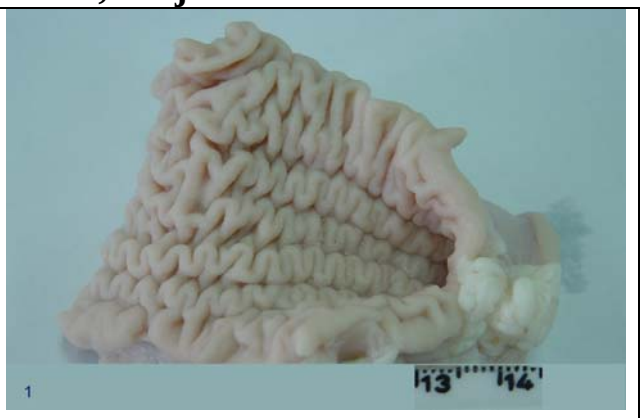
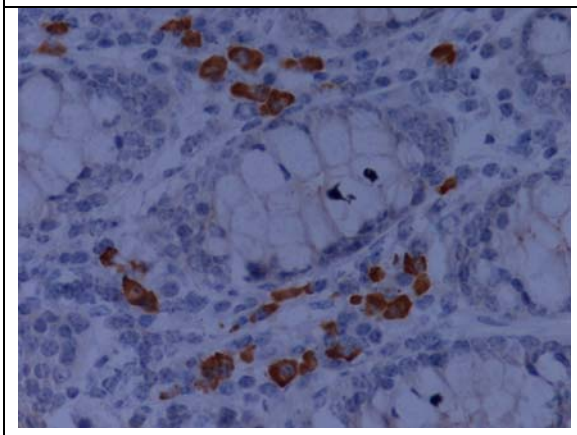
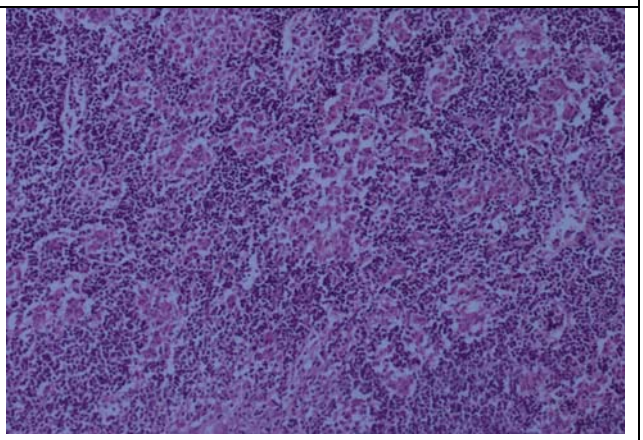
Enhanced Diagnostic Capacity and Control Measures of Some Transboundary Animal Diseases with Emphasis on Pathology and Epidemiology of Johne's Disease / Paratuberculosis in Jordan, Egypt and Algeria

7-9 August, 2007

Jordan University of Science and Technology
Faculty of Veterinary Medicine
Prepared by**: Prof. Nabil Hailat, Project Coordinator**







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Executive Summary

A three day-regional workshop on Enhanced Diagnostic Capacity and Control Measure of SomeTransboundary Animal Disease with Emphasis on Pathology and Epidemiology of Johne's Disease / Paratuberculosis in Jordan, Egypt and Algeria was held on 7-9 August, 2007. About 25 scientists from the participating countries were involved effectively in the workshop. Some were lecturers while others were participating for upgrading their capacities, those were mainly from the Ministry of Agriculture. Master and undergraduate students participated also in the workshop, in two cases the master students delivered presentations and helped in the practical sessions. The first day and a half focused on the theoretical presentations of Johne's disease and its counterpart in humans, Crohn"s disease. The Crohn's disease was presented by a professor in human medicine while the Johne's disease lectures and practical session were presented by Professor from Veterinary Faculties from the three participating countries in addition to the Palestinian Authority. The second one day and a half focused on gaining some practical skills and knowledge on diagnosing the Johne's disease using histopathological and immunohistochemical examinations. The last session was on Avian Influenza and one presentation on Bovine Keratitis/conjunctivitis. Some teaching materials which were pre-prepared and other teaching materials prepared by the participants themselves were given to the participants as reference materials. A copy of this report which includes some teaching materials is being distributed to them.

The main delivered message was that the diseases is very prevalent and needs policy and research measures. In Jordan, a report was sent to the president office for his consideration. A committee was also formed by the Minister of Agriculture to study the disease in the governmental research stations. Brochures for public education and farmers awareness were prepared. They will be distributed to them once they are finalized. In the participating countries research activities started to merge with cooperation with our laboratories for regional prevention and control program of Johne's diseases.

INTRODUCTION:

Small and large ruminants are important for the livelihood of farmers in Jordan. The small ruminants (SR) population is nearly 2.4 million sheep and goats while the large ruminants are about 65000 cattle and 15000 Camels, benefiting approximately 100 thousand families, which have an average of six members. There is an increasing demand for the products of these animals, mainly milk and meat, by the country's five and a half million people who are concentrated in urban areas (figure1).

The contribution of the agriculture sector (254 million JD, 1JD= $1.4) to the total Gross National Product (GNP) (4,364 million JD) is approximately 5.5%, most of which (58%) derives from animal production. The contribution of animal production to the GNP is 3.19% (General Directorate for Statistic 1997). The contribution of the small ruminant sector to the GNP is not known but is likely to be less than 1%. The total amount of money invested in poultry and livestock amounts to 550 million JD.

Jordan produces approximately 204,622 tons of milk and 14,972 tons of red meat per year. This represents 61.4% and 44% of its needs in milk and red meat, respectively. Sheep produce approximately 23.5% and 56% of the country's total production of milk and meat, respectively, while goats produce approximately 13% the country's milk and 18% of the meat.

Most small ruminant producers are resource – poor farmers with low income and education levels compared with other sectors of the population. Feed costs and the scarcity of feed resources, in addition to other costs such as transportation and water, results in many producers selling part of there flocks in order to be able to feed the remaining animals. The production system include migratory systems managed by Bedouins (20%), semi migratory systems also managed by Bedouins (70%), and intensive or sedentary system (10%). Since 1990's there has been a slight shift from extensive to more intensive or sedentary systems.

Small ruminant populations are decreasing (tables 1and 2). This trend is in contrast to an increasing market demand for their products that is being satisfied by imports. The amount of imported chilled and frozen red meat was 17,183 tons in 1991, increasing to 30,169 tons in 1993. Milk imports were at 101,957 tons in 1991 and rose to 178,833 tons in 2000. This presents opportunities for production intensification and the insertion of small ruminant producers into the market.

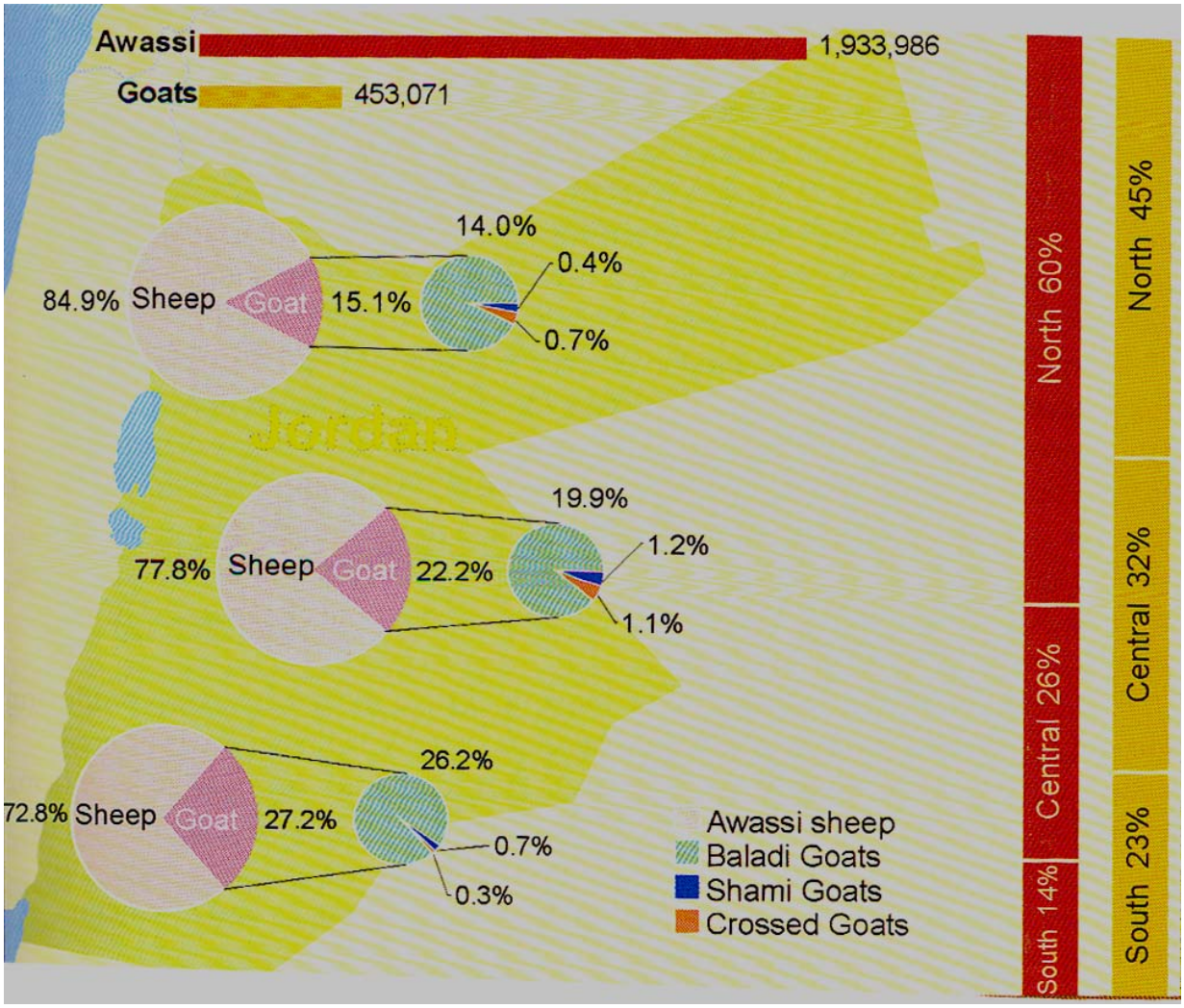
Table 1: Population of Small Ruminant Breeds (/Thousand Heads) in Jordan and Population Change in 1991-1999

Species and breed 1991-1999	1991	1995	1997	1999	changes(%)
Awassi sheep	2,671.0	2,375.0	1,935.0	1,933.0	-28
Baladi goats	0,458.0	0,821.0	0,666.0	0,419.0	-9
Shami goats	0,020.6	0,031.0	0,027.0	0,016.0	-2
Crossbred goats	----	0,053.8	0,026.0	0,030.0	NA
Foreign breed goats	----	----	0,001.0	0,000.5	NA
Total goats	0,478.6	0,905.8	0,720.0	0,461.5	-4
Total small ruminants	3,149.6	3,280.8	2,655.0	2,394.5	-24

Table 2: Population of Small and Large Ruminants (/Thousand Heads) in Jordan and Population Change in 2000-2005.

Species	2000	2001	2002	2003	2004	2005
Awassi Sheep	1,895	1,868	1,741	1,793	1,671	2,024
Goats	0,640	0,533	0,729	0,668	0,565	0,555
Camels	13	14	13	12	13	13
Cattle	65,20	66,80	69,80	66,27	69,26	71,80

Figure1: This map shows the population of sheep and goats in Jordan distributed in three regions; north, central, and south of Jordan, also it describes the distribution of sheep and goats according to there breeds. The Awassi sheep breed represents the main sheep breed in Jordan.



PROJECT DOCUMENT MODEL FORMAT

PEREZ-GUERRERO TRUST FUND FOR ECONOMIC AND TECHNICAL COOPERATION AMONG DEVELOPING COUNTRIES, MEMBERS OF THE GROUP OF 77
GOVERNMENT OF ……..

Type of project:	Regional
Title:	Int/.. /.. /.. – [Enhanced Diagnostic Capacity and Control Measures of Some Transboundary Animal Diseases with Emphasis on Pathology and Epidemiology of Johne's Disease/Paratuberculosis in Jordan, Egypt and Algeria.].
Sector:	[UNDP will insert]
Beneficiaries:	About 500000 sheep and goat farmers, 700 dairy cattle farmers and 2000 poultry farmers in Jordan, Egypt and Algeria. In addition, graduate students, technicians, laboratory research assistants and academicians in the region will be benefited.
Duration of project: December 2006 to October 2007.	
Estimated starting Date: 8 months from date of approval in September.	
PGTF inputs: [33000 US$]	
Other inputs: [35000 US$]	
Total cost of project: [68000 US $]	

Signed on behalf of:

Date: _____*[signed]*_____ UNDP
Resident Representative

Date: :_____ Government of Jordan
Name : Suhair Al-Ali
Title : Minister of Planning and International Cooperation

Implementing Institution:
Name: Prof. Wajih Oweis Date: January, 22nd / 07
Title : President, Jordan University of Science and Technology (JUST)

Part I

a. Situation Analysis

Johne's disease (JD) is a transboundary, infectious, incurable, chronically progressive granulomatous enteritis which affects domestic (sheep, goats and cattle) and exotic ruminants. The causative agent is Mycobacterium avium paratuberculosis (MAP), a facultative intracellular acid-fast bacillus. Ruminants become infected as young animals via fecal-oral transmission of the organism and may remain subclinically infected for long periods of time, shedding low numbers of organisms before progression to the terminal stage of infection. It has also been shown that the disease is zoonotic and can be transmitted to humans causing Crohn's disease, a chronic, relapsing inflammatory conditions affecting any part of the human gastrointestinal tract.

Paratuberculosis poses a significant economic and health problem worldwide, especially in the cattle and small ruminants industry. Economic losses occur due to animal culling, lowered milk production, reduced carcass value, and poor reproductive performance, and are estimated to be about $200 per infected cow per year in herds with at least 10% prevalence. The cost of the disease in ovine is approximately 90$ per clinical case. Furthermore, it has been indicated that economic losses to the cattle industry in the United States are staggering, reaching 1.5 billion $ annually. The economical losses caused by Johne's disease in the Arab World has not yet been determined. The disease also considered as one of the international trade barriers as many countries require imported animals should be free of the disease.

Limited studies on JD have been conducted in the Middle East and North African countries. Very recently, JD was reported in cattle from Egypt, in sheep in Morocco and Saudi Arabia. In Jordan, three years ago, we conducted a study on apparently healthy sheep and goats, using histopathological and immunohistochemistry IHC examinations and culture. We found that the disease is very prevalent; 80% prevalence rate in Awassi sheep and 75% in Shami and local goats. More recently, we conducted another study on apparently healthy cattle using serological screening tests and histopathology and the prevalence rate was 65%.

Early diagnosis of infected animals is essential for avoiding the spread of infection, and eradication is dependent on detection and culling of infected animals as early as possible. Several countries have established national centers to diagnose and implement control programs of the disease. It is necessary to familiarize the farmers, animal scientists as well as veterinarians with the early diagnostic signs and tests of the disease for better management and control. From our experience we have observed that farmers are not aware of the disease, its public health concern and its negative impact on their income.

Therefore, we intend to organize a regional a 3-day workshop on the pathology and prevalence of Johne's disease with the objective of enhancing the diagnostic capacity and control measures and increasing the public awareness of the disease. This workshop will also be useful to enhance the diagnosis of Avian Influenza Virus (H5N1), which has been diagnosed in poultry and in humans in Egypt and Jordan, and Infectious Bovine Keratitis as well as other animal diseases using IHC. Livestock farmers, veterinarians, animal scientists, undergraduate and graduate students are anticipated to participate in these workshops. We have long experience in organizing national, regional and international workshops in the field of animal agriculture. Just recently, we have conducted a workshop on Veterinary Accreditation where 37 faculties of veterinary medicine and regional and international experts were invited to this workshop. We, in the faculty of Veterinary Medicine will coordinate this project with close consultation with the other partners in Egypt and Algeria. The workshop is the first one to be conducted on these diseases in the region and hopefully will be a model for other countries and regions and pave the road for international conference in the region.

Part I
b. Strategy

In 2002, we were the first people to accurately diagnose the disease in sheep and goats using histopathology, immunohistochemistry and ELISA technology. These techniques were newly introduced to our laboratories and to Jordan. Later on, scientific research was conducted to assess the size of the problem in sheep, goats and dairy cattle. The veterinarians in the Ministry of Agriculture were not aware of the disease and lack the training and the capacity of diagnosing the disease using accurate laboratory tests.

Through several formal and informal meetings we have brought to the attention of our veterinary colleagues in the Ministry of Agriculture the importance and the occurrence of Johne's disease in Jordan. Furthermore, through our visitations to sheep and goats, and dairy farms, and through our post mortem examination at the Animal Health Centre in our faculty we have brought the issue of Johne's disease to the farmers. Recommendations of the above-mentioned studies were sent to the Ministry of Agriculture to consider Johne's disease as a trade-limiting factor with the objective of decreasing the occurrence of the disease in Jordan.

The target beneficiaries will be veterinarians in the Ministries of Agriculture, academicians from the veterinary faculties, undergraduate and graduate students, as well as many sheep, goats and cattle farmers. Technicians and research personnel will improve their diagnostic skills in the diagnosis of this disease. Some human doctors specialized in gastrointestinal tract and interested in Crohn's disease will be invited to participate in this workshop. Participants will be trained to use these techniques to diagnose other animal diseases such as; Avian Influenza, Rabies, Bovine Viral Diarrhea and Infectious Bovine Keratitis. Some of these diseases, such as; Avian Influenza, has been diagnosed in poultry and in humans in Jordan and in Egypt.

It is anticipated that more research will be conducted and focused on Johne's disease with emphasis on environmental issues that may have an impact on the occurrence and the high prevalence rate in livestock. Such research is expected to be extended to other animal diseases. Moreover, these workshops will stimulate countries in the region to host international conferences on animal diseases, such as Johne's disease in ruminants and Avian Influenza in poultry. Brochures and pamphlets with pictures in Arabic language will be prepared for the farmers and the animal scientists to manage and control such diseases.

Goal:

Demonstrate, through discussion and presentation, immunohistochemistry as an effective technique for improving diagnosis and benefiting animal health, public health, and trade in the Middle East and North African (MENA) region.

Objectives:

1- To enable sharing of expertise on animal disease diagnosis among the countries of Jordan, Egypt and Algeria.
2- To facilitate transfer of knowledge and new diagnostic techniques related to animal diseases with emphasis on Johne's disease.
3- To exchange information on the prevalence and status of Johne's disease. in the region
4- To increase the human resource capacity in the laboratories for animal diseases diagnosis with reference to Johne's disease.
5- To increase the awareness of farmers, animal scientists about the epidemiology and the public health concerns of Johne's disease and Avian Influenza H5N1.
6- To enhance the communication between and amongst scientists and veterinarians in the ministries of agriculture.
7- To produce a proceeding pertaining the status of Johne's disease and Avian Influenza in the region.

Your Excellency,
President of Jordan University of Science and Technology
Professor W. Oweis

Dear President:

Attached please find a summary report on Paratuberculosis in Jordan as you requested.

Please let me know if I can be of help for more details of the issue.

My best regards,

Nabil Hailat, DVM, Ph.D,
Professor of Pathology,

Summary Report Pertaining to the Present Status of Paratuberculosis (Johne's Disease) in Jordan with Reference to its Prevalence, Economic Impact and Zoonotic Potential.

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1. Background and Introduction:

Paratuberculosis (Johne's disease) is an infectious disease of ruminants, considered by many researchers to be one of the most serious infectious disease currently plaguing the world's sheep, cattle and goat industries. The disease, caused by Mycobacterium avium subsp. Paratuberculosis (MAP), a gram-positive acid-fast organism shed in the feces of infected animals, is characterized by a chronic granulomatous enteritis.

Contaminated feed and water, bedding and soiled udder are thought to be the major routes for spreading of the organism. Young animals less than six months of age are thought to be the most susceptible to infection, and older animals are more resistant to infection. Cattle become infected early as young calves via fecal- oral transmission of the infectious agent. Intrauterine infection in cattle has been well documented. Isolation of M. paratuberculosis from a sheep fetus has been confirmed, and antibody to M. paratuberculosis has been demonstrated in colostrum-deprived lambs.

The economic effect of Johne's disease is that restriction of livestock marketing and contamination of land. In Iceland, for example, measures to be taken for the control of paratuberculosis are expensive, in which hundreds of kilometers of fences were put to restrict sheep movement from infected area. Laboratory tests used to detect infected animals are also costly and with all this expenses it is very difficult to

eradicate the disease. In addition, consumers of animal products are becoming increasingly sensitive about the possible effect of livestock disease and residues on product quality and human health. The potential link between Crohn's disease and M. paratuberculosis is an example. M. paratuberculosis has been described as a co-factor in the occurrence of Crohn's disease, an intestinal disorder in humans.

During the routine postmortem examination of sheep, brought to the necropsy laboratory at the Veterinary Health Center at Jordan University of Science and Technology, we have observed that some emaciated sheep had clinical, gross and histopathological findings compatible with Johne's disease. Furthermore, during my visitations to some dairy farms in Al-Thlaile, where I performed necropsy, I observed that some cattle were emaciated, weak, and suffered from chronic shooting diarrhea and upon the necropsy I found that they had compatible pathological findings with Johne's disease. During an awareness and educational campaign that I conducted three years ago to sheep farmers in Ajloun and Al-Moagar, we also observed cases with Johne;s disease in sheep.

II. Action Plan/ Awareness, Education and Research Activities:

A. Conducted and Ongoing Research Activities on Johne's Disease in Jordan:

As a first step in understanding the size and the impact of this disease in Jordan, we conducted an interview with twenty sheep flocks, ranging from 50-500 heads, and we found that 75% and 80% of the twenty flocks had a history of emaciation and bottle jaw respectively. The farmers reported that it was rare to see improvement in bottle jaw cases after antihelminthic treatment. They also observed that the emaciation was associated with diarrhea (betherre in Arabic farmer language), and that bottle jaw was not treatable. The farmers believed that diarrhea was not commonly associated

with emaciation and bottle jaw together; rather the last two constraints were linked together in many cases. Once the intermittent diarrhea appeared after emaciation, no recovery was noted. Losses due to such cases were common in all the flocks and affected sheep never responded to treatment. Such cases are called Awaiehiat. The reported signs by the sheep farmers strongly suggested the presence of paratuberculosis among their flock for many years and are suffered economically as a result of this disease.

Therefore an action plan was conducted as follows:
First: A research proposal was funded from the Ethiopian Government (World Bank Grant) as part of the training program for an Ethiopian student in Veterinary Pathology to study the Pathology and the Prevalence of the Disease in sheep and goats in Jordan. The research also was funded partially by the Dean of Research at Jordan University of Science and Technology. Part of the results are shown later(table 1and2)
Second: Another research proposal was funded from the Dean of Research at Jordan University of Science and Technology to study the Pathology and the Prevalence the disease in Cattle and Camels in Jordan. This was for a master student from Algeria in Veterinary Pathology. Part of the results of this study are shown later (Table 3).

Third: Because we found that the disease is very prevalent in the animal species examined we wanted to know the molecular relationship between the bacterial strains among the animal species. A third proposal was submitted to the dean of research for funding. This is part of a third master student from the Al-Nagah University, The Palestinian Authority, just recently was funded from the Deanship of research at JUST. A more detailed proposal was submitted to Showman foundation for financial support.

Fourth: Because Johne's disease has severe impacts in importing and exporting life animals, and with the recent international regulations in the framework of WTO/GATT, globalization and environmental considerations, we see an urgent need for capacity development in areas of Agriculture Policies and International Trade with special reference to Transboundary Animal Diseases, we submitted a proposal on enhancing Agricultural policy, jointly with Egypt, Yamen, Algeria, and the Palestinian Authority.

Fifth: Another master student from Algeria, this coming fall, will study the disease in importing sheep and cattle. It aimed at finding out the relationship between the strains of the causative agents in imported and local animals to find the source of infection. This will help us in putting a better control program.

Sixth: A more comprehensive proposal was submitted on Johne's disease for funding to Abdel Latif Scientific Fundation in Saudi Arabia last month.

B. Capacity Development, Public Awareness and Farmers Education:

First: Because we wanted to increase the awareness among the scientists in the Arab countries regarding this johne's disease, a proposal was submitted and was funded from an American Funding Agency through the UNDP. This was jointly with Benha University in Egypt and El-Taref University in Algeria. Part of this project is organizing a regional workshop, August 7th to 9th, 2007 as the first training workshop that I am organizing for capacity building and enhancing diagnostic skills for the counterparts countries, and enhance the awareness of scientists from these countries. The Ministries of Agriculture and Health, Veterinary Syndicate, farmers

and the private sectors were invited to attend the training workshop. An administrative and technical report (scientific proceeding) will be prepared and distributed to the concerned parties. Furthermore, two brochures in English and two in Arabic regarding Johne;s disease sheep and in cattle are being prepared and will be distributed to the Ministries and scientific centers and institutions in Jordan, Egypt and Algeria. Some will also be distributed to the farmers. Additionally, several field days will be conducted to train farmers and upgrade their skills on the management of their herds to reduce the impact of the disease.

Second: Last year, a training regional workshop was organized where some veterinarians for the Ministry of Agriculture and Jordan University of Science and Technology participated, and Johne's disease was a hot issue where I presented our research results.

Third: The Ministry of Agriculture sent us few months ago some samples from a sheep farm in Al-Fgage which belonged to a governmental farm to find out if the disease was present in that farm. We diagnosed that the sheep suffered from Johne's disease. (The report is attached). The veterinarian who suspected the case was a graduate of our university.

Fourth: Our investigations were carried out to study the associated pathological lesions of Johne’s disease in the intestine and mesenteric lymph nodes and its prevalence in apparently healthy and young Awassi sheep, goats, cattle and camels in Jordan, using gross/histopathological, Acid Fast stain, culture and immunohistochemistry examinations. Part of the results are shown in the tables below.

Table 1

Histopathologicl findings, from intestine and lymph node samples in Apperently healthy Awassi sheep aged 8-24 months, at different sites of Jordan, 2002.

Site	Samples collected	Samples processed	Grade of the lesions					
			I	II	III	SP	Total +ve	Total -ve
Amman	60	45 (75)*	7 (16)	18 (40)	16 (36)	1 (2)	**42 (93)**	3 (7)
Sweleh	112	63 (56)	11 (17)	23 (36)	25 (40)	2 (3)	**61 (97)**	2 (3)
Irbid	107	94 (88)	5 (5)	29 (31)	57 (61)	1 (1)	**92 (98)**	2 (2)
Total	279	202 (72)	23 (11)	70 (35)	98 (48)	4 (2)	**195 (97)**	7 (3)

The percentage was taken by considering the decimal number >5, adding one in all the results SP = special lesion, * Numbers in parenthesis are percentiles**.**

Table 2: Immunohistochemical stain results from intestinal samples in apparently healthy Awassi sheep, 8-24 months, 2002.

Sites	No. of samples collected	No. of samples processed	Immunohistochemical stain				
			+	++	+++	Total	
						+ve	-ve
Amman	49	42 *(86)	40 (95)	-	-	**40 (95)**	2 (5)
Sweleh	63	56 (89)	39 (70)	9 (16)	7 (12)	**55 (98)**	1 (2)
Irbid	107	36 (34)	29 (81)	-	-	**29 (81)**	7 (19)
Total	219	134 (61)	108 (81)	9 (7)	7 (5)	**124 (93)**	10 (7)

*= Numbers in parenthesis are percentiles

Table 3: Distribution of positive cases of intestine (ileum) and mesenteric lymph nodes (MLNs) of cattle examined by histopathology and immunohistochemistry, Jordan 2004-2005.

Tissue	Histopathology			Immunohistochemistry		
	Tissue No.	Pos	(%)	tissue No.	Pos	(%)
Ileum	263	175	**66%**	170	110	**65%**
MLNs	263	67	**25%**	120	74	**61%**

Conclusion: The disease is found in Jordan with very high prevalence and cause economical losses to the farmers, the national herds and the national economy, although the reports in the Ministry of agriculture indicate that this disease is not found in Jordan. The same case is extended to the Arab countries. This disease will affect the trade of animals and animal products between Jordan and the rest of the Arab countries and the rest of the world.

Solutions: VERY DIFFICULT. Eradication is almost impossible. A control program is possible and will reduce the losses significantly. It needs a national plan supported by a political decision and well.

Recommendation: A four year control program.

Proposed Cost of the Program: 300000 JD.

Figure 1. A Group of Emaciated Sheep from Friday Market (Soug Al-Haramieh) at Ramtha Area.

Figure 2. Sheep Carcasses (Very Emaciated not more than 15 Kg each)Friday market.

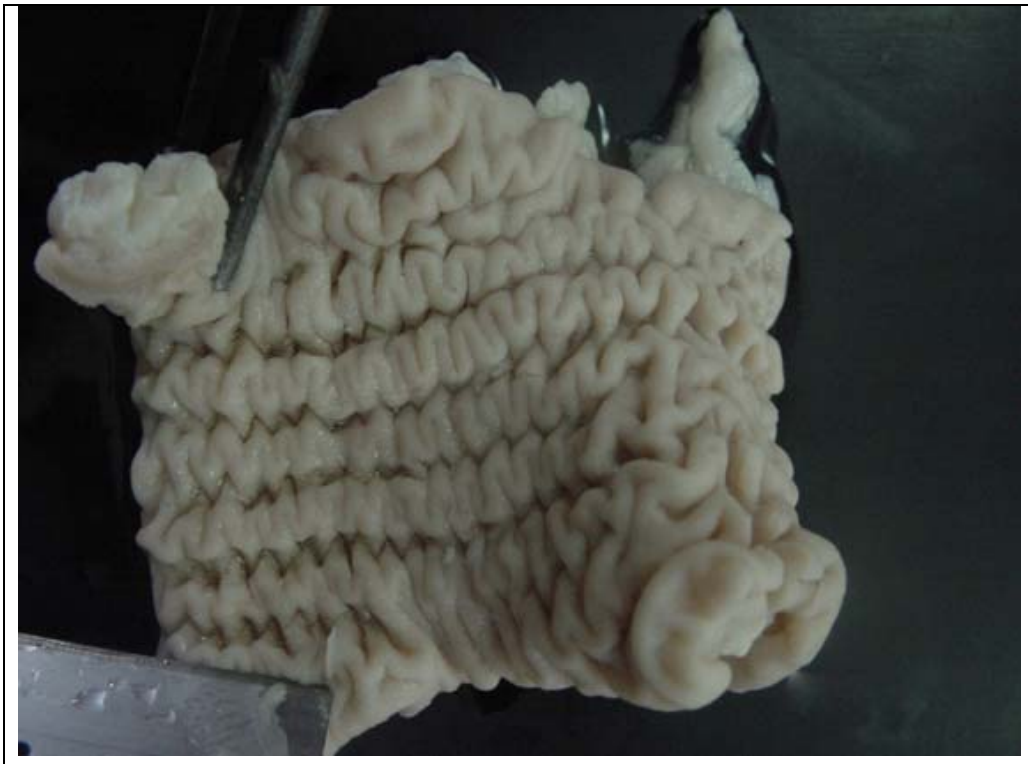

Figure 3.Thick Abnormal Ileum with Corrugations from the Above Shown Sheep

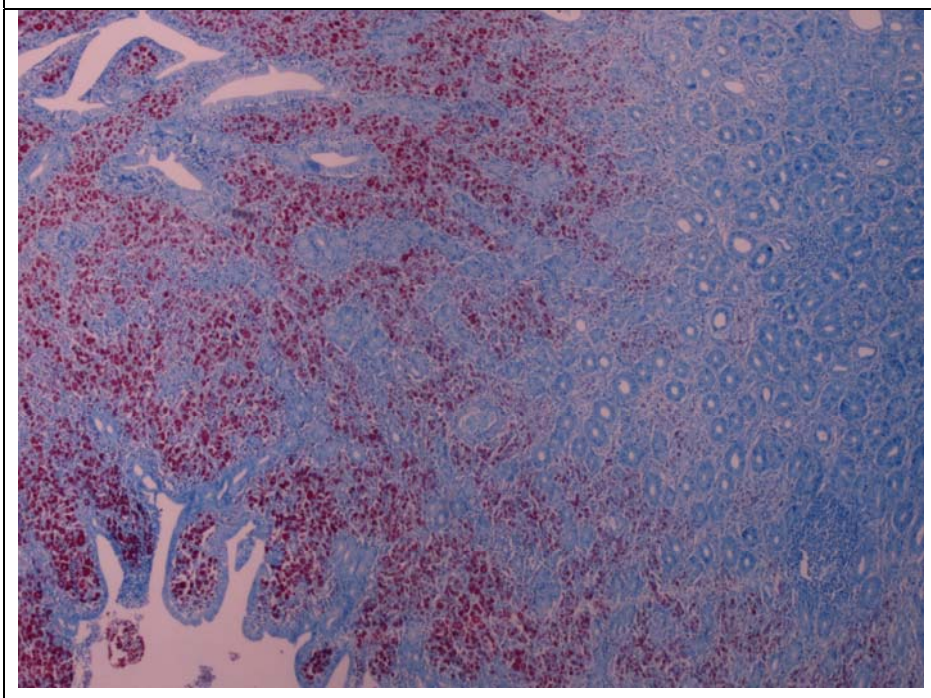

Figure 4. Histopathological Profile of the Ilium Stained with Acid- Fast Stain. Only the Macrophages Have the Bacteria which Get the Stain.

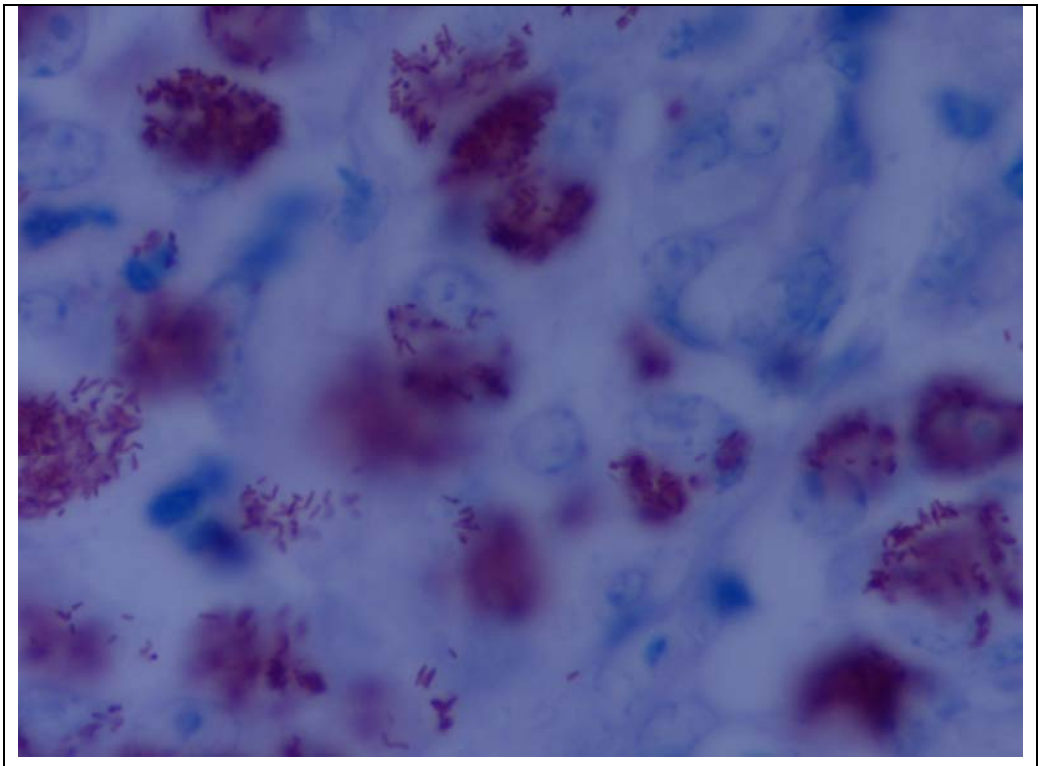

Figure 5: Higher Magnification of Figure 4. Note the Bacilli (Bacteria) Inside and Outside the Macrophages

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Ref. .....

Date. .....

الرقم : ٣/١/٦٤٦

التاريخ : ١/٧/١٤٢٨ هـ

الموافق : ١٦/٧/٢٠٠٧ م

معالي وزير الزراعة الأكرم

تحية طيبه وبعـــــد،،،

فأشير إلى كتابكم ذي الرقم 3589/1/9/5/5 تاريخ 2007/7/12 بخصوص تشكيل لجنـة فنيـة متخصصة لدراسة موضوع ظهور إصابات بمرض نظير السل Johne's Disease في محطـة الفجيج الزراعية لمعرفة نسبة انتشار المرض ووضع المقترحات المناسبة.

أرجو أن أسمي الأستاذ الدكتور نبيل هيلات للمشاركة في اللجنة الفنية المشار إليها أعلاه.

واقبلوا فائق الاحتـــرام

عميـــد كليـــة الطب البيطـــري

الأستـــاذ الدكتـــور صائب السخن

نسخة:-
- الدكتور رئيس قسم علم الأمراض وصحة الحيوان.
- الأستاذ الدكتور نبيل هيلات.

أرسلت فاكس وإيميل للطرفين
صباح ١٦/٧/٢٠٠٧

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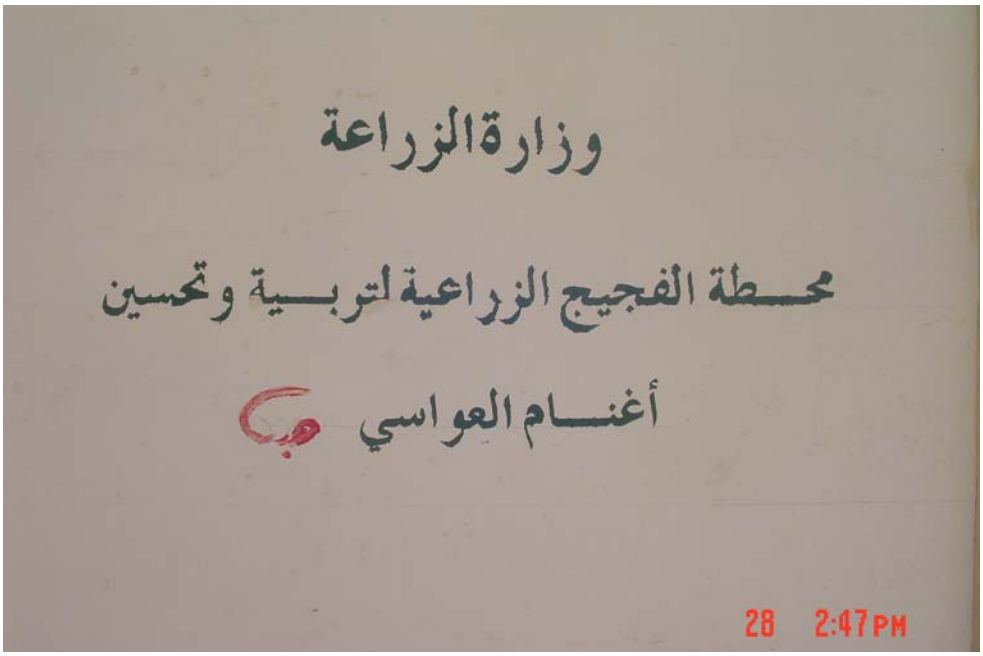
وزارة الزراعة
محطة الفجيج الزراعية لتربية وتحسين
أغنام العواسي
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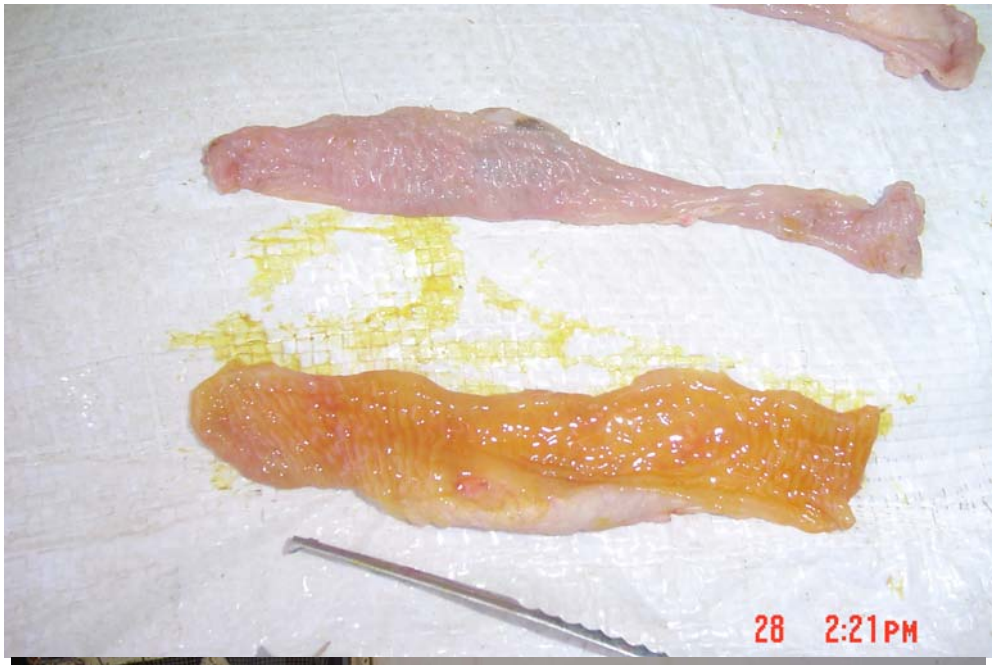
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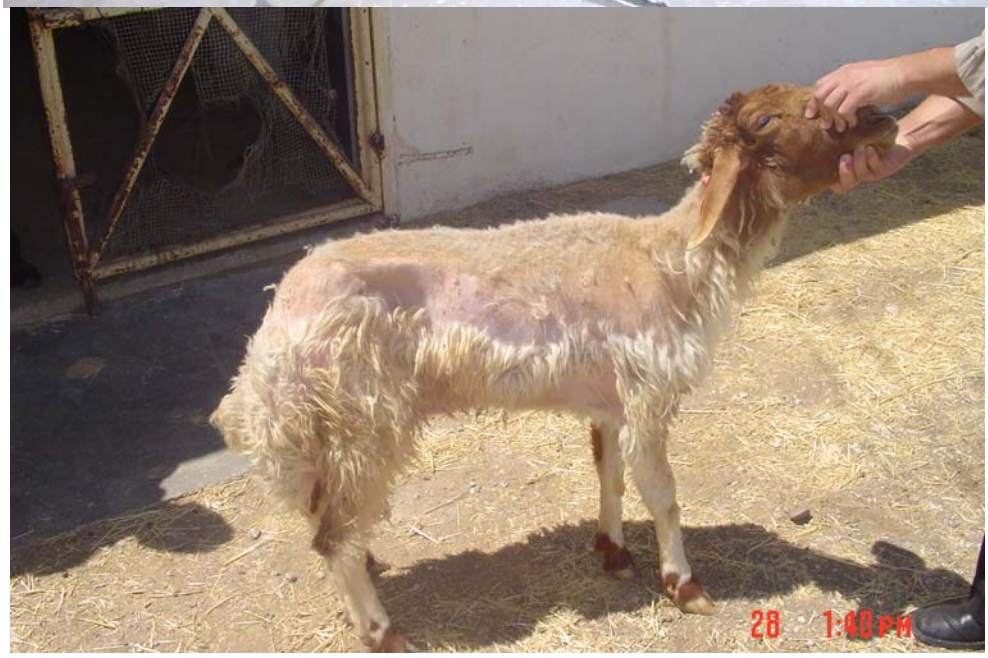
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...cy of Veterinary Medicine

كلية الطب البيطري

ص.ب (٣٠٣٠) اربد (٢٢١١٠) الأردن هاتف ٧٢٠١٠٠٠ (٢-٩٦٢) - فاكسميلي ٧٠٩٥١١٧ (٢-٩٦٢)

الرقم : ..... Ref. .....

