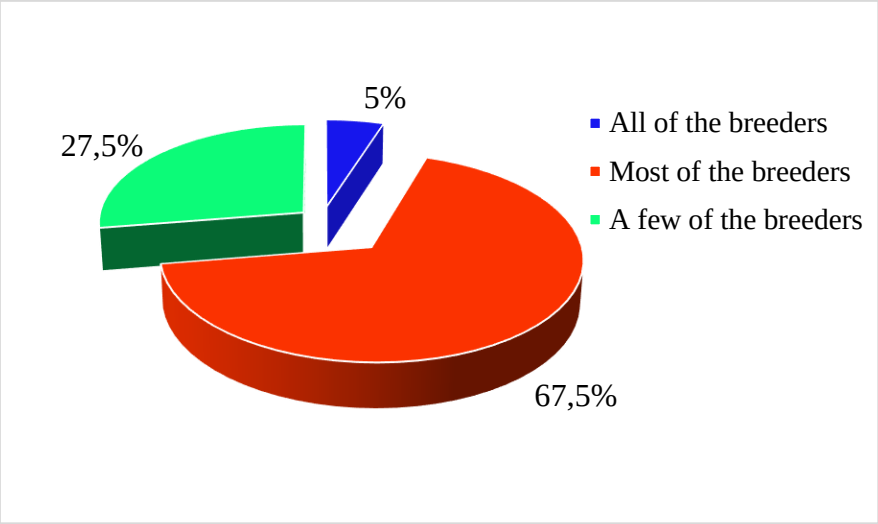

Figure 16. Frequency of responses concerning the compliance with the withdrawal periods by breeders.

The non-compliance with the withdrawal periods recommended for antibiotic treatments might be explained by the lack of awareness among the breeders about the possible risks that may arise due to the presence of antibiotic residues in dairy milk. While the most accurate reason is mere economical, breeders can not risk the financial loss engendered by discarding the non-complaint milk, instead they tend to sell it in black markets due the lack of regulation.

6.1.1.8. Antimicrobial resistance

Our survey showed that out of 40 interviewed veterinarians 55% reported that they prescribe antibiotics for prophylactic purposes (fig. 20). Where Tetracycline was the most incriminated with a rate of 86.36%, represented mainly by oxytetracycline for the prevention of respiratory diseases and chlortetracycline for the prevention of metritis (fig.21). While other antibiotics were rarely used in prophylaxis therapy with a rate of 13.63%.

According to (Mensah *et al.*,2014), the methaphylaxy remains the most common practice done by 64% of farmers in case of mastitis, 53% for respiratory problems, 39% in case of neonatal enteritis, 12% in case of umbilical disease and 4% in foot diseases.

The results are detailed in table 19 and 20; appendix 04.

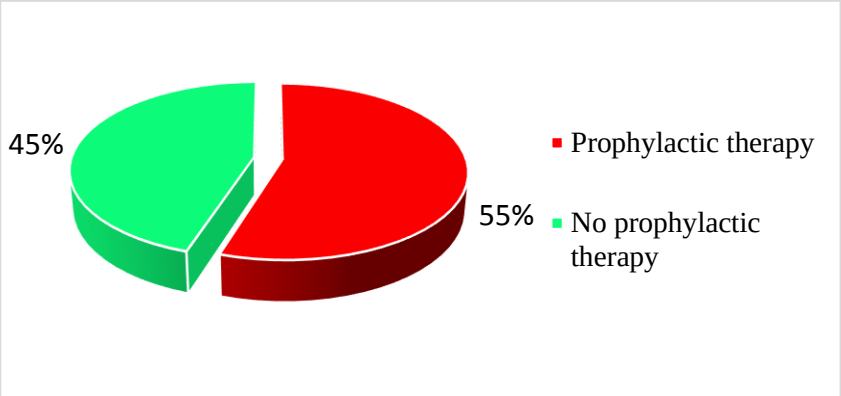

Figure 17. Frequency of prophylactic therapy prescription

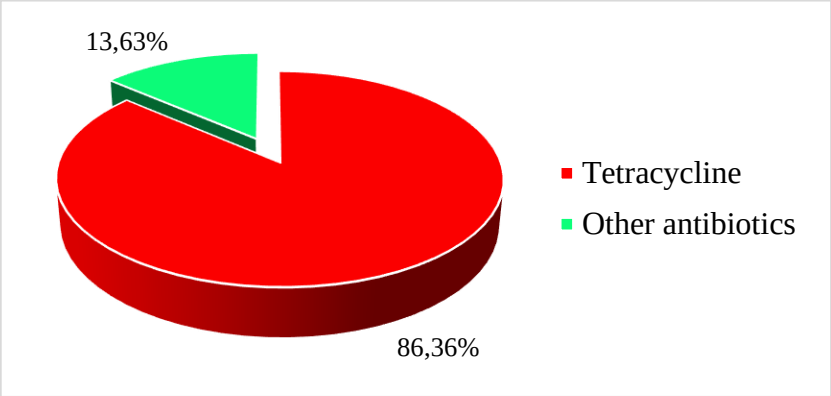

Figure 18. Frequency of the most commonly prescribed antibiotics for prophylactic therapy.

The results showed that the majority of the interviewed veterinarians (85%), have registered cases of antimicrobial resistance (fig. 22). Where, the resistance to tetracycline mainly represented by oxytetracycline was the predominate among the registered cases (fig. 23) with a rate reaching to 82.35% as for beta-lactam a rate of 14.7%cases was registered, while the resistance to Macrolide antibiotic was reported with a rate of 2.94%. The results are detailed in table 21 and 22; Appendix 04.

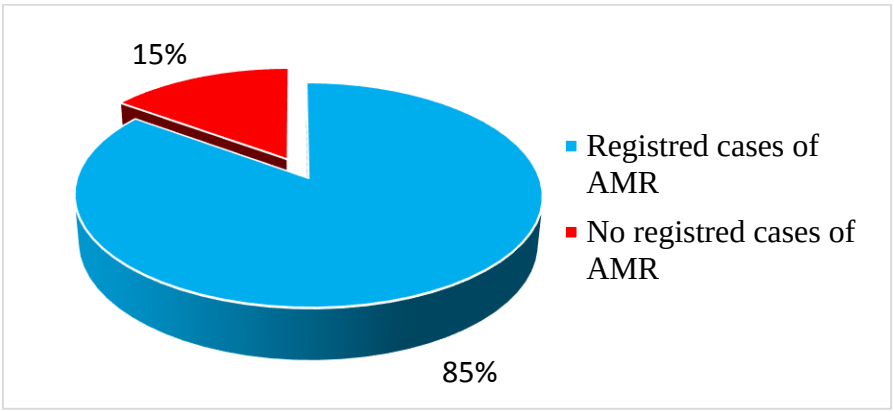

Figure 19. Frequency of registered cases of Antimicrobial Resistance (AMR)

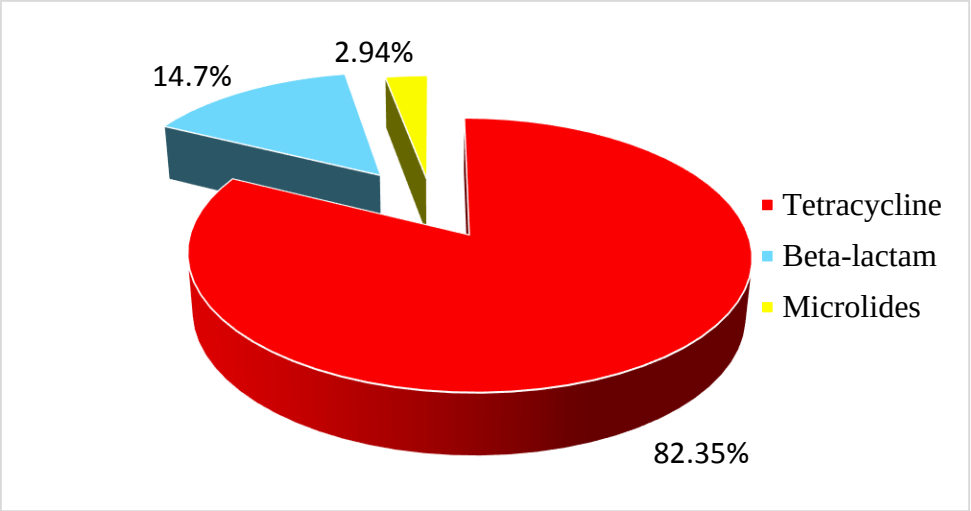

Figure 20. Frequency of the most implied antibiotics among the registered cases of AMR

The resistance to antibiotic treatment can be explained by their extensive use especially for prophylactic purposes. Therefore, there is a correlation between the extensive use of antibiotic for prophylactic purposes and the emergence of antibiotic resistant bacteria for the same misused antibiotics.

