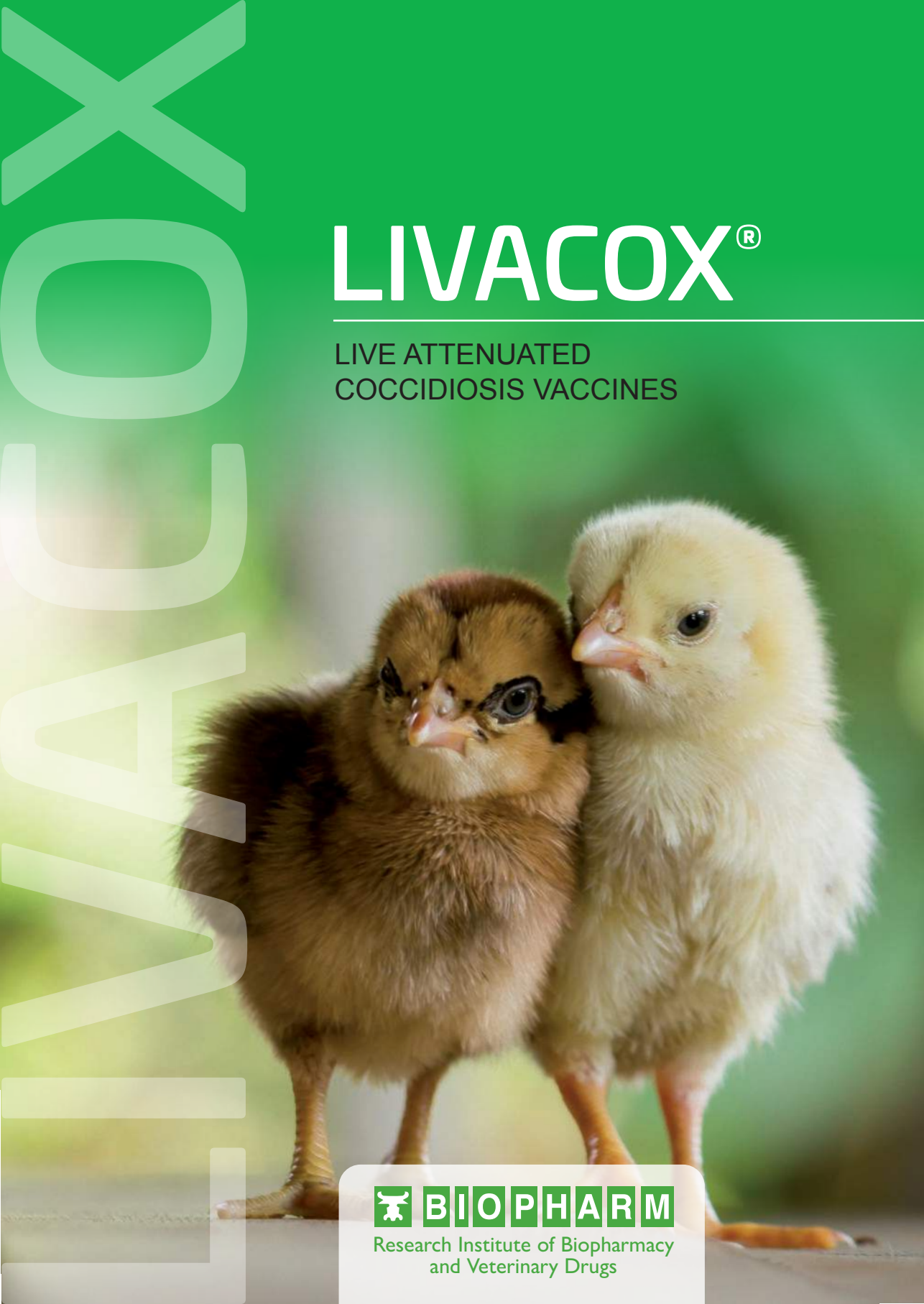
LIVACOX®

LIVE ATTENUATED
COCCIDIOSIS VACCINES



BIOPHARM
Research Institute of Biopharmacy
and Veterinary Drugs

Contents

Coccidiosis in Chickens

Coccidiosis is an infection affecting invertebrates, birds and mammals. The disease is caused by *coccidia* – microscopic, single-celled, obligate intracellular parasites. *Coccidia (syn. Coccidiasina)* belong to the *Conoidasida* class, *Apicomplexa* phylum and *Alveolata* kingdom.

Coccidia are host-specific, it means that coccidian species infecting chickens do not affect turkeys, ducks, or any other animals as their potential hosts.

Chicken coccidiosis is caused by coccidia of the genus *Eimeria*. It is manifested by inflammation to different degrees of the intestinal mucosa, which results in decreased performance to death of affected birds. Symptoms such as drowsiness, apathy, ruffled feathers, reduced feed intake or bloody diarrhoea sign the possible occurrence of coccidiosis.

Severity of the disease depends on many factors: the host (age, immunity), parasite characteristics (species, pathogenicity, reproductive potential, localisation of its developmental stages in the intestine), as well as on the farm management (zoo-hygienic conditions).

In the **clinical form of coccidiosis**, considerable damage to the intestinal tissue, bleeding, diarrhoea, dehydration, and high mortality can be observed in affected chickens. **Subclinical coccidiosis** is characterised by reduced feed intake and impaired nutrients uptake, decreased growth rate, and temporary reduction in eggs laying.

Coccidia are ubiquitous in chicken environment, their final developmental stage – oocysts are mostly disseminated by feces, litter, dust, attending personnel, birds, rodents, insects, etc.

There are seven species of coccidia belonging to genus *Eimeria* known to infect chickens (*Gallus gallus domesticus*), namely: *Eimeria acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, and *E. tenella*. Significant differences exist among them in terms of their properties and pathogenicity, thence they also differ in their economic importance in poultry farming.

TAB. 1 *– Pathogenicity and economic importance of chicken (Gallus gallus domesticus) coccidian species*

Species	Pathogenicity	Economic importance
E. praecox (Johnson, 1930)	0	*Non-significant*
E. mitis (Tyzzer, 1929)	+	*Non-significant*
E. acervulina (Tyzzer, 1929)	++	***Significant*** *– causes worsened feed conversion and retarded growth*
E. brunetti (Levine, 1942)	+++	*Non-significant – rare, local occurence; can cause mortality*
E. maxima (Tyzzer, 1929)	+++	***Significant*** *– causes mortality*
E. tenella (Reilliet & Lucet, 1891)	++++	***Significant*** *– causes mortality*
E. necatrix (Johnson, 1930)	++++	***Significant*** *– causes mortality in chickens older than 9 weeks*