التاريخ : ..... هـ ..... Date .....

الموافق : ٢٠٠٨/٨/٨ م

الدكتور رئيس قسم علم الامراض وصحة الحيوان

تحية طيبه وبعد،،،

فاشير الى شروحاتكم على حاشية كتاب معالي وزير الزراعه رقم ١١٨٢/٩/٥/٥ تاريخ ٢٠٠٧/٤/٢١م، والخاص بفحص عينات نسيجيه للكشف عن مرض نظير السل.
ارفق طياً التقرير الخاص بالحالة علماً بأن رقم الفاكس ٠٦/ ٥٦٨٦٣١٠.

واقبلوا فائق الاحترام،،،

الاستاذ الدكتور نبيل هيلات

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ص.ب (٣٠٣٠) اربد (٢٢١١٠) الأردن هاتف ٧٢٠١٠٠٠ (٢-٩٦٢) – فاكسميلي ٧٠٩٥١١٧ (٢-٩٦٢)

Ref. ..... الرقـــم : .....

Date 9-5-2007 التاريخ : ..... هـ

الموافـق : ..... م

Histopathological Report on Awassi Sheep Samples Submitted by the Ministry of Agriculture

Histopathological, Immunohistochemistry and Acid Fast Stain examination were conducted on tissue samples from the small and large intestines and their corresponding lymph nodes of Awassi sheep submitted by the Ministry of Agriculture dated 21-4-2007 with reference number 5/5/9/1182.

Findings and Results:

It was found that the mucosa was moderately to severely infiltrated with mononuclear cells. Several islands and sheets of epitheloid cells were also seen in the mucosa between the crypts and in the tip of villi which were thick because of the inflammatory cells. Epitheloid cells and lymphoid depletion in the lymph nodes were also seen. These lesions are compatible with the lesion found in Johne's Disease. Acid fast stain examination revealed red spots in the cytoplasm of the epitheloid cells indicating the presence of acid fast microorganisms. Immunohistochemistry using monoclonal antibodies against *M.paratuburculosis* reacted positively with the tissue confirming the presence of the microorganisms of *M.paratuburculosis.*

Diagnosis and conclusion: The sheep from which the samples were taken had Johne's Disease.

Comments: If there is one animal clinically sick with Johne's in a herd or a flock, this means there are another 20 animals subclinically sick with Johne's Disease. Those animals are shedding the causative agents and contaminating the environment. The disease needs a long term planning and commitment for its control.

Prof. Nabil Hailat and Dr. Wael Hananeh
Department of Pathology and Animal Health
Faculty of Veterinary Medicine
Jordan University of Science and Technology
Irbid-Jordan 22110

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Ministry of Agriculture
Al-Fjaij Agricultural Services

Our flock in detail

Data collected, reviewed, and presented upon request from the comity assigned to assess Johne's disease (Paratuberculosis) situation in Al-Fjaij Agricultural Services

By

Dr.Mothafer Al-Rjoub
الدكتور
مظفر الرجوب
Supervising Veterinarian / Al-Fjaij Agricultural Services

27/8/2007

This data was collected upon request from and handed over to the joint comity from the Ministry of Agriculture and the Jordan University of Science and Technology for the purpose of evaluating the Paratuberculosis situation in Al-Fjaij Agricultural Services and it must be used for that purpose only. It is also prohibited to publish or hand over any data to unauthorized people.

Investigation on the Prevalence and Pathology of Paratuberculosis (Johne's disease) in Apparently Healthy Cattle in Jordan

N Hailat[a,*], W Hananeh[a], H Hemida[a], JR Stabel[c], S Jaradat[b] and A Al-Saleh[a]

[a] Pathology Laboratory, Department of Pathology and Animal Health, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid, PO Box 3030, Jordan

[b] Department of Genetic Engineering, Faculty of Science and Art, Jordan University of Science and Technology, Irbid, PO Box 3030, Jordan

[c] USDA-ARS, National Animal Disease Center, Ames, IA 50010, USA

Abstract

Paratuberculosis (Johne's disease) is infectious, chronically progressive granulomatous enteritis which affects domestic and exotic ruminants. The causative agent is *Mycobacterium Avium Paratuberculosis (M Johnei)*, a slow growing mycobactin-dependent acid-fast bacillus. We investigated the occurrence of Johne's disease in apparently healthy cattle, using 263 ileum and corresponding mesenteric lymph nodes, by histopathological examination, and 170 ileum and 120 mesenteric lymph nodes by immunohistochemical examination. The prevalence of the disease

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was 65% and 66% using immunohistochemistry and histopathology techniques respectively. When ZN and ELISA techniques were implemented, the prevalence was 1% (4/120) and 3% (8/278) respectively. Grading from I-IV of histopathological lesions based on type of cellular infiltrate and severity of lesions revealed most positive cases in grades I and II. Furthermore staging I-III of immunohistochemistry results, has presented a high number of positive cases in stage I. Statistical analysis of these results showed a significant correlation between histopathology and IHC. On the other hand, ELISA showed a low prevalence (3%) in this study reflecting its low sensitivity for the diagnosis of subclinical JD. These results showed that histopathology is a very good diagnostic method for subclinical paratuberculosis in cattle especially when it is coupled with IHC. We conclude that JD is prevalent in cattle in Jordan and it is the first study of JD in the country and the results strongly suggest alarming fears of the severity of disease at the national and probably the region level. Thus a national control strategies are well founded due to its economical importance.

Key words: Cattle; enzyme-linked immunosorbent assay; histopathology; ileum; immunohistochemistry; Paratuberculosis, Jordan.

Pathology of Subcliniclal Paratuberculosis (Johne's Disease) in Awassi Sheep with Special Reference to its Prevalence in Jordan

N Hailat[a,*], W Hananeh[a], AS Metekia[a], JR Stabel[b], A Al-majali[c], and S Lafi[a]

[a] Pathology Laboratory, Department of Pathology and Animal Health, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid, PO Box 3030, Jordan

[b] USDA-ARS, National Animal Disease Center, Ames, IA 50010, USA

[c] Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Jordan University of Science and Technology, Irbid, PO Box 3030, Jordan

Abstract

Paratuberculosis (Johne's disease) is an incurable infectious, chronically progressive enteric disease affecting domestic and exotic ruminants. The causative agent is *Mycobacterium Avium Paratuberculosis (M Johnei)*. In this study, the occurrence of subclinical Johne's disease in Awassi sheep is investigated. Histopathological examination of 202 ilea and the corresponding mesenteric lymph nodes was conducted. In addition, immunohistochemical examination, using rabbit polyclonal antiserum, of 134 ilea and 83 mesenteric lymph nodes was also conducted. The prevalence of the disease was 97% and 93% using histopathology and

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immunohistochemistry techniques, respectively. When the lymph nodes were tested, it was revealed that 79% of them were positive by IHC. The histopathological lesions were graded from I-IV, I being the least severe, based on the type of cellular infiltrate (lymphocytes, macrophages and epithelioid cells) and the severity of the lesions. Analysis of the results revealed that most positive cases were in grades I and II. Furthermore, the IHC reactions were classified into three types depending on the number of stained cells and the intensity of the staining (mild, moderate and strong). Direct smears, and tissue sections obtained from the ilea and stained with ZN revealed that out of 219 and 202 samples, 53 (24%) and 22 (11%) were positive respectively. Results of the culture revealed that 22 (10%) out 219 were positive. These results showed that subclinical paratuberculosis in sheep is very prevalent in Jordan and strongly suggest alarming fears of severity of the disease at the national level.

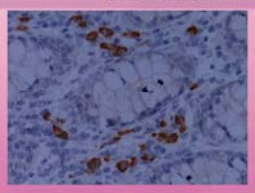
Keywords: Sheep; Paratuberculosis; histopathology; enzyme-linked immunosorbent assay; ileum; immunohistochemistry.

Enhanced Diagnostic Capacity and Control Measures of Some Transboundary Animal Diseases with Emphasis on Pathology and Epidemiology of Johne's Disease/Paratuberculosis in Jordan, Egypt and Algeria

Organized and coordinated by:

Prof. Dr. Nabil Hailat

	Time
DAY 1	
* Participants are received at the airport and taken to hotels	
* Registration and Opening ceremony	9:30-10:30
* Coffee break	10:30-11:00
* Country report of Egypt on Johne's Disease....Prof. Al-Attar	11:00-11:20
* Country report of Algeria on Johne's Disease....Prof. O. Rachid	11:20-11:40
* Country report of Jordan on Johne's Disease....Dr. Al-Bakheet	11:40-12:00
* Basic epidemiology and epidemiology of Johne's disease in the region....Prof. S.Lafi (Faculty of Veterinary Medicine (FVM)) + Discussion	12:00-01:00
* Lunch time	01:00-02:00
* Johne's disease definition, pathology and clinical signs....Prof. Nabil Hailat	02:00-02:30
* Diagnosis of Johne's disease....Dr. Wael Hananeh	02:30-03-00
* Crohne's disease in Jordan....Dr. Jad Allah/Faculty of Medicine/JUST	03:00-03:30
* Prevalence and pathology of Johne's disease in sheep & goats....Prof. Nabil Hailat	03:30-04:00
* Prevalence and pathology of Johne's disease in cattle....Prof. Nabil Hailat	04:00-04:30
DAY 2	
* Principle of histopathology ....Prof. Nabil Hailat	08:00-09:00
* Principle of immunohistochemistry....Prof. Nabil Hailat	09:00-10:00
* Practical session 1 :	
* Preparation of tissues and slides for Histopathology and Immunohistochemistry....Prof. Nabil Hailat	10:00-06:00
DAY 3	
* Practical session 2 :	
* Reading and evaluation of Histopathology and Immunohistochemistry slides....Prof. Nabil Hailat	08:00-01:00
* Contagious Bovine Keratitis....Dr. H. Al-Maghreby	02:00-02:20
* Avian influenza in Egypt....Dr. Muna Ali	02:20-02:50
* Avian influenza....Dr. Sa'ad Gharaibeh	02:50-03:20
* Avian influenza....Dr. Muh'd Al-Natour	03:20-03:50
* Discussion and Recommendations	03:50-05:00



Workshop Program

Enhanced Diagnostic Capacity and Control Measure of Some Transboundary Animal Disease with Emphasis on Pathology and Epidemiology of Johne's Disease / Paratuberculosis in Jordan, Egypt and Algeria *Organized and Coordinated by:*

Prof. Nabil Hailat

7-9 August, 2007

* Coffee Break (10:30-11:00)

* Registration and Opening Ceremony (9:30-10:30)

Day 1: Cont. , Morning Session/ Chairman: Prof. N. Hailat , Co-Chairman: Prof. Al-Maghreby			
Time	**Title of Presentation**	**Name of Speaker**	**Affiliation**
11:00-11:20	Country Report of Egypt on Johne's Disease	Prof. Al-Attar	President of Benha University
11:20- 11:40	Country Report of Algeria on Johne's Disease	Prof. O. Rachid	President of El-Taref University Center
11:40-12:00	Country Report of Jordan on Johne's Disease and Future Control Programmers	Dr. F. Al-Bakheet	General Veterinary Directorate , Ministry of Agriculture
12:00-12:30	Crohn's Disease in Jordan	Dr. Jad Allah	Faculty of Medicine, JUST
12:30-1:30	Lunch time at JUST		
Afternoon Session/ Chairman: Prof. Al-Attar, Co-Chairman: Dr. Abdallh Metai			
1:30-2:30	Basic Epidemiology and Epidemiology of Johne's Disease in the Region	Prof. S. Lafi	Faculty of Veterinary Medicine(FVM), Jordan University of Science and Technology (JUST)
2:30-3:00	Johne's Disease Definition, Pathology and Clinical Signs	Prof. N. Hailat	FVM, JUST
3:00-3:30	Diagnosis of Johne's Disease	Dr. W. Hananeh	FVM, JUST
3:30-4:00	Prevalence and Pathology of Johne's Disease in Sheep and Goats	Prof. N. Hailat	FVM, JUST
4:00-4:30	Prevalence and Pathology of Johne's Disease in Cattle	Prof. N. Hailat	FVM, JUST
8:00	Diner		
Day 2, Morning Session/ Chairman: Prof. O. Rachid, Co-Chairman: Prof. H. Bakery			
Time	**Title of Presentation**	**Name of Speaker**	**Affiliation**
8:00-9:00	Principle of Histopathology	Prof. N. Hailat	FVM, JUST
9:00-10:00	Principle of Immunohistochemistry	Prof. N. Hailat	FVM, JUST
10:00-1:00	**Practical Session 1 :** Preparation of Tissue and Slides for Histopathology and Immunohistochemistry	Prof. N. Hailat	FVM, JUST
1:00- 2:00	Lunch at JUST		
Time	**Title of Presentation**	**Name of Speaker**	**Affiliation**

2:00-6:00	**Practical Session 1 :** Preparation of Tissue and Slides for Histopathology and Immunohistochemistry	Prof. N. Hailat	FVM, JUST
8:00	Diner		
Day 3			
8:00-10:00	**Practical Session 2:** Reading and Evaluation of Histopathology and Immunohistochemistry Slides	Prof. N. Hailat	FVM, JUST
10:00-10:15	Coffee Break		
10:15-12:30	**Cont. Practical Session 2:** Reading and Evaluation of Histopathology and Immunohistochemistry Slides	Prof. N. Hailat	FVM, JUST
12:30-1:30	Lunch at JUST		
Afternoon Session/Chairman: Dr.Samir ALfuqaha, Co- Chairman: Prof. N. Hailat			
1:30- 1:50	Diagnosis, Treatment and Control of Contagious Bovine Keratitis	Dr. H.Al-Maghreby	Benha University
1:50-2:10	The Experience of Egypt Pertaining to Avian Influenza (H5N1) with Emphasis on Control Program	Prof. Amal Abd El_nassar	Benha University
2:10-2:30	Laboratory Diagnosis of Avian Influenza/H5N1	Dr. S. Gharaibeh	FVM, JUST
2:30-2:50	Virology and Epidemiology of H5N1/ Jordan Emergency plan	Dr. M. Al-Natour	FVM, JUST
2:50-3:10	Genetic Comparison of H9N2,H5N1 and H7N7	Dr. Nadim Amarin	Poultry Vaccine Technical Executive
3:10-3:20	Discussion and Recommendation		

ص.ب (٣٠٣٠) إربد (٢٢١١٠) الأردن هاتف ٧٢٠١٠٠٠ (٢-٩٦٢) فاكس ٧٢٠١٠٨٥ (٢-٩٦٢)

الرقم: ٣٢٥/٣٠/١٧٠٨ Ref. :

التاريخ: ..... هـ Date :

الموافق: ١١-٩-٢٠٠٧ م

الأستاذ الدكتور نبيل هيلات

كلية الطب البيطري

تحية طيبة وبعد،

أرجو التكرم بالإطلاع على خلاصة تقييم دورة " **الأمراض العابرة للحدود مع التركيز على مرض نظير السل وانفلونزا الطيور** " التي عُقدت خلال الفتـــرة ما بين ٧-٢٠٠٧/٨/٩، بعد تعبئتها من قبل المشاركين بالدورة.

وتفضلوا بقبول فائق الاحترام

مدير المركز الاستشاري للمعلوم والتكنولوجيا

أ.د. خليل إبراهيم عريفج

.../ر.م.

P.O. Box: (3030) Irbid 22110 Jordan Tel.: (962-2) 7201000 - Fax: (962-2) 7201085
E-mail: consult@just.edu.jo

المركز الاستشاري للعلوم والتكنولوجيا
دائرة التعليم المستمر
خلاصة تقييم الدورات التدريبية

اسم الدورة: نظير السل في الأمراض العابرة للحدود اسم المحاضر/ المحاضرون: أ.د. نبيل هيلات

تاريخ انعقاد الدورة: ٧-٢٠٠٧/٨/٩ عدد ساعات الدورة:

	عناصر التقييم		ممتاز	جيد جداً	جيد	مقبول	ضعيف
١	**أهداف الدورة ومدتها**	١- مدى وضوح الأهداف	١٢	٣			
		٢- ملائمة مدة الدورة لمواضيعها	٨	٤	٢	١	
		٣- تغطية المواضيع لاحتياجاتك التدريبية	٧	٧	١		
٢	**موضوع الدورة**	١- إعطاء الأولوية لموضوع الدورة	٨	٥	٢		
		٢ - مدى ترابط وتسلسل مواضيع الدورة	١٠	٣	٢		
		٣- مدى توافر مادة علمية تغطي مواضيع الدورة	١٢	٢	١		
٣	**برنامج الدورة**	١- تجانس مواضيع الدورة مع أهدافها	١٠	٤	١		
		٢ -الفترة المحددة لحل الأمثلة التوضيحية	٥	٨	٢		
		٣- الفترة المحددة للتطبيقات العملية(إن وجدت)					
٤	**التسهيلات والخدمات**	١-ملائمة مكان التدريب من حيث السعة والترتيب والمقاعد	٧	٧	١		
		٢- ملائمة مكان التدريب من حيث الإضاءة والتهوية والضجيج	١٤	١			
		٣- وسائل التدريب الإيضاحية	١٥				
		٤- مدى كفاءة أجهزة الحاسوب المستخدمة في الدورة	٦	٥	١		
٥	**المحاضرون**	١-سعة صدرهم وحماسهم للتدريب	١٤	١			
		٢-قدرتهم على إيصال المعلومات	٧	٨			
		٣-التزامهم بمواعيد المحاضرات	٧	٨	١		
		٤- إعطاء فرصة للنقاش	٩	٦			
		٥- قدرتهم على جذب اهتمام المشاركين	٧	٨			
٦.	تقييم ذاتي	١- مستوى المشارك بمواضيع الدورة قبل الاشتراك	٣	٨	٢	١	
		٢- مستوى المشارك بمواضيع الدورة بعد الانتهاء منها	٨	٦			
٧	**ملاحظات أخرى:**						

Recommendations:

1. Prepare and produce leaflets and brochures for farmers education and awareness.
2. Conduct more field days and workshops on how to reduce the impact of Johne's disease on animal industry.
3. Start national program for control and prevention of the disease and search for proper funding.
4. Enhance the diagnostic capacity of the pathology laboratory with emphasis on transboundary animal diseases.
5. Start regional research program for the purpose of controlling the disease.
6. Form national committees in the Ministries of Agriculture for Johne's disease control and prevention.
7. Review and update the policy of the government for animal trade.
8. Establish regional association for Johne's disease for information dissemination and education.

#Lec	*Tital of the presentation*	*Name of the speaker*	#Page
Lec.1	Johne's disease (Paratuberculosis Johne's disease (Paratuberculosis)	Prof.Hussam El-Din El-Attar	39
Lec.2	*Paratuberculosis In Algeria* Is Algeria Really Unharmed by this Pathology ?	*Prof. O. Rachid*	50
Lec.3	Country Report of Jordan on Johne's Disease and Future Control Programmers	Dr. F. Al-Bakheet	54
Lec.4	Crohn's Disease in Jordan	*Dr. Jad Allah*	58
Lec.5	Basic Epidemiology and Epidemiology of Johne's Disease in the Region	Prof. S. Lafi	70
Lec.6	Johne's Disease Definition, Pathology and Clinical Signs	Prof. N. Hailat	77
Lec.7	Diagnosis of Johne's Disease	Dr.Wael Hananeh	84
Lec.8	Prevalence and Pathology of Johne's Disease in Sheep and Goats	Prof. N. Hailat	91
Lec.9	Prevalence and Pathology of Johne's Disease in Cattle	Prof. N. Hailat	118
Lec.10	Paratuberculosis In Algeria Is Algeria really unharmed by this pathology	Dr.Ryad Bouzidc	131
Lec.11	Principles of Pathology and Technical Aspects of Immunohistochemistry	Prof. N. Hailat	137
Lec.12	Infectious Bovine Keratoconjunctivitis (Pinkeye – Contagious Ophthalmia- IBK)	Prof.Hussein El-Maghraby	147
Lec.13	Avian Infleunza	Prof Dr Amal Abdel Naser	154
Lec.13	Prevention & Control of Avian Infleunza	Prof Dr Amal Abdel Naser	168
Lec.14	Avian Influenza Surveillance and Diagnostics	Dr.Saad Gharaibeh	171
Lec.15	Avian Influenza	Dr. Mohammad Q. Al-Natour	178
Lec.16	Genetic Comparison Of H9N2 AI Viruses Isolated	Dr. Nadim M. Amarin	186

Lecture #2

Paratuberculosis In Algeria

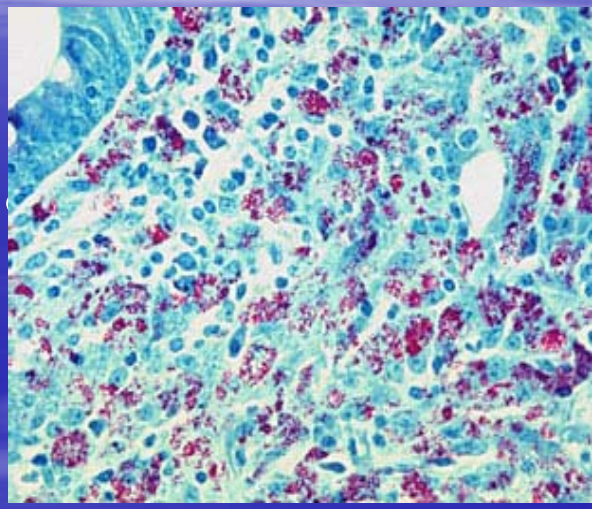
Is Algeria really unharmed by this pathology ?

PRESENTED BY
Dr BOUZID RIAD , METAI A ,SOUISSI M and OUZROUT R

L'Algerie est elle vraiment indemne ?

Paratuberculosis infectious enzootic, contagious, incurable, disease with a long period of incubation

Mycobacterium avium subspecies paratuberculosis.



Belgium, seroprevalence cattle 17,4

Netherlands 54,7 %

In the United States, 22 % of the 1008 dairy milky cattle have a prevalence of

infected animals superior to 10 % [6], and 9 % of milk-feeding cattle

,

Algeria is officially declared unharmed of

paratuberculosis and no real study has been

realized up till now

Clinical Signs

intractable chronic diarrhea
emaciation

↓Productivity

In Small ruminants. Animals lose
few

weeks of chronic evolution.

Paratuberculosis present the most chronic evolution from

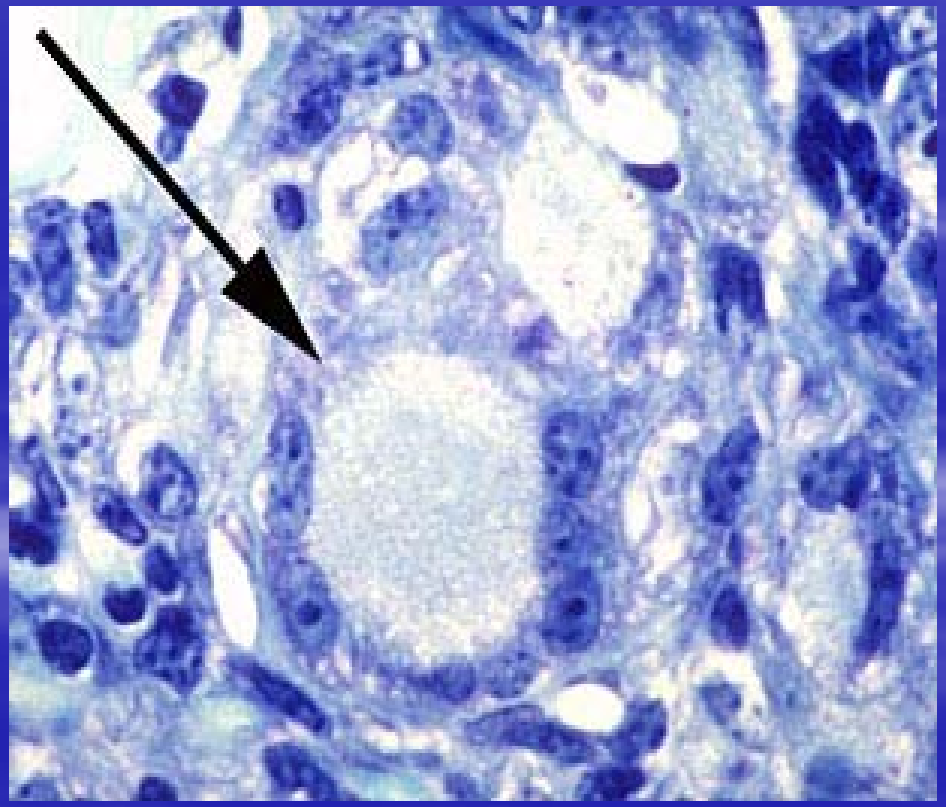
all bacterian diseases,Without any treatment

Macroscopic lesions

caudal small intestine, cecum, and proximal colon.
Mesenteric lymph nodes
The initial localization of the infections process and the small distal intestine ,especially the terminal ileum and the ileo-caecal valvula

Microscopic lesions

granulomas in the intestines, lymphatics, and lymph nodes



Diagnostic

Detection with Coproculture

it is the most reliable techniques of confirmation of the excretion

Specificity : 99.9% **Sensibility : 50 %**

Detection with PCR

Spécificité : 97-99%

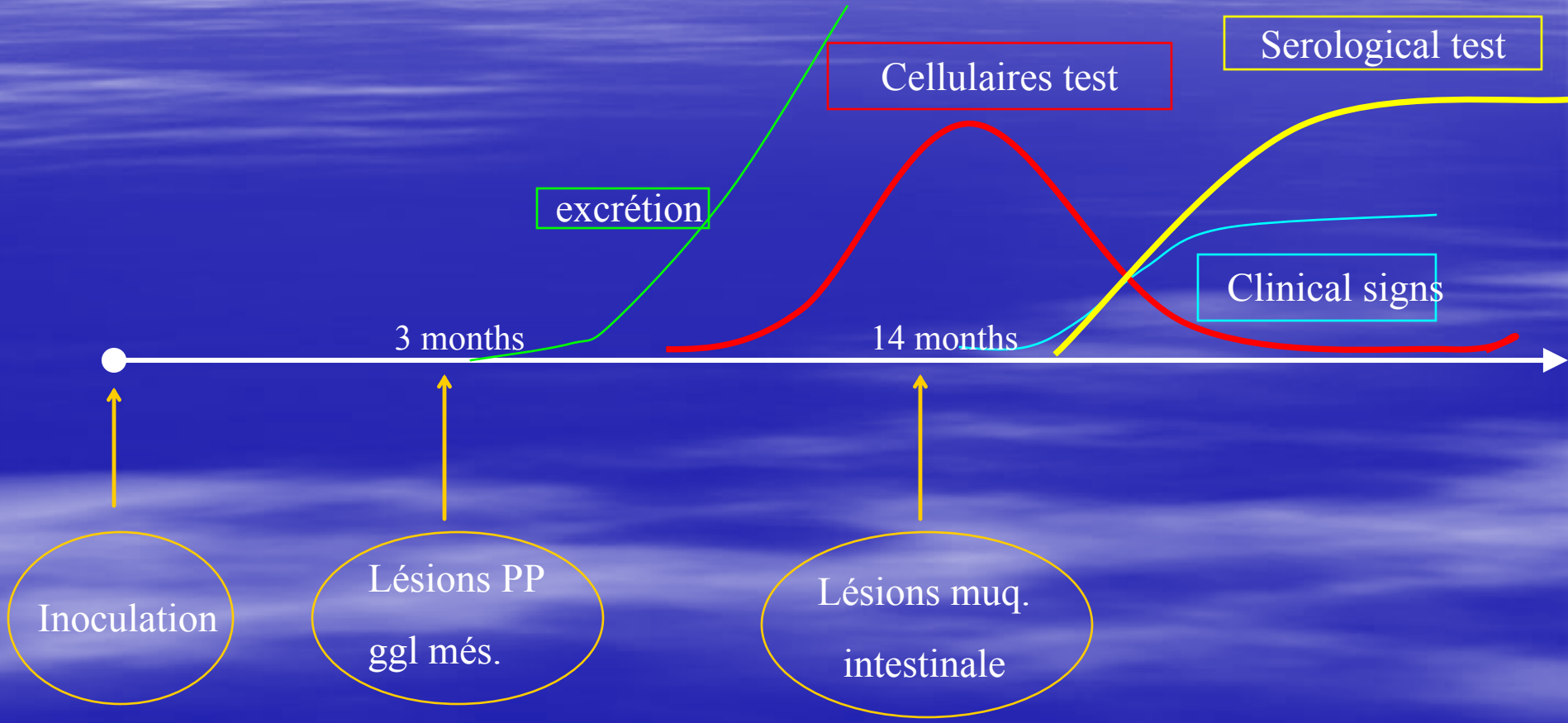
rapid but difficult and expensive technique.

Detection with ELISA

presence of anti-bodies over an infected animal by paratuberculosis is not systematic

No technique presents sufficient reliability

Limites of the tests



Bovin live-stock and the breeding system in Algeria

- **Sheep:** 8 896 919
- **goats :** 3 272 024
- **Cattle:**1 434 770
- **Camels :** 278 023

80 percent of cattle are in the North, of these 53 percent are to the east and 24 percent to the west with 23 percent in the centre

raising mode

raising in mountainuous zones

raising in interior plains

Main diseases of obligatory declaration in Algeria

Sheep-Pox / Rabies / Bovin brucellosis
Caprine brucellosis / Bovin tuberculosis

Other encountered diseases

Various infections and parasitical diseases affect our live-stock, allmixed species

Commentaries and conclusion

animal pathologies in Algeria are essentially drawn from the magazine (Santé Animale Mondiale of the Office International des Epizooties.

The reality on the ground is some thing else, the pratician veterinarians often declare paratuberculosis symptoms.

This pathology is underestimated in Algeria because it doesn't provoke attrocious mortality cases similar to big epizooties as the bovin plague ,the bovin contagious peri-pneumonia or the charcoal ,it doesn't constitue a priority.

Generally the breeders sloughter their emaciated animals and less productive before the appearance of clinical signs of the disease before its diagnostics

The paratuberculosis is an important disease regarding its economic impact over the productivity of the breedings

The precocious reform,the decreasing of milk production ,reduced fertility,the growing lateness and the increased mortalities are the main consequence of this disease

scientific community continues to search to know if it exists a relation between the disease of Crohn

this potential impact for the public health is more ans more mediatized and could become a subject of auxiety for the consumer

Lectuer # 3

Country Report of Jordan on Johne's Disease and Future Control Programmers

PRESENTED BY:
Dr. F. Al-Bakheet, Ministry of Agriculture Jordan

برامج السيطرة على الامراض الحيوانية المشتركة

خطة وزارة الزراعة للسيطرة و القضاء على مرض السل البقري

د.لورا صوالحة
رئيس شعبة الاستقصاء الوبائي

برنامج مرض السل البقري

- اهم بنود الخطة تتلخص بما يلي:

- الحد من انتشار المرض لمزارع الابقار السليمة
- فحص الحيوانات المستوردة
- منع انتقال المرض بين الحيوان و الانسان

الخطوات المتخذة لتنفيذ البرنامج

- تشكيل لجنة فنية و لجنة توجيهية لمتابعة و تقييم البرنامج
- تشكيل فرق عمل ميدانية و تامينها بالمستلزمات الضرورية
- تدريب الفرق على تنفيذ اعمال الفحص الميداني للمرض
- جمع و تحليل النتائج بواسطة برنامج احصائي محوسب

خطة العمل التنفيذية

- الحد من انتشار المرض في المزارع السليمة

- اجراء المسح الشامل لتغطية كافة الحيازات
- تحديد المزارع و المناطق الخالية من المرض
- تحرير شهادات صحية للمزارع المفحوصة و الخالية
- توعية المزارعين حول المرض و طرق انتقالة
- اصدار تعليمات خاصة بمرض السل البقري

خطة العمل التنفيذية

- فحص الحيوانات المستوردة
  - التشديد في تطبيق انظمة الحجر البيطري
  - فحص الابقار حال دخولها الى الاردن للتاكد من الشهادات الصحية المرفقة مع الارساليات
  - تصويب اوضاع المحاجر البيطرية الحكوميةو انشاء محاجر تتسع للمستوردات من الحيوانات الحية

خطة العمل التنفيذية

- منع انتقال المرض بين الحيوان و الانسان
- التكثيف من برامج التوعية و الارشاد البيطري
- تفعيل الرقابة على الذ بح العشوائي غير القانوني بالتعاون مع الوزارات و البلديات و المؤسسات ذات العلاقة
- تشديد الرقابة الصحية البيطرية على الذبائح داخل المسلخ

الية التنفيذ

نوع الفحص المستخدم :

اختبار فحص الحساسية المقارن بواسطة استخدام مادة السللين البقري و الطيري التي تحقن في منطقة الرقبة و تقراء النتائج بعد 72 ساعة اعتمادا على سماكة الجلد ما قبل و ما بعد الحقن و تعبئة النتائج على استبان خاص و من ثم ادخالها على قاعدة بيانات خاصة

الية التنفيذ

حجم العينة

تم تحديد حجم العينة اعتمادا على الدراسات الاولية التي اجريت سابقا حيث تم تحديد نسبة الانتشار 10% و معدل ثقة 95%

و تم توزيع الجداول الاحصائية على اطباء الميدان ليتمكنوا من تحديد حجم العينة حسب حجم القطيع الكلي في المزرعة اثناء الزيارة للقطيع

الية التنفيذ

النتائج التي تم الحصول عليها لغاية هذا التاريخ :

- تم اجراء الفحص على 2050 راس من الابقار في كافة محافظات المملكة و تم تحليل النتائج ,وسوف يستكمل العمل خلال 3 اشهر القادمة.
- بانتظار قرار اللجنة التوجيهية لاستكمال بنود الخطة

الية التنفيذ

- اما بخصوص المستورد

✓ تم مخاطبة الدول التي لم تلتزم بالشروط الواردة في رخصة الاستيراد و المتعلقة بخلو الحيوانات من الامراض الوبائية و المعدية و ان تكون مفحوصة ضد مرض السل البقري قبل الشحن و بنتيجة سلبية

✓ تم التشديد على اجراء الفحص على نفقة المستورد

✓ ارسال الابقار ذات النتيجة الموجبة للفحص الى المسلخ لاجراء المزيد من الفحوصات العينية و المخبرية

مرض نظير السل

- تم تشحيص مرض نظير السل من خلال التشريح في الاردن من قبل كادر محطة الفجيج الزراعية/معان في قطيع اغنام يحتوي علي 700 راس حيث تم مراقبة القطيع لفترة من الزمن من قبل الطبيب المشرف على المحطة والذي لاحظ ازدياد حالات النفوق في القطيع و عدم الاستجابة للعلاجات المتجدمة بشكل روتيني لعلاج بعض الحالات المرضية في القطيع.

مرض نظير السل

تم عمل استقصاء و بائي و تقييم وضع المحطة و ارسال عينات ليتم تشخيصها مخبريا في جامعة العلوم و التكنولوجيا .

كانت نتائج الفحوصات المخبرية موكدة لنتائج التشحيص الاولي

الفحص النسيجي

الفحص البكتيري (صبغة خاصة لبطتيري مرض جونز)

الفحص المناعي النسيجي

مرض نظير السل

تمت الاستجابة لمكافحة هذا المرض بتشكيل لجنة فنية متخصصة لدراسة النتائج المخبرية من قبل المختصين ليتم تقييم وضع المحطة التي تم تشخيص المرض بها ليتم وضع الاقتراحات لحلول ممكنة ليتم تطبيقها على مستوى المحطة ذات العلاقة.

و من ثم عمل دراسة اولية لتشمل قطاع الثروة الحيوانية(ابقار و اغنام (في المملكة. لتحديد حجم المشكلة لوضع سياسة عامة لنتمكن من القضاء و السيطرة على المرض ومنع انتشارة

المشاركة في الدورة التدريبية التي ستعقد في جامعة العلوم حول طرق تشخيص المرض .