As to the problem of self-medicating behaviours displayed by the breeders to treat their dairy cattle, the majority of interviewed veterinarians (80%) (fig. 24), have reported that the breeders tend to self-medicate their cattle herd without consulting a veterinarian and especially for the treatment of mastitis (fig. 25) with a rate of 93.75% of cases as well as for the treatment of other diseases. The results are detailed in table 17 and 18; appendix 04.

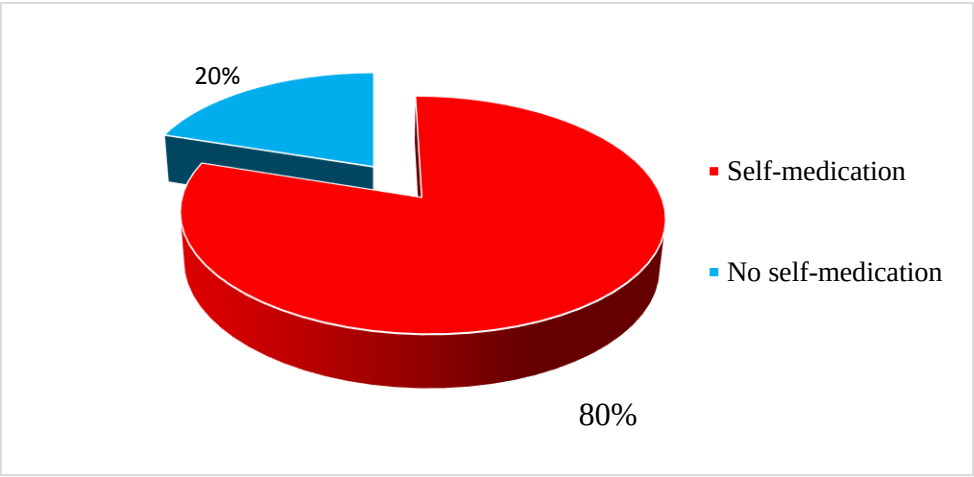

Figure 21. Frequency of self- medicating dairy cattle by breeders

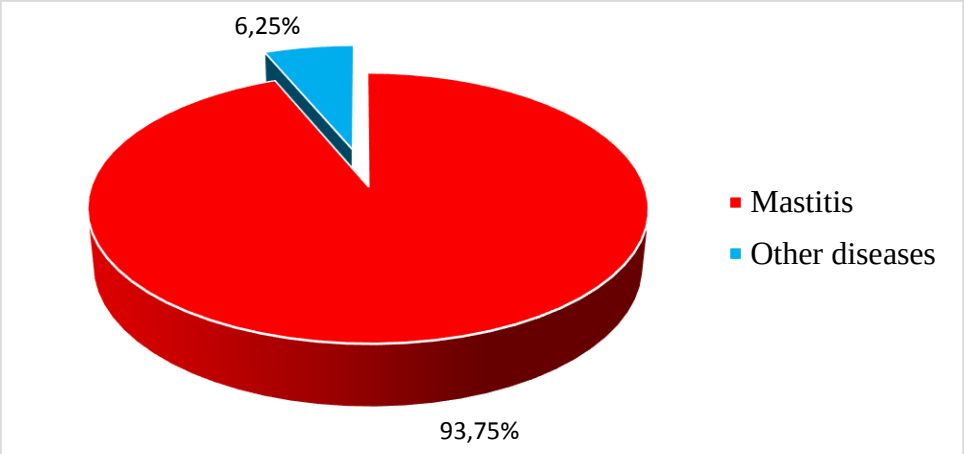

Figure 22. Frequency of the most commonly diseases implied in self-medicating cases by the breeders

The problem of the self-medicating behaviours displayed by the breeders to treat their dairy cattle, the purchase of OTC antibiotics is maybe due to the fact that when faced with the same symptoms of a recent disease, the breeders tend to use the same exact treatment previously prescribed by the veterinarian, without putting into consideration the compliance with the recommended instructions of administrating antibiotics mainly with the recommended doses which must be proportioned to the weight of the animal to be treated, any error in weight assessment would lead to administrating an overdose or an under-dose of antibiotics, the latter is more known to cause AMR in dairy cattle breeding. The extensive use or the misuse of antibiotics will lead to the selection of drug resistant bacteria and eventually to future treatment failures.

6.1.2. Results of the questionnaire Survey for dairy factory managers

6.1.2.1. Production types

The production specialties vary from one dairy establishment to another:

Factory 1 for example, the productions vary in quality, where we can find several specialities, such as pasteurized whole dairy milk, semi- skimmed pasteurized packaged recombined milk, semi-skimmed dairy milk, and many dairy products such as Lben, butter, Yogurt and Fresh cream. In addition to other non-dairy products such as cherbat (sirups)

For the second factory, the types of production consist of: pasteurized whole Dairy milk, pasteurized recombined packaged milk, Lben. In addition, they have a deferent kind of product which is pasteurized whole recombined milk.

6.1.2.2. Production capacities (Quantities)

In terms of production capacities, the obtained data are quite similar. We have noticed that the first factory had a production capacity estimated with 20000 L/ day. However, for the second, the production capacity is reaching to 22000 L/ Day as shown in the table 2.

Table 2. Production capacities of dairy factories

Dairy Factories	Quantity/ Day (Liter)	Quantity/ Month(Liter)
Factory1	20000	520000
Factory 2	22000	572000

6.1.2.3. Raw material supply (powder and dairy milk)

The source of milk powder is the same for the two dairy establishments; which is ONIL (Office National Interprofessionnel de Lait et des produit laitiers) which is the main supplier responsible for importing and distributing milk powder throughout the national territory.

For the production of pasteurized recombined milk, the 0% fat powder is recombined with the 26% fat milk powder to obtain semi- skimmed milk with 15% fat.

Milk is never mixed with cow's milk, for both the diary establishments in question.

Local milk production (dairy milk) remains minimal compared to the amounts of powder consumed, the quantities vary according to the seasons (production peaks between March and June).

6.1.2.4. Types of laboratory quality control protocols

In terms of quality control check, the data obtained are quite diversified. We have noticed that the first establishment followed a protocol that consist of analysing several parameters to ensure the quality of milk such as: organoleptic parameters, physico-chemical parameters and immune-chromatographic test for the detection of antibiotic residues (qualitative and semi-quantitative "test Beta Star Combo"). However, for the second establishment the protocol consisted of analysing physico-chemical parameters and microbiological control, the latter is reserved only for recombined milk based on milk powder.

6.1.2.5. Production problems

In all the establishments, starting culture problems during processing, exist. Antibiotic residues are the most incriminated.

According to the questionnaire survey, only one unit used a qualitative test for detecting antibiotic residues. In the later, the test is used exclusively in case of analysing raw dairy milk destined for processing, if found positive for antibiotic residues, the milk will be rejected and sent back to the collectors then to the breeders, thus sold in streets and dairy shops as raw natural milk.

For the other dairy establishment, the acquisition of the screening test for antibiotic residues remains an unfulfilled project.

However, in order to control the danger of antibiotic residues. The first dairy establishment have established a traceability plan through collectors, in aim to select, subsequently, potential breeders who are aware of the antibiotic residues. On the other hand, the second establishment had no traceability plan

6.2. Results of the qualitative analysis of antibiotic residues by BetaStar® S Combo assay for beta-lactam and tetracycline residues

6.2.1. Results

The results showed that out of a total of thirty samples; (08) are positive, a rate of positivity at 26.66% and negativity at 73.33%. The global results are detailed in table 3and illustrated in figure 26.

Table3. Global result of screening for drug residues in raw milk for both beta lactam and tetracycline antibiotics.

	Number of screened samples	Results			
		Positive		Negative	
		Number	%	Number	%
Total	30	8	26.66	22	73.33

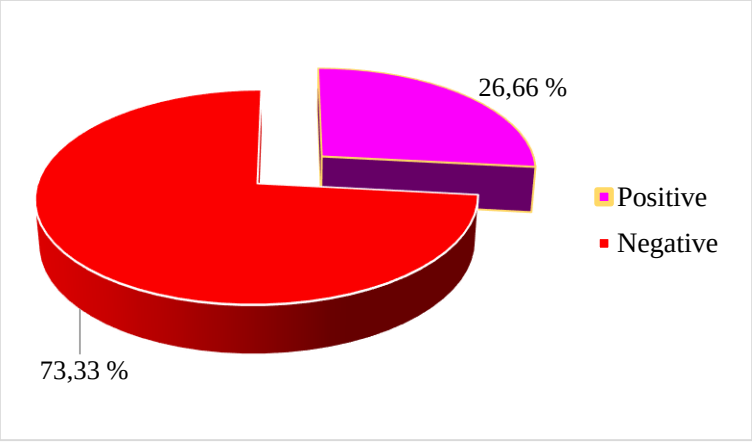

Figure 23. Contamination rate of the analyzed milk with antibiotic residues.