Etiology and Epidemiology

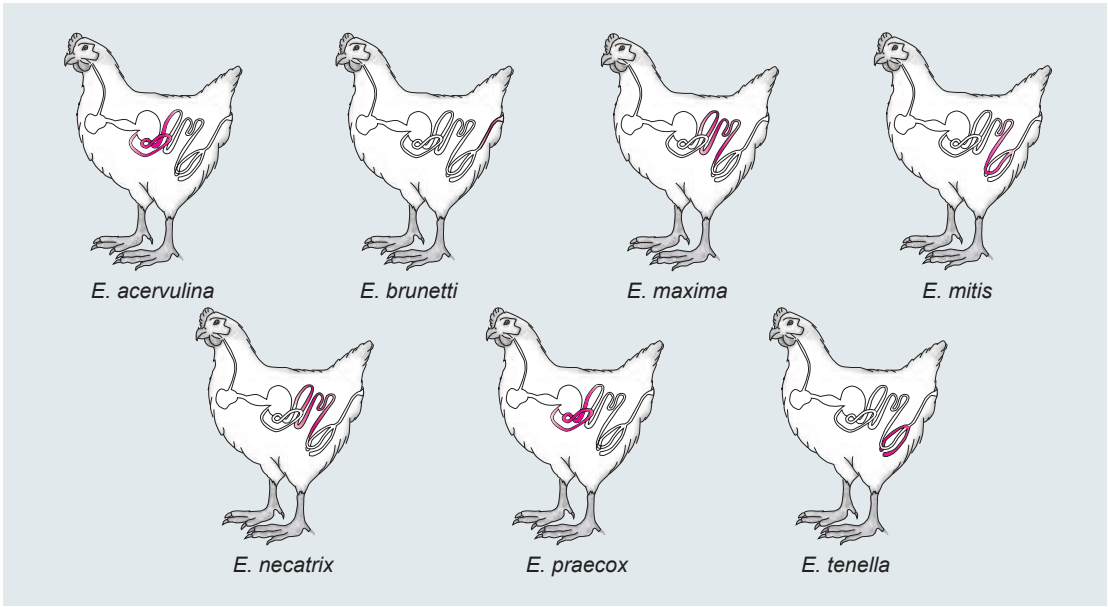
Individual species of *Eimeria* can be distinguished on the basis of the structure, morphology, size and shape of oocysts, and the localisation in individual organs or their parts (see Fig. 1). *Eimeria* species are host and tissue specific.

Only some of the chicken coccidia are considered to be economically significant in poultry farming world-wide (see Tab. 1). In broilers, breeders and layers, the most common cause of coccidiosis infection is *E. acervulina, E. maxima*, and *E. tenella*. *E. necatrix* is mostly diagnosed in chickens older than 9 weeks and therefore, is a threat to layers and breeders, and to broilers fattened longer than 9 weeks.

LIFE CYCLE

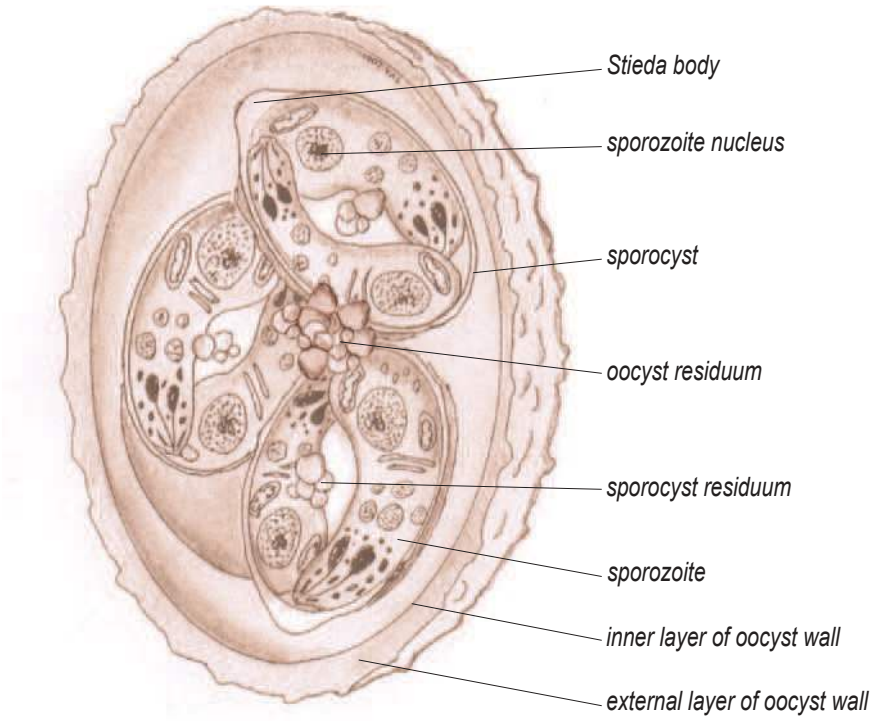
Infection starts by ingestion of infectious (sporulated) coccidian oocysts (see Fig. 2) from which sporozoites are released into the host´s digestive tract and penetrate the epithelial cells of the intestinal walls. Asexual (schizogony) and sexual (gametogony) reproduction follow within cells

FIG. 1 **–** ***Intestine localisation of chicken coccidia***



of the intestinal walls of different parts of the intestine in dependence on the coccidian species. Final phases of the cycle are excretion of unsporulated oocysts *via* feces into the environment and their maturation (sporogony) into sporulated oocysts capable of next infection (see Figs. 3 & 5).

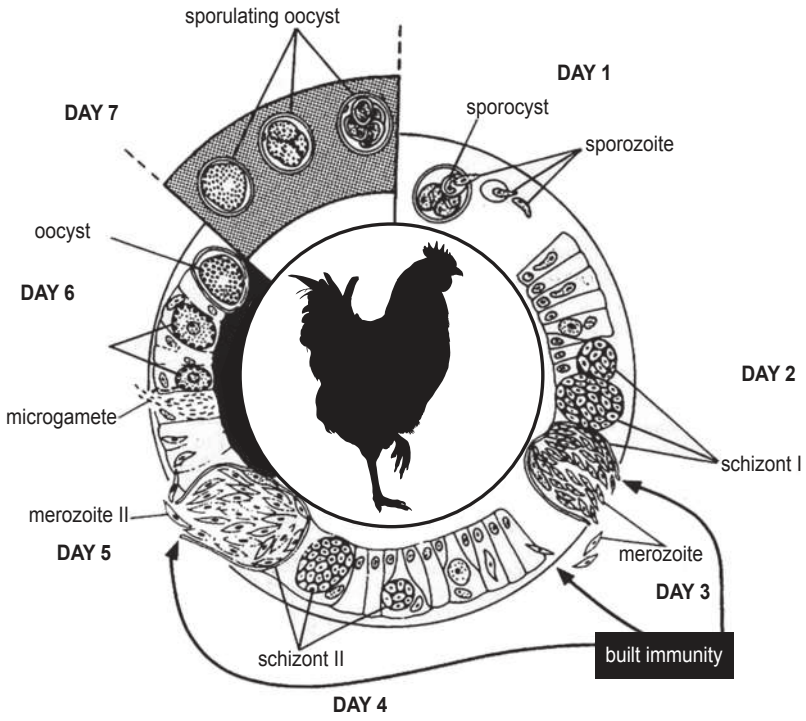
FIG. 2 **–** *Eimeria spp. sporulated oocyst (Levine, 1961)*



The minimum time required for sporulation of oocysts (Fig. 4) differs for each *Eimeria* species and ranges from 15 to 30 hours. The factors affecting the sporulation process are presence of oxygen, appropriate temperature and humidity of the environment. The high moisture content in the litter has a negative effect on the oocyst sporulation process due to the low oxygen content and higher amount of ammonia present as a metabolite of unwanted bacteria for whose growth and reproduction the wet litter is a favourable environment. Ammonia is one of few substances effective against coccidian oocysts.