برامج السيطرة على الامراض الحيوانية المشتركة

- الدكتور فارس البخيت

مدير مديرية البيطرة /قطاع الثروة الحيوانية

وزارة الزراعة

Lecture # 4

Crohn's Disease in Jordan

RESENTED BY:

Dr. Jad Allah Faculty of Medicine
Jordan University of Science and Technology

Objectives of Presentation

- Review the role of various etiologic factors in the pathogenesis of Crohn'n disease (CD)
- Review the current state of knowledge relevant to a **microbial etiology** of CD
- Discuss the case FOR and the case AGAINST the etiologic role of *mycobacterium avium paratuberculosis* (MAP) in CD
- Discuss the possible connection between CD and **Johne's Disease (JD)**
- Define needed research that could shed light on the etiology and pathogenesis of CD

Definition

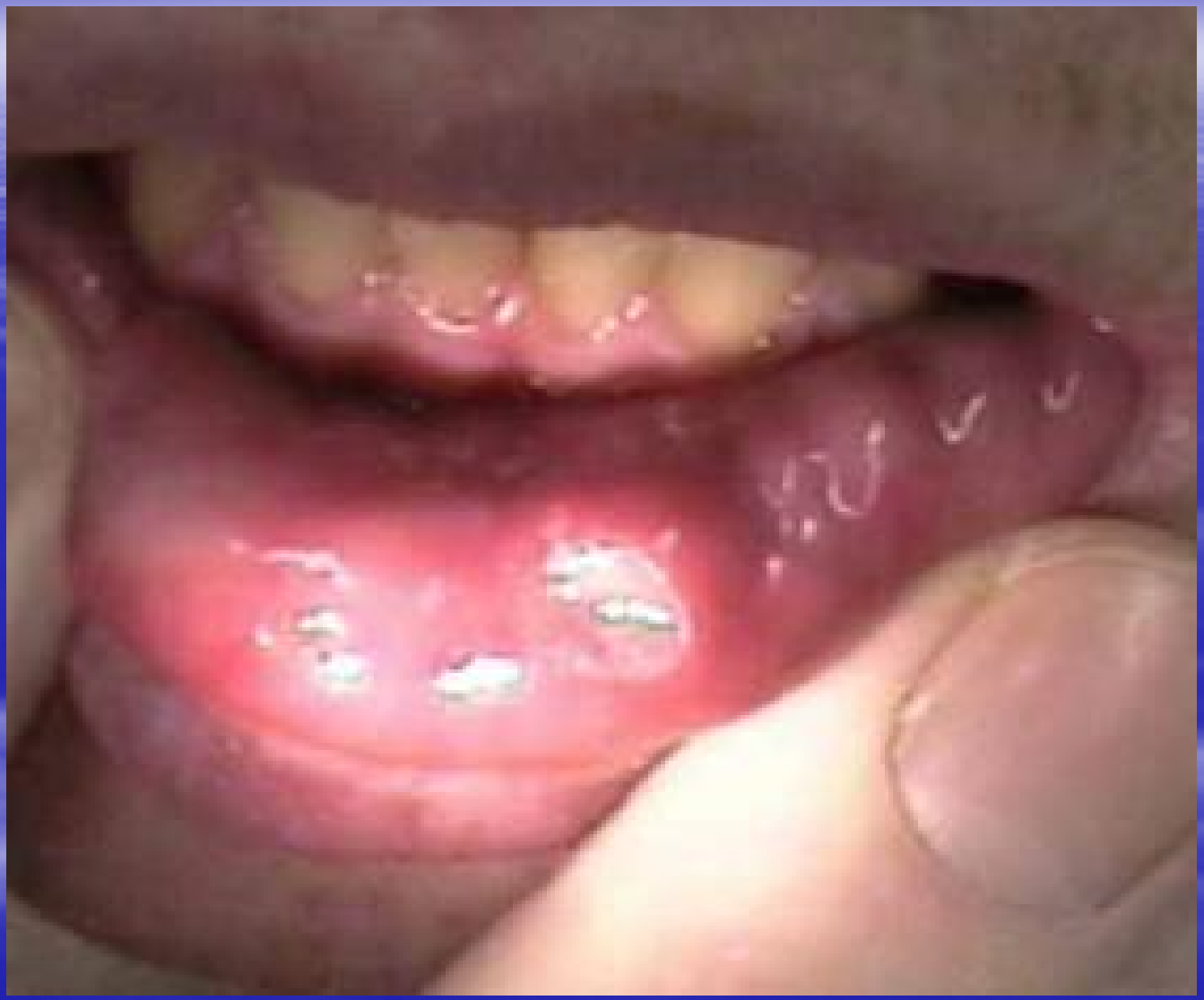
Crohn's disease (CD) is an idiopathic, chronic-relapsing, transmural inflammatory process of the bowel that can affect any part of the GI tract from the mouth to the anus

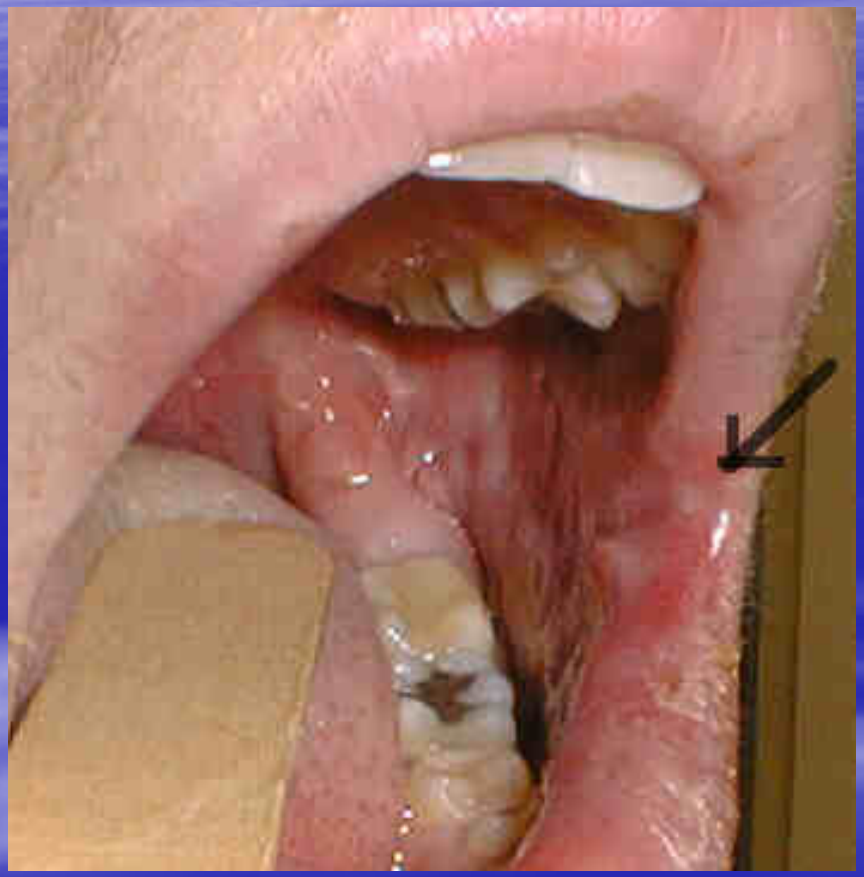
History

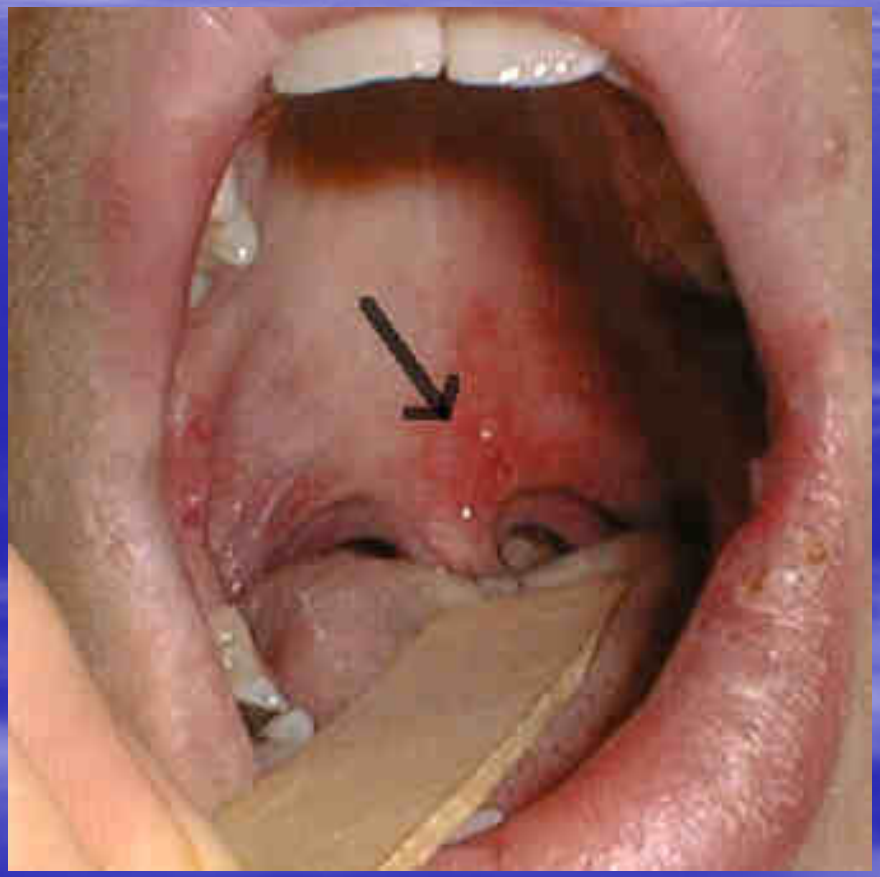
- ***1806***: First reported case of Crohn's by Combe and Sanders to the Royal College of Physicians in London, England
- ***1823***: John Abercrombie of Edinburgh clearly outlined a difference in ileal and colonic diseases
- ***1913***: Surgical evidence of the disease reported in the paper 'Chronic Intestinal Enteritis' written by Dr. Kennedy Dalziel at the Western Infirmary in Glasgow

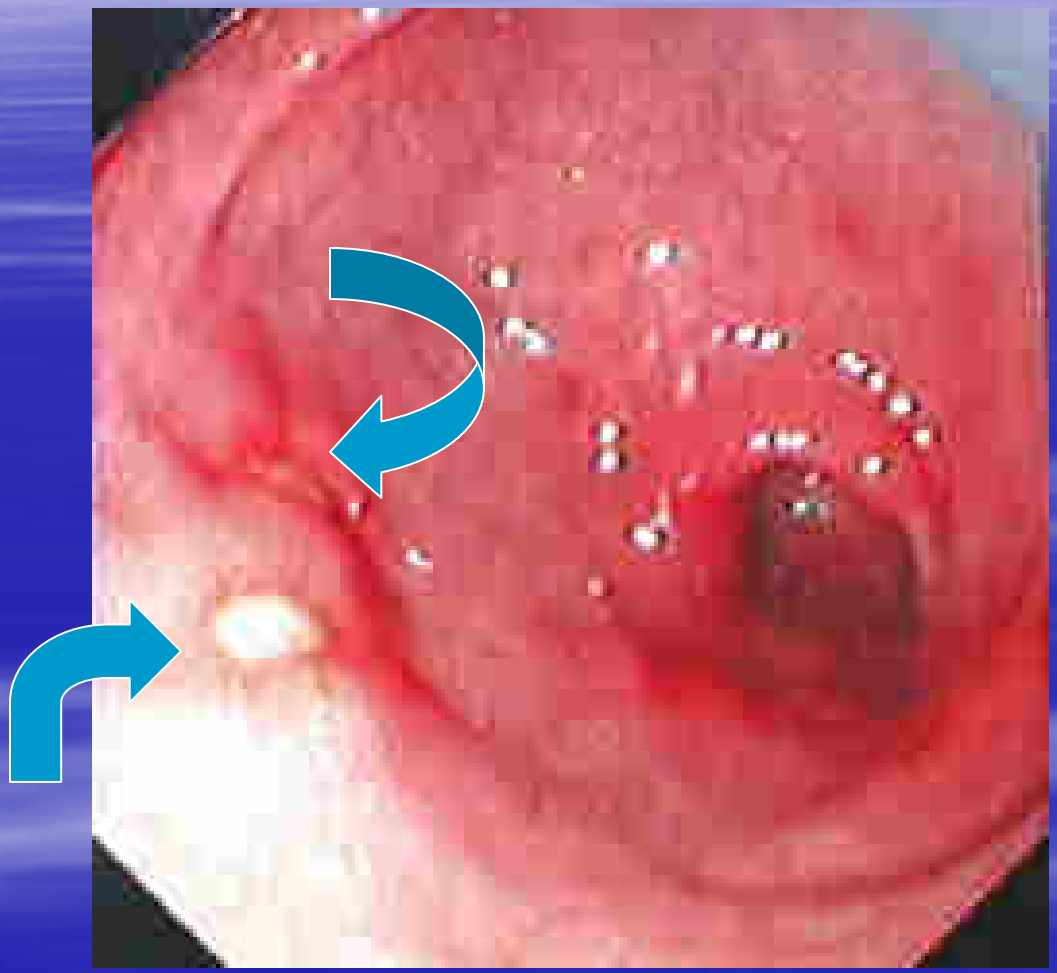
History (cont'd)

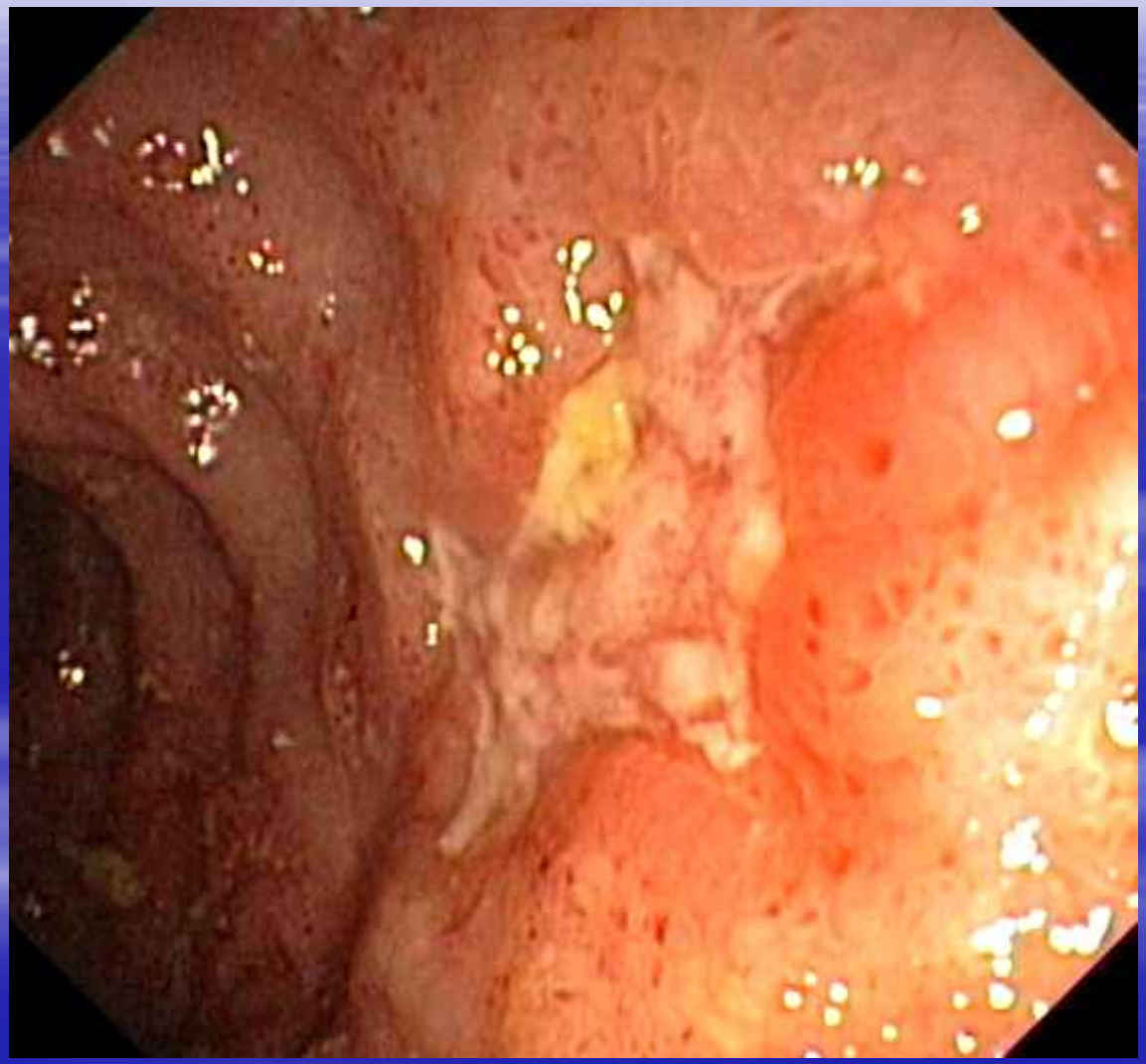
- Described in 1932 by Crohn, Ginsburg, and Oppenheimer of Mount Sinai Hospital in New York
  - Their paper 'Regional Ileitis' recorded cases of "non specific granulomas of the intestine"
  - Based on fourteen surgical cases mostly operated on by surgeon, Dr. A. A. Berg

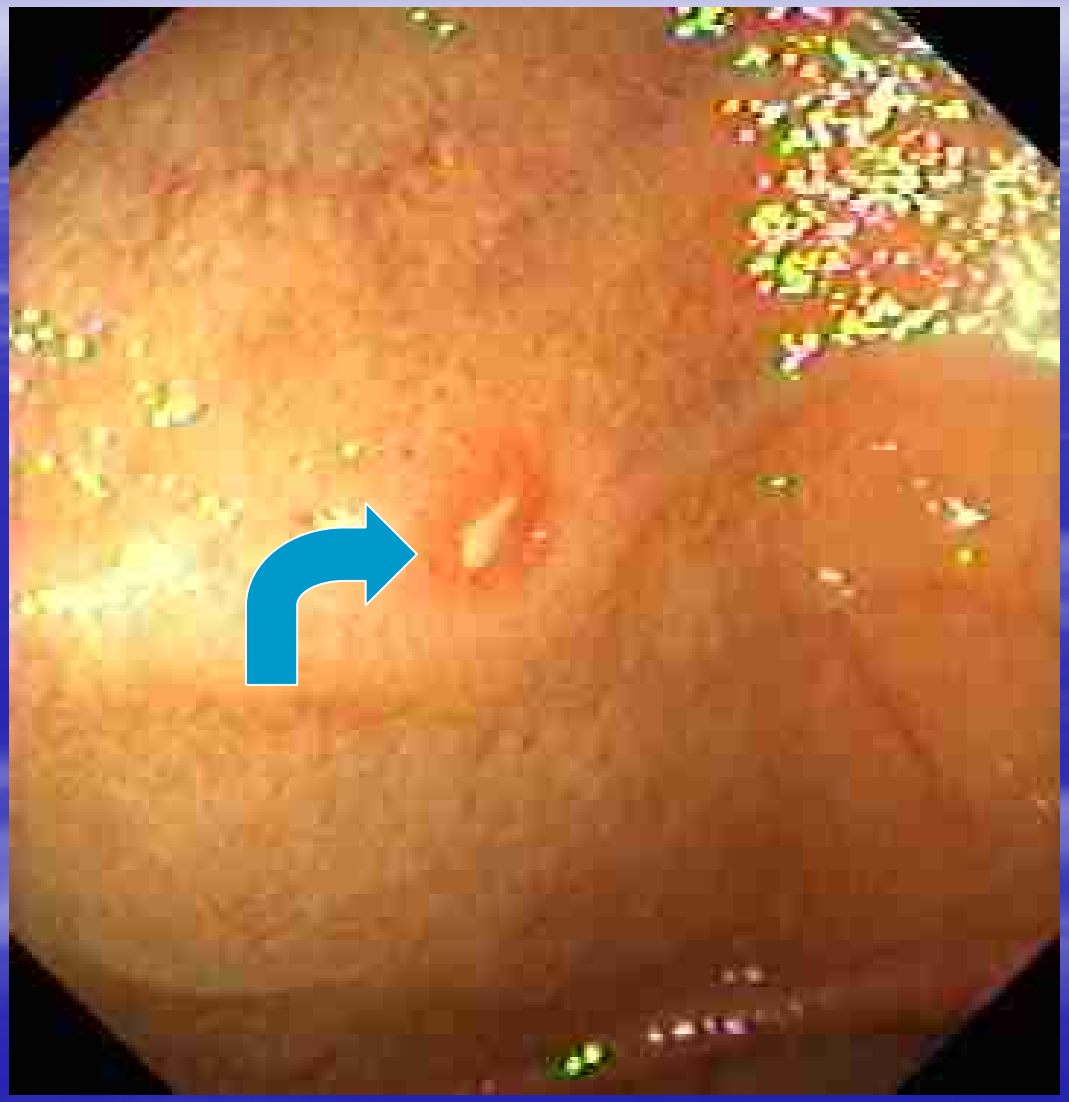


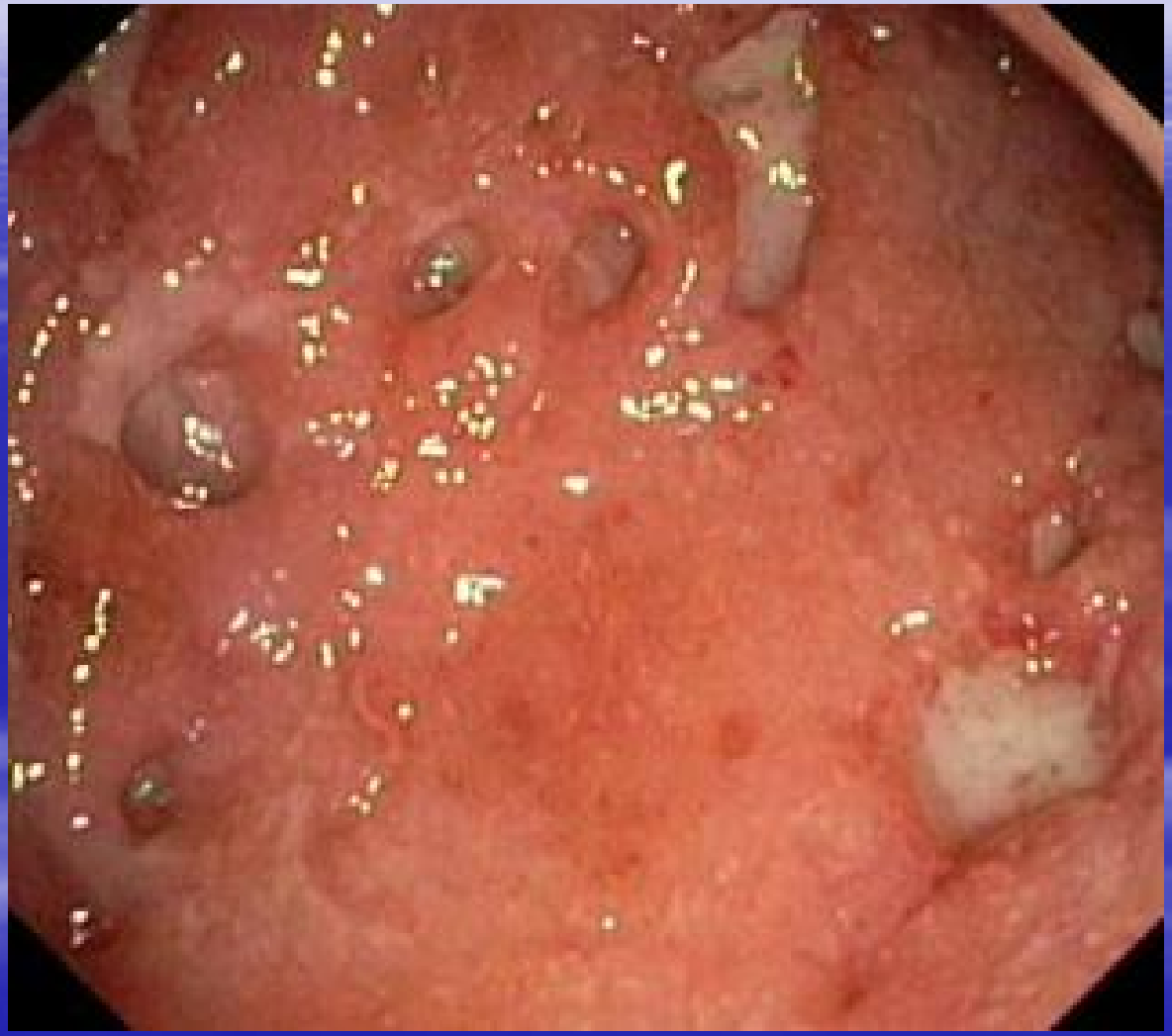


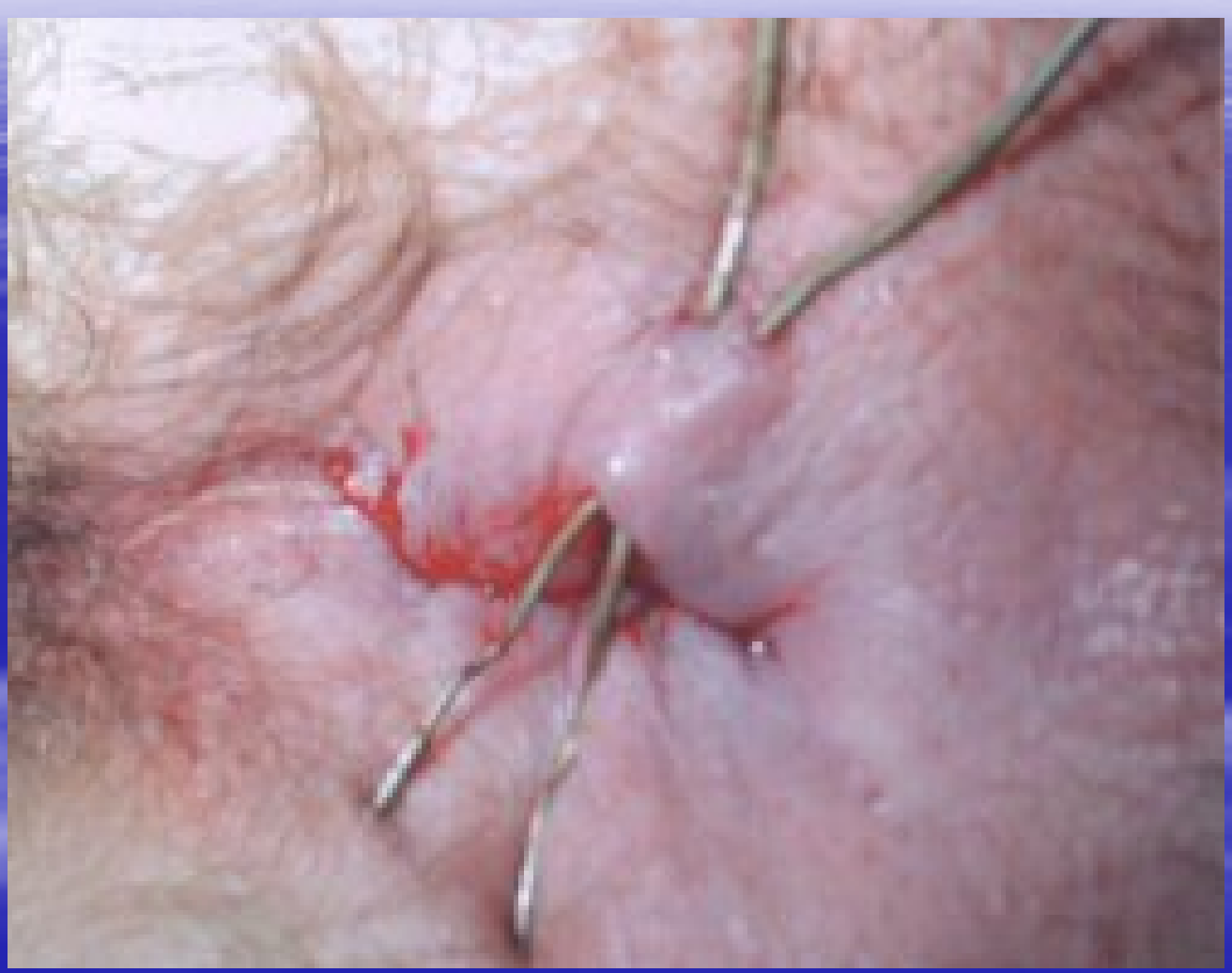










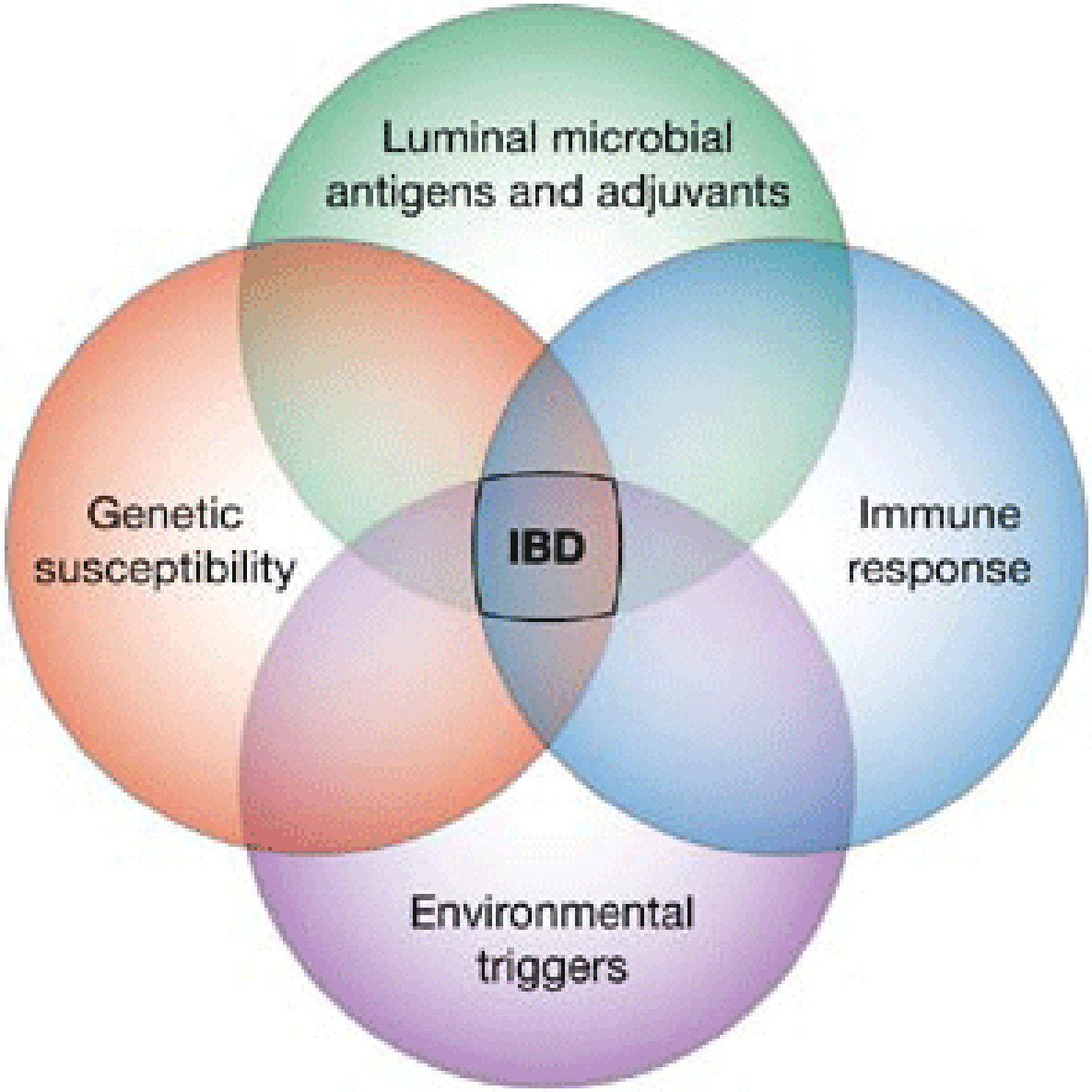


Epidemiology of CD

- European studies:
  - Northern Europe: 7-9/100.000
  - Southern Europe: 4/100.000
  - UK: 6-13/100.000
- USA: up to 133-150/100.000
- Jordan: ??.....but increasingly diagnosed
- More common in
  - Urban than in rural areas
  - Smokers
  - Certain ethnic groups
- In Wales, a 4000% increase of the incidence of CD since the 1930s
- CD is seen much more commonly in milk-drinking areas (Northern Europe, USA, Australia, New Zealand)

Etiology and Pathogenesis

- Genetic factors
- Environmental precipitants and disease cofactors
- Immune response and inflammatory pathways
- Infectiuos agents?????



Source: Nat Clin Pract Gastroenterol Hepatol © 2006 Nature Publishing Group

Genetic Factors

- The prevalence varies among different populations
- The risk is increased among first-degree relatives of affected patients
- There is greater concordance among monozygotic than dizygotic twins
- Syndromes resembling inflammatory bowel disease co-segregate in families with rare genetic disorders (e.g., glycogen storage disease type IB, Wiskott–Aldrich syndrome, Hermansky–Pudlak syndrome)
- Numerous candidate genes have been identified with putative allelic associations with inflammatory bowel disease (e.g. NOD 2 gene)

Orholm M, et al. N Engl J Med 1991;324:84-8

Environmental Precipitants and Disease Cofactors

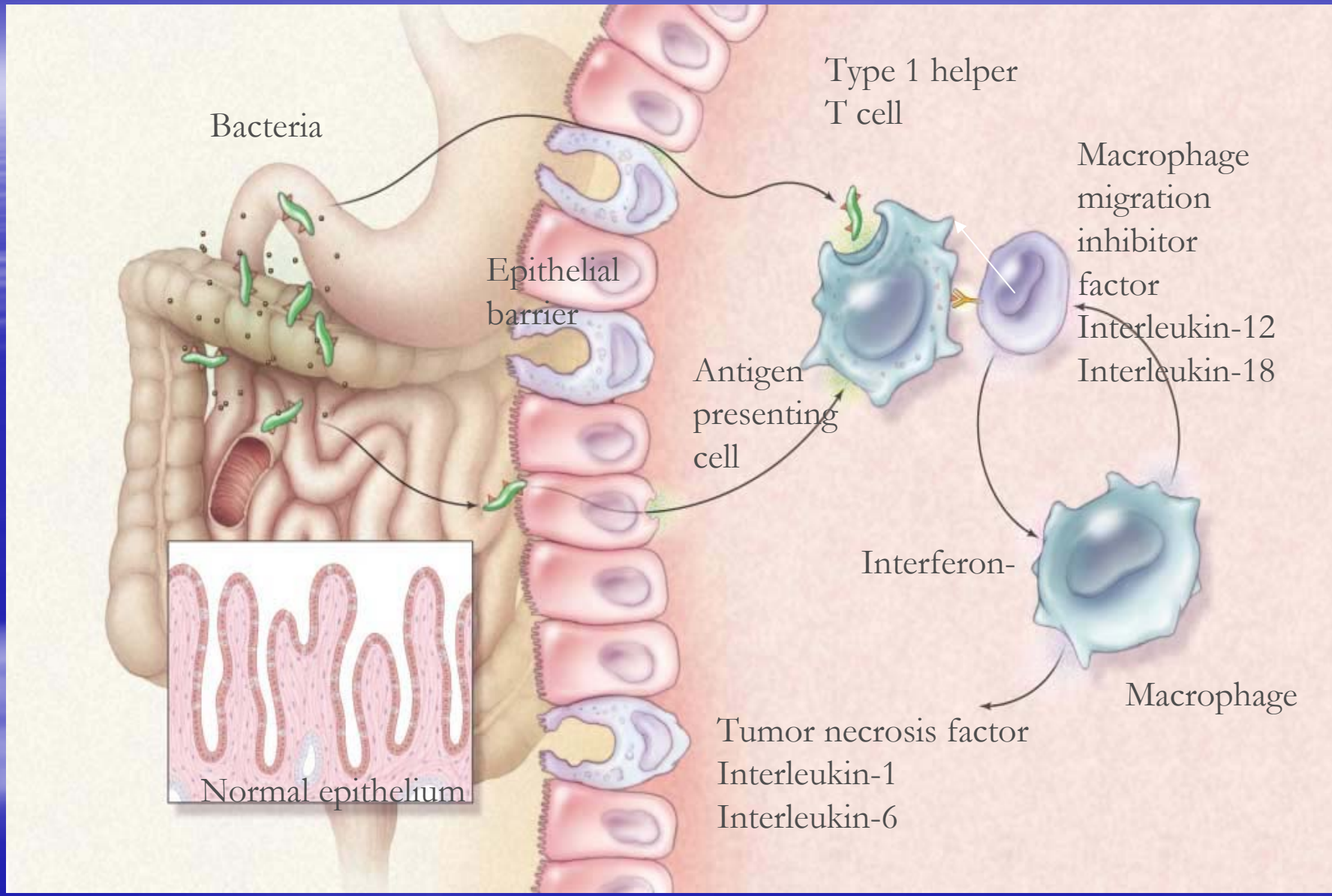
- "Only" 45 percent of identical twins are concordant for CD
- Use of NSAIDS: disease flares
- Smoking: increased risk of disease, worse natural history/complications
- Luminal flora
  - Broad spectrum antibiotics and probiotics effective in a subset of patients

Evans JM, et al. Gut 1997;40:619-22
Lindberg E, et al. Gut 1988;29:352-7
Swidsinski A, et al. Gastroenterology 2002;122:44-54

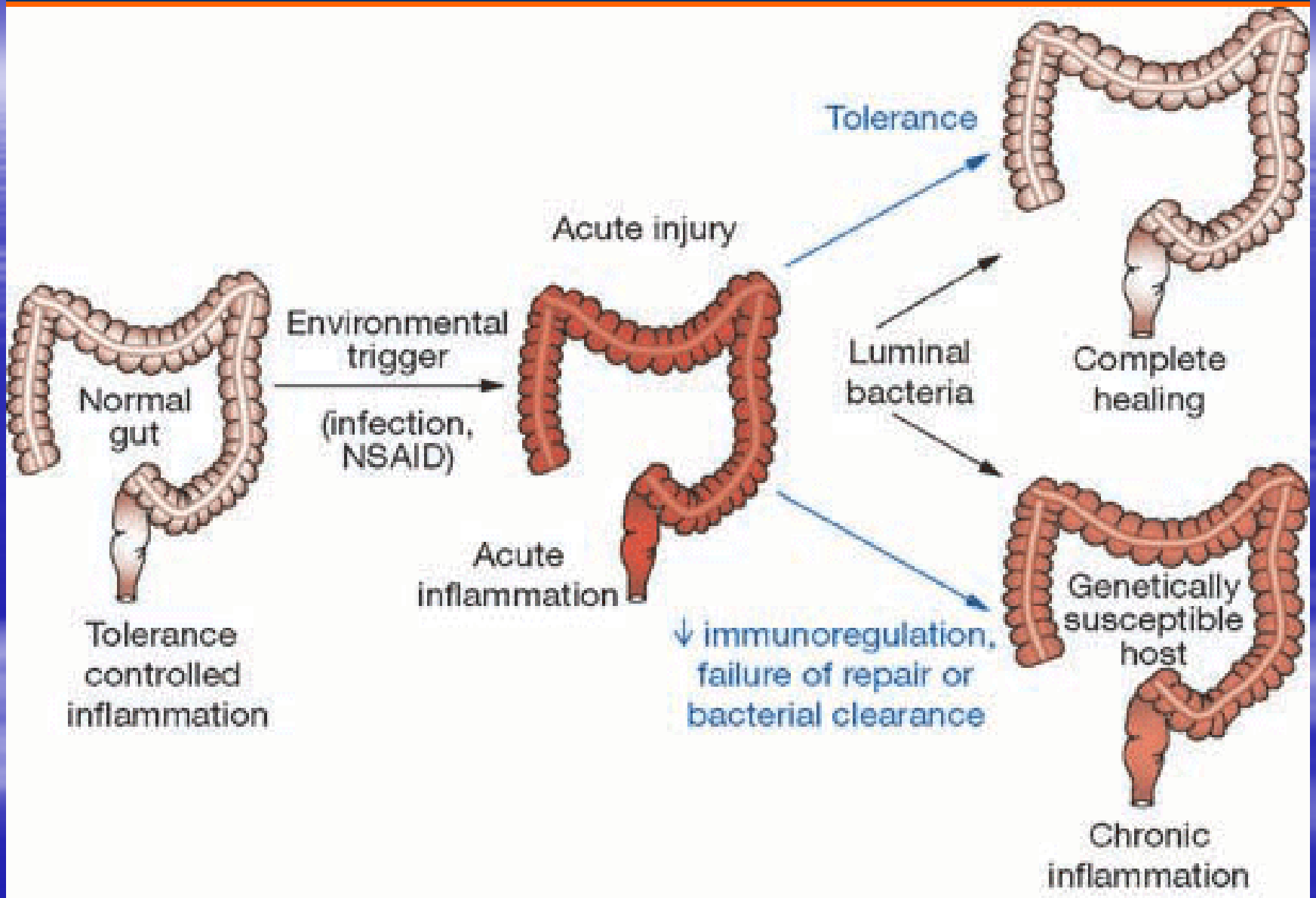
Immune Response and Inflammatory Pathways

- It remains unclear whether
  - the immune system is activated as a result of an intrinsic defect (either constitutive activation or the failure of down-regulatory mechanisms) or
  - because of continued stimulation resulting from a change in the epithelial mucosal barrier
- The mucosa of patients with established Crohn's disease is dominated by CD4+ lymphocytes with a type 1 helper-T-cell (Th1) phenotype
- The activation of central immune-cell populations is eventually accompanied by the production of a wide variety of nonspecific mediators of inflammation

Fiocchi C. Gastroenterology 1998;115:182-205



Podolsky DK. NEJM 2002; 347: 417-29



Paramyxovirus Infections in Childhood and Subsequent Inflammatory Bowel Disease

- *Background:* Measles virus has been implicated in the etiology of both inflammatory bowel diseases (IBDs), Crohn's disease and ulcerative colitis
- *Methods:* The data are from 7019 members of a nationally representative 1970 British Cohort Study. The ages of five childhood infections were recorded before onset of IBD symptoms
- *Results:*
  - Mumps infection before age 2 years was a risk for ulcerative colitis
  - Measles and mumps infections in the same year of life were significantly associated with ulcerative colitis and Crohn's disease
- *Conclusion:* Atypical paramyxovirus infections in childhood may be risk factors for later IBD

Montgomery SM, et al. Gastroenterology 1999;116:796–803

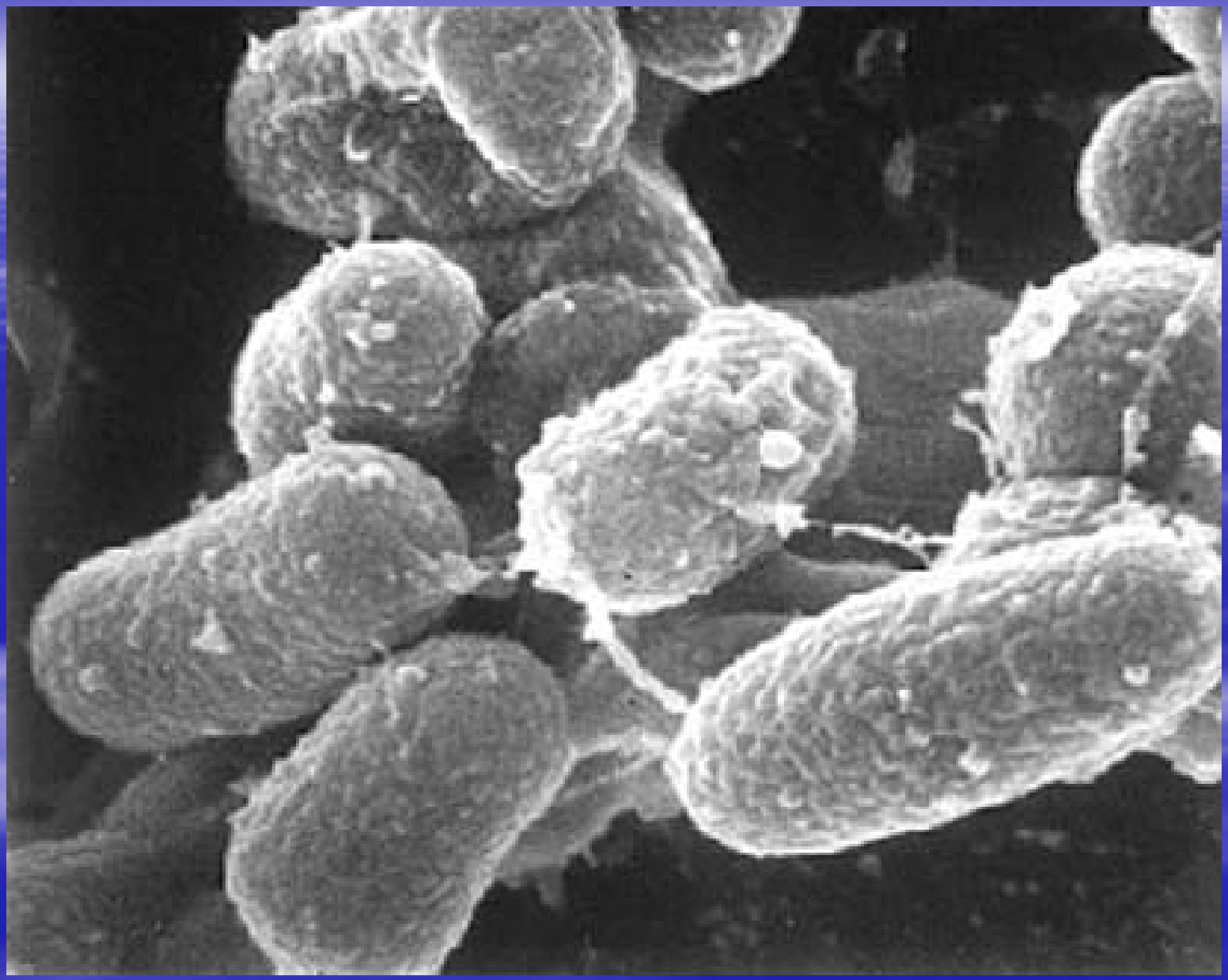
Enhanced *Escherichia coli* Adherence and Invasion in Crohn's Disease and Colon Cancer

- Background: Altered mucosal glycosylation in inflammatory bowel disease and colon cancer could affect mucosal bacterial adherence. This study aimed to quantify and characterize mucosa-associated and intramucosal bacteria, particularly *Escherichia coli*
- Methods: Mucosa-associated bacteria were isolated, after dithiothreitol mucolysis, from biopsy samples obtained at colonoscopy
- Results:
  - Mucosa-associated and intramucosal bacteria were cultured more commonly in Crohn's disease and colon cancer than in ulcerative colitis and controls
  - Mucosa-associated *E. coli,* which accounted for 53% of isolates, were more common in Crohn's disease and colon cancer
- Conclusion: There is a central role for mucosally adherent bacteria in the pathogenesis of Crohn's disease and colon cancer

Martin HM, et al. Gastroenterology 2004;127:80–93

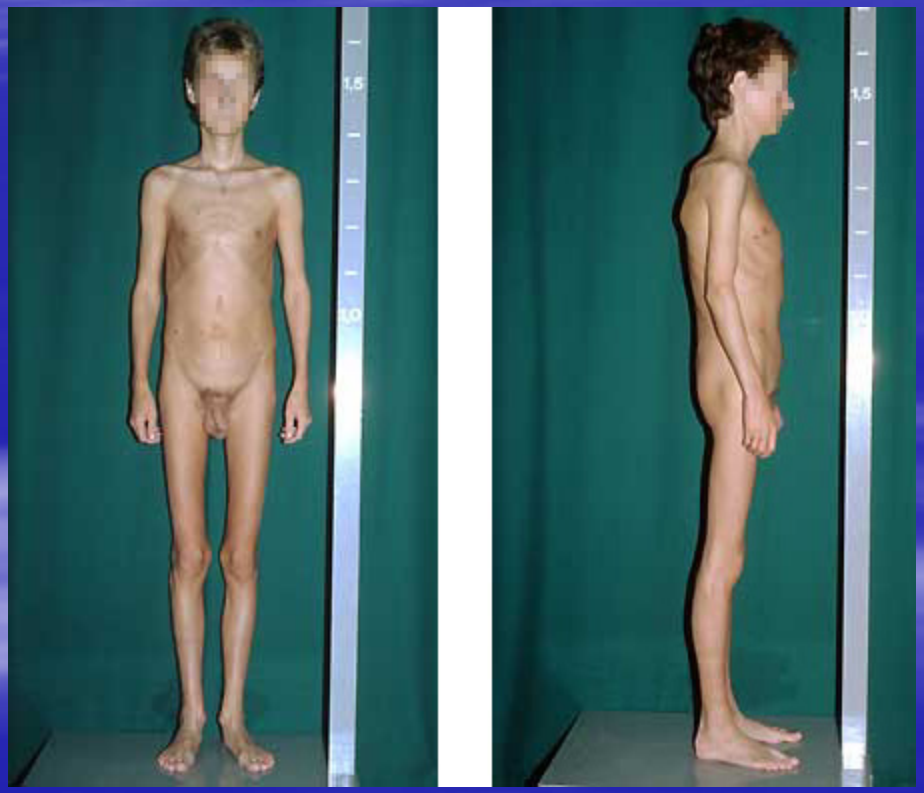
Johne's and Crohn's

Is there a connection?



