



Investigation of Buffalo Herd's Diseases-Brucellosis, Toxoplasmosis, Foot and Mouth Disease- in Some Villages of Baghdad

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Abstract

This study is to investigate Brucellosis, Toxoplasmosis and Foot and Mouth disease of buffalo herds in Baghdad villages. 114 samples (serum) were collected, including 22 for calves less than 6 months and 92 samples of females aged between three to seven years, the samples were collected by staff of Baghdad Veterinary Hospital / Veterinary Directorate, and has been working in Central Veterinary Laboratory/ Veterinary Directorate.

The ELISA diagnostic kits are selected for the detection of antibodies of Brucella abortus, Toxoplasmosis and Foot and Mouth disease (Multispecies).

The result of the research is the presence of different levels of infection in the same herd 58.18% of Brucella abortus, 11.40% of Toxoplasmosis and 44.73 % of Foot and Mouth disease (Multispecies).

The purpose of the study is to investigate the three diseases and their compatibility with the symptoms, although buffalo herds have not been previously vaccinated against the diseases, which proves the existence of different levels of infection in one herd and it adversely affect the development of livestock.

التقصي عن أمراض قطعان الجاموس - البروسيلا وداء المقوسات والحمى القلاعية- في بعض قرى محافظة بغداد

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الخلاصة

تم إجراء هذه الدراسة للتقصي عن مرض البروسيللا وداء المقوسات والحمى القلاعية في قطعان الجاموس في قرى محافظة بغداد. حيث تم أخذ العينات بصورة عشوائية من قطعان الجاموس وكان عدد العينات (114 عينة) مصل دم، منها 22 لعجول دون ال 6 أشهر و 92 عينة أناث معدل أعمارها بين ثلاثة الى سبع سنوات، حيث تم جمع العينات من قبل كادر مستشفى بغداد البيطري، دائرة البيطرة. وتم العمل بها في قسم المختبرات والبحوث البيطرية المركزي، دائرة البيطرة، وزارة الزراعة. تم اختيار العدد التشخيصية الأليزا للكشف عن الأجسام المناعية المضادة لكل من بكتريا بروسيللا أبورتس، داء المقوسات، ومرض الحمى القلاعية (بدون تحديد الصنف). وقد كانت نتائج البحث هي وجود مستويات مختلفة للأصابة في القطيع الواحد، حيث كانت نسبة الاصابة بالبروسيللا أبورتس 58.18% ونسبة الاصابة بالتوكسوبلازما 11.40% أما نسبة الاصابة بمرض الحمى القلاعية فكانت 44.73%. أن الهدف من الدراسة هو التقصي عن وجود الأمراض الثلاثة ومطابقتها مع الأعراض، علماً أن قطعان الجاموس لم تلقح سابقاً ضد الأمراض مما يثبت وجود مستويات مختلفة للأصابة في القطيع الواحد وأنها تؤثر سلباً وبشكل واضح على تنمية الثروة الحيوانية في العراق.

Introduction

Brucellosis is a contagious zoonotic disease of public health importance around the world. Disease affects domestic and wild animals and humans. It is a Contagious Bacterial Zoonotic disease. This disease has been associated with the import of livestock by breeders from unknown countries which is possible to be infected with the disease.⁽¹⁾

Brucellosis is common in low- and middle-income countries which are characterized by poor hygiene, consumption of raw animal products (e.g. milk and meat) and a lack of public health education programs. The disease causes direct socio-economic effects in societies that depend on livestock production for their livelihood. Although brucellosis is widespread in cattle in the Middle East and North Africa, it has not been studied in detail except for approximate figures on the epidemiology of infection in these regions.⁽²⁾

Among the most important clinical symptoms are miscarriages during the last three months of pregnancy, stillbirth, weak calves, and infertility in male and female, retention of fetal membranes, swollen testicles and loss of appetite. Infection in cattle depends on age, immune status, and natural resistance, mode of infection and virulence of the strain.⁽³⁾