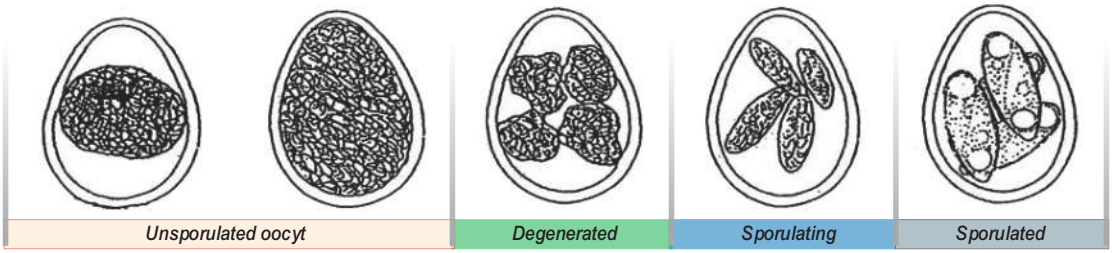
For the optimal course of the oocyst sporulation process the temperature of +29 ± 1 °C was determined under experimental conditions. In the field conditions, this process is influenced by a number of other factors.

FIG. 3 **–** *Eimeria tenella life cycle (Hammond, 1973)*



Coccidian oocysts are very resistant. Because of their high propagation potential and special composition of the cell wall they can survive for long time even in extreme environmental conditions. The maximum temperature survival limit for coccidian oocysts was determined +55 °C under experimental conditions.

FIG. 4 **–** *Process of exogenous Eimeria spp. oocyst sporulation (McDougald & Reid, 1997)*



BIOLOGICAL CHARACTERISTICS OF COCCIDIA

Prepatent Period

Prepatent period defines the endogenous phase of coccidian life cycle, it means the time from the sporulated oocysts ingestion by the host till the new oocysts production (excretion). This period ranges from 4 to 7 days. The minimum is 83 to 138 hours in dependence on the coccidian species (see Tab. 2).

TAB. 2 – The duration of the prepatent period and reproductive potential of chicken coccidia

Species	Minimal prepatent period (hours)	Reproductive potential (oocysts/bird x10^6)
E. acervulina	97	1.14
E. brunetti	120	0.38
E. maxima	121	0.23
E. mitis	93	0.82
E. necatrix	138	0.24
E. praecox	83	0.27
E. tenella	115	0.25

Reproductive potential

Reproductive potential is a very important parameter from a practical point of view, pointing out the extraordinary reproductive ability of the coccidia (see Tab. 2).

Reproductive potential expresses the amount of oocysts produced during one life cycle after the infection with only one oocyst. Individual coccidian species have very different ability of reproduction.

Chicken (*Gallus gallus domesticus*) coccidia are:

- host specific;
- tissue specific;
- their life cycle consists of asexual and sexual phases;
- their patogenicity depends on the localisation of their developing stages in the intestinal epithelium.

Immunity Against Coccidia in Chickens

Coccidiosis remains one of the most common and economically most important diseases in poultry production. Over the last 50 years, scientists were engaged in the complex immunological mechanism ensuring destruction of these parasites. Initially, they tried to fight against coccidia by stimulation of the humoral component of the immune system by vaccination with antigens stimulating antibody formation. However, the variability of the antigens of the individual developmental stages of coccidia complicated the identification of a specific antigen for the development of such a vaccine. At the same time, the research with a focus on the cellular immunity was ongoing. This type of immune response proved to be much more effective against coccidia. Direct contact between the host intestinal cells and the parasite is the necessary key for effective activation of the immune response.

Vaccination is the most natural way to protect chickens against coccidiosis. Currently, two types of vaccines against coccidiosis in chickens, **non-attenuated** and **attenuated** (for details see p. 16) are available on the market.

MODE OF ACTION OF LIVE ATTENUATED VACCINES

Immunity against coccidiosis in chickens is developed by stimulation with live oocysts of coccidian species contained in the vaccine. During the mild infection, a specific immune response is triggered and immunocompetent cells are multiplied. Immunity against coccidiosis is cell-mediated, antibodies play only a minor role.

To develop sufficient immunity, the chickens must be exposed to coccidian infection at least twice. The first infection is initiated by the vaccine itself. After completion of the first life cycle (4 – 7 days depending on the coccidian species), oocysts are produced and excreted in feces. These oocysts are the source for the second infection. After accomplishment of 2 – 3 life cycles, chickens become fully immunised.

It is important to vaccinate chickens as soon as possible to enable early colonisation of the poultry farm with attenuated vaccinal coccidian lines and to ensure that chickens have access to their feces (litter) for two weeks after the vaccination at least.

BENEFITS OF THE CONTINUOUS MULTIPLICATION OF VACCINAL *EIMERIA* LINES IN THE ENVIRONMENT

- Chickens are continuously "revaccinated" by oocysts, which serve as a permanent antigenic stimulus.
- Chickens not vaccinated with the correct dose of the vaccine are immunised by ingestion of oocysts produced by properly vaccinated chickens. (The risk occurs only with non-attenuated vaccines, when large amounts of virulent oocysts could cause coccidiosis outbreak.)
- Attenuated and to anticoccidials sensitive lines of *Eimeria* displace wild coccidia resistant to anticoccidials in the poultry house environment.

PROPER LITTER HANDLING

For the aforementioned revaccination mechanism it is necessary to ensure appropriate properties of the litter and correct handling of it. Chickens are in continuous and close contact with the litter, its quality is as important as correct temperature, light regimen, ventilation, water or feed quality. As has been already mentioned, the chickens must be allowed to access their feces (litter) after vaccination. Improper handling of the litter can result in limited access of chickens to the oocysts contained in it, what may adversely affect (postpone) the development of their immunity against coccidiosis. Chickens should have sufficient space to move, however, too much space is also undesirable, because the probability of the chicken contact with the oocysts in the litter is reduced.

An important factor affecting the process of oocysts sporulation is oxygen presence. Too wet litter with low oxygen content can negatively affect the sporulation of oocysts. The result is slow propagation of coccidian vaccinal strains and, as consequence, delayed immunity development. In areas with high relative humidity it is therefore important to keep optimum stocking density in the poultry house, above all during the first three weeks after vaccination, so that the moisture content in litter is not too high.