Crohn's & Johnes Disease

Brothers or just distant relatives?



Can Mycobacterium Avium Paratuberculosis (MAP) cause CD?

- In 1984, Chiodini succeeded in culturing live MAP germs from the gut walls of children with CD

(Chiodini RJ. Is Crohn's disease a mycobacterial diasease? Academic Publishers; 1992: P 1-15)

- Using DNA fingerprinting
  - 65% of bowel samples from CD patients came up positive
  - Only 4% of samples from patients with ulcerative colitis was positive *(Schwartz D, et al. 99th American Society of Microbiology General Meeting; 1999, Chicago, illinois)*

Detection and Isolation of MAP from Intestinal Mucosal Biopsies of Patients with and without CD in Sardinia

- METHODS: MAP was detected by IS900 PCR on DNA extracts of fresh intestinal mucosal biopsies as well as by isolation in culture using supplemented MGIT media followed by PCR with amplicon sequencing
- RESULTS: Twenty five patients (83.3%) with CD and 3 control patients (10.3%) were IS900 PCR positive. MAP grew in cultures from 19 Crohn's patients (63.3%) and from 3 control patients (10.3%)
- CONCLUSIONS:
  - Mycobacterium avium subspecies paratuberculosis was detected in the majority of Sardinian Crohn's disease patients
  - The finding of the organism colonizing a proportion of people without Crohn's disease is consistent with what occurs in other conditions caused by a primary bacterial pathogen in susceptible hosts

Sechi LA, et al. Am J Gastroenterol. 2005 Jul;100:1537-8

Connection The CD and JD

- There are data supporting MAP as a causative agent of CD
- There are data that DO NOT support MAP as a causative agent of CD…..

Is there a middle ground?

Top:- The intestine of a cow with Bovine JD
Bottom:- The intestine of a person with CD

Similarities Between Johne's disease and Crohn's disease

- Clinical manifestations of both diseases begin after sexual maturity. There is some evidence to suggest that hormonal cycling, associated with calving and lactation, plays a role
- Both diseases cluster in families. For humans this is interpreted to mean The target site of disease is the ileum for both JD and CD
- there's a genetic linkage. The same familial clustering occurs in JD but it is interpreted as a function of the high frequency of transmission between a cow and her calf
- The host response in both diseases, as seen by histopathology, is quite similar
- Clinical symptoms are essentially identical
- There are non-caseating tuberculoid granulomas in some species of animals with JD and certainly in CD

Differences between Johne's disease and Crohn's disease

- Traditionally, JD has not been considered to be segmental, while skip lesions are described for CD
- Acid-fast bacteria are not seen in tissues from CD patients while they can be found in most cases of Johne's disease, if we look hard enough
- Animals do not have the manifestations of bowel stenosis and perforation observed in people with CD
- There is focal ulceration of the Peyer's patches or the intestinal mucosa. This is rare in JD
- CD patients do not recognize MAP with their immune system

Expert Opinion

With few exceptions, world experts in Johne's and Crohn's diseases agree…

There is insufficient evidence to prove or disprove that MAP is a human pathogen or that it is a cause of Crohn's disease

Two-year Combination Antibiotic Therapy with Clarithromycin, Rifabutin, and Clofazimine for Crohn's Disease

- METHODS: Two hundred thirteen patients were randomized to clarithromycin 750 mg/day, rifabutin 450 mg/day, clofazimine 50 mg/day or placebo, in addition to a 16-week tapering course of prednisolone
- RESULTS:
  - At week 16, there were significantly more subjects in remission in the antibiotic arm (66%) than the placebo arm
  - At week 104, the figures were 26% and 43%, respectively
  - During the following year, 59% of the antibiotic group and 50% of the placebo group relapsed
- CONCLUSIONS:
  - Using combination antibiotic therapy with clarithromycin, rifabutin, and clofazimine for up to 2 years, we did not find evidence of a sustained benefit
  - Short-term improvement was seen when this combination was added to corticosteroids, most likely because of nonspecific antibacterial effects

Selby W, et al. Gastroenterology. 2007 Jun;132(7):2594-8.

Anti-mycobacterial Therapy in Crohn's Disease

Heals Mucosa with Longitudinal Scars

- PATIENTS: A retrospective review of 52 patients with severe CD. Thirty-nine patients who had at least one follow-up colonoscopy during treatment were included
- METHODS: Patients received rifabutin, clofazimine and clarithromycin for 6 months to 9 years. Ramp-up dosing was used. Colonoscopies and histological analyses monitored progress
- RESULTS:
  - In 2/6 patients (33.3%) who had > 3 years of treatment after scarring occurred, scars receded, becoming imperceptible as full healing occurred.
  - Histologically, a marked reduction in inflammation occurred in 15/39 patients (38.5%). Of these, 6/15 patients (40%) displayed restoration of normal mucosa
- CONCLUSIONS: CONCLUSIONS: The presence of scarring fading to normal mucosa on anti-MAP therapy implies a more profound healing not seen with standard anti-inflammatory and immunosuppressant drugs

Borody TJ, et al. Dig Liv Dis. 2007 May; 39: 438-44

Basic and *clinical* research should be aimed at answering the following fundamental questions

- Does MAP, or other microbial pathogen(s), cause CD?
- Do affected tissue samples from CD patients consistently contain MAP or any other pathogen?
- Can we detect specific immune reactions to a CD associated pathogen?
- What is such a pathogen's phenotype and genotype?
- Can we make the disease better by using appropriate antimicrobial drugs?

Basic Studies

- **Establish cell or organ culture models** of infection focusing on growth characteristics and gene expression of MAP in cell culture
- **Establish new animal models of** MAP **infection**.
- **Develop an improved large animal model of CD**
- **Perform in vivo expression technology (IVET) studies** in animals susceptible to JD to identify bacterial genes uniquely expressed *in vivo*
- **Compare** MAP **DNA sequences** to available genome sequences of other mycobacteria. These comparisons may yield clues to pathogenicity
- **Identify and optimize diagnostic** MAP **antigens** that can be isolated or produced by recombinant technology or other means and made widely available to researchers.

Basic Studies (cont'd)

- **Adapt antibiotic susceptibility testing methods** to deal with a species that grows even more slowly than the so-called "slow growing mycobacterial pathogens"
- **Determine the relationship** between MAP and the *M. avium* complex, whether from CD or JD
- **Develop a high-density array of ribosomal DNA or RNA** on a chip that can be used to more completely define the organisms associated with CD
- **Apply subtractive hybridization techniques** to look at the difference between CD tissues obtained by intestinal biopsy, tissues from a non-involved area of the intestine from the same CD patient, and normal tissues from controls

Clinical Studies

- **Determine potential infectious etiologies of CD** by collecting and studying biopsy tissues from the intestines of Crohn's patients (stratified into perforated and contained lesions) and controls, and using sensitive diagnostic methods to enumerate any microbial flora associated with the disease
- **Define the host immune response in Crohn's Disease**
  - What are the factors that contribute to the continuing inflammatory cascade observed in Crohn's disease? Normal flora, pathogens, diet, and stress have all been suggested as contributors to disease
- **Conduct epidemiological research** to elucidate risk factors for human infection
- **Conduct genetic studies** of families with a history of CD
- **Determine the effect of anti-MAP therapy on the natural history of CD**

Perception is Reality

If consumers adopt the PERCEPTION

that Johne's is a human health risk,

the economic impact on industry could be…

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Daily Mail

GoodHealth

Vaccine to end Crohn's misery for thousands

By MARTYN HALLE

A VACCINE has been developed that could cure more than 150,000 people suffering from the crippling digestive illness Crohn's disease.

The breakthrough comes after more than 15 years' research by a leading British expert. Professor John Hermon-Taylor — who developed the vaccine at London's St George's Hospital, says trials went well: 'This is very exciting news and could herald the end of suffering for tens of thousands of people.'

The cause of Crohn's disease is still unclear, but there is growing evidence that bacteria could be responsible for most cases.

Prof Hermon-Taylor's research shows that a bug called mycobacterium avium paratuberculosis (MAP), which is found in sheep, pigs and cows and passed into the food chain via water and milk, is the cause of most human cases of Crohn's disease.

But those with a family history of ulcerative colitis (another bowel disorder) also run a higher risk of getting the disease. And stress is thought to exacerbate the illness, which can affect any part of the digestive system.

Most people develop the disease, which costs the NHS hundreds of millions of pounds a year in drug treatments and surgery for sufferers, before they are 30 — the peak is between 14 and 24.

Three-quarters of people with Crohn's disease require surgery to repair their damaged bowel, and half of those will need a second operation within ten years.

The new vaccine helps sufferers by stimulating their immune systems to clear MAP from their bodies. It can also be used to immunise people and even animals against the disease.

The symptoms of Crohn's disease are often vague and difficult to identify. They can be chronic diarrhoea and abdominal pain, fever, lack of appetite, weight loss and a feeling of fullness in the gut.

The new vaccine was made using two safe viruses — a cold virus and one used in smallpox vaccine. The research team attached them to a fragment of MAP DNA. Once in the body, they stimulate the white blood cells to kill the bacteria.

In a patient vaccinated against Crohn's, the immune system would be armed to fight off the disease should it try to enter the body.

Professor Hermon-Taylor was the first doctor to make the link between Crohn's and MAP, which is present in between three and six per cent of pasteurised milk.

When he tested patients with Crohn's disease for MAP, he found the same bacteria in their intestines as in animals.

But the government's Advisory Committee on the Microbiological Safety of Food insists transmission of MAP from milk to humans is not proven, despite evidence of it getting into the milk supply.

Prof Hermon-Taylor disagrees: 'There really cannot be a dispute over the origins of this infection.'

'We are extremely confident that the vaccine will work, but we now have to test it thoroughly. The first stage will to be to test it on healthy human volunteers to ensure there are no side effects. If all goes well, then we move to trials on people who have the disease.'

Prof Hermon-Taylor is convinced the disease can largely be eradicated by immunisation.

There is no specific treatment for Crohn's currently, but various drugs can be taken to relieve cramps and diarrhoea, including codeine.

Some patients, especially those with abscesses and infections, may need antibiotics. Other useful drugs include steroids, although long-term use can be toxic.

'I have had patients who have lives completely messed up by this illness,' says Prof Hermon-Taylor. 'Those of us involved in the research are all excited by the prospect that we are so close to curing sufferers and preventing others becoming ill.'

LEMONS FIGHT ASTHMA

CITRUS fruits such as oranges, lemons and grapefruit may protect against asthma, a study has found.

When the diets of 515 people with asthma were compared with a group of a similar size without asthma, it was found that the people with asthma had less fruit in their diet.

Men and women who ate at least 46 grams of citrus fruit a day were 40 per cent less likely to have asthma than those who ate none.

Researchers at Cambridge University, who carried out the study, suspect the antioxidant properties of fruit may have a protective effect against asthma.

Blood levels of vitamin C, found in citrus fruit, and manganese, found in spinach, wholegrain cereals, nuts and tropical fruits such as pineapple, were found to be significantly lower in people with asthma.

Citrus fruits are already known to contain powerful cancer-preventing nutrients.

Conclusions

- Crohn's disease is a multifactorial disease or syndrome, with no one etiological factor appearing to dominate
- At present there is insufficient scientific evidence to prove or disprove a conclusive link between Johne's disease (or MAP) in ruminants and some cases of Crohn's disease in humans
- Efforts should continue to solve this controversie

Politics and Medicine

"It's not whether you have the proof of something, but whether or not the medical community wants to accept it"

Chiodini RJ, MD

Thank You

Lectuer # 5

Basic Epidemiology and Epidemiology of Johne's Disease in the Region

PRESENTED BY:

Professor Shawkat Lafi, FVM, JUST

Epidemiology of *M. Paratuberculosis* (Johne's disease) and its Public Health Concern

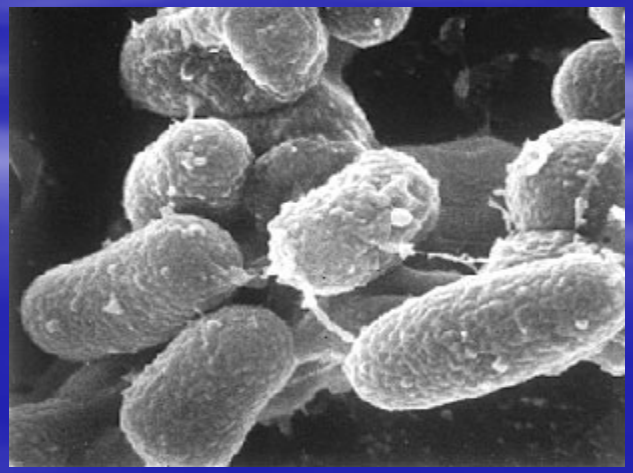
Professor Shawkat Q. Lafi (Hailat)
Faculty Of Veterinary Medicine
Jordan University of Science & Technology

What causes Johne's disease ?

- Johne's disease is a contagious bacterial disease of the intestinal tract. A German veterinarian first described the disease in a dairy cow in 1895.
- It is a relative of the bacterium that causes tuberculosis in humans (*Mycobacterium tuberculosis*), cattle (*Mycobacterium bovis*), and birds (*Mycobacterium avium*….IS900), Cattle Strain, Sheep strain and Intermediate Strain.

- It can replicate only when it is in animals (macrophages): it cannot multiply in nature, outside the animal (mycobactin…Iron) .

It can survive in the environment for over a year because of its resistance to heat, cold and drying.



How do animals get Johne's disease? Source of Infection

- Ingestion of the bacterium occurs when the newborn's environment is contaminated with manure from an infected adult animal, or by drinking milk from an infected animal.

- Young animals are far more susceptible to infection than are adults.

- The milk may become contaminated from the environment (manure-stained teats). Or lactogenically from diseased mothers.

How do animals get Johne's disease? Continue…

- In the advanced stages of the infection, the bacterium is shed directly into the milk (bacteria in the blood).
- After infection, many months or years go by until the infected animals shows signs of Johne's disease.
- Contaminated Water and Pasture

How do animals get Johne's disease? Cont…..

- Johne's disease typically enters a herd or flock of animals when an infected, but healthy-looking, animal is purchased.
- After several years, the owner recognizes signs of the disease in a number of animals.
- Individual animals get infected by close contact with other infected animals, that shed the bacterium in their manure.
- Most often, the infection is acquired by eating material contaminated with *M. paratuberculosis* when animals are very young.

Transmission of Infection

- Risk factors have not been fully studied. However, Animal age is perhaps the most well-recognized factor affecting *M. paratuberculosis* transmission. In cattle, there is an age-dependent increase in resistance to *M. paratuberculosis* infection.
- This means it takes a larger dose of the bacterium to infect an adult (over 2 years old) than it does to infect a young animal (0 to 6 months-old).

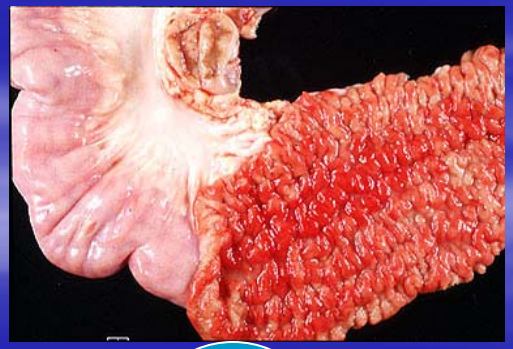
- The same could be said for other ruminants

Host Range

- It has a broad host range. The type of animals most commonly infected are ruminants.
- The disease has been reported in sheep, goats, elk, deer, bison, camels, llamas, rabbits, faxes, pigs, horses, Chickens and wild ruminants in zoos.

What are the signs of Johne's disease ?

- In Cattle, mainly diarrhea and rapid weight loss.
- In sheep and goats, diarrhea is less common, soft stool and weight loss.
- In general, animals with Johne's disease continuing to eat well.
- Infected animals maintain a normal temperature but may appear unthrifty and can become weak in later stages of the infection.
- Because of the slowly progressive nature of the infection, signs of Johne's disease are usually not seen until animals are adults.



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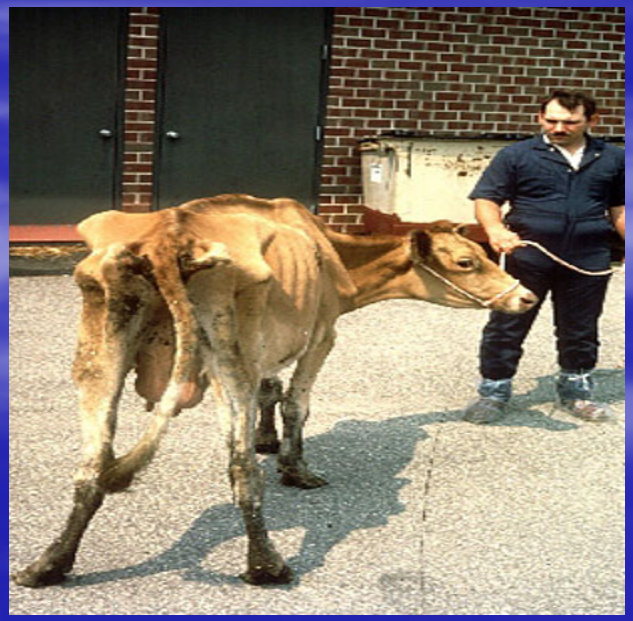
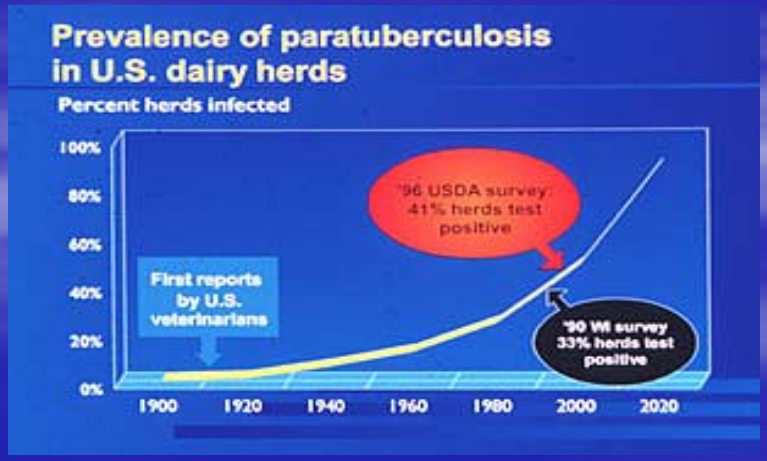


Prevalence of Paratuberculosis

- Johne's disease occurs worldwide. In the U.S. it is estimated that 7.8% of the beef herds and 22% of the dairy herds are infected with *M. paratuberculosis*. Infection rates in cattle in other countries are generally similar.
- In Jordan (Hailat et al.,2006). About 65% of the apparently healthy cattle, 60% of camels and more than 70% of sheep and goats were found to have lesions compatible with Johne's disease.
- (Sweden and some states in Australia are disease free)



Prevalence of Paratuberculosis

- Incidence of Johne's disease is reported to be increasing in the Netherlands (75%), Finland, Italy and Scotland. China, Japan, India, Korea, Kazakhstan, Nepal and the Philippines reported to have Johne's disease.

- New Zealand reports that Paratuberculosis is widespread in dairy cattle and goats plus is of concern in sheep and beef cattle as well.
- In African the disease was reported in 49 countries: Kenya, Nigeria, Sudan, Tunisia, Zambia and South Africa and Egypt were considered to be free of the disease. (A recent study in Egypt revealed that 75/160 sample were positive by culture from cow; 3/ 5 districts)
- Johne's disease is known in Israel and more than half of the herds are truly infected despite the fact that in herd sero-prevalence does not reach high.

How can you prevent your animals from getting Johne's disease?

- Animals brought into the herd are not infected with *M. paratuberculosis*. Johne's disease Free herds are the best sources of animals for purchase.
- For control two strategies must be employed at the same time:

- A. Hygienic Measures including:
- identifying and removing heavily shedding animals from pasture,
- Fed milk free of *M. Paratuberculosis or* (pasteurized) (Offspring are more susceptible than adults).
- weaning young lambs early and placing them on clean pasture without adult contact
- Avoiding commingling with other animal species of unknown Johne’s Disease status.
- Pasture rest for a year if it has been contaminated
- keeping water sources free of contamination.

How can you prevent your animals from getting Johne's disease? Continue..

- B. Vaccination Programs
- In Narway, 1967, about 131,000 goats were vaccinated over the next five years. The infection rate was reduced from 53% to 1% with most of the infections occurring in goats that escaped vaccination.
- A commercial vaccine against Johne's Disease in sheep was developed in Spain (Gudair, CSL, Australia) and is being tested in Australia (Eppleston et al., 2003; Eppleston et al., 2004).
- A vaccine was developed using organisms cultured from cattle. Mycopar (Fort Dodge Animal Health, Fort Dodge, IA) is a vaccine developed in the United States against the cattle organism.

How can you prevent your animals from getting Johne's disease? Continue..

- Sheep Trail In Australia using Gudair® Vaccine:
- vaccinated at one to three months of age. 5 years after post vaccination showed a 90% reduction in fecal shedding
- There were 75 deaths due to Johne's Disease in control sheep and 7deaths due to Johne's Disease in vaccinated sheep, representing a 90% reduction in mortality
- Among a 77 sheep culled before two years of age, 18% of vaccinated animals and 49% of control ewes were found to be infected. Thus, the results of the Australian trial with support the effect in the present trial with Mycopar®.

- **Conclusions**
- Based upon the histopathology results in this experiment, Mycopar® is a promising vaccine to help control and eventually to eradicate Johne's Disease from sheep flocks.

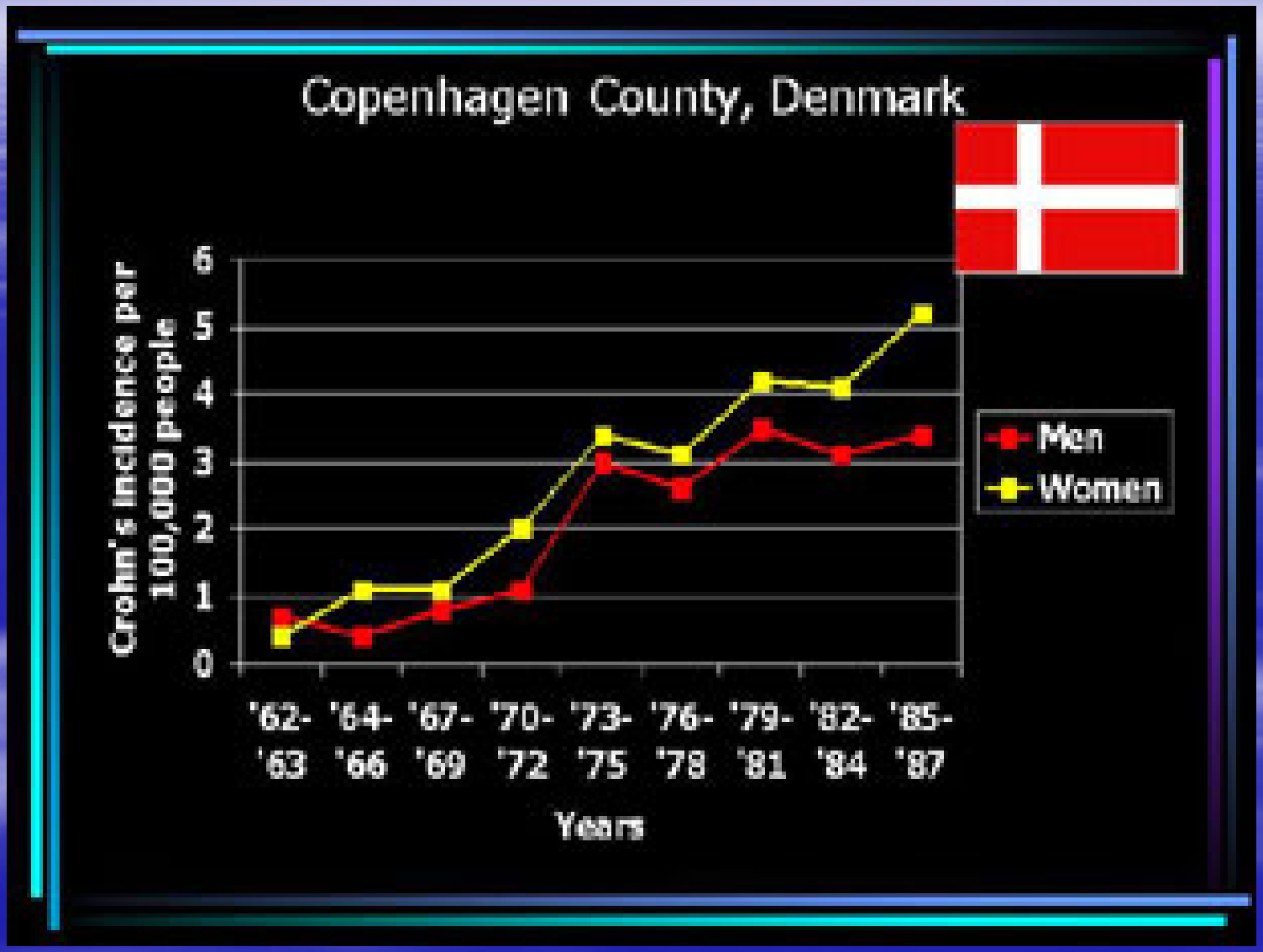
Crohn’s Disease Vs Johne’s Disease

- Etiology:
- The cause of Crohn's disease is not known. Many etiologies have been suggested, including autoimmune, genetic, dietary components plus various infectious agents such as the measles virus, *Listeria* and *M. paratuberculosis*
- Clinical Signs:
- Diarrhea and weight loss are the predominant clinical signs of both Johne's disease and Crohn's disease. Abdominal pain is a prominent feature of Crohn's disease in humans but, although difficult to measure in animals, seems absent in cattle and variable in other species. Fever is part of the constellation of signs in Crohn's disease but is not commonly seen in Johne's disease.

Crohn's Disease Vs Johne's Disease

- Epidemiology:

- Johne's disease was first described in 1895. Crohn's disease was first recognized 18 years later. The incidence of Crohn's disease in industrialized parts of the world is increasing. From the 1960s to the 1970s the incidence of Crohn's disease rose 4 folds in Scandinavian countries . In 1992 study reported a six fold increase in Crohn's disease in Copenhagen County, Denmark.
- **In USA, 500,000 cases a year.**
- In Minnesota, 1991 study, there was an adjusted Crohn's disease prevalence of 133 per 100,000, equating to **1 in 752 persons**. This was 46% higher than that seen in 1980.
- In Israel the prevalence of Crohn's disease ranges from 10 to 70 cases per 100,000 population. (more in the Southern Part of Israel)
- Crohn's disease attacks people in the prime of life: the highest incidence is found in **the age group 15-24 years**.


Copenhagen County, Denmark
Crohn's incidence per 100,000 people
0
1
2
3
4
5
6
'62-'63
'64-'66
'67-'69
'70-'72
'73-'75
'76-'78
'79-'81
'82-'84
'85-'87
Years
Men
Women

Crohn's Disease Vs Johne's Disease

- incidence of this infection is rising at a fairly rapid rate.
- In the U.S. roughly 3-10% of dairy cattle are infected with *M. paratuberculosis*. The USDA-NAHMS Dairy '96 survey concluded that 22% of U.S. dairy herds had an *M. paratuberculosis* infection prevalence of >10% based on ELISA (blood) testing.

- Crohn's disease is thought to occur early in life and then be followed by a 15-30 year incubation or latency period. Johne's disease also has a long interval between infection with *M. paratuberculosis* and onset of clinical signs (2-10 years).
- Clinical signs in both diseases are seldom seen before sexual maturity.

Crohn's Disease Vs Johne's Disease

- A lack of association between Crohn's disease and exposure to animals is often cited as evidence that *M. paratuberculosis* is not the cause of Crohn's disease. (That is, it is thought that the number of people diagnosed with Crohn's disease is no greater among dairy farmers who theoretically work with infected animals than people with no exposure to domestic agriculture species).

- Minnesota (1980) indicated that the incidence of Crohn's disease in urban residents was higher than that in rural residents. That study, however, did not characterize the occupation or life style of the rural dwellers nor did it find differences in Crohn's disease rates between urban and rural dwellers statistically significant.

- During the decade 1987–1997, the prevalence of Crohn's disease has increased in Israel and is approaching the rates in Europe and America

General Remarks

- Several studies have shown a strong familial association for Crohn's disease. In Sweden and Denmark, studies showed that first-degree relatives had a 10 to 21-times higher risk of having CD. In a Canadian study, children with inflammatory bowel disease (IBD) had significantly (P=0.001) more IBD in their families than did controls.
- In May 2000 a research group in Florida reported isolation of *M. paratuberculosis* from breast milk of woman with Crohn's disease providing a possible infectious disease explanation for the familial association observation.
- Collins et al. reported that 13.4% of 142 U.S. Crohn's disease patients were test-positive for paratuberculosis as compared to 2.6% of healthy blood donors.

General Remarks Cont….

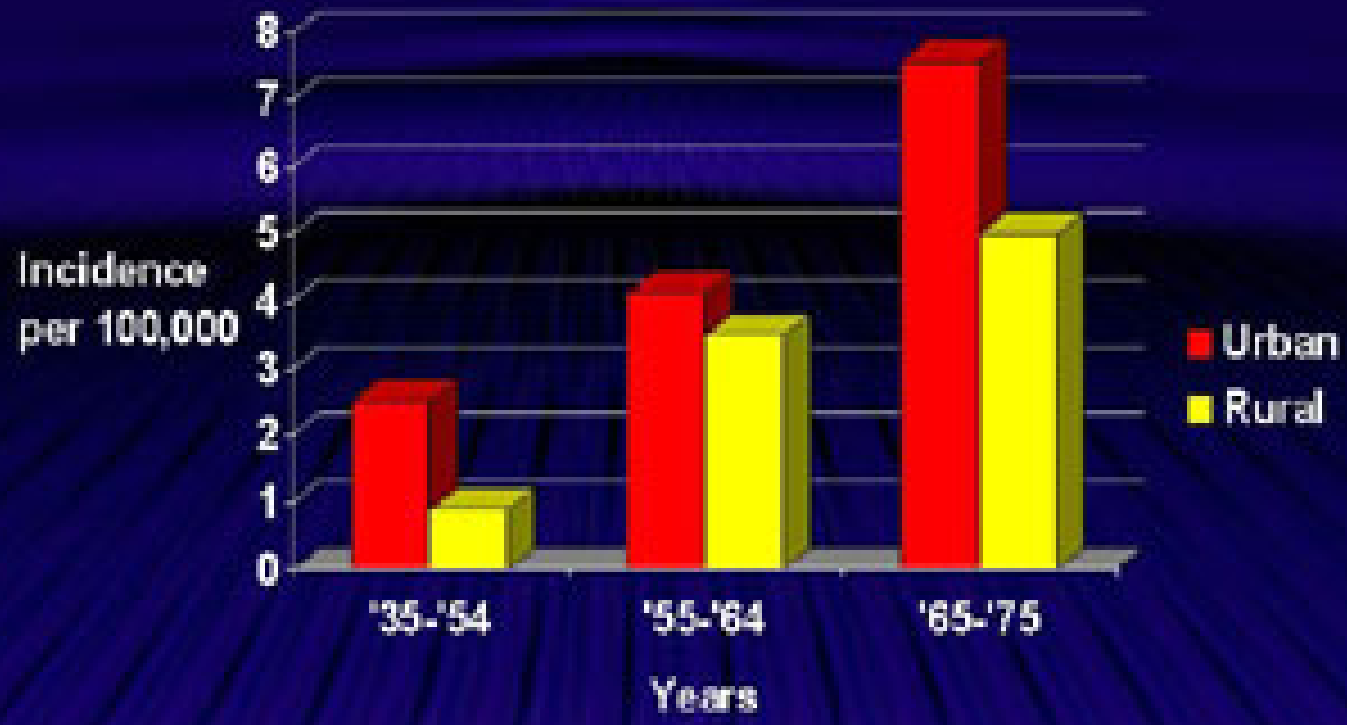
- Two studies have reported attempts to infect animals using isolates (strains) of *M. paratuberculosis* derived from humans with Crohn's disease. Infant goats were reportedly infected orally with a single strain ("Linda") of *M. paratuberculosis* in 1986.
- A 1991 report found that chickens could be infected by multiple exposure routes using the same *M. paratuberculosis* strain.

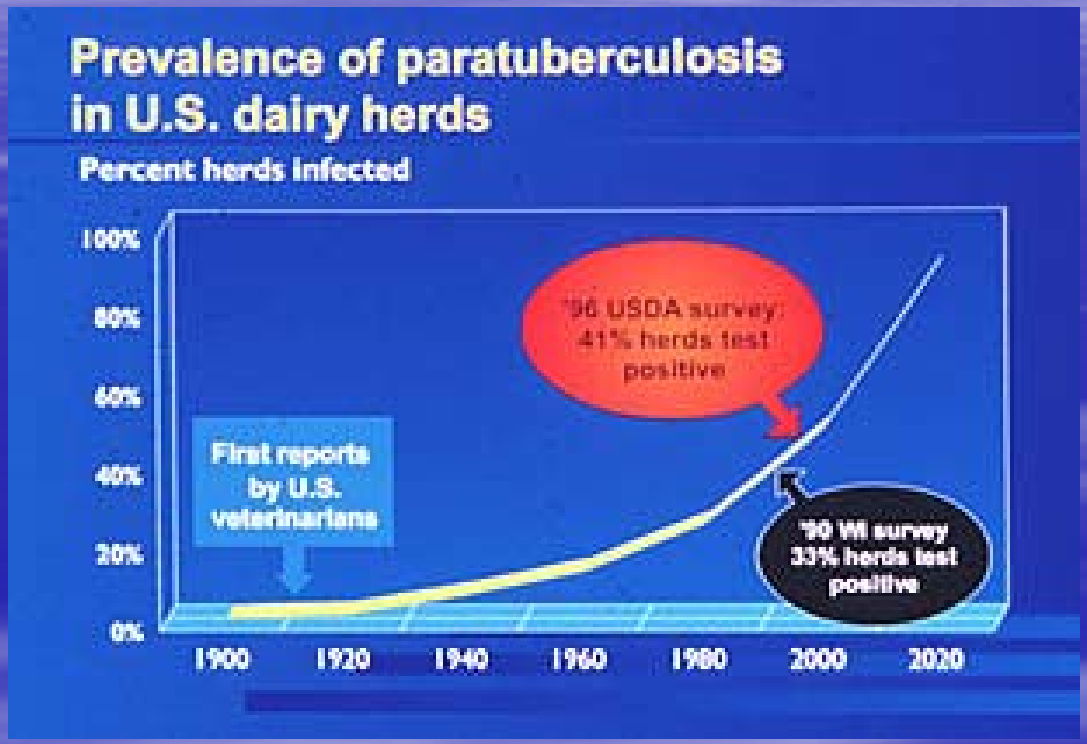
General Remarks Cont….

- Treatment
- In 1984 study of Shaffer et al. reported no improvement of 14 Crohn's disease patients treated for 12 months with rifampicin and ethambutol (two drugs used to treat tuberculosis).
- small placebo-controlled study in 1995 found prolonged remission (>1 year) in 8 of 15 CD patients treated with clarithromycin for 6 months. In 1997, Gui et al. reported a study attempting treatment of Crohn's disease patients with a combination of two drugs, rifabutin and one of two macrolides (clarithromycin or azithromycin), for >18 months. They reported that clinical remission was induced in >93% of 46 patients.

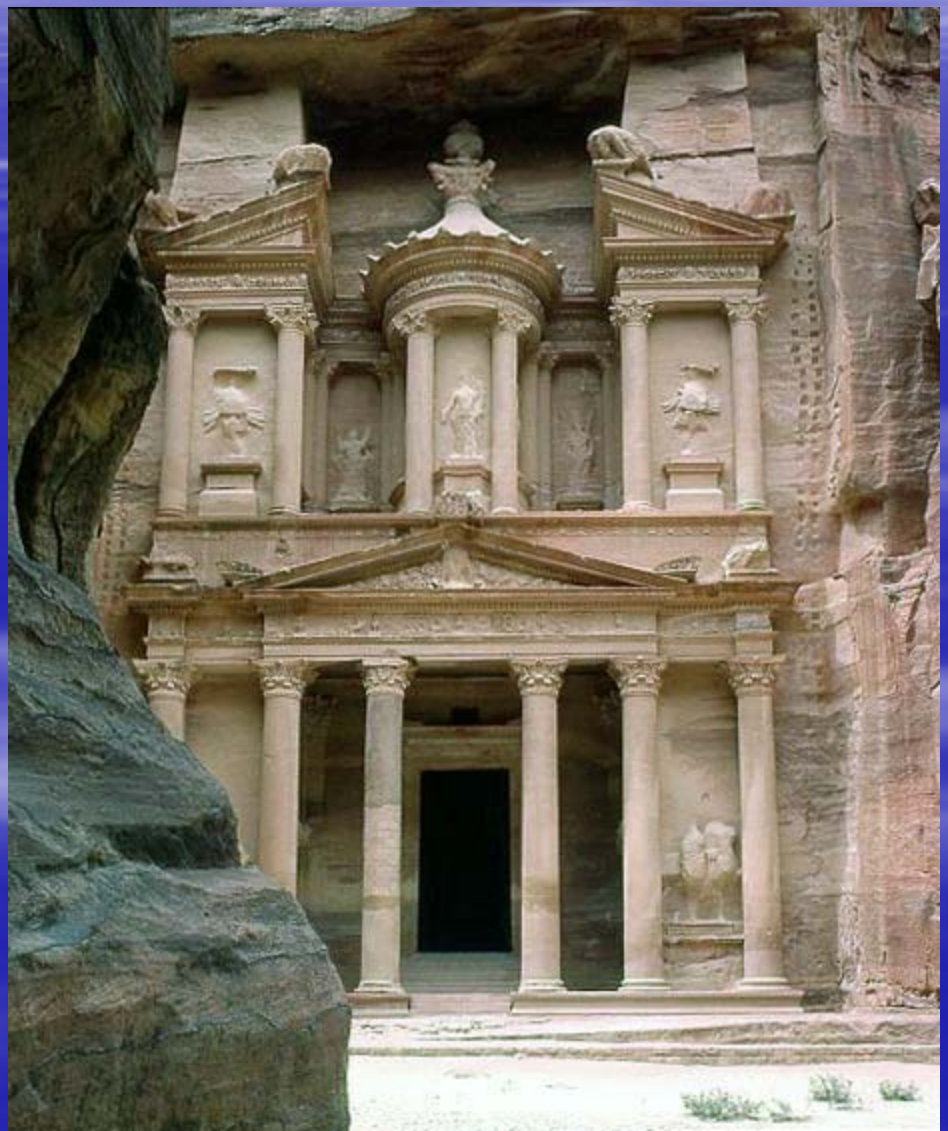
Crohn's Disease Incidence

Mayo Clinic, Olmsted Co. Minnesota




Prevalence of paratuberculosis in U.S. dairy herds
Percent herds infected
100%
80%
60%
40%
20%
0%
1900
1920
1940
1960
1980
2000
2020
'96 USDA survey: 41% herds test positive
First reports by U.S. veterinarians
'90 WI survey 33% herds test positive

Thank You for your kind attention



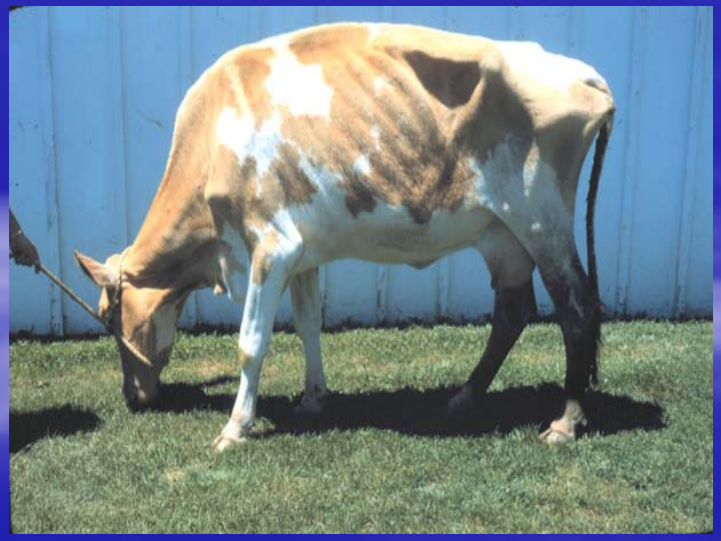
Lectuer # 6

Johne's Disease Definition, Pathology and Clinical Signs

PRESENTED BY:

Professor Nabil Hailat DVM, PhD, Project coordinator. Faculty of Veterinary Medicine (FVM), Jordan University of Science and Technology (JUST)

Johne's Disease




What is Johne's
disease?