The first identify the cause of brucellosis is the scientist David Bruce in 1887, from which the disease was named, he mentioned that it has ball-like shapes (Coccus),⁽¹⁾ the name brucellosis was gradually replaced in the nineteenth century by

the disease of Mediterranean fever and Malta fever. The disease has many synonyms, including Crimean fever, Cyprus fever, Gibraltar fever, goat fever, Italian fever, Neapolitan fever and biological warfare, and is similar to fever that is caused by other diseases such as Malaria, typhoid fever, rheumatic fever and arthritis. The disease is caused by the genus *Brucella*, in cattle is *Brucella abortus*, and in goats, *Brucella melitensis* which the main cause of the disease in humans.⁽⁴⁾

The disease affects the reproductive system of animals, which leads to significant losses in productivity, such as reduced milk production, abortion, poor offspring, weight loss, rejection and culling of infected animals due to sterility, lameness and impediment to trade and export (trade restrictions). The source of infection is infected field animals and their products, domestic and wild animals. The most vulnerable people to this disease are veterinarians, butchers, animal breeders, farmers, and workers in the meat and dairy industry. Sources of infection include aborted substances, vaginal secretions, milk and semen from infected animals.⁽⁵⁾

Infection may occur through the skin, conjunctiva or mucous membranes of the respiratory tract by inhalation but the most common route of infection in cattle is through the gastrointestinal tract. The infection spreads to the local lymph nodes, where the *Brucella* multiplies within the phagocytes, then invades the lymphatic vessels, followed by Bacteremia, which leads to systemic infection, then moves to the pregnant uterus, male reproductive organs, and the mammary gland.

Perhaps the most dangerous is the variable incubation period and the inability to determine which animals will subsequently become seropositive, approximately 5 percent of infected strains will retain the infection and become seropositive only after the first birth.

B. abortus has a strong tendency to the uterus during the third trimester of pregnancy and it is believed that the reason is the high concentrations of erythritol, which is a source of carbon and energy for the growth and reproduction of bacteria. Trophoblast cells at the base of the placental villi are the first place for bacteria to invade the tissues of the fetal placenta, then spread to Myxotrophoblasts, and thus metabolic exchanges between mother and fetus are compromised leading to miscarriage.⁽⁶⁾

Diagnosis can be made through laboratory tests of blood, serum or milk samples and through laboratory tests such as the Rose Bengal Test (RBT), Enzyme-linked Immunosorbent Assay (ELISA), these tests are for the purpose of scanning. And the polymerase chain reaction (PCR) test, Serum Agglutination Test (SAT), Complement Fixation Techniques (CFT) as confirmatory tests, or by in vitro culture of placenta, vaginal secretions, or milk of infected animals, the proper diagnosis is one of the main obstacles to the complete eradication of brucellosis.⁽⁷⁾

The World Health Organization (WHO) has classified brucellosis as one of the seventeen neglected endemic animal diseases in developing countries, where the disease is widely distributed in the Mediterranean, the Middle East, Central Asia and parts of Latin America, making it a global problem, and to control the disease we need a global strategy One World, One Health strategy, because the only approach to achieving control and eliminating it is cooperation between public and animal health authorities, and prevention cannot be achieved without disease control in animals.⁽⁸⁾ Most countries in Asia use a test-and-slaughter strategy to eliminate and control the disease, which requires effective monitoring, prompt and accurate diagnosis.⁽⁷⁾ There is wide agreement that vaccination is the most effective and practical way to reduce the incidence of disease, as effective control

requires a long-term commitment. The first effective brucellosis vaccine was based on the strain 19, and this results in reasonable protection against *B. abortus* but at the expense of a sustained sero response. The same problem occurs with the *B. melitensis* Rev.1 which is still the most effective vaccine against brucellosis in sheep.⁽⁹⁾