LITTER RECYCLING

In some countries, particularly in the South America, it is common to reuse the litter from the previous broiler fattening. With the continuous use of LIVACOX®, this practice is from the theoretical point of view useful, because attenuated strains of coccidia occur in the litter. However, in case of previous use of either non-attenuated vaccine or anticoccidials, the efficacy of LIVACOX® vaccination may be significantly reduced. Another risk of litter recycling is presence of unwanted microorganisms in the litter that may negatively affect animal health. To limit these negative factors

to the minimum, sanitation should be done before reuse of the litter. The most often used way of litter sanitation is fermentation, when during a few days without the presence of oxygen, sufficiently high temperatures reduce the microbiological and parasitic burden of the litter. Taking into account all the risks of litter recycling, it is important to house under such zootechnical conditions only chickens already vaccinated with LIVACOX®. **If you consider litter recycling, it is good idea to discuss the risks with the LIVACOX distributor in advance.**

CAGE REARING

During the last 10 years, there has been an essential change in the cage system of poultry breeding, e.g. in Europe. First and foremost, the egg-laying hens´ farmers have had to spend a considerable amount of money to change their housing technology to comply with the legislation. Outside Europe and North America, intensive cage breeding is the most common and coccidiosis still remains the problem of the day there. Nevertheless, the risk of coccidiosis outbreak in cages is lower than in the floor system of housing. The reason is limited contact of chickens with their feces. Coccidiosis can occur here with non-vaccinated poultry, it can arise from various sources (insect, dust, personnel). The chickens from the upper cage floors contaminate the birds housed on the lower floors. Another important source of coccidia contamination are the automatic manure conveyors.

As already mentioned, to develop sufficient permanent immunity against coccidiosis chickens should be provided with a permanent antigenic stimulus, i.e. coccidian oocysts. In the floor system of housing the chickens have permanent contact with their feces. However, in the cage system their access to their droppings is limited. It is therefore necessary to solve this “revaccination” problem, taking into account the specific conditions of cage rearing.

Control of Coccidiosis

ANTICOCCIDIAL MEDICATION

Anticoccidials have been successfully used since the late 1940s. Until the 1980s, they were the main means of coccidiosis control. Continuous application and mistakes in mixing anticoccidials with feed resulted in their use in incorrect concentrations. This led to the development of resistance of the parasite to most anticoccidial drugs available on the market. Negative effects of some anticoccidials on eggs laying and hatchability, their toxicity for some animals, and their residues in meat and eggs are all well-known facts that gradually led to the restriction or prohibition of some anticoccidials in many countries (e.g. in the European Union).

Anticoccidial drugs can be classified in two categories either according to their origin: **chemicals** (halofuginone, nicarbazin, diclazuril) and **ionophores** (monensin, lasalocid, salinomycin, narasin), or according to their mode of action: **coccidiocidal** (destroy the coccidia population) and **coccidiostatic** (prevent the replication and growth of coccidia population). To be effective, anticcodials must be administered throughout the whole fattening (rearing, breeding).

As a tool for minimizing the development of coccidia resistance to anticoccidials, special programs have been developed:

1. **Shuttle program** – application of several anticoccidials with different modes of action during single grow-out;
2. **Rotation program** – alternation of anticoccidials with different modes of action after 6 to 12 months of use.

Consequently, the need to move away from chemotherapeutics led to the development of coccidiosis vaccines as a natural biological means of coccidiosis control. This is the way how to avoid the shortcomings experienced when using anticoccidial drugs.

VACCINATION

Vaccination is the most natural and ecological approach to prophylaxis that gives chickens life-long immunity against coccidiosis. Vaccines do not leave residues in meat and eggs and are safe for humans, other animal species and the environment.

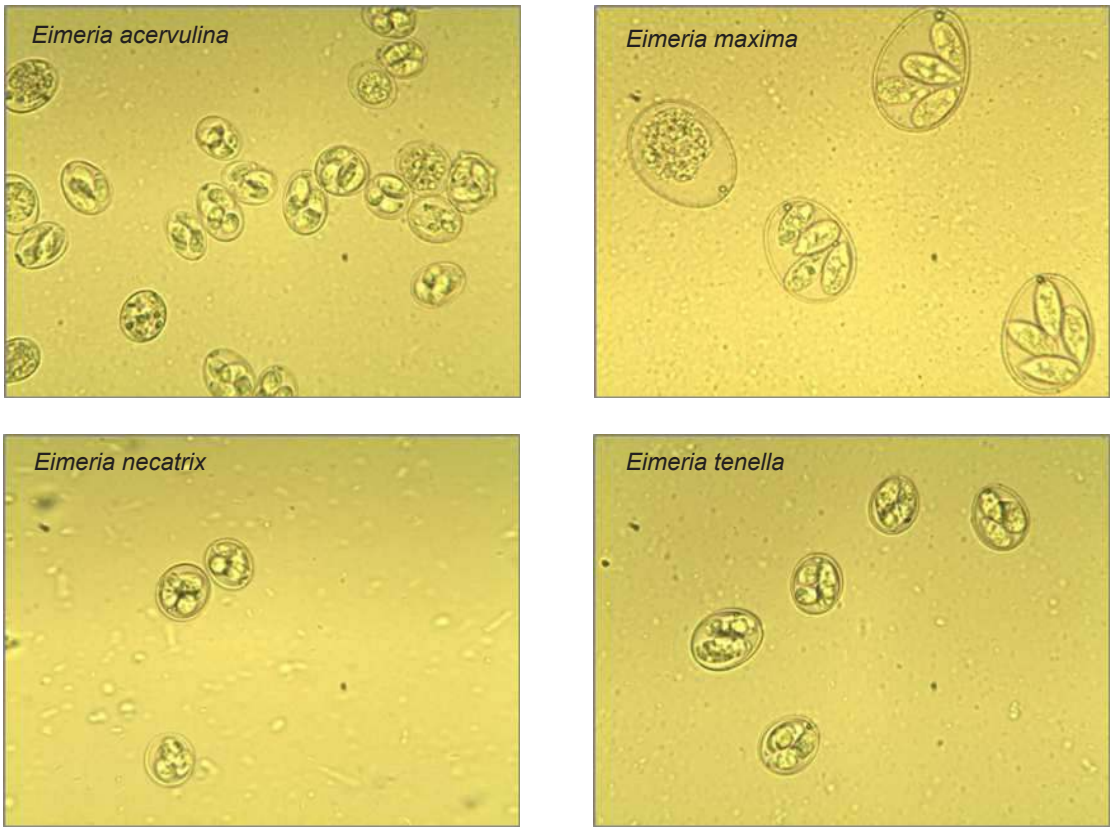
There are two types of vaccines available on the market, namely: non-attenuated (virulent) and attenuated.

Non-attenuated (virulent) vaccines contain live, virulent coccidian oocysts, hence in addition to field virulent coccidia spread in the poultry house the chickens are exposed to the fully pathogenic parasites from the vaccine. Therefore, when using these vaccines, there is a risk of strong post-vaccination reaction and clinical coccidiosis outbreak, especially in birds with compromised immunity. After administration of virulent vaccines, preventive treatment with anticoccidials is often necessary and even recommended by the manufacturers of these vaccines.

Attenuated vaccines contain live oocysts of attenuated lines of *Eimeria* species. These vaccines are much safer due to the biologically attenuated pathogenicity of the parasites. Their controlled infectious challenge minimalizes risk of coccidiosis outbreak.

LIVACOX® vaccines belong to the group of attenuated vaccines. They protect chickens against the most economically significant *Eimeria* species.