Introduction

- Pronounced: **“YO - knees”**
- Chronic infectious disease of domestic and exotic ruminants (dairy and beef cattle, sheep, goats, and camelids).
- Many wildlife ruminants (deer, antelopes, mountain goats, bisons, camels, llamas, and others) are also affected by paratuberculosis.

Historical Perspective

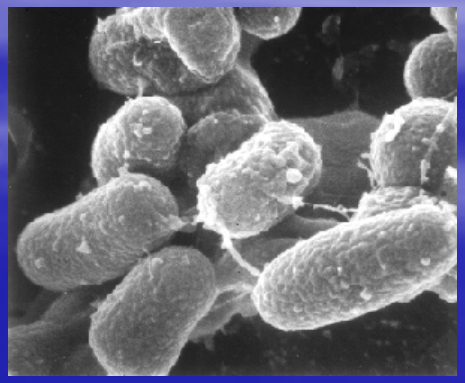
- In 1895, paratuberculosis was first described as a clinical entity by Johne and Frothingham.
  - They found:
  - thickened intestinal mucosa and enlarged mesenteric lymph nodes.
  - intestinal wall was heavily infiltrated with leukocytes and epithelioid cells.
  - abundant acid-fast (red staining) bacteria.

Heinrich Johne

Etiology

- *Mycobacterium avium* subspecies *paratuberculosis*.
- *Abbreviated MAP for short.*

- A slow growing gram positive, facultative intracellular, acid fast bacillus.
- Requiring exogenous mycobactin for growth outside of a natural host (animal).



- This organism is very hardly in the environment and is relatively resistant to many disinfectants.

  - Survives in the feces for up to 1 year

165 days - River water

520 days - Tap water

245 days - Freezing temperature

Transmission

- Primary sources of spread:
  - In utero
  - Colostrum /milk
  - Feces
- Risk of infection is highest in young stock.

Transmission

Disease Progression/Shedding

Disease Development and Bacterial Shedding

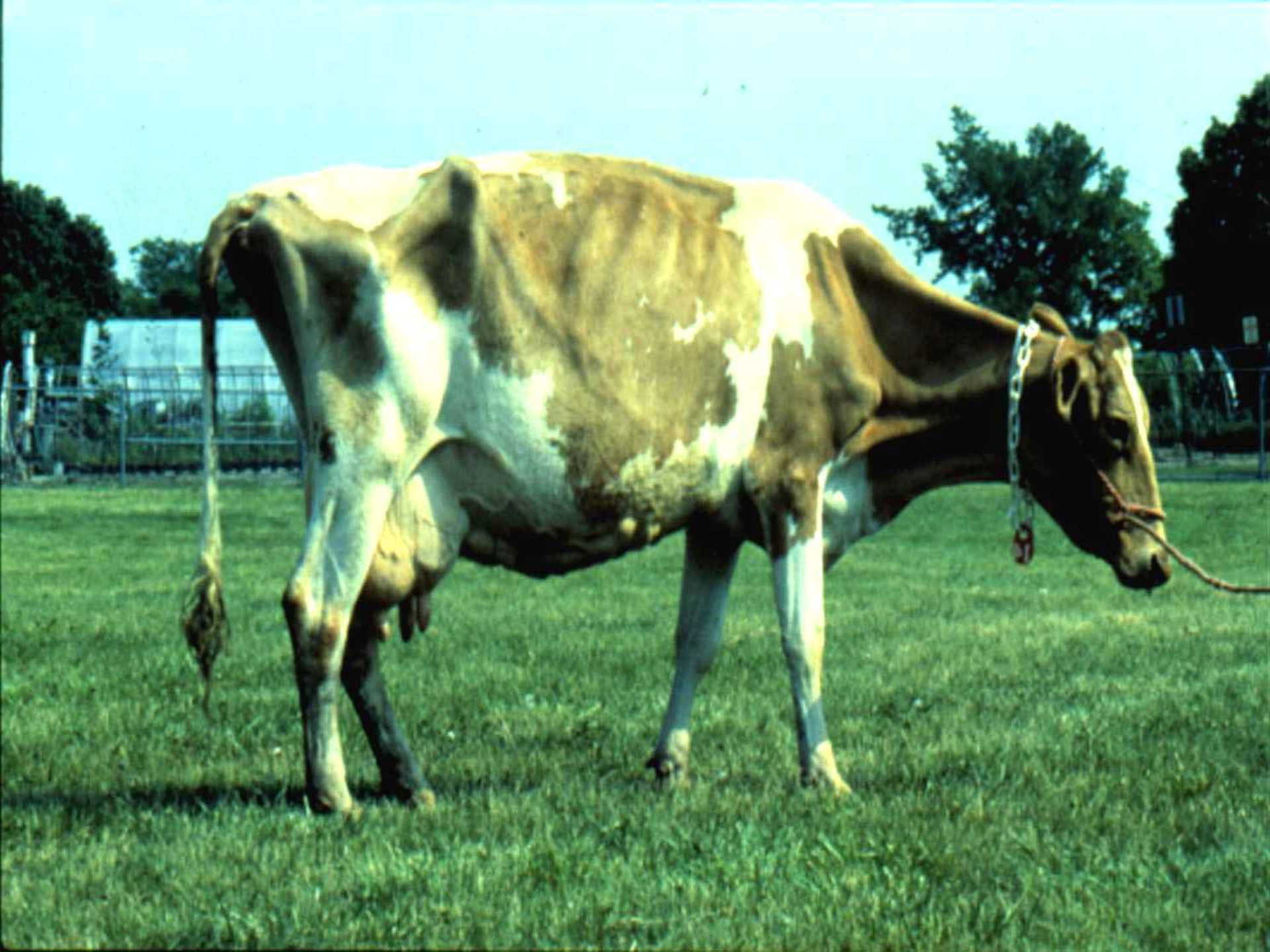
Birth

Adult

The Disease

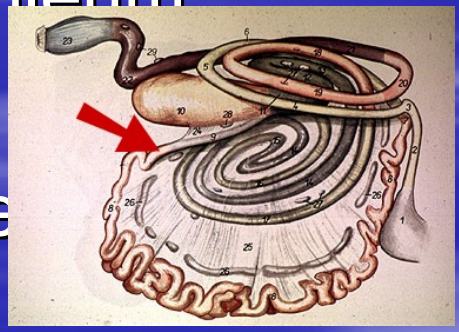
- Chronic, Non-treatable Disease.
- Long period between infection and disease.
- Incubation period is usually 2-4 years.
- Intractable diarrhea, emaciation in cattle.
- In small ruminants similar but no diarrhea.

Clinical Johne's Cow – profuse watery diarrhea



Pathogenesis

- Pathology varies among animal species and, between individual animals.
- The primary site of infections is the ileum
- Lymph nodes draining the ileum are secondary infection.
- Tertiary sites of infection (liver, spleen, and lymph nodes distant from the gastrointestinal tract).



- Grossly, there can be a complete lack of lesions - the intestine may appear entirely normal.

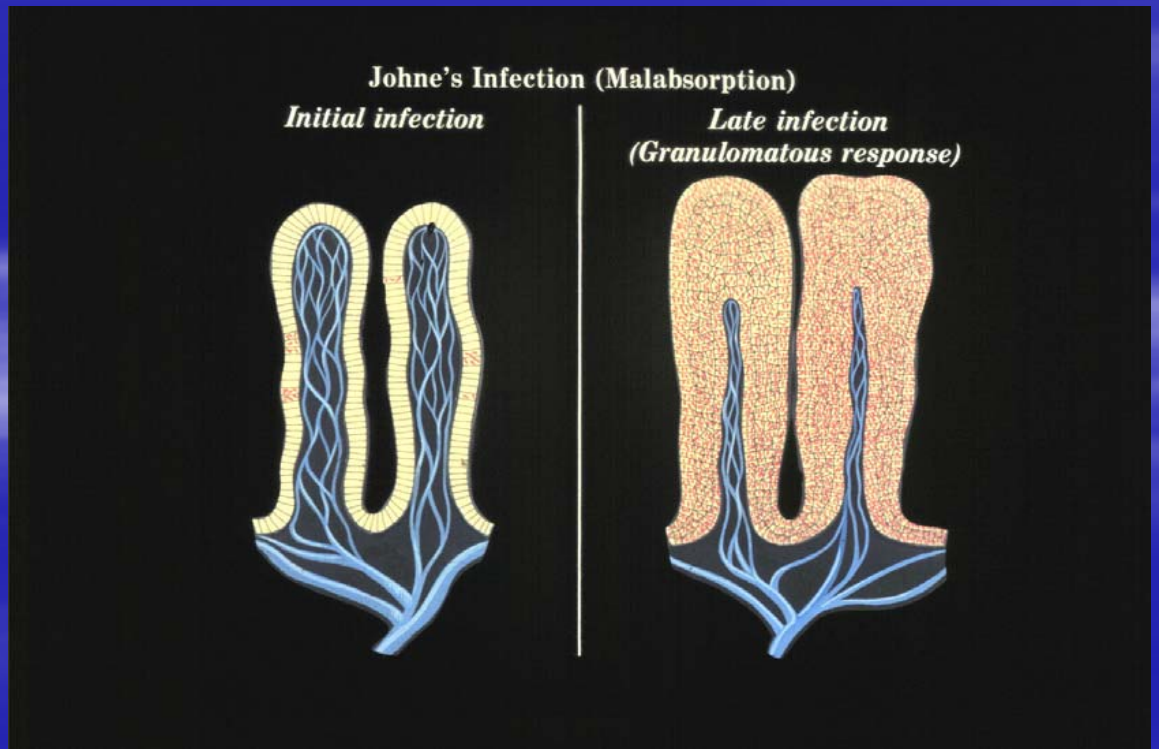
- In other cases, it appears thickened and corrugated and the neighboring lymph nodes are enlarged and edematous.

Well marked, thickened, corrugated, granular surface.

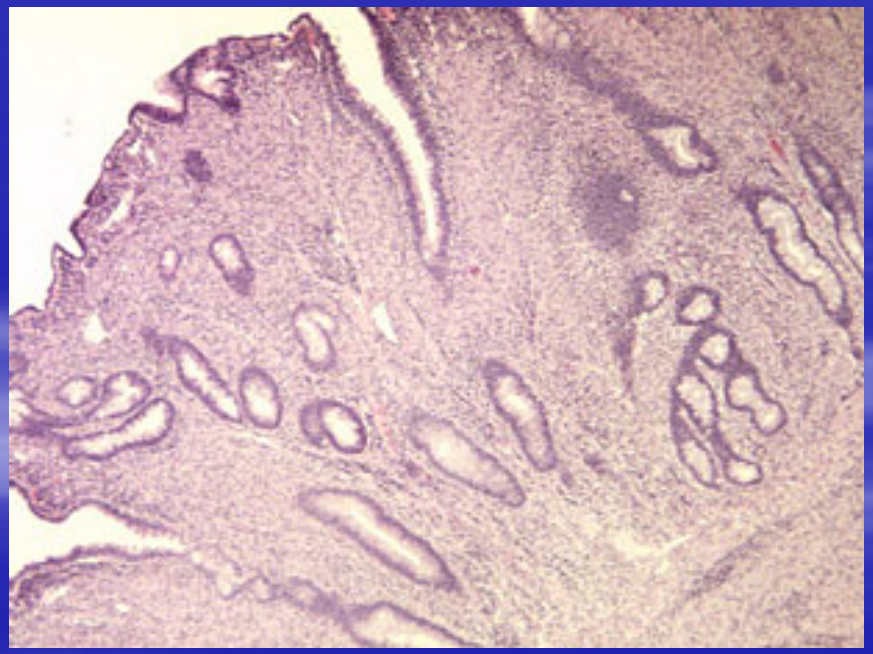
Thickened intestinal mucosa due to Johne's disease

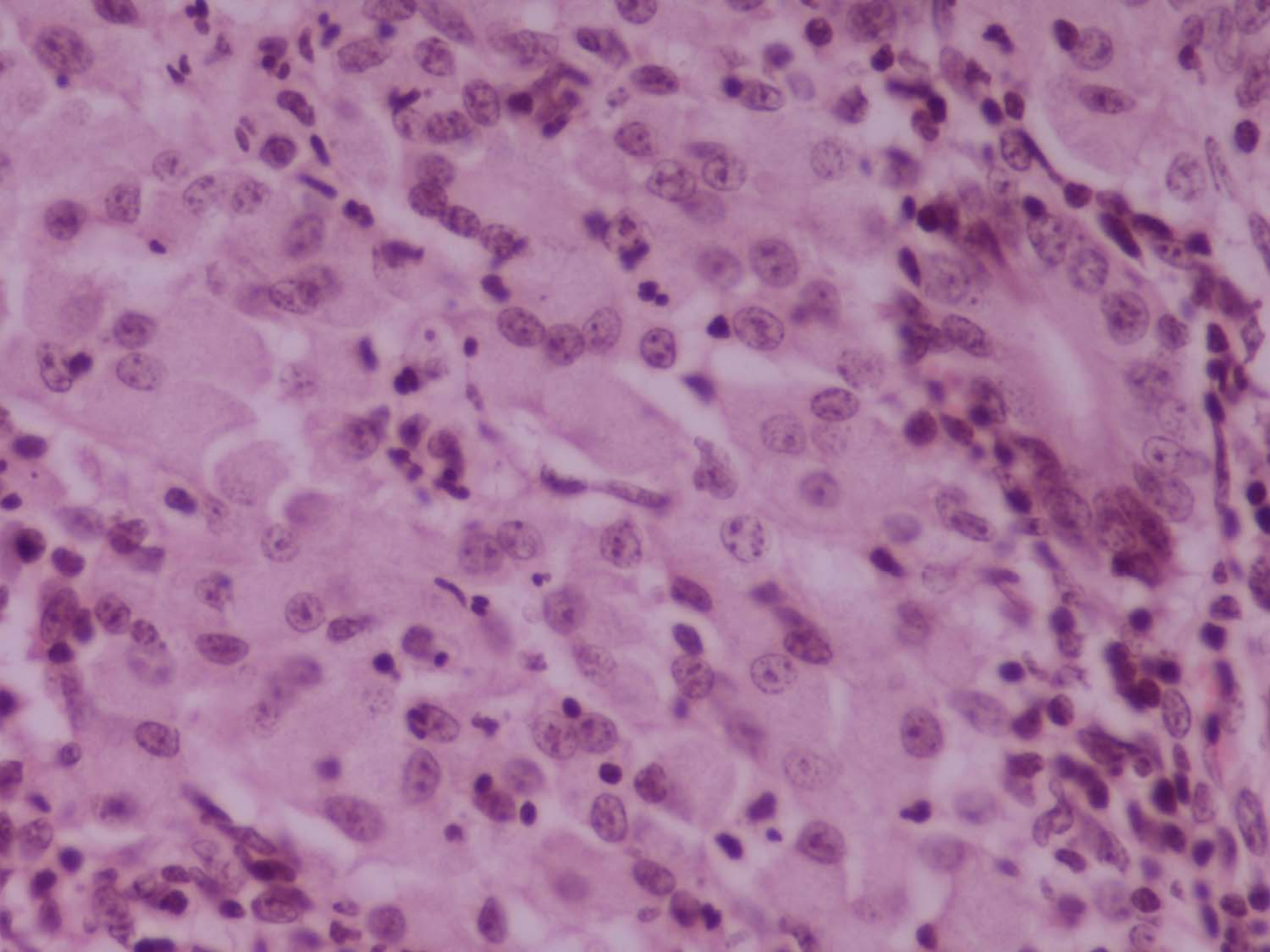
Thickened intestinal mucosa due to Johne's disease

- Thickening and enlargement is due to the influx of inflammatory cells to the infected region.

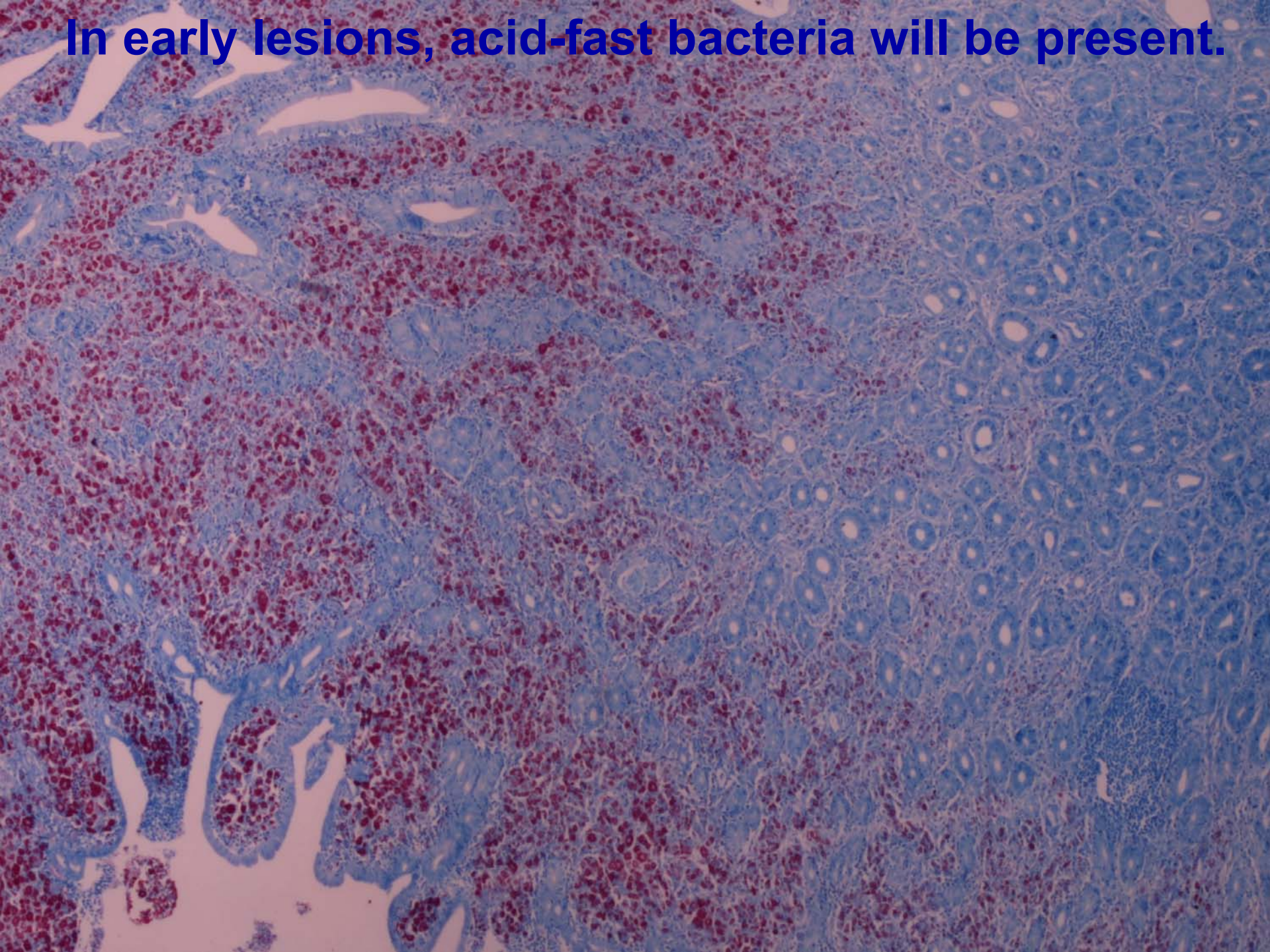


- earliest cellular changes are increased numbers of mononuclear phagocytes (macrophages) and lymphocytes.

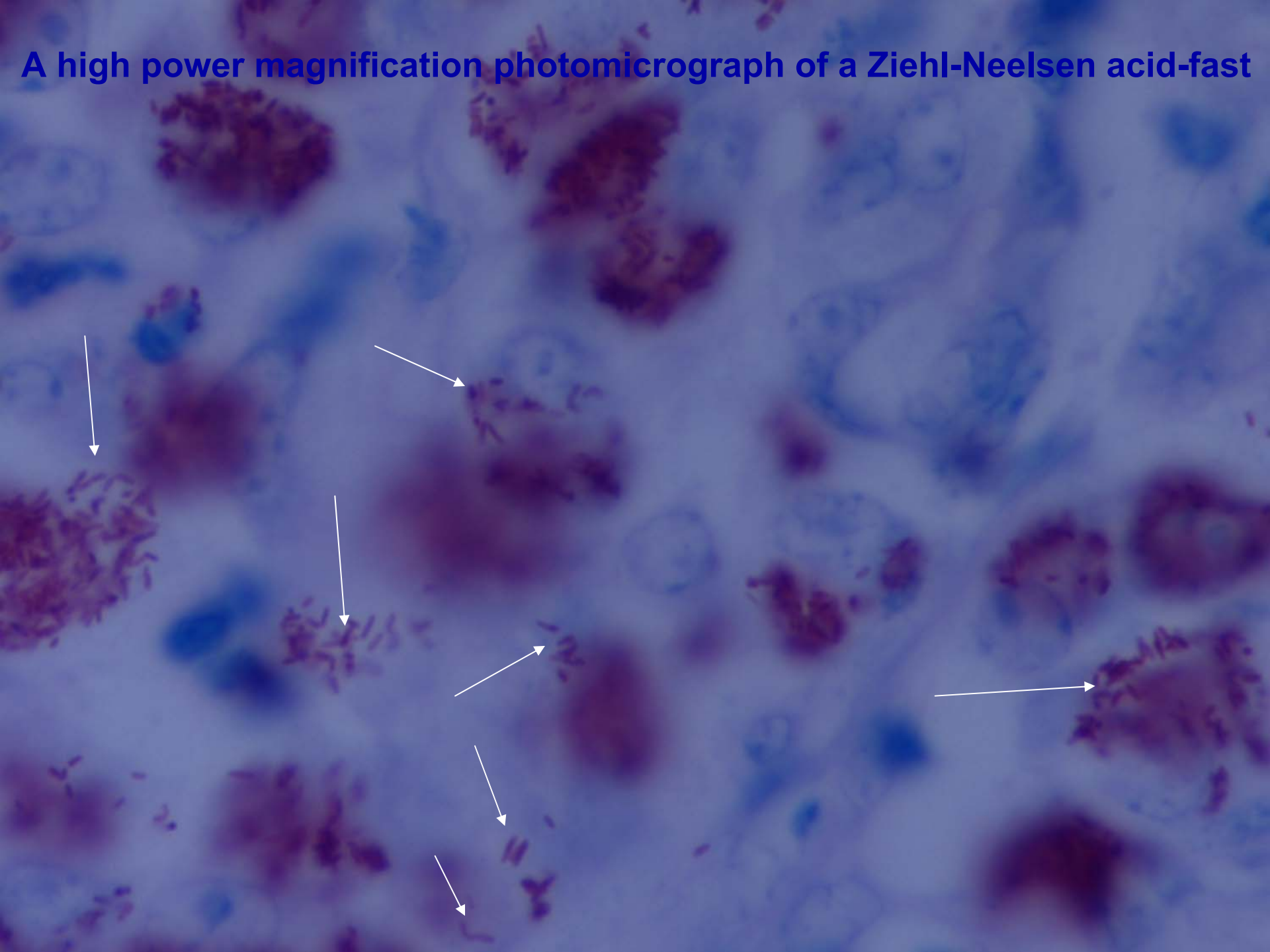




In early lesions, acid-fast bacteria will be present.

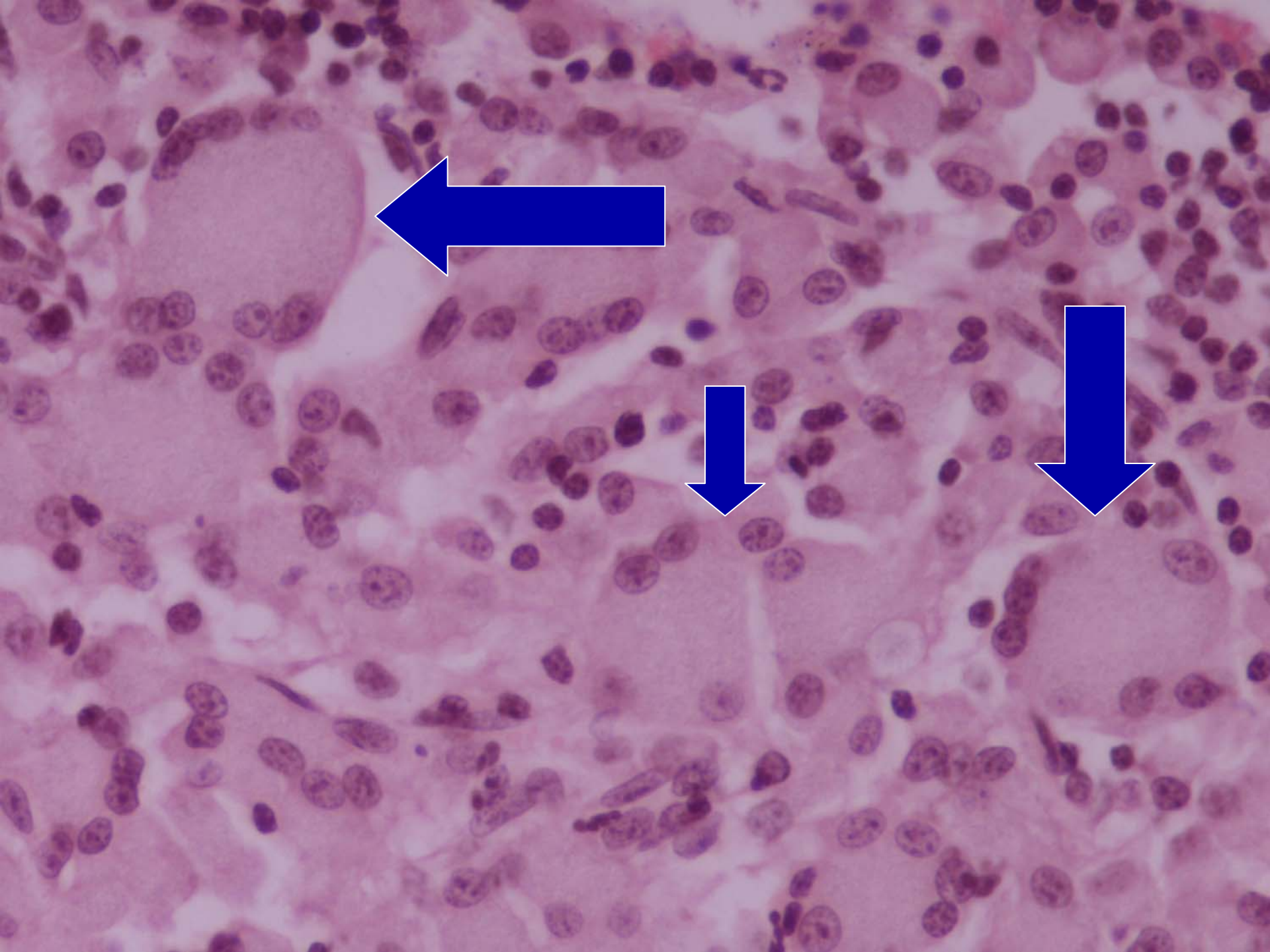


A high power magnification photomicrograph of a Ziehl-Neelsen acid-fast



- Other lesions, have few acid-fast bacteria and are referred to as paucibacillary or tuberculoid.

- As the infection progresses, the mononuclear inflammatory infiltrate becomes pronounced and giant cells become more numerous.



Lecture # 7

Diagnosis of Johne's Disease

PRESENTED BY:

Dr. Wael. Hananeh FVM, JUST .

PARATUBERCULOSIS DIAGNOSIS

BY

DR. WAEL HANANEH

Paratuberculosis diagnosis

1. Clinical signs together with the signalment:
   - In bovine: intermittent diarrhea, emaciation and hypoproteinemia in animals older than 19 months.
   - In small ruminants, the clinical disease is similar to that observed in bovine except that diarrhea does not occur.

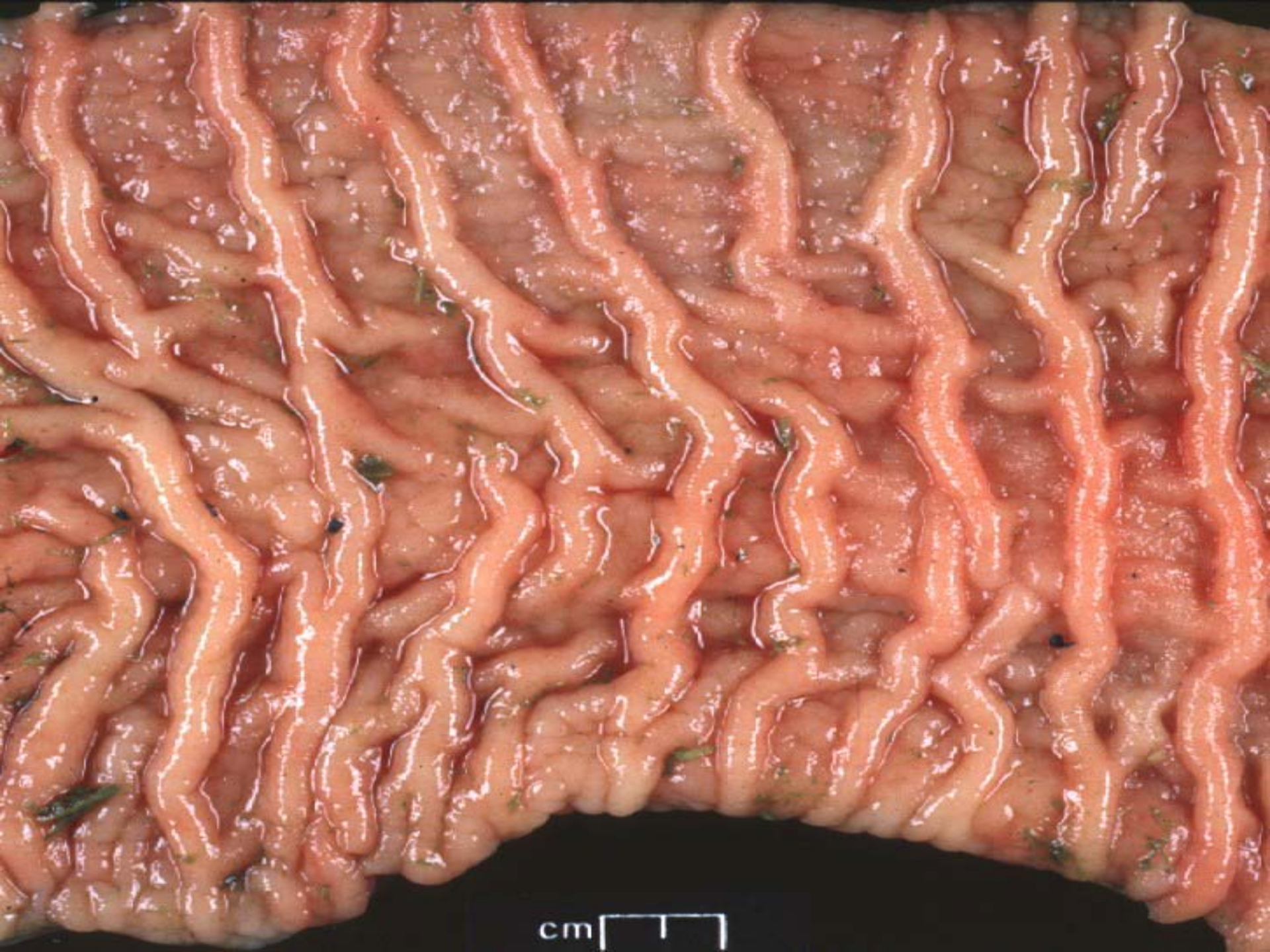
Paratuberculosis diagnosis

- In other ruminants, the disease is considered a wasting disease because of the loss of the body mass.

Paratuberculosis diagnosis

2. Gross lesions:

The gross lesion is segmental thickening of the ileum, cecum and proximal colon often accompanied with mesenteric lymphadenopathy.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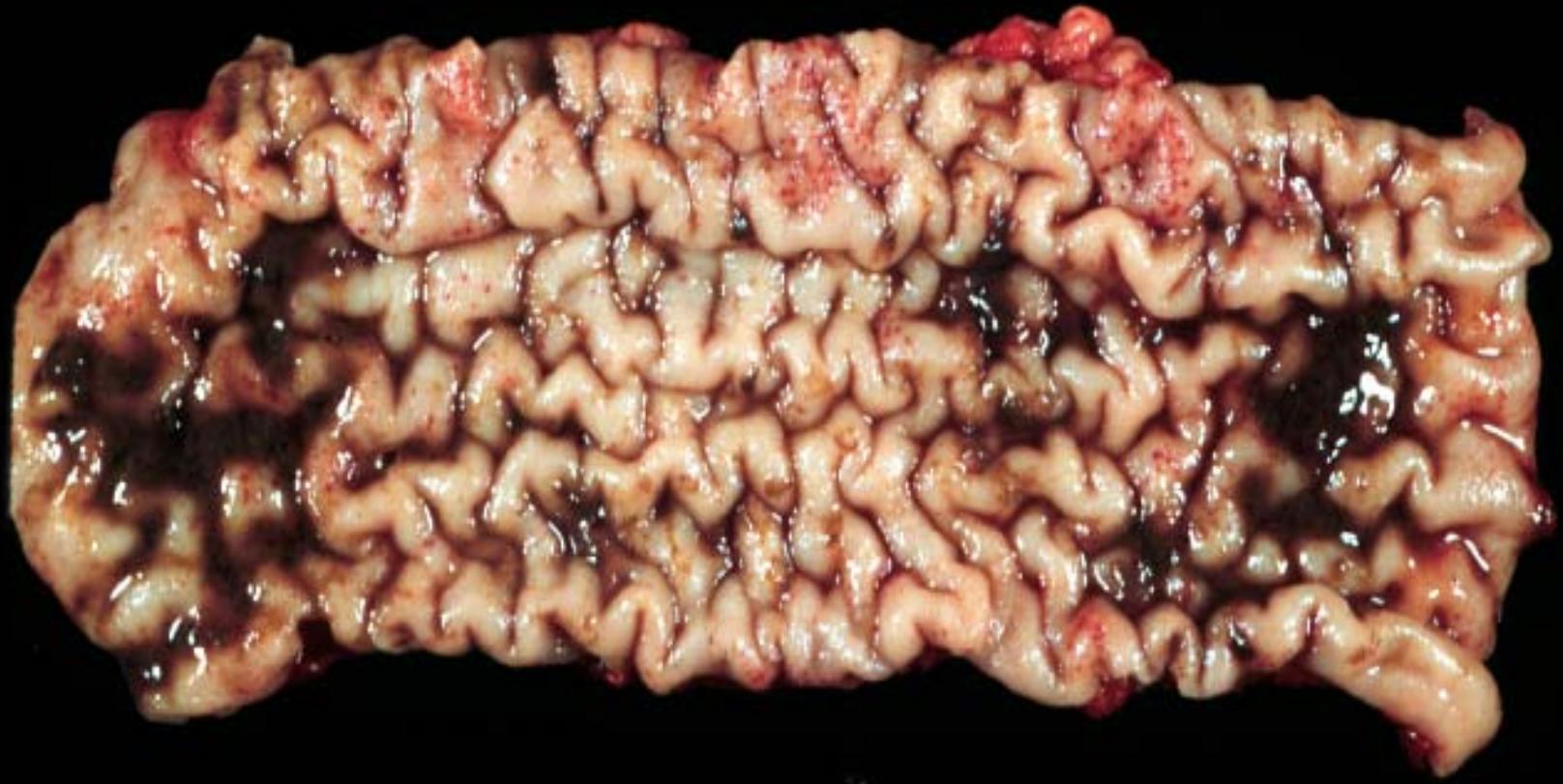


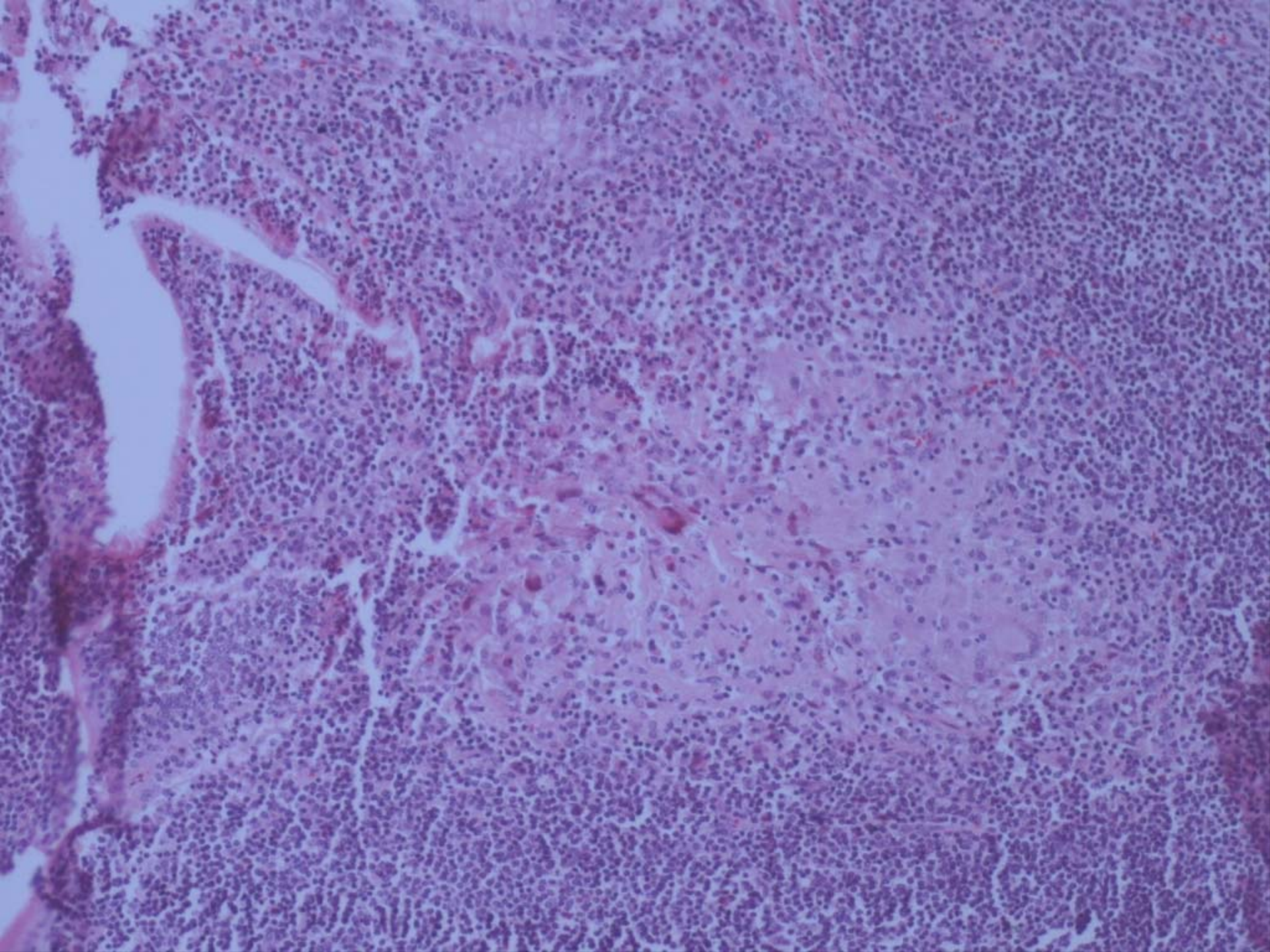
Paratuberculosis diagnosis

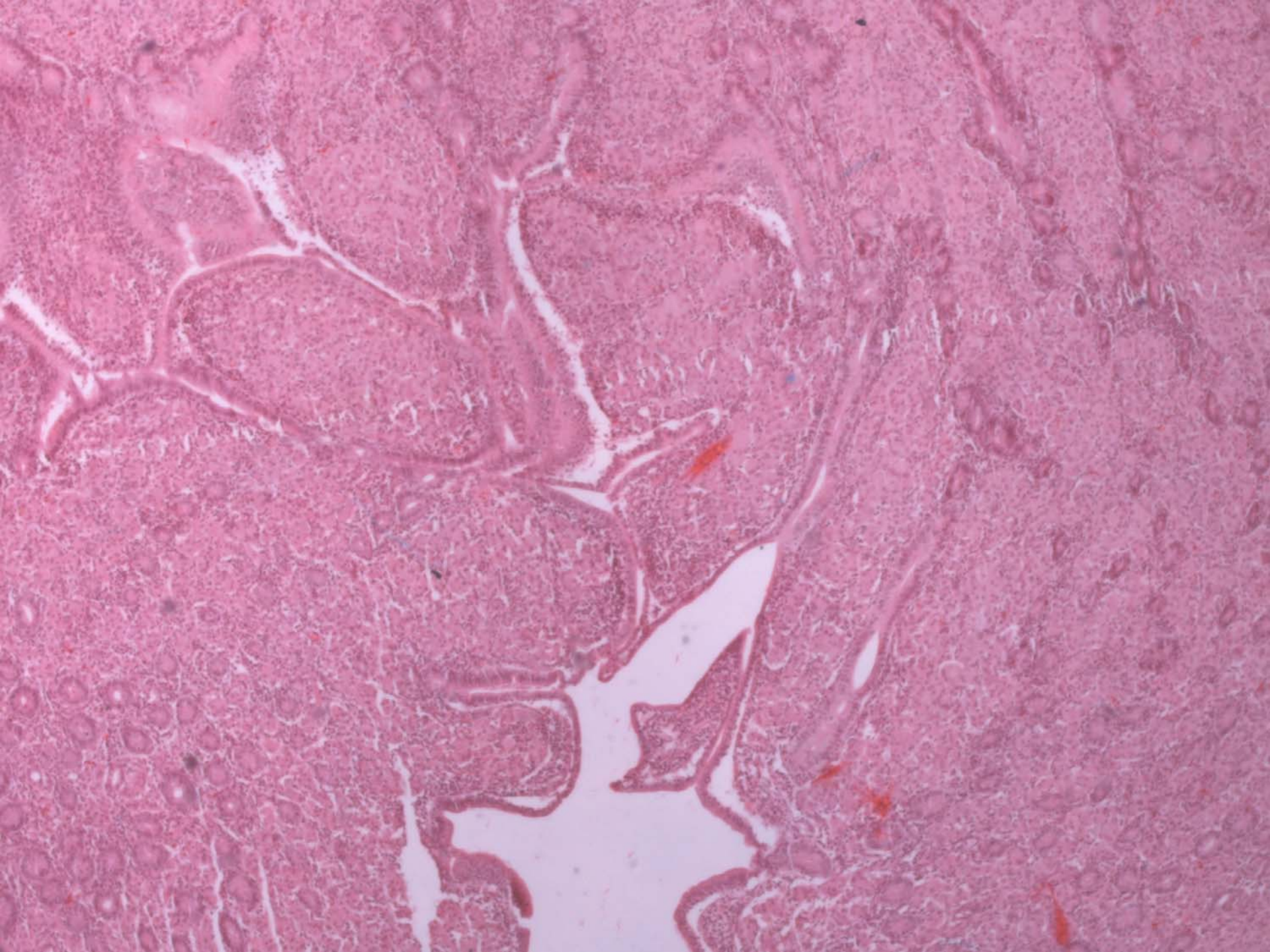
3. Histopathological lesions:

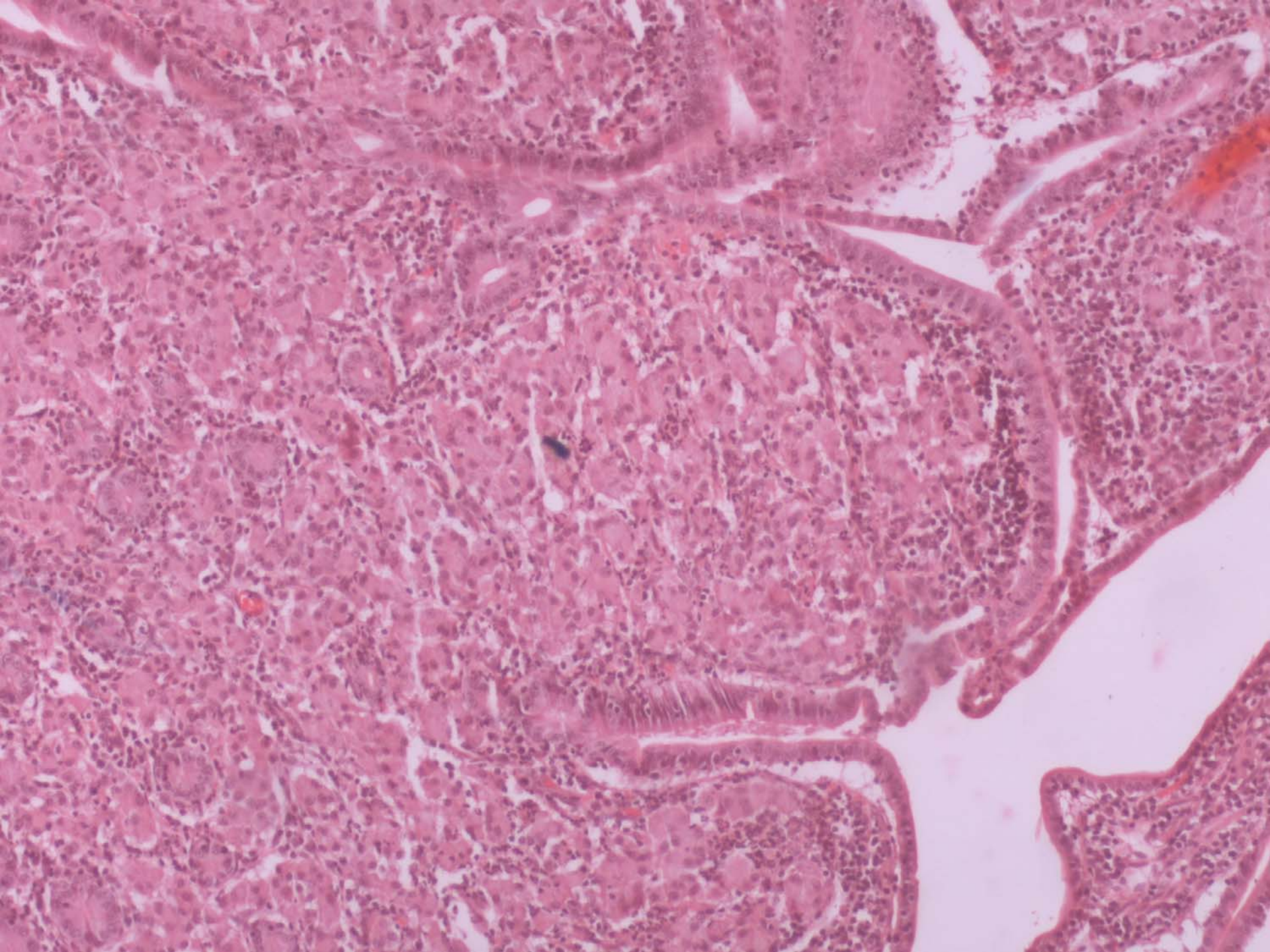
In bovine: non caseating granulomas contain numerous foamy macrophages with large number of AFB.