Toxoplasmosis is one of the common parasitic diseases between animals and humans of extremely dangerous animal origin, which causes public health problems in many countries,⁽¹⁰⁾ *Toxoplasma* parasite infects many warm-blooded animals such as cows, buffaloes, goats, birds and others⁽¹¹⁾ which are considered the intermediate host, while the final host is the Felidae Family domestic and wilderness which is the main source of disease prevalence in clinical and subclinical forms.⁽¹²⁾

In the animal, it may lead to abortion or death after birth, as it may affect the nervous system thus affected on the vital functions of the brain. An aborted fetus is often accompanied by either a weak fetus or "embalmed" fetus.⁽¹³⁾

The disease is a widespread disease caused by a parasite of the Protozoa that lives inside a single-celled host cell known as *Gondii Toxoplasma*. The disease is called Toxoplasmosis because the parasite has a curved shape similar to a banana. The disease was first discovered in Gondi Rats in Tunisia in 1908, it was estimated that approximately one third of the world's population is infected with this parasite.⁽¹⁴⁾

Toxoplasma gondii - passes in two stages without specificity in the host. The asexual stage, can parasitize in all mammals, including humans, animals and cats in addition to birds, while in the sexual stage it is limited only to the cat family as the original host. Cats ingest infected meat that carries cysts Tissue filled with eggs and parasitic spores, where the cells lining the intestines invade, the eggs are released with cat feces, and when ingested by humans or animals, infection occurs.⁽¹⁵⁾

Epithelial cells become infected with bradyzoites in infected tissues or sporozoites (in oocytes in cats feces) which are shed in the feces of another creature containing

contaminated food (or with water) or by direct transfer of oocytes. After fertilization, the female gamete then an immature zygote, then oocysts are expelled first in the intestinal lumen and then out in the feces, sporogony and maturation of the oocytes can occur, in 1-2 days, in the external environment. ⁽¹⁶⁾

Toxoplasmosis is difficult to diagnose, due to the high percentage of subclinical infection and persistence of this parasite in tissues. Serological studies of Toxoplasmosis have been recorded using several serological tests to detect Toxoplasmosis including ELISA and IFAT test (Indirect Fluorescent Antibody Test) and Latex test. The disease must be differentiated from other diseases such as Cytomegalovirus (CMV), Syphilis, Rubella, Herpes Simplex, and Herpes Zoster. ⁽¹⁷⁾

Care should be taken to cleanliness and avoid contact with cats, soil and raw meat. ⁽¹⁸⁾ Meat of any animal should be thoroughly cooked to an internal temperature of 66°C before human or animal consumption. Freezing meat to an internal temperature of -12°C is effective in killing tissue cysts. ⁽¹⁸⁾

Foot-and-mouth Disease (FMD) is a highly contagious viral disease of significant economic impact that affects cattle, sheep, goats and other cloven-hoofed ruminants. ⁽¹⁹⁾ Foot and mouth disease is characterized by fever and vesicular lesions on the tongue and lips, in the mouth, on the nipples and other soft areas. The disease causes heavy losses in production. While the majority of infected animals recover without the need for treatment, it is rarely fatal in adults but is extremely fatal in Calves due to myocardial degeneration. ⁽²⁰⁾

The disease gained a place in history in 1898 as the first animal disease caused by a virus, however and despite more than a century of active research and elucidation of many aspects of the pathogenesis of FMD, critical knowledge about host-virus interactions is still lacking. ⁽²¹⁾

Foot and mouth disease is a disease on the OIE list that must be reported to the Organization, as defined in the OIE Animal Health Act. ⁽¹⁹⁾

FMD is caused by a pet virus of the genus Aphthovirus of the family Picornaviridae. There are seven serotypes of the virus (A, O, C, SAT1, SAT2, SAT3, and

Asia) endemic to different countries around the world. Each strain requires a specific vaccine to provide immunity to the vaccinated. The seven serotypes are also found in the wild, although the latter does not play a significant role in disease conservation so far, the only confirmed reservoir in wildlife is the African Buffalo Cavallo (*Syncerus caffer*). ^{(19) (22)}