FIG. 5 **–** *Oocysts of economically important chicken coccidian species*



LIVACOX® – Live Attenuated Coccidiosis Vaccines

LIVACOX® vaccines are live attenuated coccidiosis vaccines for chickens (*Gallus gallus domesticus*). The vaccines were developed in BIOPHARM – Research Institute of Biopharmacy and Veterinary Drugs, Czech Republic, and belong to the first attenuated vaccines against coccidiosis in poultry launched on the world market.

LIVACOX® vaccines are produced in accordance with the strict requirements as stipulated by the Good Manufacturing Practice (GMP) rules for the production of immunological preparations and medicaments. Oocysts of LIVACOX® vaccines are propagated in SPF-chickens. All batches of the vaccines are controlled for efficacy, safety, and absence of pathogenic microorganisms (bacteria, viruses, mycoplasmas) before their release onto the market.

LIVACOX® vaccines are successfully used in more than 50 countries worldwide since 1990s, meeting all requirements of poultry welfare, protection of human and animal health as well as of the environment. No residues are in the final poultry products (meat and eggs) after the vaccination, which is beneficial for the organic production and healthy food.

Two types of LIVACOX® vaccines are available:

LIVACOX® T is a trivalent live attenuated coccidiosis vaccine for broilers (see p. 18).

LIVACOX® Q is a quadrivalent live attenuated coccidiosis vaccine for breeders and layers and long-term fattening broilers raised over 9 weeks (see p. 19).

Although *Eimeria* lines contained in these vaccines are attenuated, they are sufficient producers of oocysts necessary for good immune response stimulation. Their propagation potential is efficient enough even for the cage technology rearing.

LIVACOX® T

1 mL of **LIVACOX® T** (= 100 doses) contains **30,000 – 50,000 live sporulated oocysts** of each attenuated lines of *Eimeria acervulina*, *E. maxima,* and *E. tenella* in 1 % w/v water solution of Chloramine B.

1 dose of LIVACOX® T provides 300 – 500 oocysts of each of the afore-mentioned species per chicken.

LIVACOX® Q

1 mL of **LIVACOX® Q** (= 100 doses) contains **30,000 – 50,000 live sporulated oocysts** of each attenuated lines of *Eimeria acervulina*, *E. maxima,* and *E. tenella* and **10,000 live sporulated oocysts** of attenuated line of *E. necatrix* in 1 % w/v water solution of **Chloramine B**.

1 dose of LIVACOX® Q provides 300 – 500 oocysts of each species of *E. acervulina*, *E. maxima,* and *E. tenella* and 100 oocysts of *E. necatrix* per chicken.

MODE OF ACTION

The mode of action of LIVACOX® vaccines is completely natural. The vaccines work on the principle of controlled infection of chickens with live sporulated oocysts of economically significant and to anticoccidial drugs sensitive coccidian species. During mild infection – parasitaemia, specific immune response is triggered and full immunity built during 2nd to 3rd coccidia life cycles. Gained immunity is then maintained through continuous revaccination (boosting) with vaccinal oocysts present in the poultry house environment.

Clinical coccidiosis in chickens usually occurs at the age of 21 – 28 days, hence vaccination of young chickens as early as possible is strongly recommended.

PATHOGENICITY ATTENUATION

Two methods of attenuation were used, namely: selection for precociousness (artificial abbreviation of the life cycle) in chickens and long-term passaging in chicken embryos (*E. tenella*). Pathological-anatomical changes in the chicken intestinal walls, as proved in the safety tests carried out with a 10-times LIVACOX® vaccines overdose challenge, were mild to none.

EXPIRY TIME

9 months from the date of manufacture (see the ***Expiry Date*** on the bottle). Use immediately after dilution. Do not use after the expiry date.

WITHDRAWAL PERIOD

Zero days.

STORAGE

Store the vaccine in a dark and dry place between +2 and +8 °C. **Do not freeze!** Do not allow contact with freezing unit of the refrigerator. Temperature of the vaccine even during the transport must be maintained between +2 and +8 °C. Keep out of the reach of children! Used or partially used containers should be disposed according to the local requirements.

CONTRAINDICATIONS

Do not use with feed or water containing anticoccidials, sulphonamides, or any other agent with anticoccidial activity. Feed and water provided to the birds 2 days before the vaccination and during the first 14 days after the vaccination **must be free of anticoccidials and sulphonamides**.

PRECAUTIONS

- Vaccinate only healthy chickens kept under good management conditions;
- Dilute the vaccine with fresh drinking water only;
- Shake bottle thoroughly before opening and mixing with water;
- To generate a booster antigen, birds must have access to litter for a minimum of 14 days after the vaccination. The droppings of the birds serve as a reservoir of coccidia oocysts for “automatic revaccination”;
- Avoid or reduce the stress conditions during two weeks after the vaccination;
- In the case of any mortality, chicken carcasses sent to diagnostic laboratory for *post-mortem* examination must be marked **LIVACOX vaccinated** !

Methods of Administration of LIVACOX® Vaccines

LIVACOX® vaccines are peroral vaccines; there are various routes of their administration:

1. *Via* drinking water;
2. By coarse spraying on chickens;
3. By coarse spraying on feed;
4. Individually into the crop.

GENERAL PRECAUTIONS

- Make sure that the vaccine is kept in a refrigerator between +2 and +8 ºC. If the vaccine was frozen, do not use it.
- Check and record the date of expiration. If expired, don't use it.
- Prepare and clean all equipment needed for the vaccination: buckets, graduated flasks, syringes, etc.
- Calculate doses of the vaccine and the required amount of water for vaccination.
- Before opening the vaccine bottles always shake them thoroughly. Rinse the bottles to ensure emptying all the vaccine into the water.
- Only healthy birds should be vaccinated.
- Supervise correct course of the vaccination.

1. Administration *via* drinking water

A single vaccine dose is given to chickens 1–10 days old. The earliest possible vaccination is recommended (preferably on 3rd day of age). It can be performed even earlier (on 2nd day), but it is necessary to pay attention to the low water consumption of young chickens and to adjust the vaccine dosage accordingly. LIVACOX® vaccines can be administered either *via* fountain and bell drinkers or *via* central watering tank connected to automatic waterers including nipple drinkers.

Procedure

1. Perform the vaccination during the morning hours, when birds are more active.
2. Provoke thirst in birds by discontinuing water supply for approx. 2 hours before the vaccine application, depending on the environmental conditions, especially the ambient temperature.
3. Before use, shake always the vaccine thoroughly and rinse the bottles with the water intended for dilution to ensure emptying all the vaccine volume. Mix the vaccine and water properly in dependence on the way of administration (fully diluted, if fountain drinkers are used, or pre-diluted, if dosing equipment is used).
4. Administer LIVACOX® vaccines in such amount of drinking water to ensure all the chickens receive full vaccine dose. Therefore, the medicated water should be drunk within approx. 2 hours, not in less than 1 hour and not in more than 3 hours, because of the risk of vaccinal oocysts sedimentation. Actual water consumption is influenced by age, sex and breed of the chickens, local environmental conditions; customers should consider field experience and requirements for vaccination under local conditions.
5. Check the consumption of medicated water. About 1 hour after application of the vaccine, slowly walk through the poultry hall and rouse resting chickens to ensure that all the chickens drink the medicated water. For visual control of the number of vaccinated chickens, an approved food dye or a chlorine neutraliser containing colourant may be added to the water before mixing it with the vaccine. In the case of a drinking system with dosing equipment, it is recommended to stir the pre-diluted vaccine several times during vaccination (approximately after every 15 minutes).