The results show that out of 8 positive results, five (5) samples were positive for beta lactam; at the rate of 62.5%, two samples were positives for tetracycline, at the rate of 25% and one (01) sample was positive for both penicillin and tetracycline, at the rate of 12.5%.

The results are mentioned in table 4 and illustrated in figure 27.

Table 4. Total of positive results for the screening of drug residues in raw milk for both beta lactam and tetracycline antibiotics.

Total	Penicillin		Tetracycline		Penicillin + Tetracycline	
	Number	%	Number	%	Number	%
8	5	62.5	2	25	1	12.5

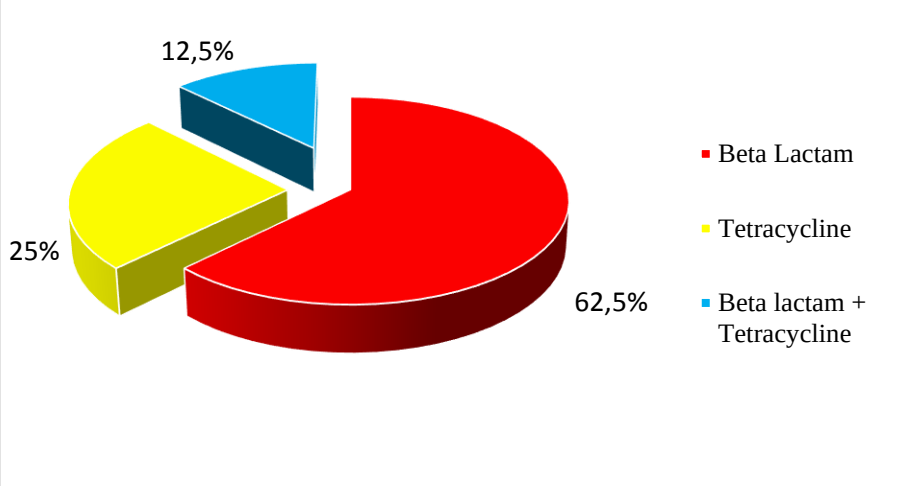

Figure 24. Contamination rate of raw milk with beta-lactam and tetracycline residue.

The study showed a heterogeneity of contamination between the districts (tab. 5), where one district has a total absence of contamination which is Foughala and in others, the contamination is greater; the frequency of positive results is 0.6 for Ouled Djellal and 0.4 for Sidi Okba. The results are detailed in table 5.

Table 5. Frequencies of positive and negatives results /Daira

Daira	Biskra	Foughala	Ouled Djellal	Ourlal	Sidi Okba	Tolga
Frequencies for positive samples	0.2	0	0.6	0.2	0.4	0.2
Frequencies for negative samples	0.8	1	0.4	0.8	0.6	0.8

6.2.2. Discussion

The screening for beta lactam and tetracycline residues in raw milk was carried out using Beta Star Combo test as a method for the detection of the two most used classes of antibiotic in dairy cattle farming.

The choice of the screening test was the Beta Star Combo a rapid and simple screening test that allow the control of the collection tanks before filling or to meet any urgent request for screening or confirmation of the presence of Beta lactam and/or tetracycline below the MRL (maximum residue limits) in a sample pf milk.

The results showed that out of a total of thirty samples; (08) are positive; i.e. a rate of 62.5% of positive samples for Beta lactam and 25% for tetracycline and 12.5% for a sample that contains both Beta lactam and tetracycline residues and 22 samples are negative with a rate of 73.33%.

Therefore, out of all the 30 samples the results show a rate of positivity at 26.66% and negativity at 73.33%.

- **Screening for beta lactam**

Our results show that out of eight (08) samples found positive, five (05) samples are positive for Beta lactam, at a rate of 62.5% and 3 are negative, at a rate of 37.5%. Of all the milk (30 samples) 16.66% are positive for beta lactam.

Based on the question 7, 8, 9 and 10 included in our survey for veterinary practitioners, Beta lactam antibiotics despite the gradual appearance of new and modern antibiotic molecules, they still represent the most active antibiotics and the most used in veterinary medicine, which explains a high rate of residues of these molecules in raw milk compared to other molecules.

Antibiotic residues originating from antibiotic therapy for animals are sometimes incriminated in human allergology. Among the most often implicated antibiotic, are penicillin belonging to the beta lactam class, these molecules used in human medicine, are involved in the majority of drug allergy cases (Demoly *et al.,* 2000).

- **Screening for tetracycline**

Out of the eight (08) samples of the contaminated raw milk, two (02) samples are positive at a rate of 25% and 6 are negative at a rate of 75%. Out of all the milk samples (30 samples) 6.66% are positive for tetracycline.

Despite the ancient discovery of tetracycline, they remain the most widely used antibiotics in veterinary medicine due to their broad spectrum of activity, their low toxicity and

their good distribution in tissues, this gives them the advantage of being used against various diseases and against many germs (Boultif, 2009).

Various studies have reported the presence of beta lactam and tetracycline residues in both tank and farm milk and even at the point of sale for the informal circuit. These studies used for the screening for these two classes, different tests and methods which are represented with the Beta Star Combo and Rosa test.

On a national level we can cite many studies; Tarzaali *et al.* (2008), reported a rate of positivity of 65,46% for beta lactams and 98% for tetracycline residues using a Rosa test. (Titouche *et al.,* 2013) reported a high percentage of contamination with penicillin and/or tetracycline (88.75%) in Freha Area (Tizi-Ouzou). A master study performed by Morsli et Beldjoudi, (2017), reported that out of 117 samples of raw dairy milk analysed using a Delvo test, 14 are found contaminated with antibiotic residues, out of which 21,42% were positive for beta lactam and 14;28% were positive for tetracycline these results were confirmed using the Beta Star Combo test.

On an international level; Pogurschi *et al*. (2015) using Beta Star Combo test reported that out of 210 analysed samples of raw milk, 42 samples were contaminated with antibiotic residues, 12 samples were positive for beta lactam at a rate of 28,57% and 30 samples were positive for tetracycline at a rate of 71,42%. Alzuheir in 2012 used a rapid screening test (IDEXX Snap test kit) for the detection of antimicrobial residues of beta-lactam and tetracycline, reported that out of 34 raw dairy milk analysed samples; 18 samples were tested for beta-lactams residues, of which 22.2 % (4 of 18) appeared to be positive, and 16 samples tested for tetracycline of which 18.7 % (3 of 16) were positive. (Bagre *et al.*, 2015) followed a microbiological method of screening for antibiotic residues in dairy products, reported a contamination rate at 51,72% of raw milk with tetracycline and 17,24% with beta lactam residues.

Conclusion

Conclusion

Antibiotic therapy in veterinary medicine has contributed significantly to the health improvement of food-producing animals and resulted in healthier, more productive animals, with lower disease incidence. In spite of these benefits, the extensive use of antibiotics for the treatment and prevention of diseases and the non-compliance with the recommended withdrawal periods leads to the presence of antimicrobial residues in milk, which constitute a major concern for public health as well as for the economy.

Milk the remains the most consumed animal product due to its highly nutritional value and low cost. Therefore, it is necessary for dairy industries to adopt sensible screening methods for the detection of antibiotic residues in milk, in order to prevent public health risks that may arise from passive consumption of these residues.

At the end of the first investigation, it turned out that the most common diseases are respiratory infections and mastitis. These are often treated and prevented with antibiotics, mainly represented by tetracycline and beta-lactam. These two antibiotics were the main source of antibiotic resistance and consequently of treatment failures, which is somehow correlated with their extensive and misuse. On the other hand, there was an increase rate of self-medication of dairy cattle displayed by breeders and a non-compliance with the recommended withdrawal periods.

At the end of the second investigation we gathered that, Milk production in Biskra is mainly based on Milk powder. The local production of dairy milk is represented with minimal quantities. Also there was technological problems in starting cultures that cause major losses in production sometimes due to the lack of traceability means and a policy for monitoring antibiotic residues.

The results of the screening for tetracycline and beta-lactam residues using Bata star Combo, showed high rate of contamination, which is mainly represented by beta-lactam followed by tetracycline residues. Which reflects in a way the questionable situation of local production of raw dairy milk, that is mainly due to the non-compliance with recommended instructions by breeders, and the lack of awareness of the possible risks on public health.