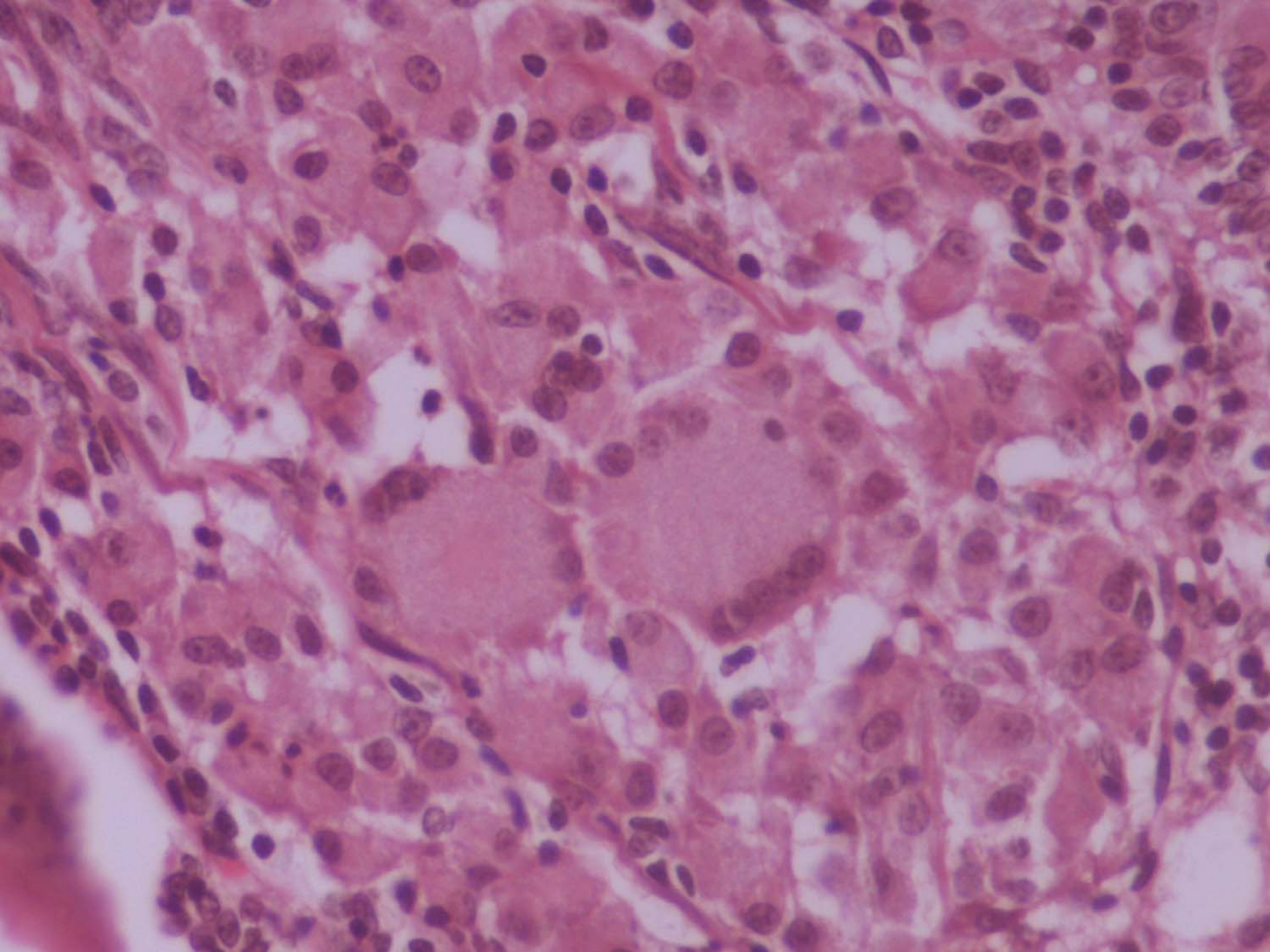
In small ruminants: caseating granulomas in the intestines, lymphatics and lymphnodes

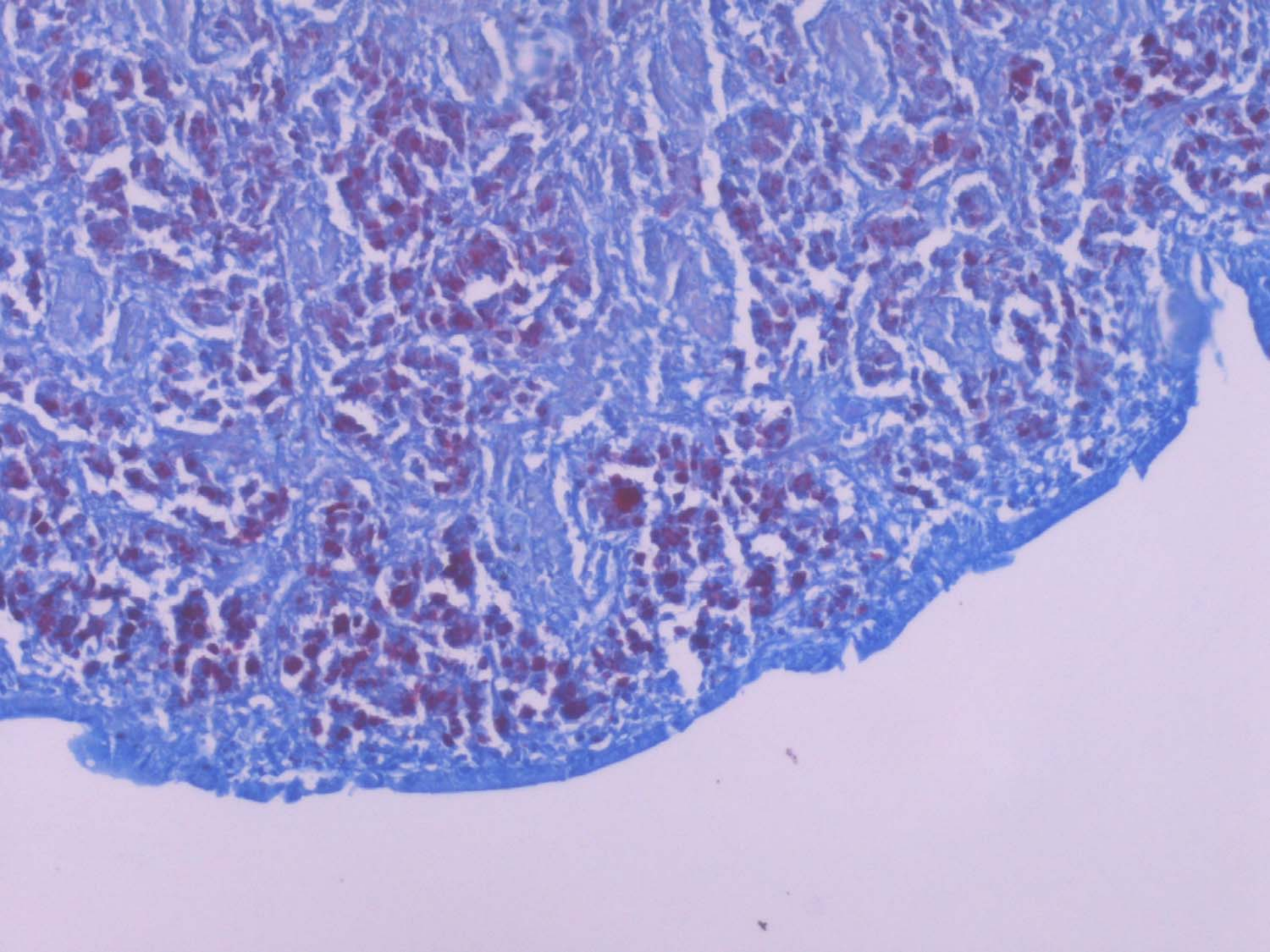
Sometimes mineralized and contain whorled accumulation of epithelioids with variable numbers of giant cells. In these granulomas, it is difficult to find AFB

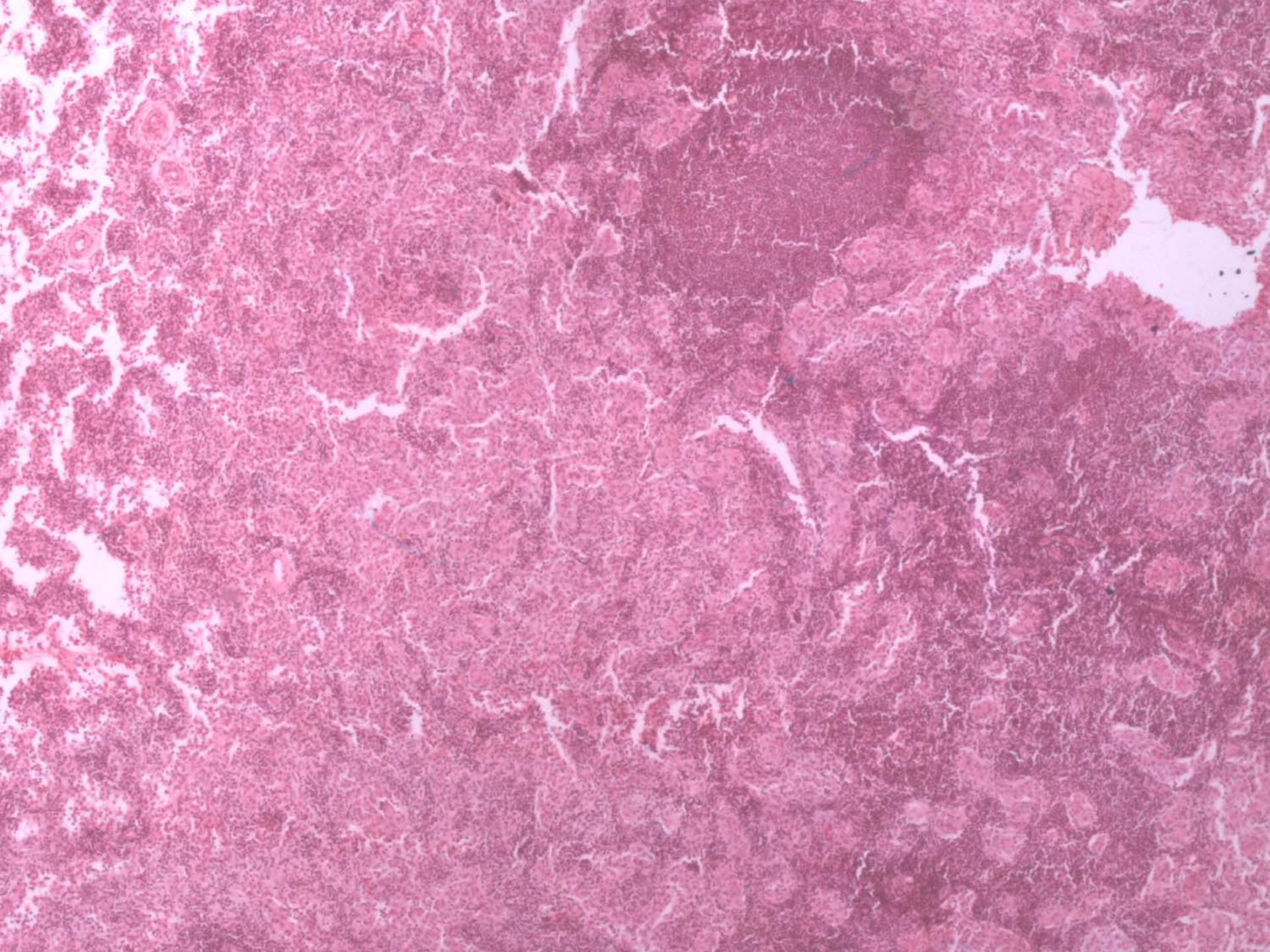


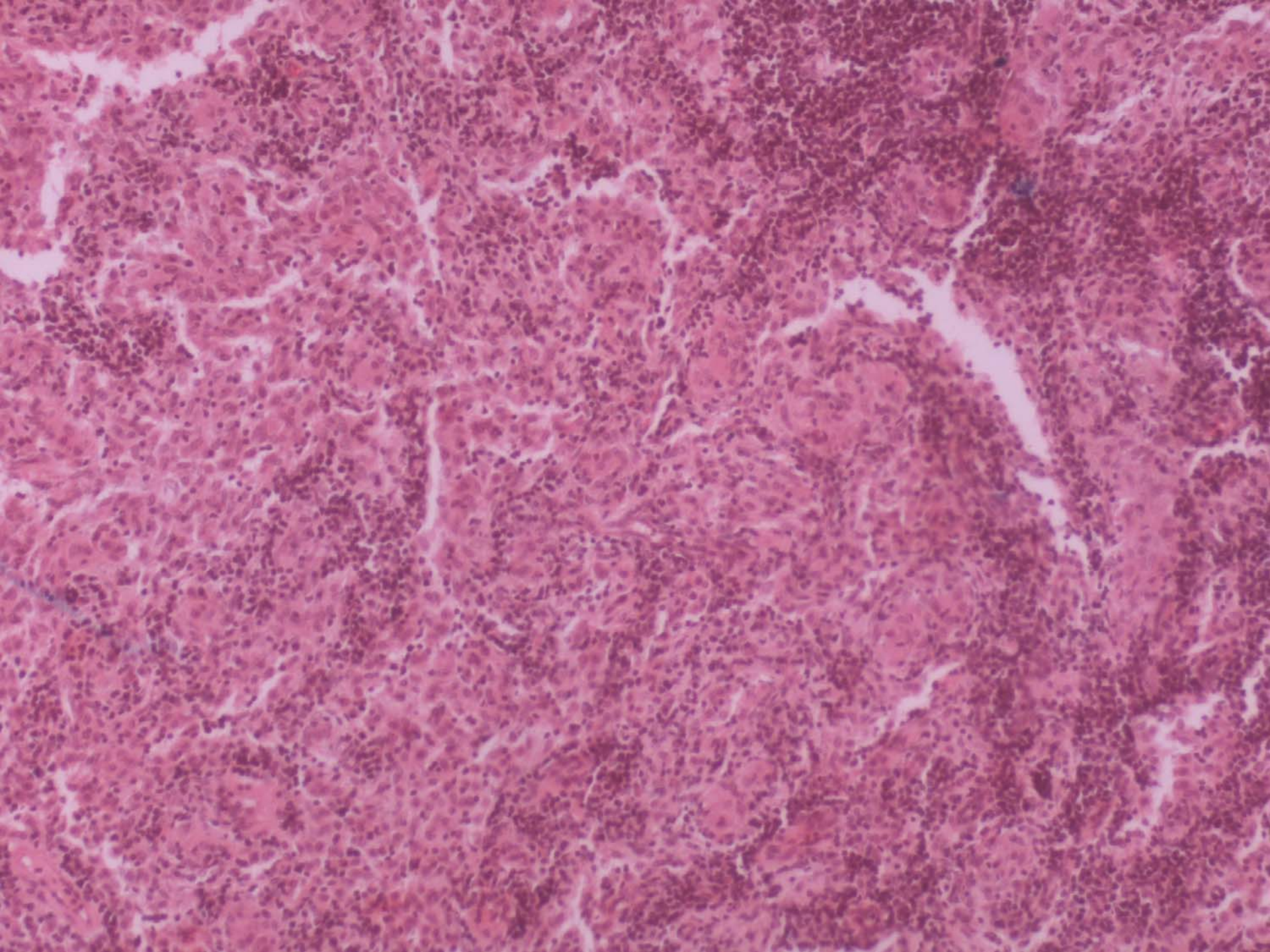


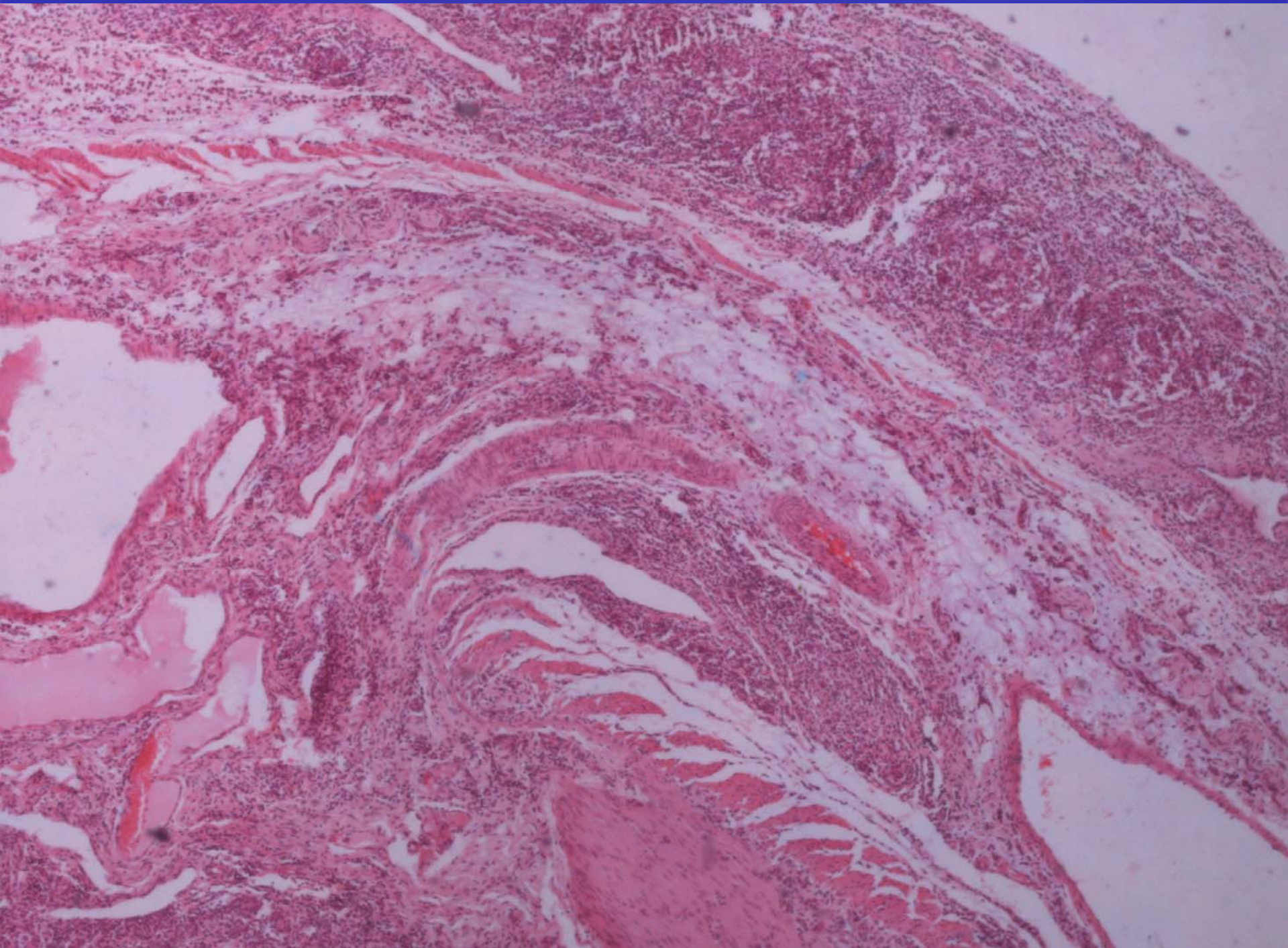


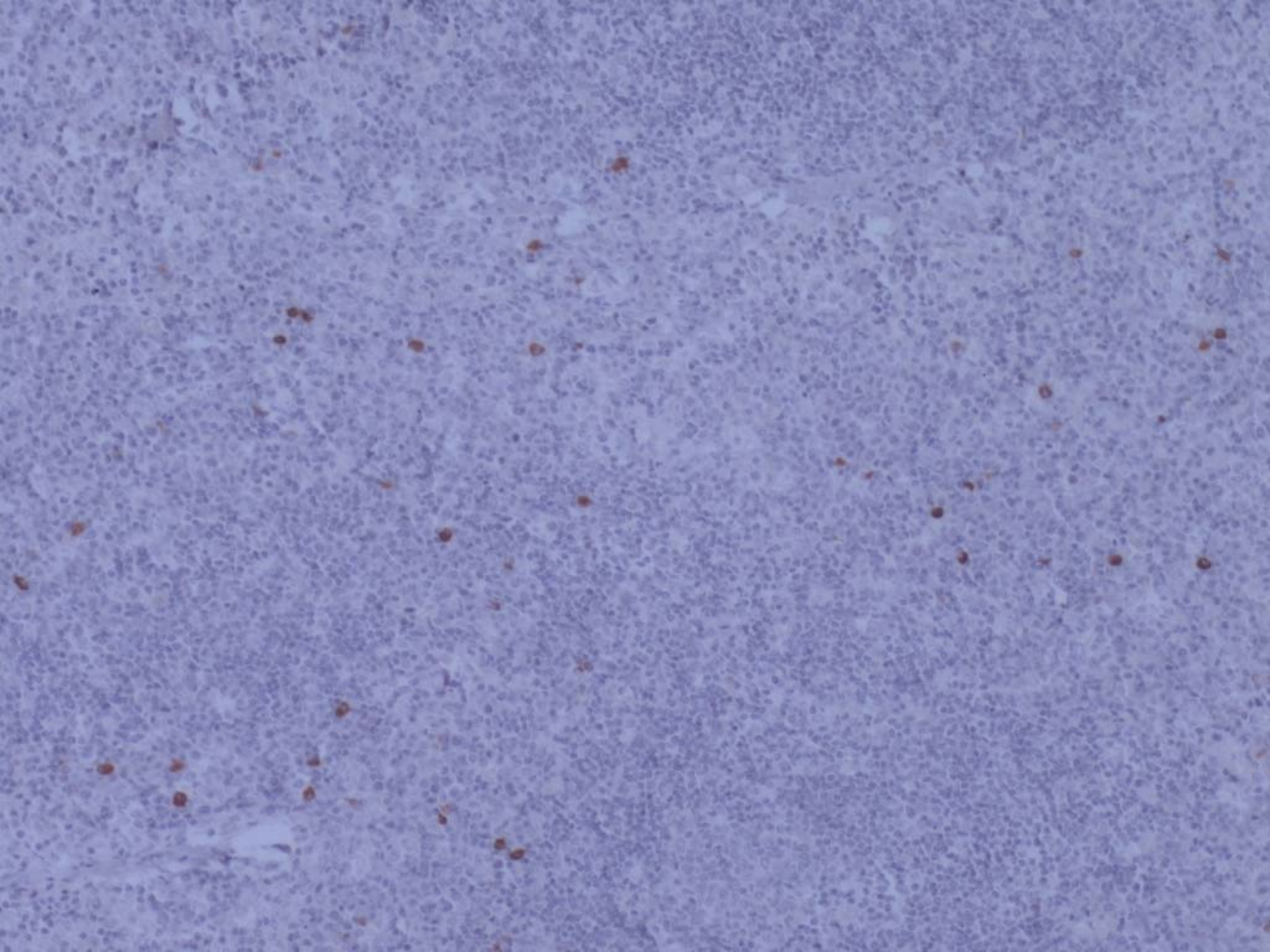










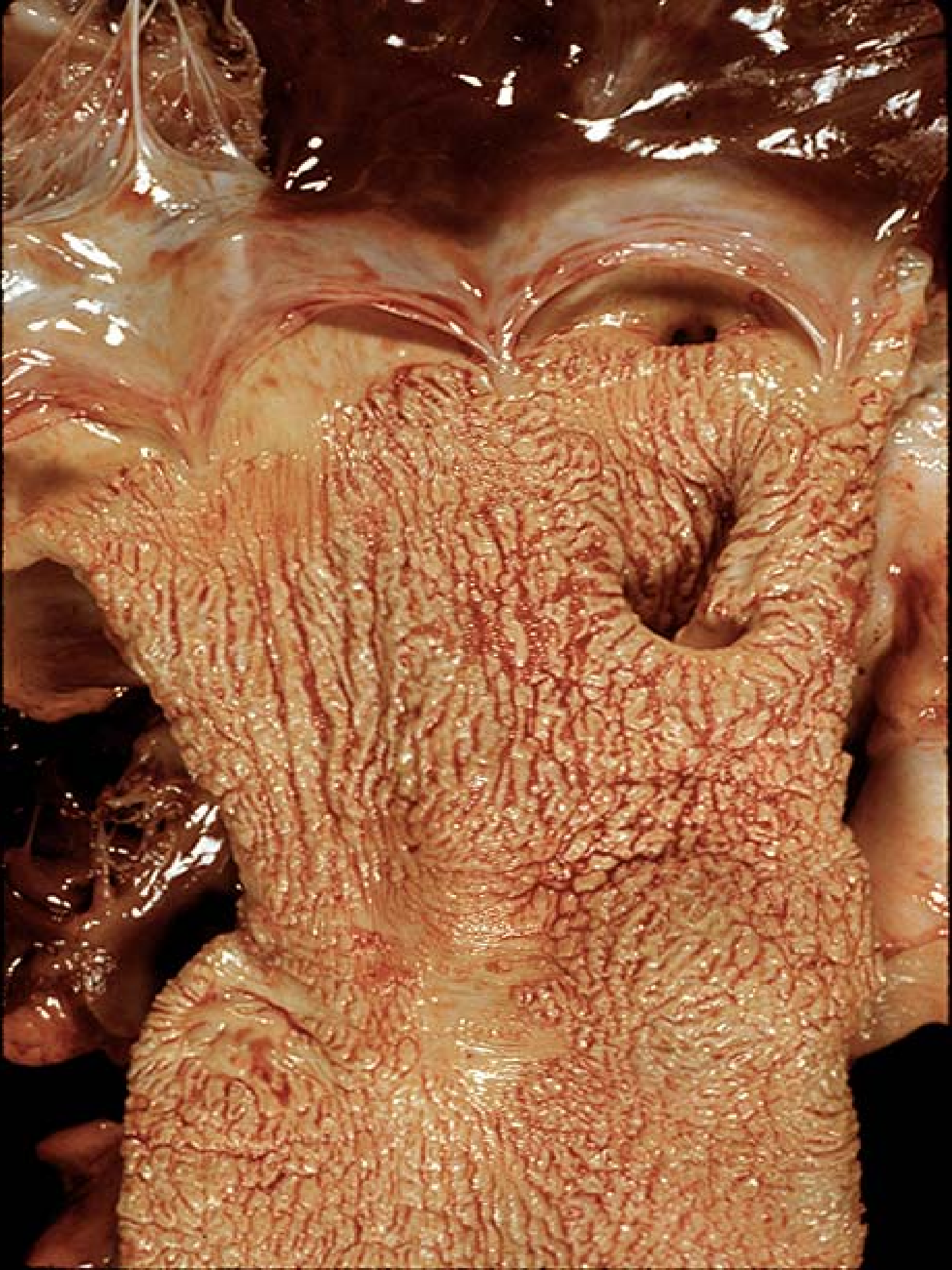


Paratuberculosis diagnosis

4. Extra intestinal lesions

A. Hepatic micogranulomas occur in about 25% of the affected animals.

B. Aortic mineralization in bovine



Tests for Para TB diagnosis

1. **Those that detect the organism:**
   A. Fecal smear and acid-fast stain
   B. Culture
   C. Polymerase chain reaction (PCR)
2. **Those that assess the host response to infection**:
   A. Antibody response to *M. Paratuberculosis* (serology).
   B. Delayed-type hypersensitivity (DTH) reaction
   C. Lymphocyte proliferation.
   D. Increased cytokine (IFN-γ) production

Information on disease diagnosis was gathered by experimental infection trials because detection is dependent on sensitivity and specificity of examination methods as well as on progress of the disease in the animal, which affects shedding of bacteria and seroconversion.

*Disease in Goats

- Bacterial culture showed poor sensitivity
- ELISA and AGID were 100 % sensitive after 180 DPI
- Also it was found that LPT on blood was a sensitive test as early as 60 DPI.

* *Veterinary pathology, July 2005*

*Disease in American Bison

- PCR was found to be 100% sensitive

**Veterinary pathology, Jan 2005*

*Disease in sheep

- In a study conducted to compare between the histologic lesion development and bacterial isolation in MPTB infected lambs, it was found that histopathology was better than bacteriology in sheep

*_Veterinary pathology,_ July 2004

*Disease in Water Buffalo

- In Water Buffalo with naturally occurring PTB.
- 20 out 405 had PTB lesions
- 14 out 20 were PCR +ve
- 6 out 20 cultured +ve

*_Veterinary pathology_ July 2006

*Disease in Cattle

- Histopathology and IHC had the same same efficacy in PTB diagnosis in cattle.

- Master student thesis 2005

Lecture# 8

Prevalence and Pathology of Johne's Disease in Sheep and Goats

RESENTED BY:

Professor Nabil Hailat DVM, PhD, Project coordinator. Faculty of Veterinary Medicine (FVM),

Jordan University of Science and Technology (JUST)

Epidemiology and Pathological Diagnosis of Mycobacterium Paratuberculosis in Awassi Sheep & Goats in Central & Northern of Jordan

Nabil Hailat, DVM, Ph. D, Department of Pathology and Animal Health, Faculty of Veterinary Medicine

Farmers interview

- Twenty farmers were visited and interviewed to collect a basic knowledge about the farming system and small ruminants management in the country .
- The interview was done to assure from the long experienced farmers the health problems encountered in their flocks, as they see them and how they handled these problems.

Interview Cont.

- In Jordan, the original farmers are Beduens, they move from one area to another for the sake of feed for their sheep and goats and therefore live in tent during the summer season.
- Their tents have two compartments one for guests, where men sit with their Arabic coffee accompanied by cups and thermus. Their hospitality and affection is one of their cultural feature, in which a person who visit the Beduen people should drink first Arabic coffee, seconded by tea.
- They receive any guest with smile and respect. They came from Mafrak (East and North Badia), where the small ruminant's population is high. The farmers have stone and bricks build houses in Mafreq areas.

Interview Cont.

- Sheep and goats are raised together, by mixed system.
- Early in the morning they are on the grazing land that is rented by the farmer for the summer time.
- When the sun become hot they return back to the their tents with their animals, and feed them barley, bran and straw hay prepared on a trough done for this purpose, and water is also available.
- Around 2:30 pm they are again on their grazing land.
- Water and feed are transported with the farmers and are available closed to their tents.

Sample Collection and Gross Examination

- During a period of 5 months August –December 2001, a total of 320 intestines were examined from Awassi sheep and local goats which were slaughtered at Amman (49 sheep), Sweleh (63 sheep and 28 goats), and Irbid (103 sheep and 13 goats) slaughter houses of Jordan.
- The age of the animals was ranging from 8-24 months for sheep and 6-12 months for goats.
- Samples were collected according to intestinal mucosa thickness, which was recorded from mild to severe .

Sample Collection and Gross Examination, cont.

- Site for sample collection was selected according to small ruminant's distribution in Jordan.
- Amman and Sweleh abattoirs were being selected representing the central part of the country, and Botchers around Irbid as the northern part.
- The study was carried out in Awassi sheep and Baladi goats between the age of 8-24 months and 6-12 months respectively.
- A total of 320 intestinal samples were examined and 260 were collected (279 sheep and 41 goats), considering any slight thickening of the mucosa.
- All animals were derived from areas of no research being done concerning Johne's disease.

Sample Collection and Gross Examination, cont.

- The intestinal samples were opened using scissor for the observation of any mucosal lesion.
- The gross examination was carried out to have an over iew of all portion of the small intestine.
- According to the small intestine gross appearance the large intestine was subjected to gross examination.
- Samples were collected from the ileum, ileo-cecal valve and respected lymph nodes.
- In few cases in which gross lesion was observed, tissue samples from the cecum and colon were collected. All the samples were trimmed in small size (4mm-1cm thickness) fixed in 10% buffered formalin, and subjected for histopathological examination and immunohistochemical stain.
- Other tissue samples were partially transported on ice to the laboratory for direct smear and culture. Samples for the culture were kept under -20°c until needed

Direct Smear

- Scrapings of the intestinal mucosa and lymph node parenchyma were carried out using scalpel blade and smears were done on microscopic slides.
- Samples were taken, especially from the ileo-cecal valve and from respective lymph nodes parenchyma.
- Slides were dried by air, fixed with methanol (100%) and stained by Ziehl Neelsen (ZN) staining method according to Coles (1986).
- Stained slides were observed using oil immersion100) x objective). Each slide was scanned for 30 minutes in order to make very high the chance to detect acid-fast bacteria.
- The findings were registered according to the bacterial appearance, in which observation of bacteria's in clumps taken as positive, in dispersed form considered suspected and as negative if neither of the two forms observed.

Histopathological Examination

- All the trimmed samples were processed for Histopathological examination, according to Bancroft (1990). Briefly subjected to the following steps:

Dehydration

- Dehydration was carried out using a tissue processor (Histokintte ,Leica ,Germany).
- The fixed tissue were passed through graded ethyl alcohol to remove excess water from the tissue (60%,70%, 95%, 95%,100%,100%),
- 2 hours the first two steps and the rest 1.5 hrs.
- Additionally alcohol is the only chemical, which is miseable with xylene that is used to clear alcohol.

Clearing

After dehydration samples were treated with xylene by passing through (Xylene I and II) During clearing the tissue become hard and translucent, 2 hrs each step.

Impregnation (Infiltration)

- The infiltration was done by two changes of melted paraffin this is to clear the xylene used during the previous step and to make infiltration of paraffin through the tissue to facilitate the sectioning by hardening, and to make ready, the tissue for paraffin wax embedding for 2 hrs. each.

Embedding

- Embedding was conducted by using paraffin wax method melted at 56-59ºC in paraffin dispenser (MEDAX, Germany) .
- The melted paraffin wax was dispensed in to the mould gently, the tissue is introduced to the base of the mould using warmed forceps, giving attention to tissue orientation and pressed gently.
- The mould was filled with the paraffin wax to the maximum capacity and allowed to cool at room temperature.
- Then transformed to –20°c to make the block stronger and to make the sectioning easier.

Trimming of blocks (Tissue sectioning)

- The molded blocks after solidification were removed and subjected to trimming at 5µm using a microtome (Leica, Germany).
- The sectioned tissue ribbon were floated on warmed water in the water bath (Leica, Germany) at 38-40ºc, to stretch the tissue properly.
- Finally the tissue taken by microscopic slides and allowed to dry using a hot plate (Leica, Germany)

Staining (Haematoxylin and Eosin stain)

Five micron-sectioned tissues were stained as follows:

- Deparafinization by passing the slides in xylene two changes 5 minutes each
- Hydration of the sections by dipping them through degraded alcohol (100%, 100%, 95%, and 70%), and running water.
- The tissues sectioned were stained by Mayers Haematoxylin for 2-5 minutes depending of the age of the stain.
- Washed in tap water, and stained by aqueous Eosin for 3 minutes
- The sectioned tissue were washed in tap water and dehydrated by dipping them in graded alcohol (70%, 95%, 100%, 100%)
- Clearing was done using xylene two changes 5 minutes each
- Finally the sectioned tissues were mounted using DPX with cover slides 22x40mm

Ziehl-Neelsen (ZN) staining of tissue for the detection of acid-fast bacteria

The staining procedure was done according to Edna et al., (1994). Briefly:

- Tissues sectioned were deparafinized and hydrated to distilled water.
- Immersed in carbol fuchsin solution for 30 minutes.
- Washed well in running water.
- Decolorized with acid alcohol solution or sulfuric acid solution until sections are pale pink.
- Wash with tap water for 8 minutes.

Ziehl-Neelsen (ZN) staining of tissue for the detection of acid-fast bacteria

- Counter stained by dipping one slide at a time in a working methylene blue solution. Sections should be pale blue.
- Washed with tap water, then rinse in distilled water.
- Dehydrated in 95% alcohol, and clear in xylene, two changes each.
- Mounted using DPX and cover slides 22x40mm
- Tissues stained by ZN were observed for 30-45 minutes each slide under100x for bacterial observation, and the results were recorded considering the presence of even one acid-fast bacilli intra-cytoplasmic or extra- cellularlly

Grading criteria for histopathological lesions

- The H&E stained sections were observed under 4x, 10x, and 40x objectives.
- Lesions are classified depending on cellular infiltration.
- Tissue was considered positive when there are macrophage infiltration or epitheloid cell is obvious in the lamina propia of the villus and between crypts and involvement of the payer's patch in which pale cell (macrophages) micro-granuloma present.
- The scoring of tissue lesion was as follows:

Grades of Histopathological features found in the last part of the Ileum and the ileo-cecal valve and respective lymph nodes

Grade	and lesions Type of inflammatory cells				
	Lymphocytes	Macrophages	Epitheloid cells	P.P.proliferation& Cr.repleciment	Microgranuloma
I	++++	++	+	-	-
II	+++	++++	+++	Moderate	Yes/No
III	+	+++	++++	Severe	Yes
SP	+	++++	++++	Calcification or caseous necrosis in the LN	Granuloma with Langhans giant cells

P.P.= Payer's patches; Cr.= Cryptes; SP= special grading; LN= Lymph Node

5. Immunohistochemical (IH) stain

- All direct smear positive sample, suspected and from the negative ones randomly selected equivalent number to the positive samples, were subjected to IH stain.
- 1- The tissue samples, from paraffin-wax embedded blocks, were sectioned at 2-3 μ to develop immunohistochemical stain.
- 2- The sectioned tissue samples were laid on vecta bond (DAKO A/S.Glostrup, Denmark) coated slides, dried by air and then allowed in oven at 55°c for 2 hours.
- 3- Tissue sections were deparaffinized in xylene and hydrated by sequential immersion of slides in degraded concentration of ethanol (100%, 95% and 70%) for one minute each.
- 4- Wash in distilled water for 5 minutes
- 5- After washed in PBS, the tissue sections were immersed in citrate buffer solution pH=6, 10mM and antigen retrieval was carried out by autoclaving the tissue section at 120°c in 15%pressure for 15 minutes (Express, Italy).