Infection with one type does not give immunity or prevent infection with the rest of the patterns, in many FMD-endemic areas, more than one serotype may spread, creating waves of infection when different serotypes enter. Infection of susceptible animals with serotypes may persist in the region and is rarely seen clinically with mild infection as the maternal antibody is lost. ⁽²³⁾

FMD virus is excreted in milk in dairy cows before clinical signs appear, so there is a chance for the virus to spread from farm to farm and from cow to calf via raw milk. FMD virus persists up to 20 weeks on hay bedding or in dry feces for as long as it reaches 14 days in summer and 6 days in winter, in urine for 39 days and in soil for 3 days in summer to 28 days in winter. ⁽¹⁹⁾

The main site of infection and reproduction of FMD virus is in the mucous membrane of the pharynx, upper respiratory tract or in dermal and subcutaneous tissues. The virus may also enter through skin lesions or the gastrointestinal tract. The virus enters the blood or binds to mononuclear cells and is distributed throughout the body to glandular tissue sites where secondary replication, cell rupture and edema occur. Vesicles that aggregate to form the thrushes and bullae characteristic of FMD develop and rupture within 48 hours. In young animals, the virus invades the heart muscle cells and appears as striated cells (tiger skin). ⁽²³⁾

The incubation period for FMD is variable and depends on the host, environment, mode of exposure, and virus strain. After infection with FMD virus, the average incubation period for sheep and goats is 3-8 days and 2-14 days in cattle, especially under intense direct contact. ⁽²⁴⁾

The disease may be suspected based on clinical signs. However, FMD cannot be distinguished clinically from other vesicular

diseases, such as swine vesicle disease, vesicular stomatitis and vesicular rash. Confirmation of any suspected FMD case will be by in vitro serological tests such as (ELISA) and (PCR).⁽¹⁹⁾

The primary measures outlined in the Global FMD Control Strategy are that detection and early warning systems are in place and effective surveillance is implemented in accordance with the detailed OIE guidelines as they help monitor the occurrence and spread of disease, an emergency vaccine will prevent or reduce local virus replication.⁽²⁵⁾

Materials and working methods

Samples were collected from 20/08/2013 to 10/09/2013 and were completed at the end of 2014. Blood samples were taken from (animals) aged 3-7 years and calves aged 3-6 months, the number of samples was (114), including 22 calves under six months, and 92 female samples with an average age between three to seven years.

Serum was isolated from blood samples in the Central Veterinary Laboratories and preserved at -20 Celsius degrees. Samples were collected by the staff of Baghdad Veterinary Hospital, the same samples passed three times, once for each diagnostic kit.

Samples were taken from villages with a high density of buffalo-breeding in Baghdad (Al-Fudhaliyah, Al-Taji, Al-Shuala, Al-Wahda, AL- Thahab AL-Abyedh and Jurf Al-Naddaf) and based on the data of symptoms in the buffalo herds, some of which were

suffering from respiratory symptoms of different severity and different ages, reproductive status, abortions and fetal death early.

Materials that must be provided: Brucella abortus Antibody Test- Brucellosis Serum X2 Diagnostic Kit, ID Screen® Toxoplasmosis Indirect Multi-species kit, Indirect ELISA, Third Diagnostic Kit was Foot and Mouth Disease (FMD) Multispecies Antibody Test Kit, Indirect ELISA .The examination process was carried out according to the instructions of the diagnostic kit for each disease. Generally, the serum containing the immune antibodies to be diagnosed is placed and incubated with the antigen covering the pits of the examination dish of diagnostic kit. After that, the remaining associated substances are removed by washing, and then monoclonal antibodies are added. Associated with peroxidase enzyme for detection with the addition of the reactant (chromagen dye solution) and that the adsorption of the color formed from the reaction of the active substance and chromogen is measured by a spectrophotometer (ELISA device reader) and on the single wave at 450nm according to the instruction manual for each disease.⁽²⁶⁾

Results

As shown in tables (1, 2 and 3), the first table represents the prevalence of Brucella abortus disease, the second table represents the prevalence of Toxoplasmosis, and the third table represents the prevalence of Foot-and-Mouth disease in Baghdad.