Recommendation

In order to ensure a better control of the presence of antimicrobial residues in milk, a number of measurements must be followed:

✓ **At a farm level**

- Identify all sick or under treatment animals using a coloured rings or cattle markers
- Antibiotic treatments must be applied according to the instructions with a proper calculation of drug doses, and it is mandatory to respect the withdrawal periods required for each antibiotic.
- The milk collected from cows under antibiotic therapy during the withdrawal period must be systematically eliminated.
- The milking units and the equipment used to milk treated cows must be cleaned and sanitize between uses to ovoid the contamination of the tank.

✓ **At a veterinarian level**

- Reduce prophylactic use of antibiotics in dairy cattle breeding
- Veterinarians and veterinary pharmacists must be more careful in dispensing of prescriptions and sale of antibiotics.
- Veterinarians must equip themselves with microbiology laboratories in order target antibiotic-based treatments
- Veterinarians must convince the breeders to eliminate the milk during antibiotic therapy and adequate them about the risks engendered by the non-compliance to the WP.

At a dairy industry level

- It is recommended to investigate the presence of antimicrobial residues which belong to the other used antimicrobial groups in dairy treatment.

- Contaminated milk with antibiotic residues must be discarded and banned from sale in informal circuit.

- reinforcement of the capacity of screening in laboratories by providing them with sensible methods such as HPLC for the detection of various antibiotics residues used in veterinary medicine

At a public level

- National monitoring of antimicrobial residues in foods and establishing of the maximum residue limits of these residues is highly recommended.

- the establishment of strict legislations by applying coercive measures towards breeders and certain veterinarians whom do not comply with regulatory measures.

-Launching of awareness campaigns about human health hazards associated with antibiotic residues in food of animal origin by concerned authorities (Ministry of Agriculture, Ministry of Health, consumer protection organizations, breeders).

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Appendix

Appendix1

Table 1. physical properties of dairy milk (Carole, 2002)

Density at 5°C	1,032
Specific heat	0,93
Freezing point	-0,550°C
pH (20°C)	6,7
Acidity (Dornic degree)	15-18
Refractive index (20°C)	1,35
Boiling point	100,5°C

Arrêt interministériel du 29 Safar 1414 correspondant au 18 aout 1993 relatif aux spécifications et à la présentation de certains laits de consommation.

ARTICLE 1 : le présent arrêté a pour objet de définir les spécifications de certains laits destinés à la consommation ainsi que les conditions et les modalités relatives à leur présentation et à leur étiquetage.

ARTICLE 2 : la dénomination ‘ lait’ est réservée exclusivement au produit de sécrétion mammaire normale, obtenue par une ou plusieurs traites, sans aucune addition ni soustraction et n’ayant pas été soumis à un traitement thermiques.

ARTICLE 3 : le lait est le produit intégral de traite totale et ininterrompue d’une femelle laitière bien portante, bien nourrie et non surmené. Il doit être recueilli proprement et ne pas contenir de colostrum.

ARTICLE 4 : la dénomination ‘lait’ sans indication de l’espèce animale de provenance, est réservée au lait de vache

Tout lait provenant d’une femelle laitière autre que la vache, doit être désigné par la dénomination ’lait’ suivi de l’indication de l’espèce animal dont il provient.

ARTICLE 5 : le lait destiné à la consommation ou à la fabrication d’un produit laitier, doit provenir de femelles laitières en parfais état sanitaires.

ARTICLE 6 : le lait ne doit pas :

- Etre coloré, malpropre ou malodorant ;
- Provenir d’une traite opérée moins de sept (07) jours le port ;
- Provenir d’animaux atteints de maladies contagieuse ou de mammites ;
- Contenir notamment des résidus antiseptiques, antibiotiques et pesticides ;
- Coaguler à l’ébullition ;
- Provenir d’une traite incomplète ;
- Subir un écrémage partiel.

Appendix 02

Table 2.the major classes of antibiotic (anonymous,2012).

Classes	Mechanism of action	Specter of activity
Beta lactam	Inhibition of cell wall synthesis.	Large specter of activity: Gram positive (+) bacteria.
Macrolide	Interfering with protein synthesis.	Large specter of activity: Gram positive (+) bacteria.
Quinolone	Inhibition of DNA synthesis.	Large specter of activity: Gram positive (+) and Gram negative (-) bacteria.
Sulfonamide	Blocking folic acid synthesis by interfering with a bacterial enzyme responsible for the process.	Large specter of activity: Gram positive (+) and Gram negative (-) bacteria.
Tetracycline	Inhibition of protein synthesis.	Large specter of activity: Gram positive (+) and Gram negative (-) bacteria, Aerobic and anaerobic.

Appendix 03

Table 3. characteristics of different tests for the detection of antibiotic residues in milk (Abidi, 2004).

Screening test	Detection method	characteristics
Acidification method	-Microbiological-Qualitative.	- test with *streptococcus thermophilus.* - affirmation with *Bacillus steathermophilus.*
Delvo test SP	-Microbiological-Qualitative.	- test with *Bacillus streathremophilus var. calidolactis.* - large specter of detection - incubation period from 2 h 30 min to 3 h - high degree of sensibility.
Delvo X Press	-immuno-enzymatic-qualitative.	- Specific for beta lactam antibiotics. - Rapid test (10 min).
Copan Milk test	-Microbiological-Qualitative.	test with *Bacillus streathremophilus var. calidolactis.* - large specter of detection - incubation period from 2 h 30 min to 3 h - high degree of sensibility.
Valio T101	-Microbiological-Qualitative.	- test with *Streptococcus thermophilus.* - high degree of sensitivity. - a long operation.
Beta star	-Immune-colorimetric, qualitative and semi quantative.	- Attest with specific receptor linked to gold particles. - Rapid test (5 min to 50 min).
Penzym test	-Enzymatic-colorimetric. Qualitative;	.- a test with an enzyme DD- carboxypeptidase. -rapid test (20 min) and easy to use.
Charm test	Immune-competition-quantitative.	- a test with radioactive molecules (C4 or H3). - large specter. - large investment
HPLC	Physico- chemical-qualitative.	- Highly sensitive with a grand exactitude - Very expensive - Easy to manipulate - Take a long period of time for the preparation of the sample.

Appendix 04

Questionnaires d'enquête auprès des vétérinaires praticiens en français

Date de l'enquête : ……
Nom de vétérinaire : ……
Localisation : …….....

Introduction au questionnaire

Les traitements vétérinaires, essentiellement les antibiotiques, utilisée à des fins thérapeutiques ou bien préventifs en élevage bovin laitier peuvent être à l'origine de la présence de résidus d'antibiotique dans le lait.

Or. Ces résidus constituent un problème majeur tant pour le consommateur sur le plan sanitaire que sur les industriels sur le plan technologique.

Dans le cadre de la réalisation d'un mémoire de fin d'étude, nous aimerions identifier les pratiques thérapeutiques adoptées par vous les vétérinaires praticiens de la Wilaya de Biskra lors de traitement des pathologies chez les vaches laitières. Votre expérience nous intéresse. Ce questionnaire est anonyme. Pour les questions, une seule réponse est demandée sauf indication contraire.

Nous vous remercions de prendre une vingtaine de minutes de votre précieux temps pour rependre à ce partage d'expérience.

1. **Etes-vous ?**

   ☐ Un homme ☐ Une femme

2. **Depuis combien d'années exercez-vous en pratique rurale ?**

   ☐ 1 à 5 ans
   ☐ 6 à 10 ans
   ☐ 11 à 15 ans
   ☐ 16 à 20 ans
   ☐ Plus de vingt ans

3. **Vous intervenez en élevages bovin laitier ?**

   ☐ Toujours ☐ Rarement ☐ Jamais

4. **Quel est le nombre estimé d'éleveurs de votre clientèle bovine ?**

   ☐ <25
   ☐ 25-50
   ☐ 51-75
   ☐ 76-100
   ☐ >10

5. **Quelle est la distribution moyenne de la taille des troupeaux bovins dans votre clientèle (de bovins) ?**

- ☐ < 5
- ☐ 5-10
- ☐ 11-20
- ☐ 21-50
- ☐ >50

6. **Quelles sont les maladies les plus fréquemment rencontrées, traités, par les antibiotiques ? (cochez un seul type de maladie le plus consomatrices d'antibiotiques dans votre cabinet**

- ☐ Problèmes de mamelle
- ☐ Problèmes digestifs
- ☐ Problèmes respiratoires
- ☐ Métrites
- ☐ Entérites néonatales
- ☐ Autres

7. **Quel est l'antibiotiques que vous utilisez pour le traitement de mammite ? (Choisissez une réponse dans chaque ligne).**

	Très Souvent (> 80% des Cas	souvent (40-80% des cas)	parfois (10-40 % des cas)	Rarement (1-10% des cas)	Jamais (0 % des cas)
Tétracycline	☐	☐	☐	☐	☐
Pénicillines (exp : Amoxicilline, Pénicilline G Ampicilline ….	☐	☐	☐	☐	☐
Céphalosporine (Exp : Ceftiofure …	☐	☐	☐	☐	☐
Macrolides (Exp : Erythromycine, Tylosine, …	☐	☐	☐	☐	☐
Quinolone (Marbofloxacine, Enrofloxacine)	☐	☐	☐	☐	☐
Sulfamide	☐	☐	☐	☐	☐
Phenicoles (florfenicol)	☐	☐	☐	☐	☐
Aminoglycosides (Spectinomycine, Streptomycine, …	☐	☐	☐	☐	☐

8. Quel est l'antibiotiques que vous utilisez pour le traitement des maladies respiratoires ? (Choisissez une réponse dans chaque ligne).