6. If the poultry house with the central drinking system is split into areas with various stocking density, use additional drinkers during the vaccination if necessary.
7. When all medicated water is consumed, the drinking water supply can be restarted.

Example: For vaccination of 10,000 four-day-old chickens, the vaccine is to be administered in 40 L (litres) of drinking medicated water within approx. 2 hours. The required 10,000 vaccine doses are contained in 100 mL of the vaccine. These 100 mL of the vaccine should be diluted with 39.9 L of fresh drinking water therefore, the total volume of the medicated water will be 40 L.

If dosing devices are used, the vaccine shall be first pre-diluted and then added proportionally to drinking water with the dosing machine. For vaccination of 10,000 four-day-old chickens, the vaccine is to be administered in 40 L of drinking water within approx. 2 hours. 10,000 vaccination doses are contained in 100 mL of the vaccine. These 100 mL should be prediluted to 400 mL to be further diluted by the dosing equipment in ratio 1 : 99 (final volume of medicated water is 40 L).

2. Administration by coarse spraying on chickens

2.1. Spraying chickens in hatcheries

LIVACOX® vaccines can be used not only *via* drinking water, but also *via* the spraying device. Considering that LIVACOX® vaccines are oral vaccines, the aim of using the spray cabinet for their administration is to enable chickens to swallow the vaccine and deliver it to the gastrointestinal tract.

This administration method is very effective when performed properly. One-day-old chickens are vaccinated directly in hatcheries. The vaccine is diluted with drinking water containing approved food dye for optical control of the application (to be sure that all chickens are sprayed) and for better visibility of the vaccine droplets to chickens. The vaccine should be applied by a coarse spray (the size of droplets at least 200 μm) so that the vaccine droplets will be visible to chickens on their feathers. After the vaccination, it is necessary to keep the chickens for about 2 hours in the crates to have enough time to vaccinate themselves by preening and pecking.

Dilution

The total volume of the medicated solution (containing a food dye to enable visual control of the application) to be sprayed (1 crate = 100 chickens) is **20 mL**, i.e. the vaccination doses for **100 chickens** in one crate are contained in 20 mL of the solution (1 mL vaccine = 100 doses + 19 mL of drinking water with dye). The chickens in every crate should be sprayed with the volume of 20 mL of medicated solution even if the crate contains less than 100 chickens.

Homogeneity

Before opening the vaccine bottle always shake it thoroughly and rinse the bottle to ensure emptying all the vaccine into the water. To avoid sedimentation of the vaccinal oocysts in the solution container during spraying, use laboratory magnetic stirrer or any other device that ensures continuous homogeneity of the vaccine solution during the course of vaccination.

Spray machine

Consult your spray machine selection with the vaccine manufacturer!

Generally, most spray machines are designed for multipurpose spraying (application of different types of vaccines, like ND, IB, coccidiosis vaccines, etc.). Therefore, any of the spray machines can also be used for application of LIVACOX®, provided a nozzle of an appropriate size is fitted to the machine in order to enable coarse spraying (droplet size larger than 200 μm). Nozzles of different sizes can be obtained from the manufacturers of spray machines and they usually specify

suitability of each nozzle size for the purpose of use. For the coccidiosis vaccine, orange nozzles are recommended. With these nozzles, air pressure 3 bar (44 psi) creates droplets of approximate size 260 µm. Air pressure 3.5 bar (51 psi) provides better spreading over the crate.

Note: This procedure provides general rules for the use of a spray machine for administration of LIVACOX® vaccines in hatcheries. Ask the manufacturer of your spray machine for further specific instructions!

Warning: Please note, that due to the presence of Chloramine B in LIVACOX® vaccines it is strongly advised NOT to carry out two or more different vaccination programs in close succession without washing the spray machine with a chlorine neutralising agent. Other live vaccines are not to be administered in the same solution together with LIVACOX® vaccine.

2.2. Spraying chickens on farms

LIVACOX® vaccines can also be applied by coarse spraying even on farms before chickens are placed in the poultry house. The aforementioned procedure for the vaccine handling is the same for spraying on farms. Considering that hand spraying devices are mostly used for the vaccine application on farms, it is necessary to focus on the uniformity of the vaccine application and the prevention of the vaccine oocysts sedimentation during spraying.

3. Administration by coarse spraying on feed

Another way of administration of LIVACOX® vaccines is their application by coarse spray on feed in the poultry houses.

Procedure:

1. Spread recommended quantity of milled granulated feed (with min. 80 % of particles larger than 1 mm) onto a surface of 30 – 70 cm^2 so as to achieve the optimal layer of 0.75 – 1.00 cm of the feed.
2. Shake the vaccine well before use, dilute the vaccine with drinking water in the ratio of 1 : 50 and evenly spray on the feed (0.5 mL of diluted vaccine = 1 vaccinal dose). Volume ratio of the diluted vaccine *vs.* feed should range between 1 : 50 – 1 : 100.
3. Pressure spray equipment with calibrated nozzle and hollow spray cone is recommended for the application of the vaccine. The spray pressure in the tank is to be set at 2 bars (29 psi), and spraying on the feed should be carried out such that the spray covers the entire width of the feed layer evenly.
4. Shake the tank with the medicated solution periodically to prevent vaccinal oocysts sedimentation.

5. Ensure the chickens the access to the medicated feed immediately after the vaccine application on it. Chickens should consume the medicated feed within 24 hours.
6. The ambient temperature should not exceed +32 °C during spraying.
7. Consult your spray machine selection with the vaccine manufacturer!

4. Vaccination into the crop

This administration method is more time-consuming and requires more precision. Nevertheless, if the application is done correctly, it ensures uniform vaccination of the whole flock.

Procedure

1. Use calibrated pipette and follow manufacturer's directions for precise use.
2. Dilute the vaccine with drinking water in ratio 1 : 19. Dosage of medicated solution after dilution is 0.2 mL per chicken.

   Example: 1 mL of vaccine LIVACOX® (vaccine volume for vaccination of 100 chickens) is diluted with 19 mL of drinking water and mixed properly (total 20 mL of medicated solution for 100 chickens). The dose of vaccine solution per one chicken is 0.2 mL.
3. Shake the vaccine well before dilution and then again just before drawing it into the pipette and application to the chicken.
4. Always draw the dose into the pipette from the centre of the vaccine solution.
5. Administer the dose from the pipette into the **crop** of the chicken (not only into the mouth) to be sure that the chicken takes the vaccine.

Frequently Asked Questions

1. What are the differences among vaccines against coccidiosis?

Two types of commercial vaccines are available on the world market:

1. **Non-attenuated** (virulent) vaccines – contain live virulent strains of coccidia;
2. **Attenuated** vaccines – contain live attenuated lines of coccidia (e.g. LIVACOX® T and LIVACOX® Q).