Table (1): The prevalence of Brucella abortus disease in buffaloes in different regions in Baghdad

Village	Number of samples	Positive samples	Percentage
Fudhaliyah	30	17	56.60%
Taji	9	0	0.00%
AL- Shuala	23	17	73.91%
AL- Wahda	30	22	73.33%
AL-Thahab AL-Abyedh	10	2	20%
Jurf Naddaf	12	6	50%
Total	114	64	58.18%

Table (2): The prevalence of Toxoplasmosis disease in buffaloes in different regions in Baghdad

Village	Number of samples	Positive samples	Percentage
Fudhaliyah	30	7	23.33%
Taji	9	2	22.22%
AL- Shuala	23	2	9.13%
AL- Wahda	30	0	0.00%
AL-Thahab AL-Abyedh	10	0	0.00%
Jurf Naddaf	12	2	16.67%
Total	114	13	11.40%

Table (3): The prevalence of Foot-and-Mouth disease in buffaloes in different regions in Baghdad

Village	Number of samples	Positive samples	Percentage
Fudhaliyah	30	14	46.66 %
Taji	9	1	11.11 %
AL- Shuala	23	14	60.87 %
AL- Wahda	30	9	30 %
AL-Thahab AL-Abyedh	10	7	70 %
Jurf Naddaf	12	6	50 %
Total	114	51	44.73 %

Discussion

The prevalence of brucellosis was 56.18%, and some of it was a newborn at the age of one to three weeks. Nowadays, young females and males of buffalo and cows (after the research was done) are vaccinated with the Brucella S19 vaccine, and this only causes a problem during the laboratory examination between the vaccinated and the infected one, it is preferable to vaccinate with type RB51, cause what distinguishes it from infection is that it decreases the type O polysaccharide antibodies, unlike what is found in the antibodies when infected, so it can distinguish the infected from the vaccinated. ⁽²⁷⁾

Toxoplasmosis affects all, but the infection differs from one species to another. In cows and buffaloes, it does not cause abortion but it is excreted in milk. Bloit (1983) reviewed the sera surveys conducted on domestic animals and concluded that the antibodies were present in cattle by 12.5% and also assumed that different animals differ in their susceptibility to infection and the rate of loss of antibodies. He concluded that cattle return to serostatus much more quickly than sheep, which is the result of a more reliable indicator of the actual spread of infection.

At autopsy or at massacres, the most common findings are multiple necrotic granulomatous foci in internal organs, that what was appeared similar approach to Bloit and what he found in cows, and he did not reveal the buffaloes, which we had antibodies at a rate of 11.40% .⁽²⁸⁾

As for FMD, the prevalence of FMD was 44.73% at the time, which was synchronized with the end of the vaccination campaigns, and the number for the immunological assessment was close. The reason for detecting the infection despite the campaigns was the refusal of breeders to vaccinate pregnant females, and most samples taken in this investigation were females of average age between 4-12 years, this reason is attributed to the fact that the breeder is afraid of vaccination campaigns against FMD, mistakenly believing that the vaccine causes abortion in pregnant animal, despite the guidance and clarification by veterinary staff that FMD is not considered a major cause of abortion, but it is considered an accidental cause, the abortion is considered as a secondary symptom as a result of high temperature and high prostaglandin in the blood. The infected herd of pregnant animal

does not undergo abortion except for some of it, and after the occurrence of other bacterial complications that cause abortion or others, where it was found in the research that many animals are carriers of both infections such as foot-and-mouth disease and brucellosis at the same time. ⁽²⁷⁾

Conclusion

Although buffalo herds have not been previously vaccinated against the diseases, which proves the existence of different levels of infection in one herd and it adversely affect the development of livestock.

Recommendation

Raising the awareness level of the breeder in the form of guidance and clarifying the picture to him about the losses related to refrain from vaccinating.

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