5. Immunohistochemical (IH) stain ,cont.

- 6- The sections were cooled at room temperature and washed in PBS for 5'.
- *All of the subsequent incubations were performed at room temperature, and all washings were with PBS 7.4 PH, (adjusted using Welihein, PH meter 1999,WTW, GmbH, Germany), using three steps for 5 minutes each.
- 7- Endogenous peroxidase was inactivated by immersion of the slides in a solution of 15% hydrogen peroxide in methanol for 30 minutes.
- 8- After washing, non-specific adherence of proteins to tissue sections was blocked using 1% bovine serum albumin (BSA), (Sigma Chemical Co., PO. Box14508, St.Louis, MO63178) incubated for 2 hrs. .
- 9- The solution was drained from the slides and the polyclonal *M. paratuberculosis* antiserum, raised in rabbit diluted 1:500 in PBS, applied and the slides were incubated for 2hrs.The rabbit antibody was kindly provided by Dr. Stabel from Iowa State University USA
- 10- Then washed and universal biotinylated anti-goat, anti-rabbit, and anti-mouse immunoglobulin (DAKO A/S, Glostrup, Denmark) diluted at 1:20 were applied as secondary antibody, and the slides were incubated for 15 minutes.

5. Immunohistochemical (IH) stain ,cont.

- 11- After washing, Strepto Avidin biotin complex peroxidase (DAKO, A/S, and Glostrup, Denmark) was applied, and incubated on the tissue section for 15 minutes.
- 12- The slides were washed and were exposed to chromogen 3,3 diamino- benzidin-4HCL (DAB, electron microscopic product, DAKO) 1mg /ml in PBS supplemented with hydrogen peroxide (10 ul of 3% hydrogen peroxide for 2ml of DAB). Incubated at room temperature for 3- 5 minutes, and slides were washed in distilled water for 5 minutes.
- 13- Then counter stained in haematoxylin 2-3 minutes

5. Immunohistochemical (IH) stain ,cont.

- 14- Washed in distilled water in three passes 5 deeps each
- 15- Immersed in bluing water for 30 seconds.
- 16- Washed in water three passes 5 deeps each.
- 17- Finally, dehydrate in degraded alcohol (70%, 95%, 100% three passes) one minute each
- 18- cleared in xyelen (3 passes one minutes each) and mounted using DPX for further observation.

5. Immunohistochemical (IH) stain ,cont.

- Slides were observed using 4x, 10x and 40x objectives.
- Sections were considered positive according to the color observation that is indication of antibody antigen reaction, and manifested by intra-cytoplasm or extra-cellular brown coloration in different areas of the stained tissue section.
- The findings were registered by counting the number of reaction, accordingly starting from one cell reaction recorded as positive, and 1-10 as 1+, more than 10 reaction, as 2++, reaction in 5 or more cells from one field was graded as 3+++.
- Additionally the intensity of the reaction was considered and in all cases only strong brown color was recorded as positive reaction .
- In all cases positive and negative control slides were processed together from the same known group of tissue sections, in order to avoid false positive and negative reaction .

Culture

Culture

Media preparation

- Middle Brook 7H10 agar base M199 (Himedia Laboratories, limited Mumbai)Bombay) 400086,India) is used with a supplement as a slant in tubes of 20ml.
- The media was prepared as OIE (2000). 9.73g in 450Lts of distilled water and 5ml of glycerol and the mixture was boiled to mix, autoclaved at 121°c, at 15% pressure for 15min., cooled at 45-50°c in water bath(GFL 1083 ,Germany).
- Fifty m/liters of the supplement (Middlebrook OADC growth supplement FD 018 ,India) was added.
- For the suppression of contaminants Penicillin of 1,000,000IU a 0.1 ml/Lt. of media and as anti-fungal agent Nystatin 50mg/Lt. was added.
- The media containing mycobactin was prepared by adding mycobactin J 2mg/Lt. (Allied Monitor IAC ,Fayette ,MO, 660 248-2823) of the medium dissolved in 4ml of ethyl alcohol with the agar base before autoclaving.

Culture , cont.

6.2. Sample preparation and inoculation

- Approximately 3-5g of intestinal mucosa scrapped and lymph node parenchyma was taken and grounded together using mortar, and allowed with trypsin 0.5% at 4°c overnight PH=7.2-7.4 (Adjusted using 4% NaOH).
- After 16-18 hours the mixture was filtered using gauze (folded), and centrifuged at 400xg for 20 minutes (PK110, ALC, Italy).
- The supernatants decanted and decontaminated adding to the pellet, 5% oxalic acid.

Culture , cont.

- The samples were allowed without disturbance for 24-30hrs. at room temperature.
- Finally 0.1ml(100µl) of inoculum taken carefully from the bottom of the tube and inoculated and evenly distributed on the media.
- Each sample was inoculated in 3 tubes (one tube without mycobactin J and 2 with mycobactin J) and incubated at 37°c (Binder GmbH, Germany) with loss screw and inclined to facilitate the evaporation of excess moisture and inoculum fluid for one week.
- After one week tubes returned vertical with tithed screw and incubated for 16 weeks.

Culture , cont.

Culture reading

- Starting from 8 weeks of inoculation,
- cultures were observed for the presence of any growth.
- At 16 weeks smears were taken from cultures that showed a growth, and stained by ZN stain.
- Culture was considered positive when white spot colonies were seen and it was confirmed by ZN stain.
- Slides were observed under 100x objective for the detection of acid fast bacilli.
- Results were recorded considering the long incubation period, the colony appearance, and acid-fastness of the bacteria.

Statistical Analysis

- Correlation, chi squares (x^2) and measure of agreement (Kappa) statistical analysis for histopathological findings and immunohistochemical stain was carried out to see the extent of relation between these laboratory techniques.
- Also sensitivity and specificity values were done for all the methods used during this study, considering histopathological method as a reference.

Results

Results

Farmers interview results

- Table 1 show the health constraints, as they were identified by the small ruminants farmers by their traditional follow up as a result of their interview in September 2002.
- About 75% of the flocks visited have history of emaciation.
- According to the farmers understanding, the cause of emaciation is old age, but some of them said that there is no age difference for the weight loss and they never knew the cause.
- We also found that the farmers are familiar with antihelimenthic drugs, like Ivomec and vaccines of sheep and goats against certain disease, such as Anthrax, Clostridial infection. .
- Bottle jaw is a constraint of all the flocks, except two flocks out of 20 .

Results ,cont.

- Their observations were that very rarely to see improvement after antihelminthic treatment.
- They complained that diarrhea (betherre in Arabic) associated with emaciation and bottle jaw is not treatable.
- Most of the time it is not very common to see diarrhea with emaciation and bottle jaw, rather the last two associates together in many cases.

Results ,cont.

- Once the intermittent diarrhea appears after emaciation, no recovery occur, losses due to such a case is common in all the flocks and never responded for treatment.
- Some elders explained that in the previous years they were observing more cases than now.
- Nowadays because farmers decreased their flock size for breeding due to high price of feed, and they sell the rest to the market for slaughter, not as before in which all females were for breeding.
- As a result the chance to observe repeated cases decreased. In some visits we were able to observe isolated animals which was due to emaciation and weakness, with bottle jaw.

	Flock size ((sheep	Flock size (goats)	Emaciation)Hazil(	Bottle Jaw)Jarjar(	Diarrhea(Bethe (rre	Flock health management		
						Deworming*	** Vaccination	Remark
	10	45	N	N	Y	Y	N	Lambing problem
	60	1	N	N	N	N	N	-
	100	1	N	Y	N	Y	Y	-
	100	10	Y	Y	Y	Y	Y	-
	100	20	Y	Y	Y	Y	Y	-
	100	20	Y	Y	N	Y	Y	-
	200	15	N	Y	N	Y	Y	-
	200	20	N	Y	N	Y	Y	-
	200	20	Y	Y	Y	Y	Y	-
0	200	30	Y	Y	N	Y	Y	-
1	200	30	Y	Y	Y	Y	Y	-
2	200	50	Y	Y	Y	Y	Y	-
3	250	6	Y	Y	N	Y	Y	-
4	300	2	Y	Y	Y	Y	Y	-
5	300	20	Y	Y	N	Y	Y	-
6	400	N	Y	Y	Y	Y	N	-
7	400	40	Y	Y	Y	N	N	-
8	450	50	Y	Y	Y	Y	Y	-
9	600	10	Y	Y	N	Y	Y	-
0	1450	150	Y	Y	Y	Y	N	-

Results ,cont.

Direct smear

- Examination of the direct smear of ZN preparation revealed the presence of acid-fast bacteria.
- The bacteria was observed in both clump and dispersed form Figure 1,2 and 3.
- Out of 320 intestine examined 260-tissue samples were collected, and subjected for ZN examination. From these 55(21.2%) were positive, 145(55.8%) were negative and 60 (23.1%) were suspected (Table 2).
- Out of 55 samples positive 53 (96.4%) of them were from sheep and 2 (3.6%) were from goats. Eighty-five percent goat samples showed the dispersed form of the bacteria and recorded as suspected, where in sheep there were 50% of the samples were suspected Table 3 and 4.

Table 3.Zeihl Neelsen stained direct smear positivity of small intestine and lymph nodes from sheep at different sites of Jordan, 2002.

Sites	No. of samples examined	No. of Samples collected(%)	+ve (%)	-ve (%)	Suspected (%)
Amman	60	49(82)	18 (37)	21(43)	10(20)
Sweleh	112	63(56)	18(29)	27(43)	18(29)
Iribid	107	107(100)	17(16)	8(7)	82(77)
Total	279	219(78)	53(24)	56(26)	110(50)

Table 4.Zeihl Neelsen stained direct smear positivity of small intestine and lymph nodes from goats at different sites of Jordan, 2002.

Sites	No. of samples examined	No. of samples collected	(%) ve+	(%) ve-	Suspected (%)
Amman	-	-	-	-	-
Sweleh	28	28	1(4)	4(14)	23(82)
Irbid	13	13	1(8)	12(92)	-
Total	41	41	2(5)	4(10)	35(85)

Gross and Histopathological Examination

Gross examination

- Gross examination of the intestine of sheep and goats in this study revealed thickening and congestion of the mucosa in the ileum portion.
- There was no apparent corrugation or ulceration in the examined tissue samples.
- The surrounding mesenteric lymph nodes were enlarged and edematous in few cases, and they appeared like cords. Fig. 4 and 5.
- This was almost seen in all the samples examined during our study.

Histopathological examination

Intestine

- Out of 231 intestinal samples 223(97%) showed an increase in the thickening and congestion of the mucosa due to inflammatory cells infiltration The mucosa was severely infiltrated with macrophages (MP) and lymphocytes, which was the most dominant cell reaction in most of the tissues.
- Epitheloid cells forming nests were also observed in some cases, but in many tissue sections scattered form of the epitheloid cells found in the lamina propia of villi and between the crypts.
- However, in many cases cellular infiltration was more obvious in the lamina propia of the villi and less between crypts Figure 6.
- The payer's patch's proliferation was observed in many tissue sections, in which the lymphatic nodule capsule involvement was clear.

Histopathological examination ,cont.

Intestine

- The proliferation ends with pale cells (macrophages) aggregated together and some times with many plasma cells, and macrophages beared in side the smooth muscle immediate to the payer's patche and also in between them forming non- capsulated microgranuloma was observed Figure 7 .
- In most cases the crypts were replaced by inflammatory cells, especially macrophages and some times mixed with lymphocytes. In most of the intestinal sections the villi were highly packed and infiltrated with epitheloid cells and macrophages and they became short and thick as a result of this infiltration figure 8.

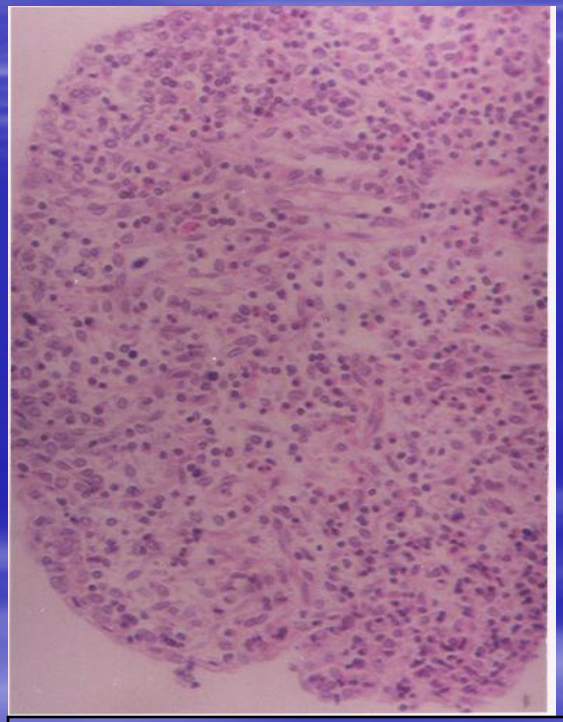


Fig. 6: intestine, Villi, many epiteloid cell infiltration with lymphocytes, lesion type 3+,H&E stain, Mag.205x

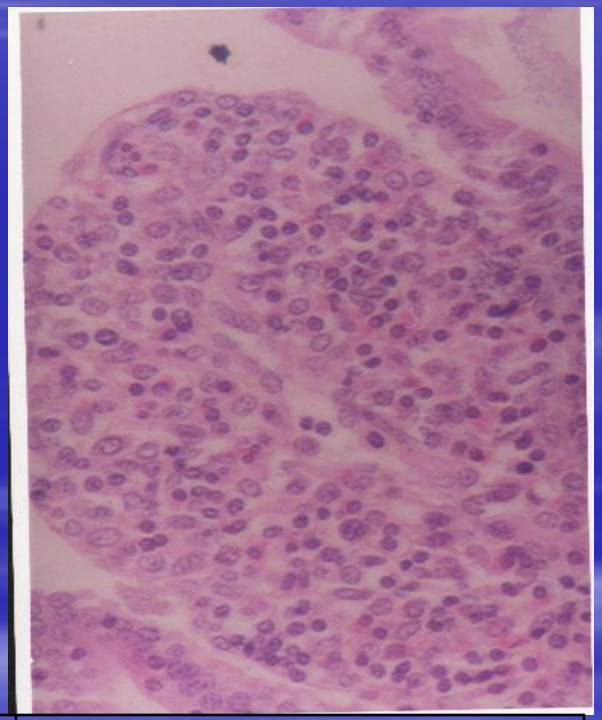


Fig.6.Intestine, villi, high infiltration of macrophages(Epitheloid cells) with some lymphocytes, lesion type 3+, H&E stain, Mag. 410x

Histopathological examination ,cont.

- Depending on the above pathological findings found in the intestine and lymph node,
- Table 6. shows that out of the 223 samples, 108(48%) were graded as III.
- This grading was based on many epitheloid cells infiltration forming nests as well as scattered in the lamina propia of the villi and between the crypts Figure 9.
- The crypts were replaced by macrophages and some lymphocytes and also by payer's patch proliferation Figure 10.
- The payer's patch proliferation was observed towards the mucosa with the lymphoide follicle capsule involvement, and the proliferation was mainly contained pale cells (macrophages) and plasma cells forming like microgranuloma figure 11 .

Table 6. Histopathological findings, from intestine and lymph node samples in sheep and goats at different sites of Jordan, 2002

Site	Sample collected	Sample processed) (%	Grade of the lesions					
			(%)I	(%)II	(%)III	(%)SP	Total positive (%)	-(%)
Amman	49	(92))45	(16)7	(40)18	(36)16	(2)1	(93))42	(7)3
Sweleh	91	(88)80	(16)13	(40)32	(37)30	(2)2	(96)77	(4)3
Irbid	120	(88)106	(5)5	(34)36	(58)62	(1)1	(98)104	(2)2
Total	260	(89)231	(11)25	(37)86	(47)108	(2)4	(97)223	(3)8

The percentage was taken by considering the decimal number >5, adding one in all the results SP= special lesion

Table 7. Histopathological findings from intestine and lymph node samples in sheep aged 8-24 months, at different sites of Jordan, 2002.

Sites	Sample collected	Samples processe (%)d	Grade of the lesions					
			(%)I	(%)II	(%)III	(%)SP	Total positive (%)	-(%)
Amman	60	(75)45	(16)7	(40)18	(36)16	(2)1	(93)42	(7)3
Sweleh	112	(56)63	(17)11	(36)23	(40)25	(3)2	(97)61	(3)2
Irbid	107	(88)94	(5)5	(31)29	(61)57	(1)1	(98)92	(2)2
Total	279	(72)202	(11)23	(35)70	(48)98	(2)4	(97)195	(3)7

The percentage was taken by considering the decimal number >5, adding one in all the results

Histopathological examination ,cont.

- In the other hand 86 of the positive samples (37%) were graded as II.
- This was represented by the same pathological pattern of grade III, except that the severity of the lesion was moderate with less epitheloid cells , but there were more lymphocytes infiltration Figure 12.
- Twenty five of the positive samples (11%) were scored as I , showing less cellular infiltration which was consisted more of lymphocytes, few epitheloid cells and some macrophages Figure 13.
-

Histopathological examination ,cont.

- A special grading (SP) of samples in sheep 4(2%) was done, in which the samples showed a sever reaction characterized by calcification of the mesenteric lymph nodes showing typical granulomatous lesion Figure 14, surrounded by macrophages and Langhans giant cells Figure 15 ,. and encapsulated by fibrotic tissue Figure 16. SP grade type lesion was not detected from goat's sample.

Histopathological examination ,cont.

- Table 7. shows the number of samples that were graded, as III were high 98 (48%) in sheep.
- Where as, samples from goats graded as III lesion were 10(34%) , and 16(55%) were graded as II Table 8., which exceeded the number of sheep samples for this grade 70(35%)

Lymph Node

- In the lymph node some mild lesions were observed such as : subcapsular cellular infiltrates, consisting mainly macrophages and some epitheloid cell Figure 17.
- In few cases calcification in the cortical and some times in the paracortical area, was evident, some of them were encapsulated with fibrotic tissue and the area infiltrated with macrophages and epitheloid cells.
- In few more cases calcification or casious necrosis surrounded by langhans giant cell with macrophages and epitheloid cells encapsulated by fibrotic tissue.

Lymph Node

- Although it was obvious in some cases Some tissue sections showed fibrotic tissue infiltration which was clearly manifested by many fibrocytes infiltrating limited areas in the cortical part of the lymph nodes Figure 18, some times accompanied by calcification Figure19. The presence of neutrophils was also observed mixed with macrophages Figure 20 .

Table 6. Histopathological findings, from intestine and lymph node samples in sheep and goats at different sites of Jordan, 2002

Site	Sample collected	Sample processed) (%	Grade of the lesions					
			(%)I	(%)II	(%)III	(%)SP	Total positive (%)	-(%)
Amman	49	(92))45	(16)7	(40)18	(36)16	(2)1	(93))42	(7)3
Sweleh	91	(88)80	(16)13	(40)32	(37)30	(2)2	(96)77	(4)3
Irbid	120	(88)106	(5)5	(34)36	(58)62	(1)1	(98)104	(2)2
Total	260	(89)231	(11)25	(37)86	(47)108	(2)4	(97)223	(3)8

The percentage was taken by considering the decimal number >5, adding one in all the results SP= special lesion

Table 7. Histopathological findings from intestine and lymph node samples in sheep aged 8-24 months, at different sites of Jordan, 2002.

Sites	Sample collected	Samples processe (%)d	Grade of the lesions					
			(%)I	(%)II	(%)III	(%)SP	Total positive (%)	-(%)
Amman	60	(75)45	(16)7	(40)18	(36)16	(2)1	(93)42	(7)3
Sweleh	112	(56)63	(17)11	(36)23	(40)25	(3)2	(97)61	(3)2
Irbid	107	(88)94	(5)5	(31)29	(61)57	(1)1	(98)92	(2)2
Total	279	(72)202	(11)23	(35)70	(48)98	(2)4	(97)195	(3)7

The percentage was taken by considering the decimal number >5, adding one in all the results

Table 8. Histopathological findings, from intestine and lymph node samples in goats, aged 6-12 months, at different sites of Jordan, 2002.

Sites	Samples collected	Samples processed (%)	Grade of the lesions					
			I(%)	II(%)	III(%)	SP(%)	Total positive (%)	- (%)
Amman	-	-	-	-	-		-	-
Sweleh	28	(61)17	(12)2	(53)9	(29)5	-	(94)16	(6)1
Irbid	13	(92)12	-	(58)7	(42)5	-	(100)12	-
Total	41	(71)29	(7)2	(55)16	(34)10	-	(97)28	(3)1

The percentage was taken by considering the decimal number >5, adding one in all the results

The data collected from histopathological examination is presented below by a figure. As it is shown high number of samples were grade III followed by grade II Figure 21 .

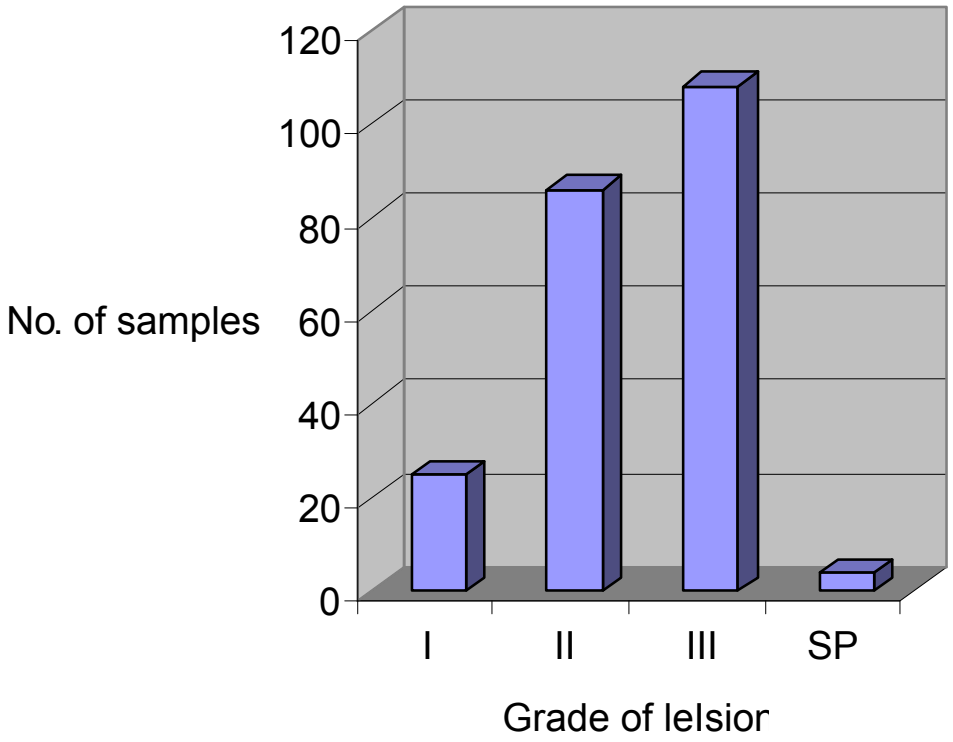


Figure 21. Distribution of the Histopathoilogical lesion grading from sheep and goats intestine and lymph nodes tissue sectior according to their grading,2002

SP= Special Grading

Other Pathological findings

- From all the inspected intestinal samples only six goats were highly infested by Cestod (Tanea), and a large amount of worm was encountered in their small intestine.
- Although in some cases, the histopathological result revealed the presence of eosinophils in the lamina propia, mostly accompanied with the presence of coccidia in which out of 231 samples 11(5%) of them showed the coccidia in the epithelial cells of the villi.
- In addition neutrophils infiltration was seen in the lamina propia and at the same time in the lumen of the crypts Figure 22. Further more neutrophils were seen in the cortex of the lymph nodes. .

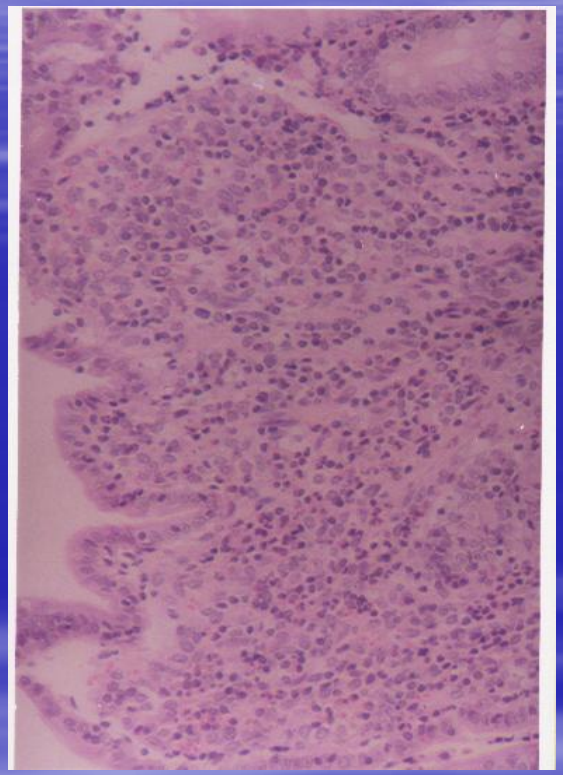


Fig.8: Intestine , short villi, due to macrophages and lymphocytes infiltration, lesion type 3+, H&E stain, Mag. 205x

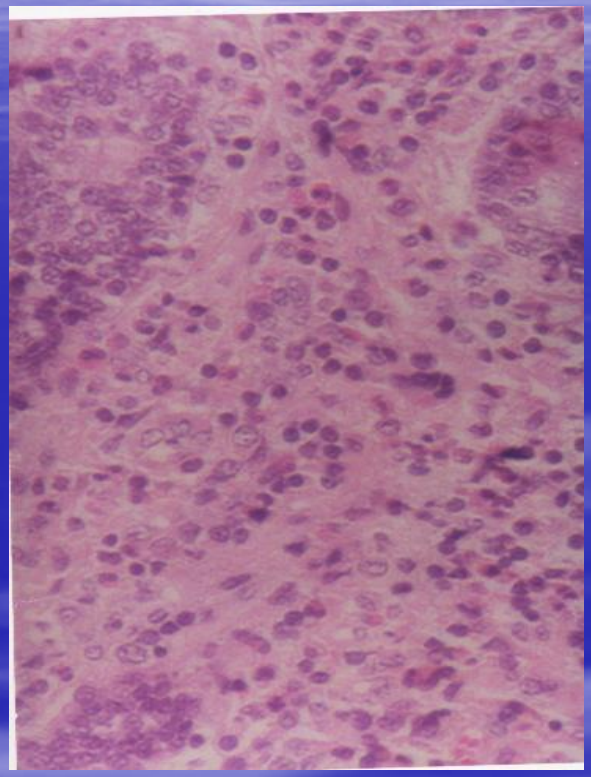


Fig. 10 Intestine , lamina propia, lymphocytes and macrophages infiltration, and replaced cryptes remnant, H&E stain, Mag. 410x

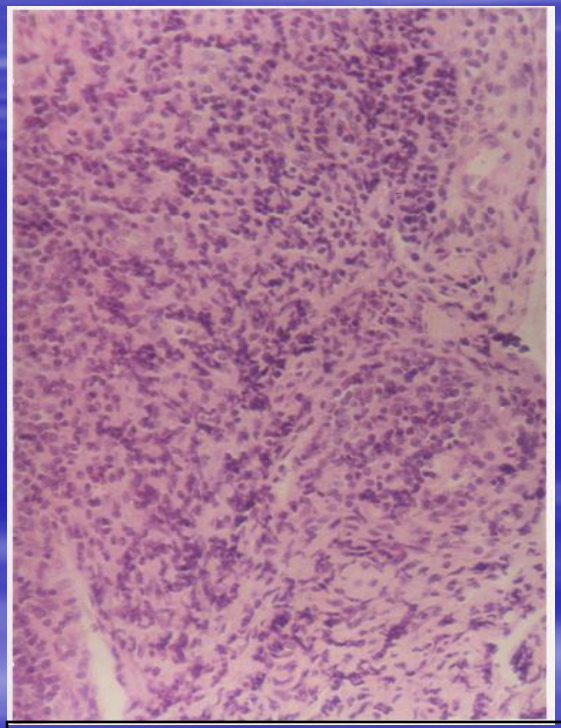


Fig. 10a. Intestine, lamina propia, many fibrocytes together with the inflammatory cells, H&E stain, Mag. 205x

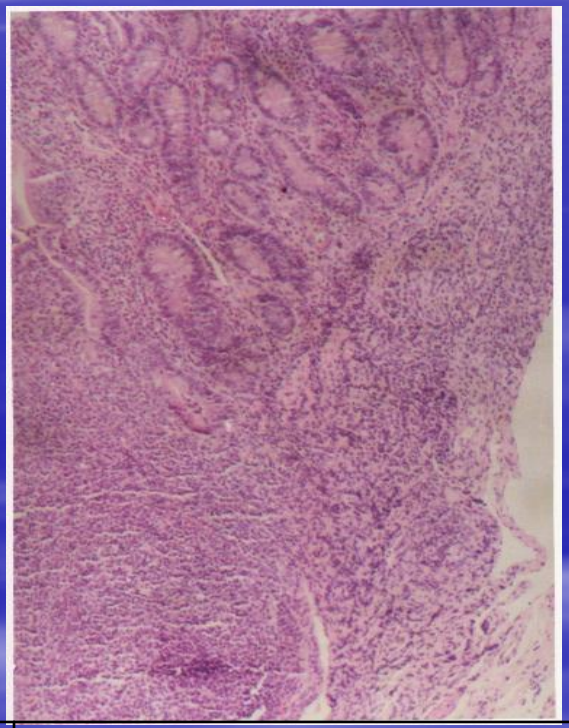


Fig. 10a :Intestine, lamina propia, crypts replacement by payer's patches, and fibrotic tissue infiltration H&E stain, Mag. 82x

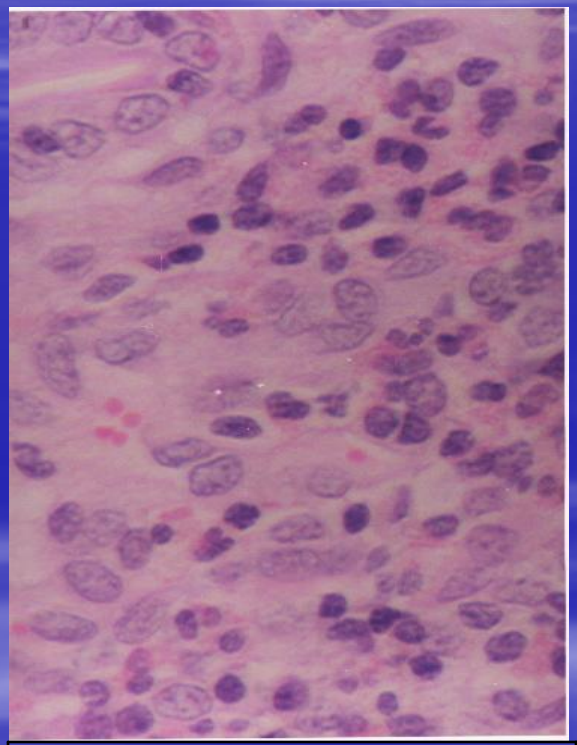


Fig. 9: Intestine, lamina propia, Epitheloid cells forming nests and some lymphocytes, H&E stain, Mag. 820x

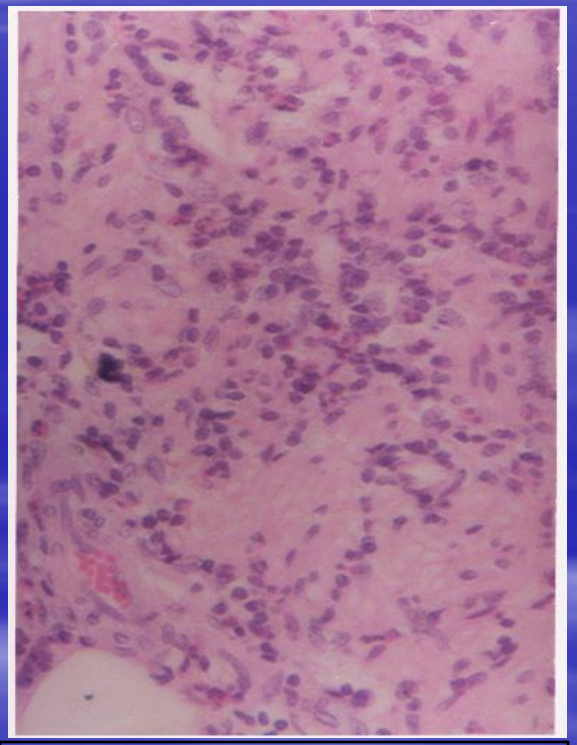


Fig. 50. Intestine lamina propia , infiltration of Eosinphils , lymphocytes and some macrophages, H&E stain, Mag. 205x

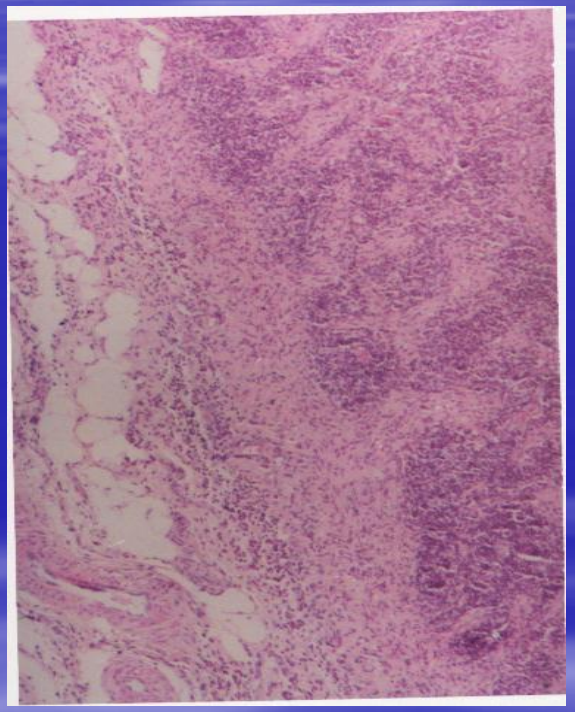


Fig. 17: mesenteric lymphnode, subcapsular inflammatory cell infiltration, H&E stain, Mag.82x

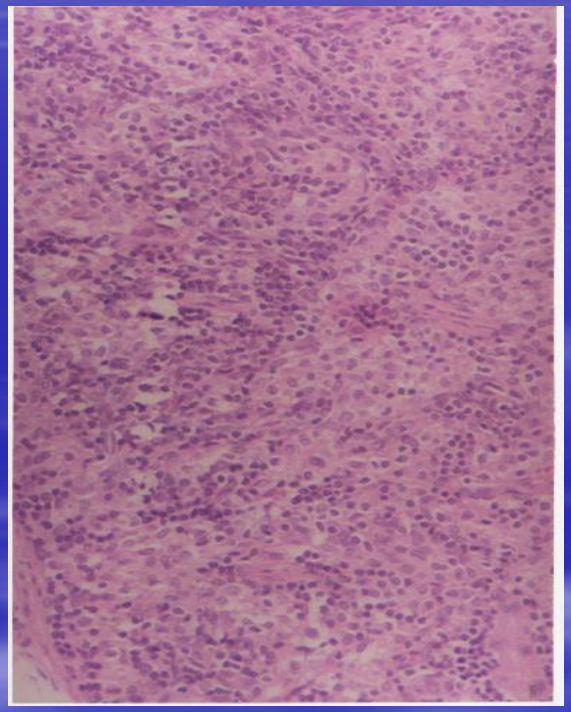


Fig. 17b. Mesenteric Lymph node, cortex, pale cell infiltrates in different areas, H&E stain, Mag. 205x

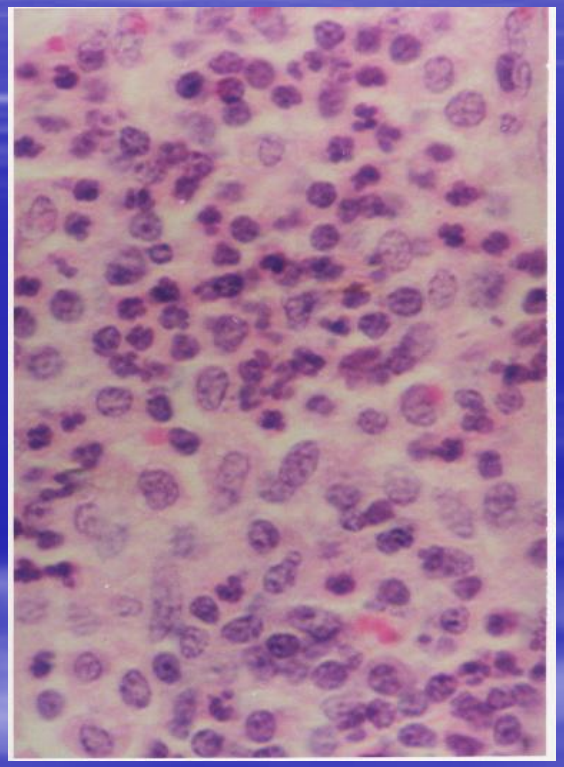


Fig.17a: Mesenteric lymph node , subcapsular area infiltrated by macrophages , neutrophils and some lymphocytes, H&E stain, Mag. 820x

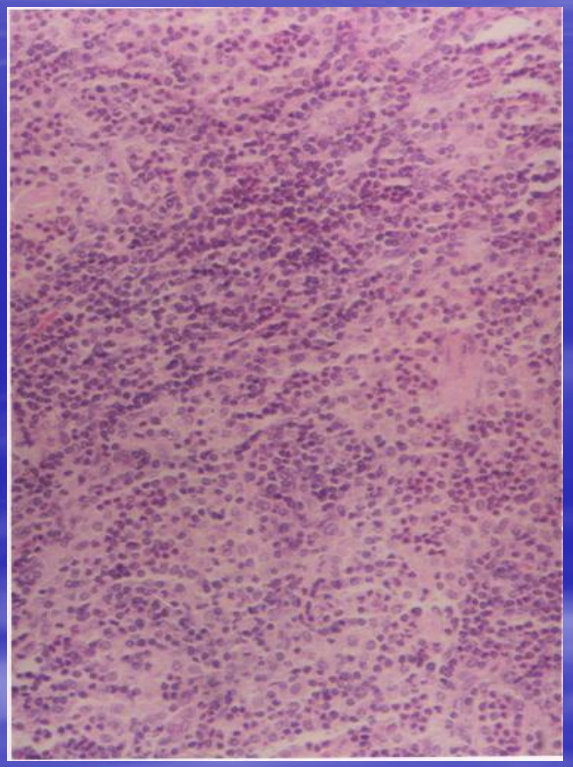


Fig. 17c. Lymph node , some pale areas composed of macrophages, H&E satin , Mag. 205x

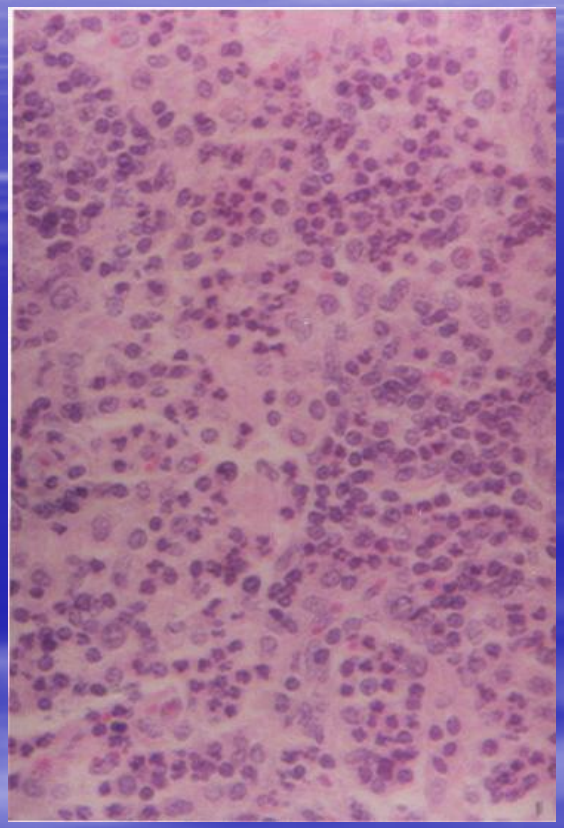


Fig.20:Mesenteric lymph node, cortex, infiltration of macrophages with neutrophils, H&E stain, Mag.410x

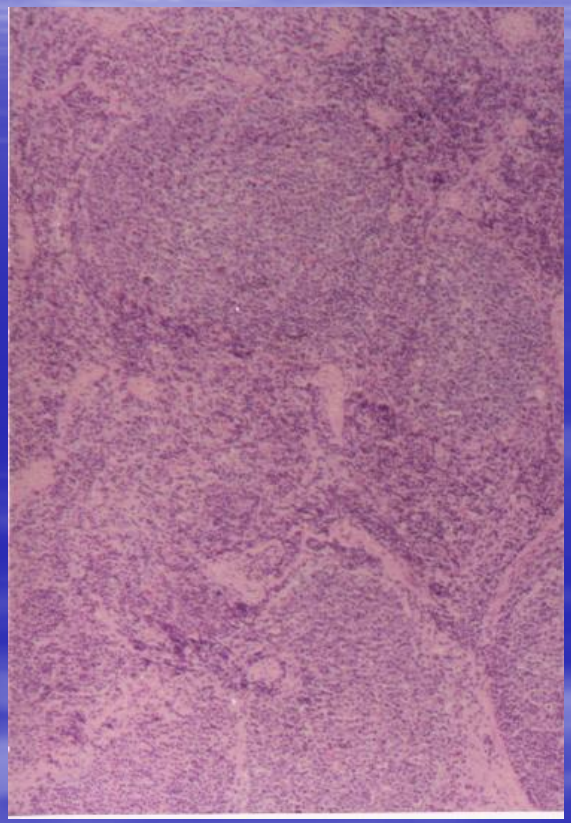


Fig. 18: Lymph node cortex, fibrotic tissue infiltration, necrosis pocess and spots of basophilic material H&E stain, Mag.82x