	Très Souvent (> 80% des Cas	souvent (40-80% des cas)	parfois (10-40 % des cas)	Rarement (1-10% des cas)	Jamais (0 % des cas)
Tétracycline	⬭	⬭	⬭	⬭	⬭
Pénicillines (exp : Amoxicilline, Pénicilline G Ampicilline ….	⬭	⬭	⬭	⬭	⬭
Céphalosporine (Exp : Ceftiofure …	⬭	⬭	⬭	⬭	⬭
Macrolides (Exp : Erythromycine, Tylosine, …	⬭	⬭	⬭	⬭	⬭
Quinolone (Marbofloxacine, Enrofloxacine)	⬭	⬭	⬭	⬭	⬭
Sulfamide	⬭	⬭	⬭	⬭	⬭
Phenicoles (florfenicol)	⬭	⬭	⬭	⬭	⬭
Aminoglycosides (Spectinomycine, Streptomycine, …	⬭	⬭	⬭	⬭	⬭

9. Quels sont les antibiotiques les plus utilisées par voie intra mammaire ?

A/ En lactation	**délais d'attente (dans le lait)**
1er ……	……
2éme ……	……
3éme ……	……
4éme ……	……
5éme ……. ……	……

B/ Hors lactation	**délais d'attente (dans le lait)**
1er ……	……
2éme ……	……
3éme ……	……
4éme ……	……
5éme ……. ……	……

10. Quels sont les antibiotiques les plus utilisées par voie générales ?

	A /les antibiotiques	**B /délai d'attente (dans le lait)**
1er	……	……...
2éme	……	……
3éme	……	……...
4éme	……	……...
5éme	...…… ……	……...

11. Sur quels critères faites-vous le choix des antibiotiques que vous prescrivez ?

- ☐ Par habitude
- ☐ En fonction du stock disponible
- ☐ Après antibiogramme
- ☐ Délai d'attente plus cours
- ☐ Efficacité
- ☐ Disponibilité
- ☐ Longue durée d'action
- ☐ Le cout (moins cher)
- ☐ Moins d'effets secondaires
- ☐ Autres ….

12. Réalisez -vous un antibiogramme avant la prescription des antibiotiques ?

- ☐ Oui
- ☐ Non

13. Apres l'administration antibiotiques, conseillez-vous l'éleveur à respecter le délai d'attente ?

- ☐ Toujours
- ☐ La plupart de temps
- ☐ Quelques fois
- ☐ Jamais

14. Informez-vous les éleveurs sur les risques engendrés par le non-respect de délai d'attente ?

- ☐ Oui
- ☐ Non

15. Vos éleveurs respectent le délai d'attente ?

- ☐ Tous
- ☐ La plupart d'entre - eux
- ☐ Peu d'entre eux
- ☐ Aucun

16. Vos éleveurs traitent -ils eux même par des antibiotiques ?

A / les mammites

- ☐ Oui
- ☐ Non

B/ Autres pathologies

- ☐ Oui
- ☐ Non

17. Prescrivez - vous des antibiotiques à titre préventif ?

☐ Oui ☐ Non

- **Si oui, lesquels, et dans quelle(s) indication(s) ?**

……
……
……
……

18. Avez-vous enregistré des cas d'antibiorésistance ?

☐ Oui ☐ Non

- **Si oui, quel Famille d'antibiotique ?**

☐ Tétracycline ☐ Macrolides

☐ Bêtalactamines ☐ Céphalosporine

19. Avez-vous un commentaire supplémentaire à nous donner ?

……
……
……
……
……
……
……
……
……

Merci de votre participation.

Questionnaire Survey for Veterinary practitioners in English

Date of the survey: ……
Name of veterinarian: ……
Location: ……

Introduction to the questionnaire

Veterinary drugs, mainly antibiotics which are becoming widely used for prophylactic or therapeutic purposes in dairy cattle farming, although their use has improved significantly the health and the production of dairy cattle , but improper use of these drugs by veterinarians or farmers and the non-respect of the withdrawal time for treated animals can results in antibiotics residues in milk which has an inhibitory effects that can cause undesirable effects on the quality of milk and its technological properties , and most importantly on human health .

As part of the realization of a Master thesis, we would like to identify the therapeutic practices adopted by you veterinary practitioners of Biskra when treating infectious diseases in dairy cows, your experience interests us, this survey is anonymous, as for the questions, only one answer is requested unless otherwise indicated

It Would you be so generous if you can grant us 20 minutes of your precious time to share with us your experience.

1. are you

☐ Male ☐ Female

2. For how many years have you been practicing in rural practice?

☐ 1 to 5 years
☐ 6 to 10 years
☐ 11 to 15 years
☐ 16 to 20 years
☐ More than 20 years

3. Do you interfere with dairy cattle treatment?

☐ Rregularly ☐ Rarely ☐ Never

4. What is the estimated number of dairy farmers under your supervision?

☐ < 25
☐ 25-50
☐ 51-75
☐ 76-100
☐ >100

5. About how much is the average distribution size of cattle herds among your clients?

- ☐ < 5
- ☐ 5-10
- ☐ 11-20
- ☐ 21-50
- ☐ >50

6. what are the most common diseases treated with antibiotics? (tick only one type of diseases that is the most consuming of antibiotics in your clinic).

- ☐ Udder Problems (exp: mastitis ...)
- ☐ Digestive problems
- ☐ Respiratory problems
- ☐ Metritis
- ☐ Neonatal enteritis
- ☐ Others

7. which antibiotics do you use for the treatment of mastitis? (tick one answer in each line)

	Very Often (> 80% of Cases)	Often (40-80% of cases)	sometimes (10-40 % of cases)	Rarely (1-10% of cases)	Never (0 % of cases)
Tétracycline	☐	☐	☐	☐	☐
Penicillins (exp : Amoxicillin, Penicillin G Ampicillin ….	☐	☐	☐	☐	☐
Cephalosporins (Exp : Ceftiofur …	☐	☐	☐	☐	☐
Macrolides (Exp : Erythromycin , Tylosin , …	☐	☐	☐	☐	☐
Quinolone (Marbofloxacin , Enrofloxacin)	☐	☐	☐	☐	☐
Sulfonamide	☐	☐	☐	☐	☐
Phenicols (florfenicol)	☐	☐	☐	☐	☐
Aminoglycosides (Spectinomycin , Streptomycin , …	☐	☐	☐	☐	☐

4. which antibiotics do you use for the treatment of respiratory illnesses? (tick one answer in each line)

	Very Often (> 80% of Cases)	Often (40-80% of cases)	sometimes (10-40 % of cases)	Rarely (1-10% of cases)	Never (0 % of cases)
Tétracycline	☐	☐	☐	☐	☐
Penicillins (exp : Amoxicillin, Penicillin G Ampicillin ….	☐	☐	☐	☐	☐
Cephalosporins (Exp : Ceftiofur …	☐	☐	☐	☐	☐
Macrolides (Exp : Erythromycin , Tylosin , …	☐	☐	☐	☐	☐
Quinolone (Marbofloxacin , Enrofloxacin)	☐	☐	☐	☐	☐
Sulfonamide	☐	☐	☐	☐	☐
Phenicols (florfenicol)	☐	☐	☐	☐	☐
Aminoglycosides (Spectinomycin , Streptomycin , …	☐	☐	☐	☐	☐

9. what are the most commonly used antibiotics by intramammary route?

A/ during lactation	**withdrawal period (in milk)**
1st ……..……	……...
2nd ……	……
3d ……	……...
4th ……	……
5th ...……	……...

B /during the dry period	**withdrawal period (in milk)**
1st ……..……	……...
2nd ……	……
3d ……	……...
4th ……	……
5th ...……	……...

10. what are the most commonly used antibiotics by general route?

	A /antibiotics	**B / withdrawal period (in milk)**
1st	……..……	……...
2nd	……	……
3d	……	……...
4th	……	……
5th	...……	……...