Both types of vaccines differ in their biological properties as well as in their composition. Non-attenuated vaccines can even cause coccidiosis, if stress conditions compromise chickens´ immune system. Coccidiosis outbreak at the end of fattening has usually significant impact on profitability, because there is no time left for the growth compensation. Non-attenuated vaccines are therefore used more often in breeders than in broilers. The use of non-attenuated coccidiosis vaccines is not allowed in the EU.

2. Why LIVACOX® vaccines do not contain all seven chicken coccidian species?

LIVACOX® vaccines contain **only economically significant coccidian species**. Other species – economically non-significant – are not contained in these vaccines to prevent colonisation of poultry farms by other unwanted coccidian species and to maintain the reasonable prices of the vaccines.

3. What is the optimal age of chickens for LIVACOX® vaccination via drinking water?

LIVACOX® vaccination *via* drinking water is usually recommended starting the 3rd day of chicken age, however, vaccination can be performed even on 2nd day. It is necessary to pay attention to the low water consumption of young chickens and carefully adjust the vaccine dosage accordingly. In no case is it recommended to prolong the prescribed duration of vaccine intake because of the risk of vaccinal oocysts sedimentation (see p. 24).

4. What is the benefit of LIVACOX® vaccines administration by coarse spray in hatcheries?

Early vaccination of one-day-old chickens is used in hatcheries that prefer supplying farmers with already vaccinated chickens. It is also recommended for farms where broilers are raised on built-up litter, or where young chickens are often immune-compromised by MDV, IBDV, CAV, mycotoxins in feed, etc.

By means of early vaccination, the poultry house is populated with attenuated coccidian lines from the very beginning and hence the multiplication of the virulent field coccidian strains can be suppressed.

5. Broilers are usually slaughtered at 5 weeks of age. During such a short life span, can actually good immunity develop to protect broilers against coccidiosis?

Yes, after accomplishment of 2–3 life cycles of coccidia (2–3 weeks) sufficient immunity against coccidiosis is developed. If the vaccination with LIVACOX® vaccines is performed early and properly, there is no risk of clinical coccidiosis outbreak.

6. What is the risk of coccidiosis outbreak, if some chickens in the flock do not receive the initial dose of LIVACOX® vaccine?

The risk is very low with LIVACOX® vaccination, because such chickens would pick up from the litter vaccinal oocysts produced by properly vaccinated chickens and develop immunity against coccidiosis.

7. What is the risk of coccidiosis outbreak during the first 14 – 21 days of chicken age until the immunity is developed?

The multiplication of the virulent field coccidia in the flock starts during the first week of the chicken life. Production of such amount of oocyst able to cause severe infection takes at least three development cycles, i.e. at least 2–3 weeks. Therefore, clinical coccidiosis does not usually occur before the age of 3 weeks.

Prior delivery of new chickens, poultry houses should be thoroughly cleaned and disinfected. The amount of field coccidia oocysts surviving these procedures is usually very low at the beginning of a new chicken grow-out cycle. After administration of LIVACOX® vaccine, the attenuated coccidian lines multiple and the number of their oocysts becomes high enough to depress the multiplication of the virulent field coccidia. The probability of ingestion oocysts of attenuated coccidia from the litter is thence substantially higher than oocysts of virulent ones.

8. Is it necessary to vaccinate caged chickens?

Cage system technology greatly reduces the possibility of circulation of coccidian oocysts, but does not completely prevent it. That is why coccidiosis can occur even with this technology, especially after manure belt conveyors failures. Vaccination is safe measure against such accidents.

9. Chickens protection against coccidiosis depends on their immunity. What happens, when change in the immune status during the grow-out occurs?

Following stress or disease, the immunity against coccidiosis may be decreased temporarily. However, there is no reason for panic, if clear-cut clinical coccidiosis with high mortality is not observed. In most cases, chickens raised under good management conditions re-establish the immunity soon.

10. Is there a need to use anticoccidial drugs with LIVACOX® vaccination?

No, it is crucial to avoid it.

11. What to do, when, by some mistake, chickens got feed with anticoccidial drugs after vaccination with LIVACOX®?

Development of chicken immunity against coccidiosis is based on the propagation of sufficient amounts of attenuated coccidian oocysts, whose maturation is significantly affected by presence of any anticoccidials. If chickens are found to be fed with anticoccidials during the immunity development period (at least 14 days after the vaccination), it is necessary to switch to use of anticoccidials in feed.

12. What can negatively affect LIVACOX® vaccination?

1. Presence of anticoccidials in feed. Anticoccidials prevent multiplication of attenuated lines of coccidia, what is essential for the development of proper immunity against coccidiosis.
2. Severe chicken disease at the time of vaccination (MDV) or strongly stressed immune system (high ambient temperature, lack of water, space stress, mycotoxins in feed).
3. Improper storage and handling of the vaccine, improper administration, incorrect dosage.
4. Poor hygiene and sanitation, short down time between successive grow-outs.
5. Poor quality of chickens.

13. Is it possible to use LIVACOX® vaccines in chickens raised on built-up litter?

The decision depends on the situation of the farm:

1. **Yes**, if LIVACOX® vaccines were administered to the chickens of the previous cycles and the vaccination was performed as early as possible, optimally on the first day of age in the hatchery.
2. **No**, if anticoccidials or **non-attenuated** vaccine were used for coccidiosis control in previous cycles. In such case, chickens are threatened not only by field coccidia, but also by virulent coccidia contained in such vaccines.

14. Is there any risk of reversion to pathogenicity of the attenuated lines of Eimeria species contained in LIVACOX® vaccines?

LIVACOX® vaccines are the result of many years of exhaustive research both in the laboratory and in the field. The reversion to pathogenicity has not yet been noted down. Each batch of the vaccine is thoroughly tested for efficacy, safety and microbiological safety. Even 10x vaccine overdose is proved to be safe for the chickens.

15. What type of LIVACOX® vaccine is recommended for use in slow-growing broilers?

If the fattening period is longer than 9 weeks, LIVACOX® Q is recommended. LIVACOX® Q contains, in addition to *Eimeria tenella*, *E. acervulina*, and *E. maxima* – coccidian species parasitizing in broilers, also *E. necatrix* – a species attacking chickens older than 9 weeks.

16. May probiotics be used together with LIVACOX® vaccination?

Yes. Probiotics help maintain the proper balance of the intestinal microflora of chickens, reduce the colonisation of the intestine by pathogenic microbes, and stimulate the immune system of the poultry. Performed field trials proved positive effect of the use of probiotics with LIVACOX® vaccines on the development and weight gains of chickens.

17. Do LIVACOX® vaccines affect skin pigmentation of broilers?

No. Field trials proved, that vaccination of broilers with LIVACOX® vaccines does not affect the pigmentation of the skin.

18. In broiler operation, should LIVACOX® T be used alone continuously or is there any advantage of using LIVACOX® T in rotation with anticoccidials?

LIVACOX® T used continuously alone:

LIVACOX® T can be safely recommended for continuous use in any broiler operation. It does not matter, whether the birds are slaughtered at the age of six weeks or younger, or later as older heavy broilers and roasters. However, broilers raised over 9 weeks of age should be vaccinated with LIVACOX® Q.