11. Based on which criteria do you choose the antibiotics you prescribe?

- ☐ By habit
- ☐ Depending on available stock
- ☐ After antimicrobial susceptibility testing
- ☐ Briefest withdrawal period
- ☐ Efficiency
- ☐ Availability
- ☐ Long period of action
- ☐ The cost (price)
- ☐ Minimum side effects
- ☐ Others …

12. Do you perform an antimicrobial susceptibility testing before prescribing antibiotics?

- ☐ Yes
- ☐ No

13. After administrating antibiotics, do you advice the farmers to respect the withdrawal period?

- ☐ Always
- ☐ Most of the times
- ☐ Sometimes
- ☐ Never

14. Do you inform the farmers about the risks caused by not respecting the withdrawal period of antibiotics?

- ☐ Yes
- ☐ No

15. Do your clients respect the withdrawal period of antibiotics?

- ☐ All of them
- ☐ Most of them
- ☐ Few of them
- ☐ None of them

16. Do your clients self-medicate their dairy cattle using antibiotics treatments? And if yes for which cases?

A / Mastitis

- ☐ Yes
- ☐ No

B/ other diseases

- ☐ Yes
- ☐ No

17. Do you prescribe antibiotics for Prophylactic therapy purposes?

☐ Yes ☐ No

- **If yes, which one (s), and for which indication(s)?**

……..
……..
……..
……..
……

18. Have you registered cases of antibiotic resistance?

☐ Yes ☐ No

- **If yes, for which groupe of antibiotics?**

☐ Tetracycline ☐ Macrolides

☐ Beta lactam ☐ Cephalosporines

19. Have you any additional comments?

……..
……..
……..
……..
……..
……..
……..
……..
……

Thank you for your participation.

Processing of questionnaire survey data

Table 4. Distribution of the interviewed veterinarians per district (Daira)

Daira	Number of veterinarians	Percentage %
Biskra	8	20
Djemourah	1	2.5
El Kantara	1	2.5
El Outaya	2	5%
Foughala	3	7.5
M'chounche	0	0
Ouled Djellel	9	22.5
Ourlal	3	7.5
Sidi Khaled	1	2.5
Sidi Okba	0	0
Tolga	9	22.5
Zeribet El oued	3	7.5

Table 5. Distribution of the responses according to the veterinarian's gender

Gender	Male	Female
Number	39	1
Percentage %	97.5	2.5

Table 6. Distribution of the number of veterinarians according to years of practice

Years of practice	1 to 5 years	6 to 10 years	11 to 15 years	16 to 20 years	More than 20 years
Number	11	12	5	6	6
Percentage	27.5	30	12.5	15	15

Table 7. Distribution of the responses according to the frequency of veterinary intervention in dairy cattle farming.

Frequency of intervention	Regularly	Rarely	Never
Number	15	25	0
Percentage %	37.5	62.5	0

Table 8. Distribution of the estimated number of dairy cattle farms under your supervision

Dairy farmers	< 25	25-50	51-75	76-100	>100
Number	26	10	2	2	0
Percentage	65	25	5	5	0

Table 9. The average distribution size of cattle herds among clients

Distribution of cattle herds	**< 5**	**5-10**	**11-20**	**21-50**	**>50**
Number	21	16	3	0	0
Percentage	52.5	40	7.5	0	0

Table 10. The most common diseases treated with antibiotics

Disease category	**Number of responses**	**Percentage %**
Udder Problems (exp: mastitis ...)	14	35
Respiratory problems	19	47.5
Digestive problems	1	2.5
Metritis	2	5
neonatal enteritis	3	7.5
others	1	2.5

Table 11. The most common prescribed antibiotics for the treatment of mastitis.

Antibiotic Classes	**Number of responses**	**Percentage %**
Tetracycline	4	10
Penicillin	19	47.5
Cephalosporine	6	15
Macrolide	4	10
Quinolone	2	5
Sulfonimide	2	5
Phenicole	0	0
Aminoglycosides	3	7.5

Table 12. The most prescribed antibiotic for the treatment of respiratory diseases

Antibiotic Classes	**Number of responses**	**Percentage %**
Tetracycline	21	52.5
Penicillin	7	17.5
Cephalosporine	5	12.5
Macrolide	4	10
Quinolone	0	0
Sulfonimide	3	7.5
Phenicole	0	0
Aminoglycosides	1	2.5

Table 13. The most common used prescribed antibiotics by intramammary route

During lactation			During the dry period		
Active molecules	**Number of responses**	**Percentage %**	**Active molecules**	**Number of responses**	**Percentage %**
Penicillin	4	10	Penicillin	5	12.5
Amoxicillin	8	20	tetracycline	11	27.5
Ampicillin	14	35	Ampicillin	4	10
Tetracycline	2	5	Amoxicillin	4	10
Cloxacilin	1	2.5	Streptomycine	3	7.5
Rifaximin	3	7.5	Cloxacillin	2	5
Cephalosporine	8	20	Cephalosporine	10	25
			Quinolone	1	2.5

Table 14. The most common used antibiotics by general route

Active molecules	Number of responses	Percentage %
Penicillin	6	15
Amoxicillin	8	20
Tylosin	5	12.5
Tetracycline	7	17.5
Oxytetracycline	9	22.5
Sulfonamide	1	2.5
Cephalosporine	2	5
Quinolone	2	5

Table 15. The Criteria of antibiotic prescription

Criteria of antibiotic prescription	Number of veterinarians	Percentage %
By habit	4	10
Depending on available stock	1	2.5
After antimicrobial susceptibility testing	0	0
briefest withdrawal period	24	60
Efficiency	39	97.5
Availability	10	25
long period of action	14	35
the cost (cheaper)	3	7.5
with the least side effects	7	17.5
others	2	5

Table 16. Frequency of compliance with withdrawal period by breeders

Compliance with WD by breeders	**All of them**	**Most of them**	**A few of them**	**None of them**
Number of veterinarians	2	27	11	0
Percentage %	5%	67.5%	27.5%	0%

Table 17. Frequency of self-medicating Dairy cattle by breeders

Self-medication	**Yes**	**No**
Number of responses	32	8
Percentage	80	20

Table 18. Frequency of the most frequent diseases treated by breeders without prescription

Diseases	**Number of responses**	**Percentage**
Mastitis	30	93.75
Other diseases	2	6.25

Table 19. Frequency of Prophylactic therapy prescription

Prophylactic therapy	**Yes**	**No**
Number of responses	22	18
Percentage	55	45

Table 20. The most incriminated antibiotic molecules for prophylactic therapy

Antibiotic molecules	**Number of responses**	**Percentage**
Tetracycline	19	86.36
Other antibiotics	3	13.64

Table 21. The registered cases of antimicrobial resistance and treatment failure

Registered cases	**Yes**	**No**
Number of responses	34	6
Percentage	85	15

Table 22. Frequency of the most incriminated antibiotic molecules among the registered cases of AMR

Antibiotics	**Number of responses**	**Percentage %**
Tetracycline	28	82.35
Beta-lactam	5	14.70
Macrolide	1	2.94
Cephalosporin	0	0

Appendix 5

Questionnaire d'enquête auprès des gérant de Laiterie en Français

Nom de Laiterie : …… **Date** :……

Nom du gérant : …….. **Localisation**:…….

Introduction au questionnaire

Les traitements vétérinaires, essentiellement les antibiotiques, utilisée à des fins thérapeutiques ou bien préventifs en élevage bovin laitier peuvent être à l'origine de la présence des résidus d'antibiotique dans le lait.

Or. Ces résidus constituent un problème majeur tant pour le consommateur sur le plan sanitaire que sur les industriels sur le plan technologique.

Dans le cadre de la réalisation d'un mémoire de master en microbiologie appliquée. Un questionnaire est conduit dans le but de collecter des informations afin d'avoir une idée sur la situation de la production et la transformation laitière à Biskra et d'identifier les procédures et les méthodes adoptée par les laiteries en les laboratoires pour assurer la sécurité sanitaire de leur produit final vis -à- vis des résidus d'antibiotiques. Votre expérience nous intéresse. Ce questionnaire est anonyme et confidentiel, il sera utilisé seulement à des fins académiques. Pour les questions, une seule réponse est demandée sauf indication contraire. Votre véritable réponse aux questions jouera un grand rôle dans les résultats des projets de recherche final.

Nous vous remercions de prendre une vingtaine de minutes de votre précieux temps pour rependre à ce partage d'expérience.