For the best results, always vaccinate at least two consecutive grow-outs in the same poultry house to allow the attenuated coccidia of LIVACOX® T displace the wild field coccidia there. The second and subsequent grow-outs show usually better performance than the first vaccinated grow-out or previous grow-out on anticoccidials.

LIVACOX T in rotation with anticoccidial drugs:

There is a long tradition of using anticoccidial drugs in the broiler industry. Despite many problems associated with the use of anticoccidials (e.g. resistance), their use continues. So far, the only possibility to delay the occurrence of anticoccidial-resistant coccidia are regular changes among anticoccidials with different modes of action. However, there is much better choice now: to alternate anticoccidials with LIVACOX® T.

The LIVACOX® T/anticoccidial rotation program may consist in:

- Vaccination with LIVACOX® T of 2 – 3 grow-outs followed by
- the use of anticoccidials in next 2 – 3 grow-outs followed by
- vaccination with LIVACOX® T of the next 2 – 3 grow-outs, and so on . . .

The advantages of the LIVACOX® T/anticoccidial rotation program are:

- Reduction of virulence of the field coccidian population in the broiler operation.
- Restoration of sensitivity to anticoccidials what helps overcome the resistance problem.

Field tests confirmed that vaccination with LIVACOX® T improves the overall performance of broilers even in the “anticoccidial” grow-outs of the rotation program.

19. What is the effect of high and low temperatures during transport and storage on the efficacy of LIVACOX® vaccines?

It is strictly advised to observe the stated storage conditions for LIVACOX® vaccines. Coccidian oocysts are sensitive to temperatures below the freezing point and the temperatures above +40 °C. Temperature above +50 °C is lethal for the oocysts, pay attention to the vaccine during its transport in summer!

20. Does the water pH or hard water used for LIVACOX® vaccines dilution affect the vaccine efficacy?

Water pH or water hardness do not affect the efficacy of LIVACOX® vaccines. However, the high pH of drinking water promotes growth of anaerobic bacteria such as *Clostridium*, which can lead to the outbreak of necrotic enteritis.

21. Necrotic enteritis and coccidiosis are often misdiagnosed. What are the reasons for a necrotic enteritis outbreak?

Gram-positive bacteria *Clostridium perfringens* are commonly present in the digestive tract of chickens. Under favourable conditions they can multiply and cause necrotic enteritis. Favourable

conditions for multiplication are anaerobic environment in the intestine, supported by undigested starch-rich feed, and increase of pH>7.0. The quality (mycotoxin content) and the composition of the feed significantly affect the incidence of necrotic enteritis, too. There is higher occurrence of necrotic enteritis with feed rich on wheat, compared to feeding corn-based mixtures. Also, water quality (pH should be lower than 6.0), adequate ventilation, and housing of chickens have an effect on the elimination of this disease. Farmers prevent the occurrence of necrotic enteritis by acidification of water. In case of an outbreak of this disease, the chickens should be immediately treated with appropriate wide spectrum antibiotics (e.g. amoxycillin).

22. How can be differentiated coccidiosis from necrotic enteritis?

Generally, gross lesions caused by **necrotic enteritis** and **coccidiosis** differ from each other.

Coccidiosis

In broilers, acute coccidiosis followed by mortality is caused by *Eimeria tenella* only, in chickens older than 9 weeks by *E. necatrix*, too. *E. maxima* can rarely cause mortality in broilers, but mostly in replacement pullets. The first symptom of coccidiosis caused by *E. tenella* or *E. necatrix* is the presence of bloody feces. The dead chickens have pale muscles and typical parts of the intestine are affected.

- *E. tenella* infection is confined to caeca, which are distended with blood and caseous cores. Haemorrhagic debris may also be found in the caeca.
- *E. necatrix* infection is usually midintestinal and characterised by haemorrhage and ballooning. Typical “salt & pepper” lesions, which are result of both petechia and white plaques, are observed.
- *E. maxima* typically invades the middle part of the intestine and orange mucus is often present in the lumen.
- *E. acervulina* invades the upper part of the intestine and is characterised by white elongated and transversally oriented lesions found on the intestinal walls. Most infections are limited to the duodenal loop. *E. acervulina* does not cause mortality, but reduces daily gain and causes worse feed conversion and growth retardation.

In the field, coccidiosis does not occur in chickens younger than three weeks, whereas necrotic enteritis occurs even in younger 2-week-old chickens.

Necrotic enteritis

Necrotic enteritis is an acute or chronic bacterial disease caused by *Clostridium perfringens*. In the affected flock, the disease is manifested by sudden mortality within a few hours, or prolonged course with general clinical signs, i.e. slow growth, emaciation, and diarrhoea. The first sign of

the acute course of necrotic enteritis is the feed intake decrease immediately followed by death. Dead chickens usually have pale kidneys, slightly swollen and prominent lobular outlines. The liver is enlarged and may have yellow dark brown discolouration with regular pattern of small grey "islets" surrounded by liver coloured network in the parenchyma, discoloured extrahepatic bile ducts and a moderate discolouration of the gall bladder. The muscle of dead chickens is congested. Mucosa of affected parts of the small intestine is covered with necrotic plaques, which can be peeled off. Particular attention should be paid to the presence of blood in the caeca. Blood is only sucked up from the bleeding small intestine and the caeca are not pathologically changed.

Correct diagnosis can also be confirmed by the result of an antibiotic treatment. While broad-spectrum antibiotics (e.g. amoxycilin) treat necrotic enteritis very quickly, they are ineffective against coccidiosis at all. Reversibly, coccidiocides (e.g. amprolium or toltrazuril) are ineffective against necrotic enteritis.

23. What is the sense of monitoring oocysts in the chicken feces and the litter?

Oocysts counts may be used to identify imminent coccidiosis problems. However, several points should be considered:

1. *Pathogenicity of Eimeria species*

   Chicken coccidia differ not only in pathogenicity, but also in their reproduction capability. As an example, *E. acervulina* is a great producer of oocysts, however, even hundred thousand of oocysts do not cause mortality. Erosion of the duodenum mucosa decreases the proper absorption of nutrients resulting in retarded growth and deteriorated feed conversion. Therefore, consideration of *Eimeria* species classification is necessary.

2. *Age of vaccinated chickens*

   The quantity of oocysts in the feces and consequently in the litter increases approximately until 3rd week of age of the chickens. Later the number of oocysts gradually decreases because of developed immunity. For this reason, the oocyst count in the first three weeks of age should not be used as the only tool for monitoring coccidiosis.

   If non-attenuated vaccines are used, there is a risk of high contamination of the litter with virulent coccidia and hence a risk of coccidiosis outbreak with a sudden decrease of immunity.

3. *Occurrence of oocysts in chickens on in-feed anticoccidials*

   High oocyst counts indicate insufficient coccidiosis control or resistance development. Used anticoccidials should be changed, or even better, the strategy for coccidiosis control should be changed.

BIOPHARM
Research Institute of Biopharmacy and Veterinary Drugs

Pohoří-Chotouň 90
CZ-254 01 Jílové u Prahy
Czech Republic
www.livacox.com • e-mail: export@bri.cz, livacox@bri.cz
phone: +420 261 395 236, +420 261 395 315