I. Partie concernant les cordonnée gérant de l'entreprise

1. **Etes -vous ?**

☐ Male ☐ Femelle

2. **Depuis combien de temps vous engager-vous dans la production laitière ?**

☐ 1 à 5 ans ☐ 11 à15 ans

☐ 6 **à 10**ans ☐ Plus de vingt ans

3. **Quel est le nombre de collecteurs responsables de la livraison de lait cru ?**

☐ Un seul ☐ 6 à 10 collecteurs

☐ 2 à 5 collecteurs ☐ Plus de 10 collecteurs

4. Quel est la distribution moyenne de nombre de fermes que vous collecteurs faire la collecte ?

☐ < 5 ☐ 5-10

☐ 11-20 ☐ 21-50

☐ >50

5. Quel est votre capacité de production de lait en litre par jour et par mois ?

Capacité de production	
Quantité/ Jour(Litre)	**Quantité/ Mois (Litre)**

6. Votre produits ?

☐ Lait de vache entier pasteurisé ☐ Yaourts

☐ Lait de vache demi écrémée ☐ Beurre

☐ Lait recombiné pasteurisé conditionné ☐ Fromage

☐ Lben ☐ Crème fraiche

☐ Raib ☐ Autres

7. Ou provient la poudre de lait que vous utilisez (votre distributeur) ?

……

8. Quel est le type de poudre de lait que vous utilisez concernant son taux en matière graisse ?

……

9. Additionnée- vous le poudre de lait lors la fabrication de lait crue de vache ?

☐ Oui ☐ Non

- **Si oui, a quel pourcentage ?**

……
……
……
……

10. Quelle sont les différents paramètres analyser pour la gestion de la qualité du lait ?

- ☐ Paramètres physico-chimique
- ☐ Paramètres microbiologique
- ☐ Paramètres organoleptique
- ☐ Test de détection des résidus

11. Avez-vous un programme de gestion de la qualité du lait par rapport aux résidus des antibiotiques ?

- ☐ Oui
- ☐ Non

12. Quels est la méthode que vous – utilisez pour la détection des résidus d'antibiotiques au niveau de votre laiterie ?

- ☐ Test biologique (microbiologique)
- ☐ Test immuno--enzymatique
- ☐ Test physico- chimique (HPLC)
- ☐ Test immuno- chromatographique (Beta Star …)

13. Dans quel cas utilisez-vous le test de détection des résidus des antibiotiques ?

- ☐ Lait crue de vache
- ☐ Lait crue de vache destiné à la transformation
- ☐ Lait reconstituée
- ☐ Lait crue reconstituée destiné à la transformation

14. Sur quels critères faites-vous le choix de technique de détection des antibiotiques que vous utilisez ?

- ☐ Efficacité
- ☐ Sensibilité (précision de lecture)
- ☐ Le cout (moins cher)
- ☐ Specificité
- ☐ La rapidité
- ☐ Le seuil de détection
- ☐ Test à large spectre
- ☐ Autres …

15. Que fait – vous des laits non conformes (qui contient des résidus d'antibiotiques) ?

- ☐ Vous renvoyer le lait non conforme au collecteur
- ☐ Vous écartez et détruisez le lait non conforme.
- ☐ Vous le mettez dans la chaine de production de lait en sachet.
- ☐ Autres

16. Tenez-vous un registre des problèmes concernant les résidus d'antibiotiques dans le lait ?

☐ Oui ☐ Non

- **Si oui, à quoi sert-il ?**

……
……
……
……
……

17. **Donnez-nous des exemples des problèmes technologiques que vous avez rencontrés dans votre unité de production à cause des lots contaminés aux antibiotiques**

……
……
……
……
……
……

18. Avez-vous un commentaire supplémentaire à nous donner ?

……
……
……
……
……
……

Merci de votre participation

Questionnaire Survey for Dairy factory managers in English

Name of the dairy factory:……. **Date** :……...

Name of the manager: …… **Localisation**:……

Introduction to the questionnaire

Veterinary drugs, mainly antibiotics which are becoming widely used for prophylactic or definitive therapy purposes in dairy cattle farming, although their use has improved significantly the health and the production of dairy cattle, but improper use of these drugs by veterinarians or farmers and the non-respect of the withdrawal time for treated animals can result in antibiotics residues in milk which has inhibitory effects that can cause undesirable consequences on the quality of milk and its technological properties, and most importantly on human health.

This study is conducted for the fulfilment of a master thesis in applied microbiology, The purpose of this questionnaire is to collect information to have an idea about the situation of dairy production and processing in Biskra and to identify the procedures and laboratory methods followed by dairy factories to ensure the sanitary security of the final product, your experience interests us, this survey is anonymous and confidential and I guarantee you that it will never be disclosed to a third party. Only it will be used for academic purposes. as for the questions, only one answer is requested unless otherwise indicated. Your genuine response to the questions will have a great role in the outcome of the final research project.

It Would be so generous if you can grant us 20 minutes of your precious time to share with us your experience.

1. are you

☐ Male ☐ Female

2. how long have you been engaging in dairy production?

☐ 1 to 5 years ☐ 16 to 20 years

☐ 6 to 10 ☐ More than 20 years

☐ 11 to 15 years

3. How many collectors are responsible for delivering raw milk to your factory?

☐ One collector ☐ 6 to 10 collectors

☐ 2 to 5 Collectors ☐ More than 10 collectors

4. **What is the average distribution of the number of farms that your collectors deliver raw milk from?**

☐ < 5 ☐ 5-10

☐ 11-20 ☐ 21-50

☐ >50

5. What is your milk production capacity per day and per month in liter unit?

Production capacity	
Quantity / Day(Litre)	**Quantity/ Month (Litre)**

6. Your products?

☐ Pasteurized whole dairy milk ☐ Yogourt

☐ Semi-skimmed dairy Milk ☐ Butter

☐ Recombined and pasteurized packaged milk ☐ Cheese

☐ Lben ☐ Fresh cream

☐ Raib ☐ Others

7. From what source you are importing the milk powder (your distributor)?

……**.....**

8. What type of milk powder are you using in terms of its fat content?

……

9. Additionnée- vous de poudre de lait lors la fabrication de lait crue de vache ?

☐ Yes ☐ No

- **If yes, to what percentage?**

……
……
……

10. What are the parameters do you monitor for quality control check?

- ☐ Physico-chemical parameters
- ☐ Microbiological parameters
- ☐ Organoleptic parameters
- ☐ Screening for residues tests

11. **do you have a milk quality management program concerning antibiotic residues?**

- ☐ Yes
- ☐ No

12. What type of screening method do you use for the detection of antibiotic residues in milk?

- ☐ Biological test (microbiological)
- ☐ Immuno—enzymatic test
- ☐ Physico- chemical test (HPLC)
- ☐ Immuno- chromatographic test (Beta Star …)

13. In what case do you use the screening test for the detection of antibiotic residues?

- ☐ Raw dairy milk
- ☐ Raw dairy milk destined for transformation processes
- ☐ Recombined Milk
- ☐ Recombined milk destined for transformation processes

14. Based on what criteria do you choose the detection techniques for antibiotics residues screening?

- ☐ Efficiency
- ☐ Sensitivity (reading accuracy)
- ☐ Cost-effectiveness (cheaper)
- ☐ Specificity
- ☐ Rapidity
- ☐ Detection rate
- ☐ Broad spectrum screening Test
- ☐ Others …

15. What do you do with non- compliant milk (which contains antibiotic residues)?

- ☐ The non-complying milk will be rejected and sent back to the collectors.
- ☐ The non-compliant milk discarded and destroyed.
- ☐ The non-compliant milk is processed in the production line of Milk bags.
- ☐ Others.

16. Do you keep a record of problems concerning antibiotic residues in milk?

☐ Yes ☐ No

- **If yes, for what purpose?**

……
……
……
……
……
……

17. **Give us examples of the problems you encountered in your production unit due to contaminated lots by antibiotic residues**

……
……
……
……
……
……

18. Have you any additional comments?

……
……
……
……
……
……
……

Thank you for your participation.

Appendix 6

Material of screening for beta lactam and tetracycline residues in raw milk using a Beta Star Combo®

✓ Material for sampling

- Sterile steel ladle for sample collection.
- Inedible marker for the identification of samples
- sterile plastic bottles (capacity 0.5 L), labelled hermetically sealed closed
- a cool container for sample transport.

✓ Material and laboratory devices

- Refrigerator
- Incubator at 47.5 °C
- Beta Star Combo® Kit

Appendix 07

Results of the screening for beta lactam and tetracycline residues in raw dairy milk.

Table 23. Global results for screening for beta-lactam and tetracycline residues in raw Dairy Milk

Daira	Samples	Results			
		Positive	%	Negative	Percentage %
Tolga	S 1			-	
	S 2			-	
	S 3			-	
	S 4	+			
	S 5			-	
Total tolga	5	1	20	4	80
Ourlal	S 6	+			
	S 7			-	
	S 8			-	
	S 9			-	
	S 10			-	
Total Ourlal	5	1	20	4	80
Biskra	S 11			-	
	S 12			-	
	S 13			-	
	S 14	+			
	S 15			-	
Total Biskra	5	1	20	4	80
Sidi Okba	S 16	+			
	S 17			-	
	S 18	+			
	S 19			-	
	S 20			-	
Total Sidi Okba	5	2	40	3	60
Foughala	S 21			-	
	S 22			-	
	S 23			-	
	S 24			-	
	S 25			-	
Total Foughala	5	0	0	5	100
Ouled Djellal	S 26	+			
	S 27	+			
	S 28			-	
	S 29			-	
	S 30	+			
Total Ouled Djellal	5	3	60	2	40
Total	30	8	26.66	22	73.33

+: **Positive result**, -: **Negative result**.

Table 24. Detailed positive results of screening for beta-lactam and tetracycline antibiotic residues in contaminated raw Dairy Milk

Contaminated samples with antibiotic residues	**Results**	
	Penicillin	**Tetracycline**
S 4	-	+
S 6	+	-
S 14	+	-
S 16	+	-
S 18	+	+
S 26	+	-
S 27	+	-
S 30	-	+

+: **Positive result**, -: **Negative result**

ملخص

الاستعمال المفرط للمضادات الحيوية في الطب البيطري للأغراض الوقائية او العلاجية في معالجة البقر الحلوب. قد يكون مسؤولا عن تواجد بقايا المضادات بالإضافة إلى أن تلوث الحليب ببقايا المضادات الحيوية يجعله غير آمن للاستهلاك البشري بالإضافة إلى الخسائر الاقتصادية لمصنعي الألبان، صار الحيوية في الحليب. تركز الدراسة الحالية على عنصرين، أحدهما تحقيق ميداني موجه البياطرة الممارسين من الضروري الكشف عن بقايا المضادات الحيوية في الحليب في كل تجميع. للمهنة بهدف فهم حالة العلاج بالمضادات الحيوية للأبقار الحلوب، والثاني تم إجراؤه للإبلاغ عن وضعية انتاج الألبان وتصنيعها في بسكرة. المكون الآخر كان الكشف عن أظهر تحليل الاستبيان الذي أجري على 40 بقايا المضادات الحيوية بواسطة بيتا ستار كومبو لدى حليب الابقار الطازج باعتباره النوع الأكثر استخداما في صناعة الألبان. %، غالبا ما يتم علاجها بالمضادات بيطريا ممارسا للمهنة أن أكثر الأمراض شيوعا في هذا المجال هي وامراض الجهاز ال تنفسي47.5% والتهابات الضرع35 الحيوية، والتي تتمثل خاصة في والتيتراسيكلين 70% بيتا لاكتام 57.5% بالترتيب، واللذان يمثلان أكثر المضادات الحيوية استخداما نظرا لكفاءتهما، توفرهما وتكلفتهما تحليل الاستبيان الثاني الذي أجري على المنخفضة. من ناحية أخرى، وجدنا ان فترات انسحاب بعد إعطاء المضادات الحيوية غالبا لا يتم احترامها من قبل المربين. مصانع الالبان وجدنا أن إنتاج الحليب في بسكرة يعتمد بشكل أساسي على الحليب المجفف، الكميات الأدنى، التي تمثل الإنتاج المحلي من حليب الأبقار، مخصصة لتصنيع مشتقات الحليب. يضاف إلى ذلك وجود مشاكل في التصنيع التي تسبب خسائر كبيرة في الإنتاج، خاصة بسبب عدم وجود سياسة لتتبع وتحاليل الكشف على بقايا المضادات الحيوية. تم فحص عينات من حليب الأبقار الطازج للكشف عن بقايا بيتا لاكتام والتيتراسيكلين باستخدام بيتا ستار كومبو، أظهرت النتائج ما يلي من إجمالي ثلاثين عينة، ثمانية (08) كانت موجبة بمعدل 26.66%, منها 62.5% عينات إيجابية لبيتا لاكتام 25% للتيتراسيكلين و12.5% لعينة احتوت على بقايا كل من بيتا لاكتام وتيتراسيكلين. 22 عينة كانت سلبية بمعدل 73.33%.

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الكلمات المفتاحية بقايا المضادات الحيوية، حليب الابقارطازج، مثبطات، بيتا ستار كومبو.

Abstract

The extensive use of Antibiotics in veterinary medicine for prophylactic or therapeutic purposes in dairy cattle farming maybe responsible for the presence of antibiotic residues in milk. However, the contamination of milk with these residues makes it unsafe for human consumption as well as the economic loss to the dairy industry. Therefore, it is necessary to detect antibiotic residues in milk at each collection. The present study focuses on two components, a field surveys one conducted to veterinary practitioners to report the situation of antibiotic therapy for dairy cattle and the second was carried out to assess the situation of dairy production and processing in Biskra. The other component was detection of antibiotic residues by Beta Star Combo for Raw Dairy Milk. Analysis of the questionnaire survey conducted for 40 veterinary practitioners showed that the most common pathologies are respiratory infections (47.5%) and mastitis (35%). These are often treated with antibiotics, mainly represented by tetracycline 70% and beta lactam 57.5% respectively. which are the most used antibiotics due to their efficiency, low cost and availability. on the other hand, we found that withdrawal periods of antibiotic are often not being respected by breeders. Analysis of the second questionnaire survey conducted to dairy factories we found that milk production in Biskra is mainly based on milk powder. The local production of dairy milk is represented in minimal quantities, added to this is the existence of transformation problems that cause significant production losses, especially due to the lack of a traceability policy and screening. tests. Samples of raw dairy milk were screened for the detection of beta lactam and tetracycline residues using Bata star Combo, the results showed that: out of a total of thirty samples; eight (08) were positive with a rate of 26.66%, out of which 62.5% were positive samples for Beta lactam and 25% for tetracycline and 12.5% for a sample that contained both Beta lactam and tetracycline residues and 22 samples were negative with a rate of 73.33%.

Keywords: Antibiotic residues, raw dairy milk, inhibitors, Beta Star Combo.

Résume

L’utilisation extensifs des antibiotiques en médecine vétérinaire à des fins prophylactiques ou thérapeutiques en élevage bovins laitiers, peut être responsable de la présence des résidus d’antibiotiques dans le lait. Cependant, la contamination du lait avec des résidus d’antibiotiques le rend dangereux pour la consommation humaine ainsi que la perte économique pour l’industrie laitière. Par conséquent, il est nécessaire de détecter les résidus d’antibiotique dans le lait à chaque collecte. La présente étude se concentre sur deux volets, une enquête sur terrain menée auprès des vétérinaires praticiens dans le but de rendre compte de la situation de l’antibiothérapie pour les bovins laitiers et la seconde a été réalisée pour mieux comprendre la situation de la production et de la transformation laitières à Biskra. L’autre volet était pour la détection des résidus d’antibiotiques par Beta Star Combo dans le lait cru de vache. L’analyse de l’enquête par le questionnaire mené auprès de 40 vétérinaires praticiens a montré que les pathologies les plus courantes sont les infections respiratoires (47.5%)et les mammites (35%).Ceux-ci sont souvent traités avec des antibiotiques, principalement représentés par les tétracyclines 70% et les bêta-lactames 57.5% respectivement, qui sont les antibiotiques les plus utilisé en raison de leur efficacité, de leur faible cout et disponibilité. D’autre part, nous avons constaté que les délais d’attente après l’administration d’antibiotiques sont souvent non respectés par les éleveurs. L’analyse de la deuxième enquête par le questionnaire mené des usines laitières, nous a permis de constater que la production de lait à Biskra est principalement basée sur le lait en poudre. La production locale de lait est représentée avec des quantités minimales. A cela s’ajoute l’existence de problèmes de transformation qui entrainent des pertes de production importantes, notamment en raison de l’absence de plan de traçabilité et de tests de dépistage des résidus des antibiotiques. Des échantions de lait crue ont été analysés pour la détection de beta-lactamine et de tétracycline à l’aide de beta Star combo, les résultats ont montré que : sur un total de trente échantillon ; huit (08) étaient positifs avec un taux de 26.66% ; dont 62.5% étaient des échantillons positifs pour le beta- lactamine et 25% pour le tétracycline et 12.5% pour un échantillon contenant à la fois des résidus de beta- lactamine et de tétracycline. 22 échantillons entaient négatifs avec un taux de 73.33% Par conséquent, il est obligatoire que des réglementations sérieuses soient mises en œuvre pour améliorer la qualité du lait.

Mots clés : résidus des antibiotiques, Lait crue de vache, inhibiteurs, Beta Star Combo.