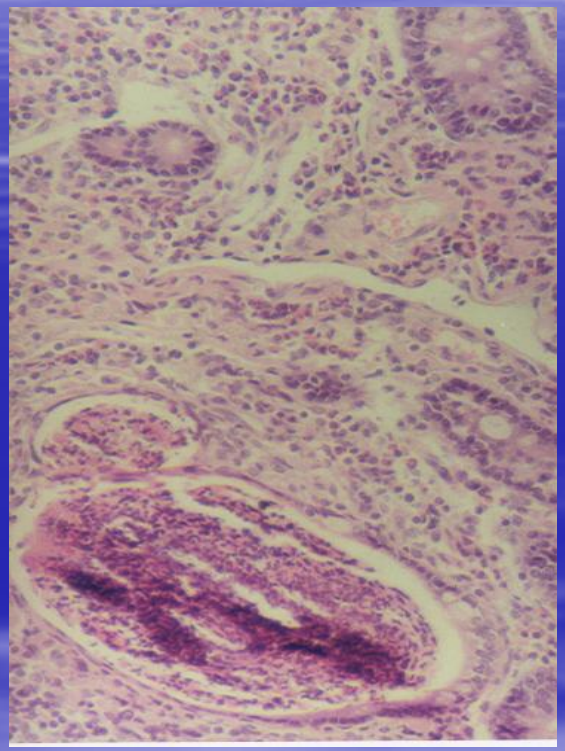


Fig.22. Intestine, lamina propia, crypts packed with polymorphonuclear cells, H&E stain, Mag. 205x

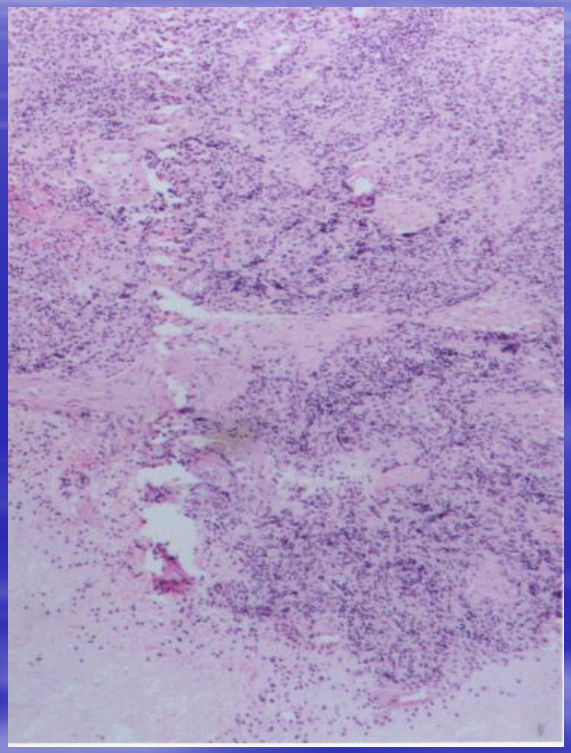


Fig. 18a: Lymph node cortex,on the way of necrosis, and starting of basophilic material accumulation H&E stain, Mag.82x

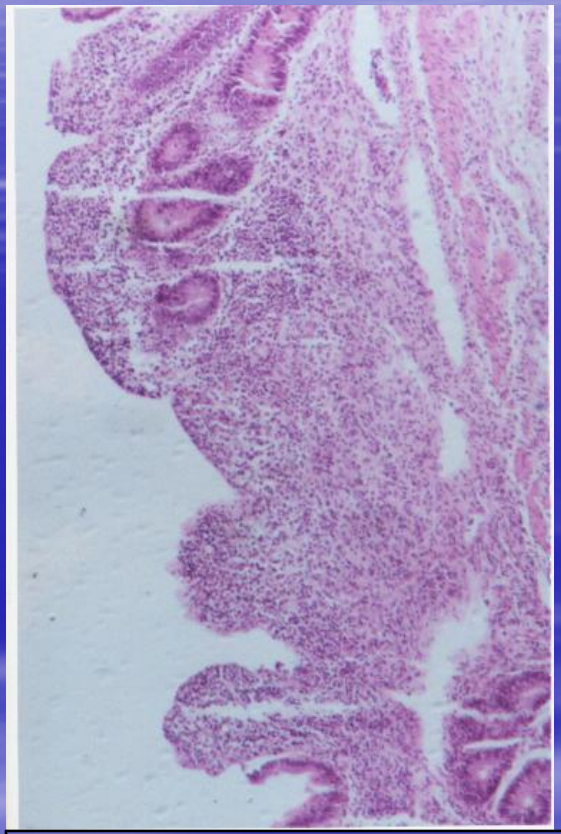


Fig.10a : Intestine, short villi and replacement of the crypts by mononueclear cells, H&E stain, Mag. 82x

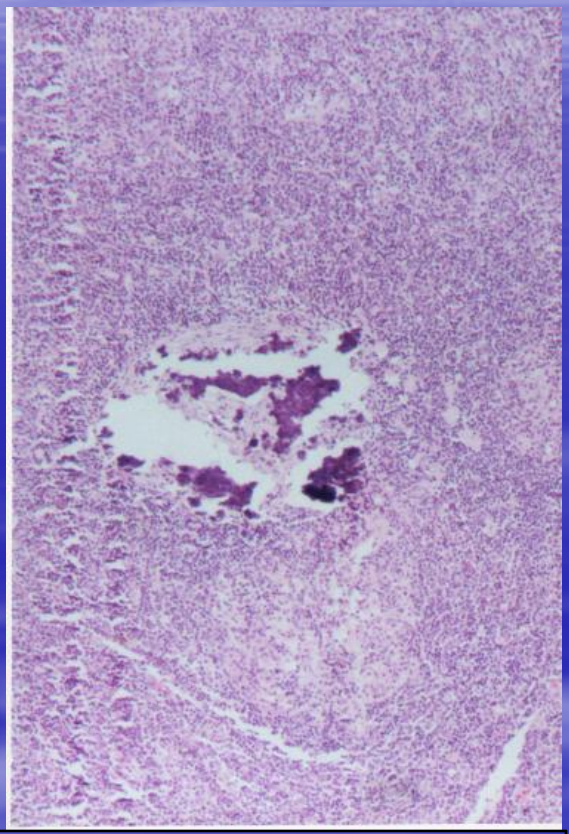


Fig 19: Mesenteric, lymph node, cortex, basophilic material (calcification) , H&E stain, Mag. 82x

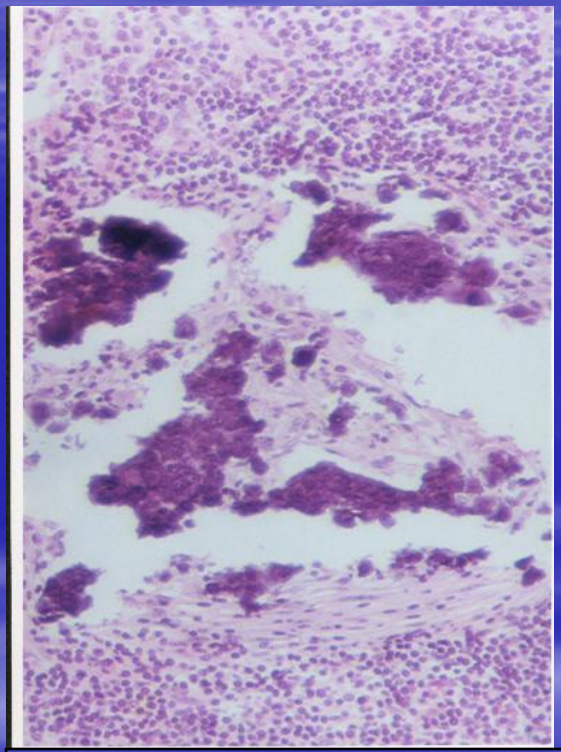


Fig. 19 : Lymph node, cortex, basophilic material (calcification) , H&E stain, Mag. 205x

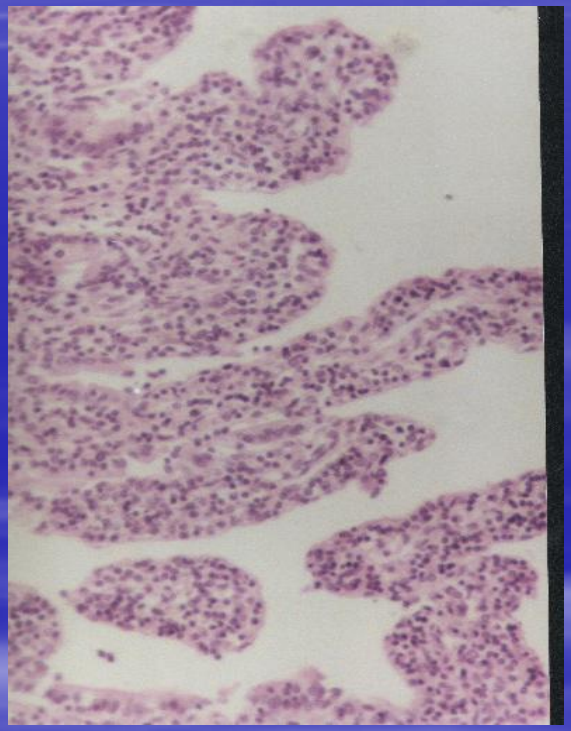


Fig.12a: Intestine ,villus, infitrated mainly by lymphocytes, grade 1,+ lesion H&E stain, Mag.205x

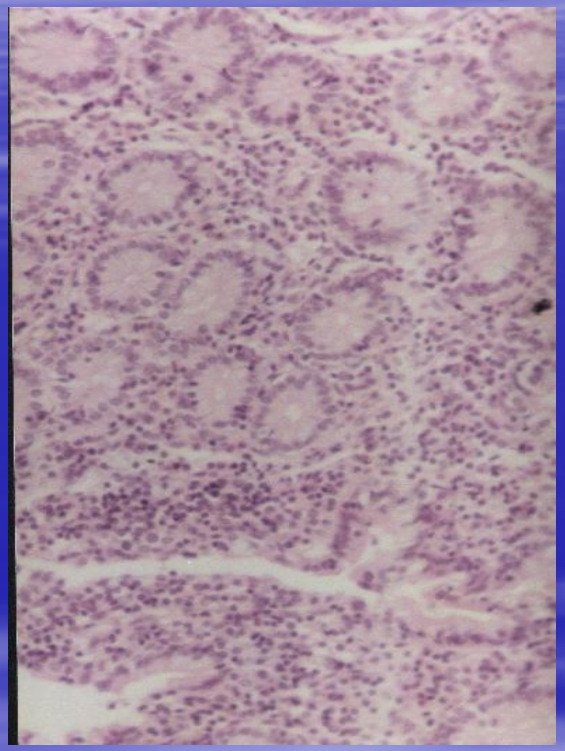


Fig. 12: Intestine, lamina propia, many lymphocytes infiltration, grade 1+ lesion, H&E stain, Mag. 205x

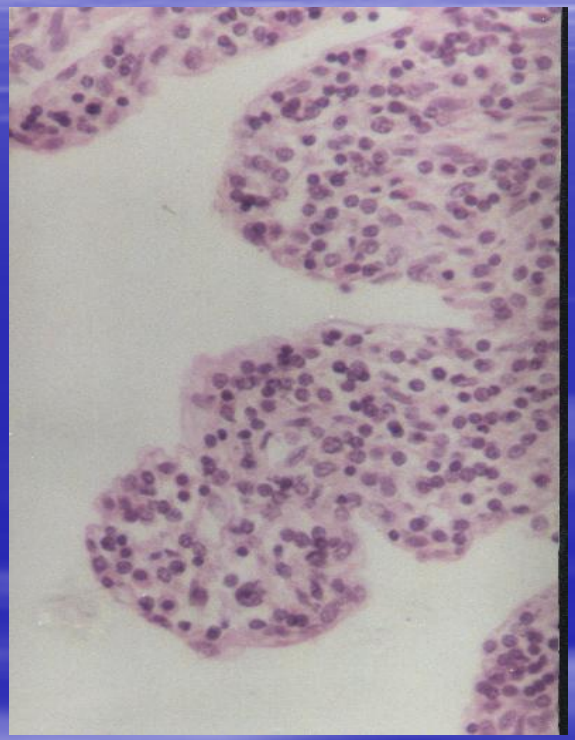


Fig.: Intestine, villi , infiltrated by lymphocytes, macrophages (some are epiteloid), lesion type 1+, H&E stain, Mag. 205x

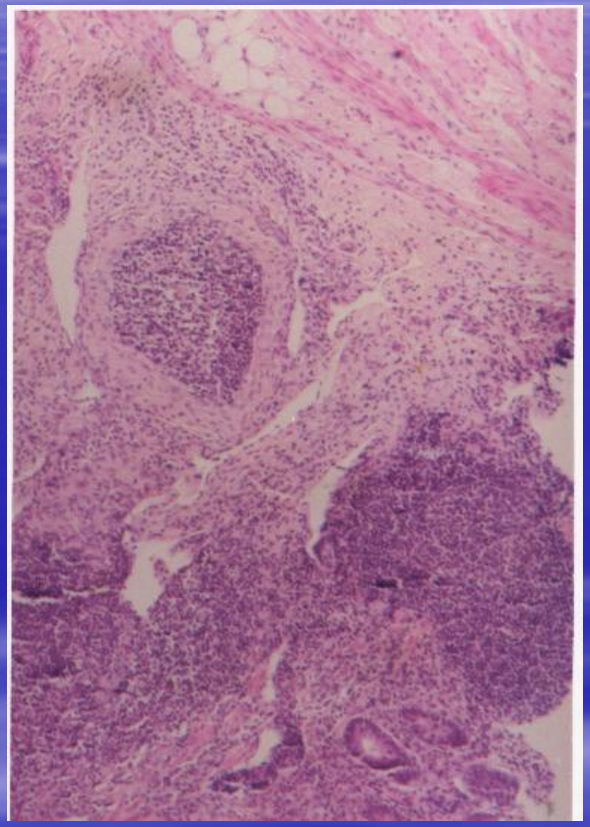


Fig. 11: Intestine, between the payer's patches, aggregation of macrophages and some lymphocytes in the smooth muscle, Mag. 205x

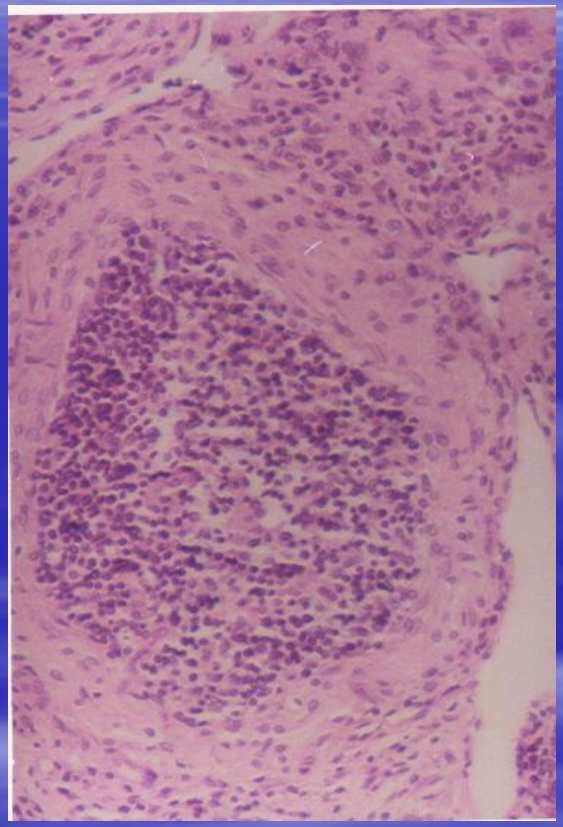


Fig. 11: Intestine, between the payer's patches, aggregation of macrophages and some lymphocytes in the smooth muscle, Mag. 410x

Tissue section stained by ZN

- Tissue sections stained by ZN were observed for the presence of acid-fast bacteria.
- Out of 202 samples examined, 22 (11%) were positive and 180 (89%) were negative for acid fast bacteria Table 9.
- In the positive samples bacteria were seen inside the cytoplasm of macrophages and, in some of them, bacteria were seen outside the macrophages or extracellulaly Figure 23.

Table 9. ZN stain tissue result in sheep and goats, 2002

Sites	No. of samples collected	No. of samples processed (%)	Positive (%)	Negative (%)
Amman	49	(%92)45	(%13)6	(%87)39
Sweleh	91	(%%69)63	(%11)7	(%89)56
Irbid	120	(%78)94	(%10)9	(%90)85
Total	260	(%78)202	(%11)22	(%89)180

Immunohistochemical staining and examination

- **Immunohistochemical stain grading**
- Examination of immunohistochemical stained tissue sections revealed, that out of 150 samples processed, 138(92%) showed positive reaction.
- This was manifested by the presence of brown color around the macrophage nucleus (intra-cytoplasmic reaction) Figure 24.
- Only 12 (8%) of the total samples were negative.
- One hundred nineteen (78%) of the samples showed mild reaction which was graded as 1+ as it was shown by the stain of 1-10 cells Figure 25.
- Eleven (7%) reacted moderately, and were graded as 2+ Figure 26.
- This was shown by the density of the staining of more than 10 cells, while 6(4%) of the samples reacted strongly as it was shown by the stain of 50% of the macrophages in the section and were graded as 3+ Figure 27., and Table 10

Table 10. Immunohistochemical stain results from intestine and lymph node samples in sheep and goats, 2002

Sites	No. sample collected	No.of samples processed (%)	Immunohistochemical stain					
			+ (%)	++ (%)	+++ (%)	+++ + (%)	Total positive (%)	- (%)
Amman	49	(86)42	(95)40	-	-	-	(95)40	(5)2
Sweleh	91	(71)65	(69)45	(16)11	(9)6	(2)1	(97)63	(3)2
Irbid	120	(35)43	(88)35	-	-	-	(81)35	(19)8
Total	260	(58)150	(80)120	(7)11	(4)6	7.)1 (	(92)138	(8)12

10-1= +cells showed cytoplasmic reaction, ++ = >10, +++ = 50% of the MP ,++++= >50% of the MP

Table 11. Immunohistochemical stain results from intestinal and lymph node samples sheep, 6-24 months, 2002

Sites	No. of samples collected	No. samples processed (%)	Immunohistochemical stain					
			+ (%)	++ (%)	+++ (%)	++++ (%)	Total positive (%)	- (%)
Amman	49	86)42 (	95)40 (	-	-	-	95)40 (	(5)2
Sweleh	63	89)56 (	70)39 (	)9 (16	)6 (10	2)1 (	98)55 (	(2)1
Irbid	107	))36 (34	81)29 (	-	-	-	16)29 (	)7 (19
Total	219	)134 (61	)108 (81	(7)9	(4)6	1)1 (	)124 (93	)10 (7

Table12.Immunohistochemical stain result from intestine and lymph node samples in goat, aged 8-12 months, 2002

No. of samples collected	Sites	No. of samples processed (%)	Immunohistochemical stain					
			+ (%)	++ (%)	+++) % (	++++ (%)	Total Positive (%)	- (%)
-	Amman	-	-	-	-	-	-	-
28	Sweleh	(31)9	(67)6	)2 (22	-	-	(89)8	(11)1
13	Irbid	(46)6	(83)5	-	-	-	(83)5	(17)1
41		37)15 (	)11 (73	)2 (13	-	-	(87)13	(13)2

Immunohistochemical stain results is presented by Figure and it is shown that high number of samples 120(80%) were graded as 1+, followed by 2+ grade, which was seen in 11(7%) of the samples Figure 28

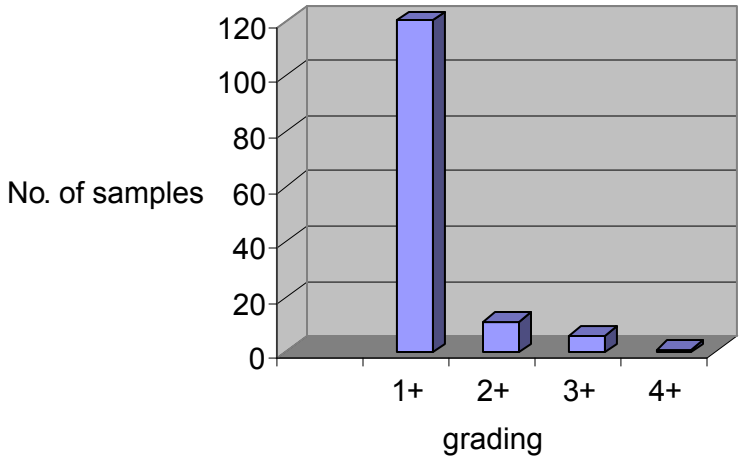


Figure 28. Distribution of the immunohistochemical staining from sheep and goats intestinal and lymph node tissue section according to the intensity of the stain1+ being the lowest and 4+ being the higest

Localization of immunohistochemical staining

- The immunohitochemical stained tissue sections were studied to find the location of the stained macrophages in different parts of the tissue.
- In the intestinal tissue section, reaction was observed more in the macrophages infiltrated in the lamina propia between the crypts, which was manifested by intense brown color Figure 29.
- The payer's patches showed less stain than the lamina propia Figure 30.,
- while rarely reactions were seen in the lamina propia of the villi Figure 31. Reactions in the lymph nodes were always accompanied with the intestinal reaction,

Localization of immunohistochemical staining

- whenever there is reaction in the lymph nodes also reaction was present in the intestine.
- The reaction in the lymph node commonly observed in the paracortex area dispersed between the cortex and medulla Figure 32.
- Some times stained macrophages in the cortex (lymphoid follicle) areas were also observed Figure 33.
- In few cases the medullar area of the lymph nodes showed reaction., the same result was observed in the sub-capsular area Figure 34.

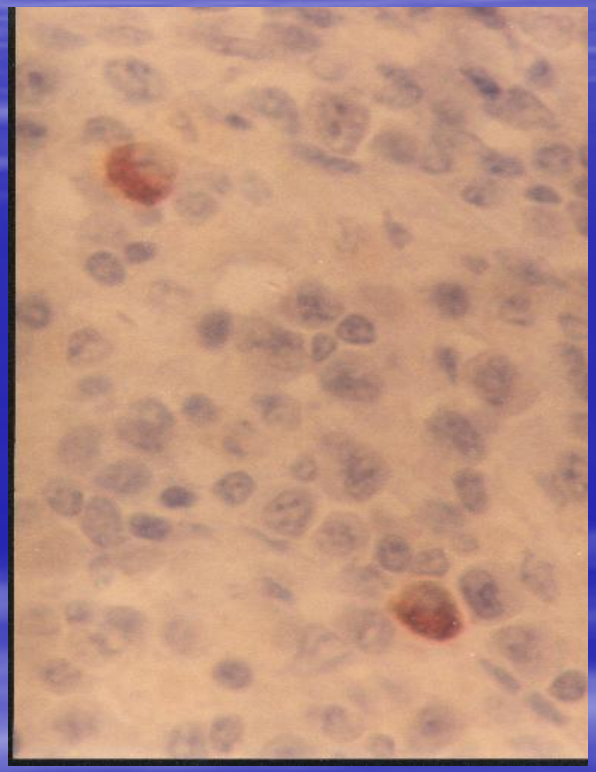


Fig. 24: Intestine , lamina propia, macrophage, intra-cytoplasmic reaction, immunohistochemical stain, Mag. 820x

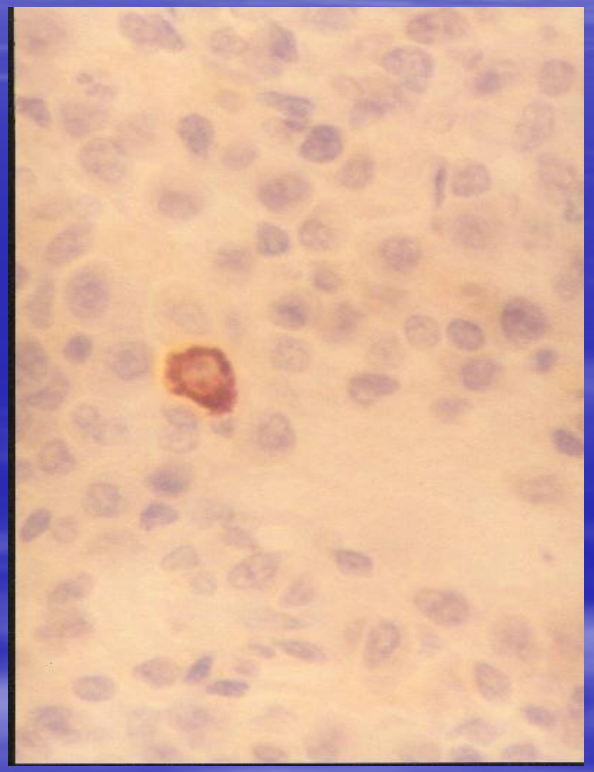


Fig. 24: Intestine , lamina propia, macrophage, intra-cytoplasmic reaction, immunohistochemical stain, Mag. 820x

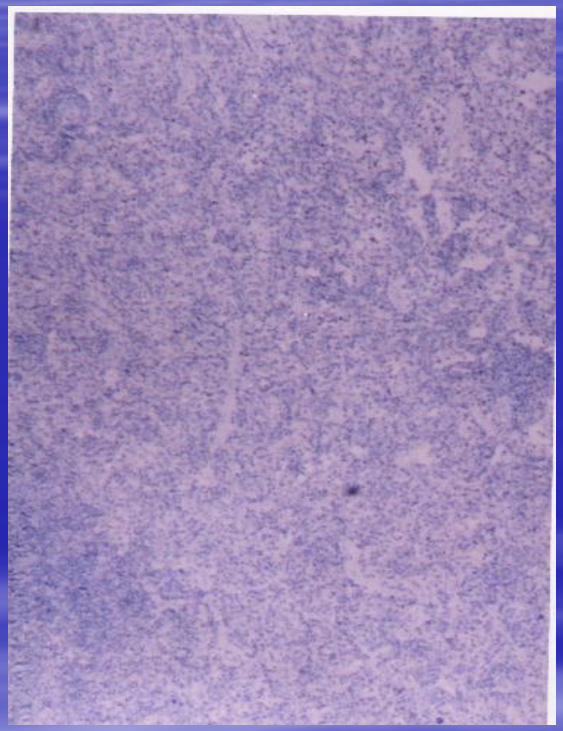


Fig 21 Control

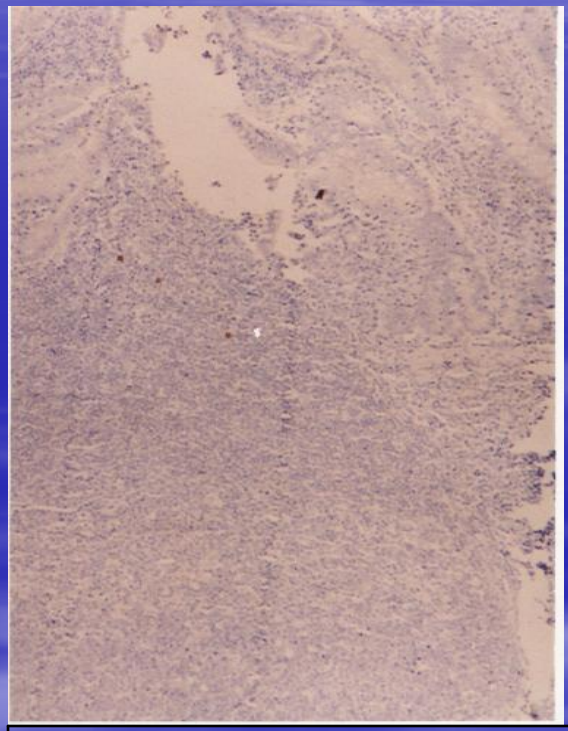


Fig. 28: Intestine, payer's patches, intra-cytoplasmic reaction, immunohistochemical stain, Mag. 82x

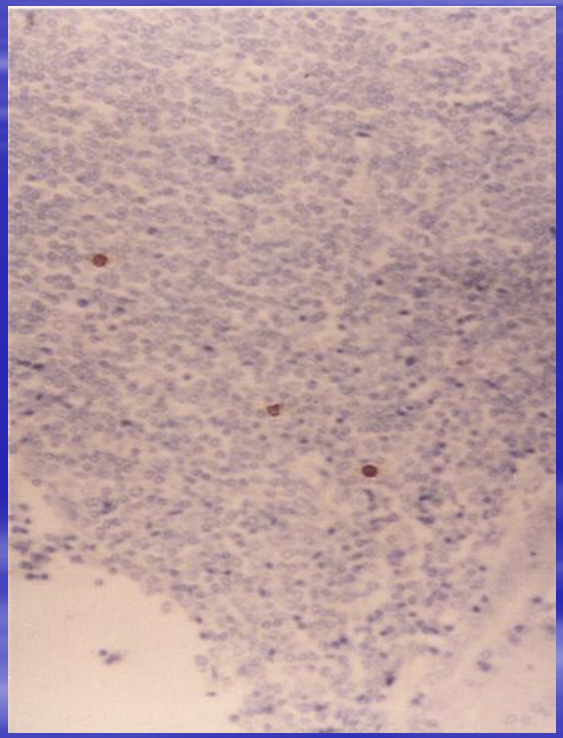


Fig. 28a: Intestine, payer's pacthes, cellular reaction, immunohistochemical stain, Mag.205x

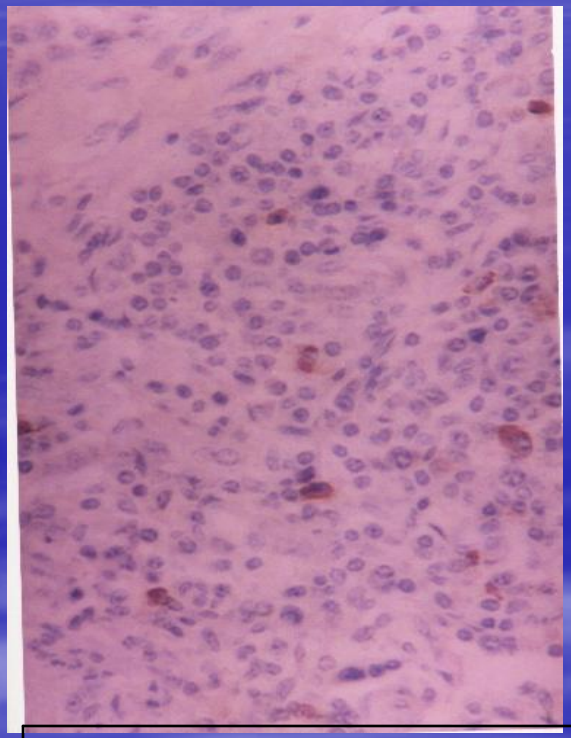


Fig. 31: Mesenteric lymph node, paracortex, cytoplasmic reaction, immunohistochemical stain,Mag. 205x

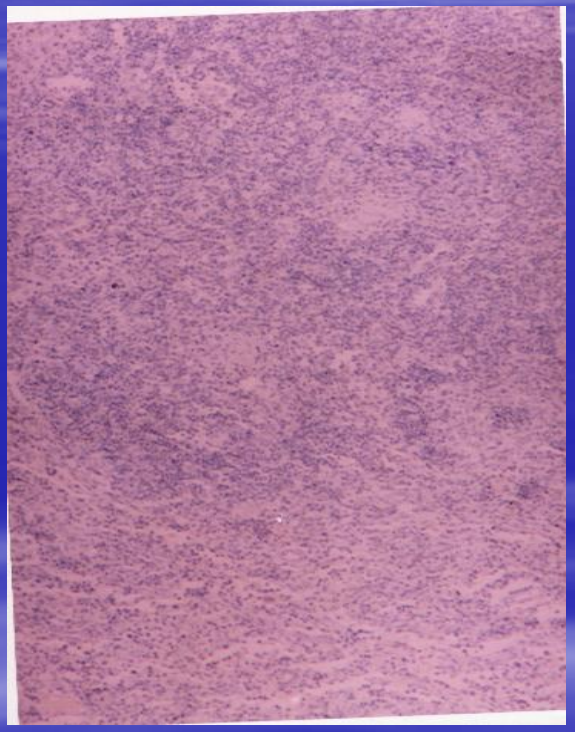


Fig. 25 control

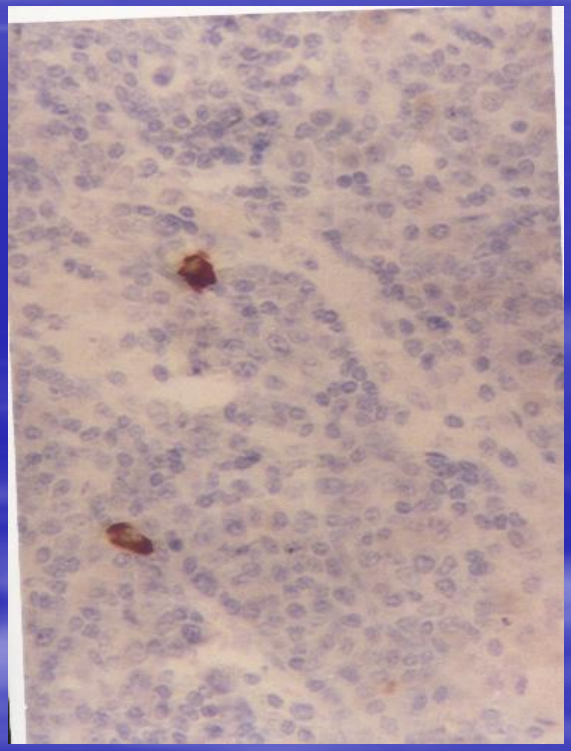


Fig. 23: Mesenteric lymph nodes interacytoplasmic reaction in the macrophages, immunohistochemical stain, Mag.410x

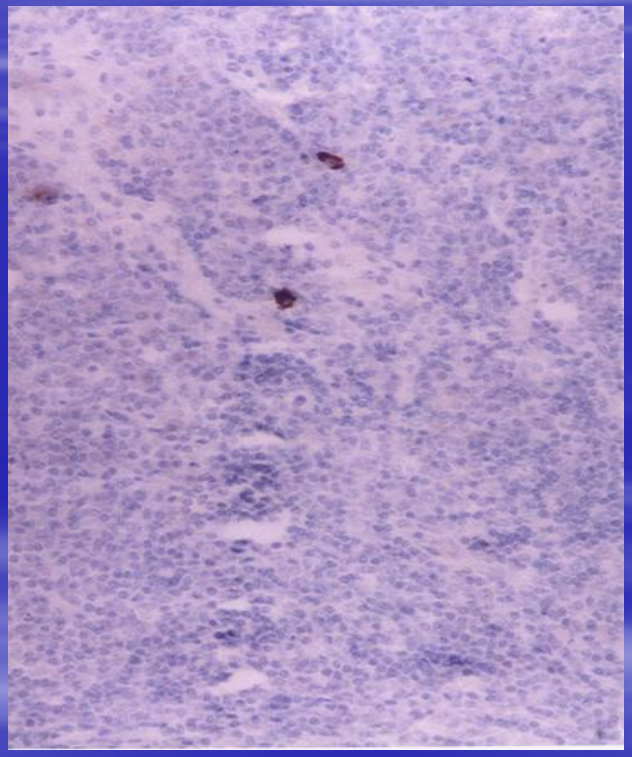


Fig. 31b: Mesenteric lymph node , paracortex, cytoplasmic reaction , immunohistochemical stain, Mag.205x

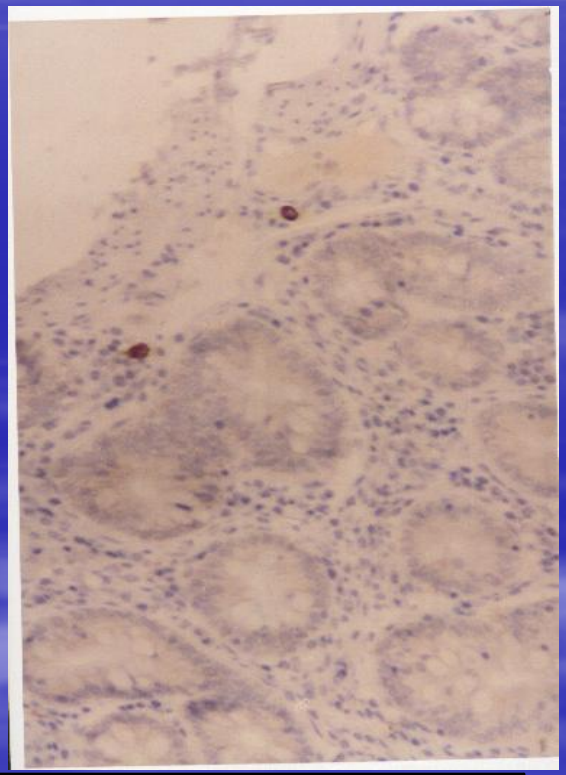


Fig. 29: Intestine, lamina propia, cytoplasmic reaction, immunohistochemical stain, Mag.205x

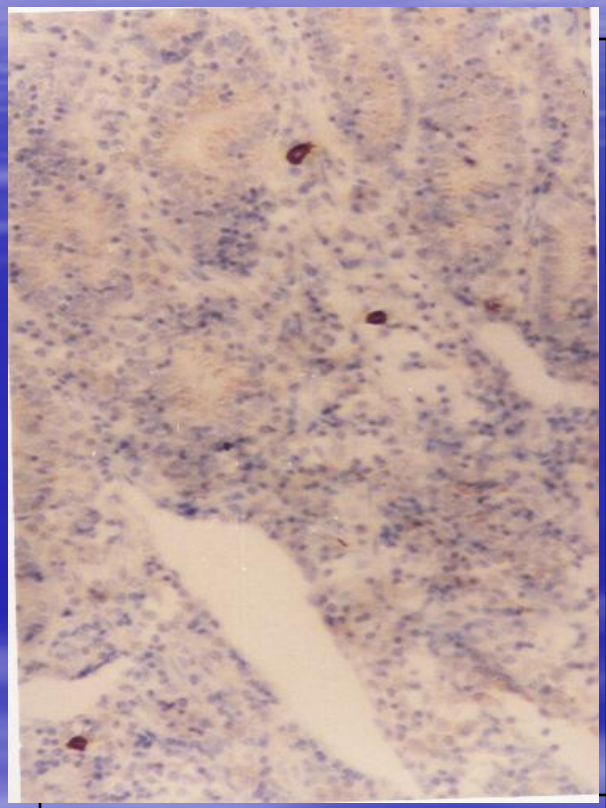


Fig. 29 Intestine , lamina propia, intera-cytoplasmic reaction in the macrophage ,immunohistochemical stain Mag.205x

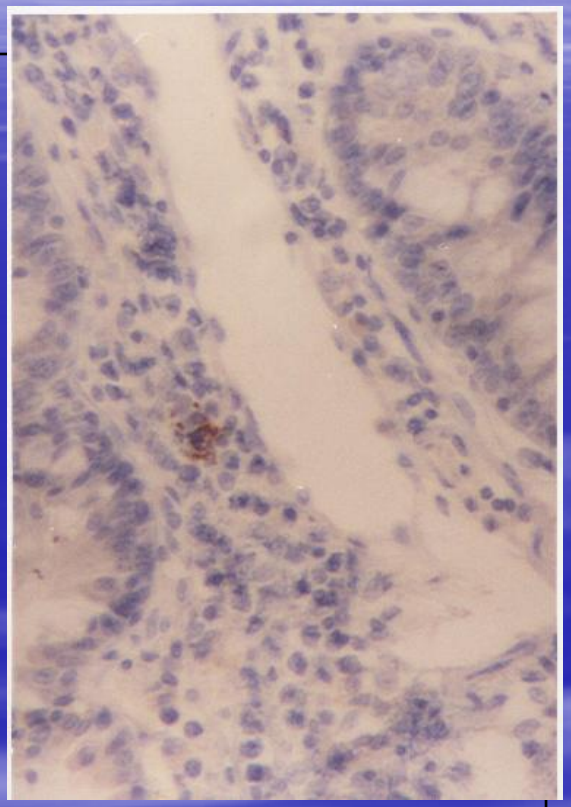


Fig.30 Intestine, villi, intra-cytoplasmic raction in a macrophage immunohistochemical stain, Mag.205x

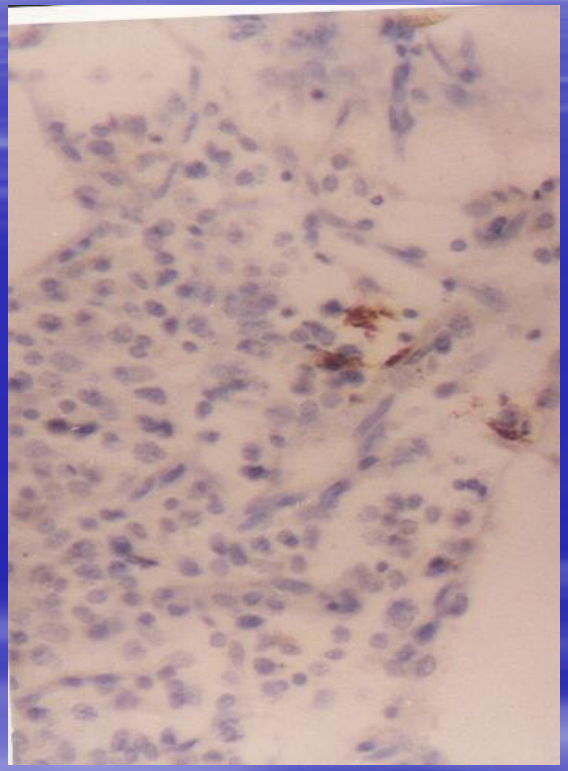


Fig.33:Mesenteric lymph node, sub-capsular area, intra-and extra-cellular reaction immunohistochemical stain, Mag. 205x

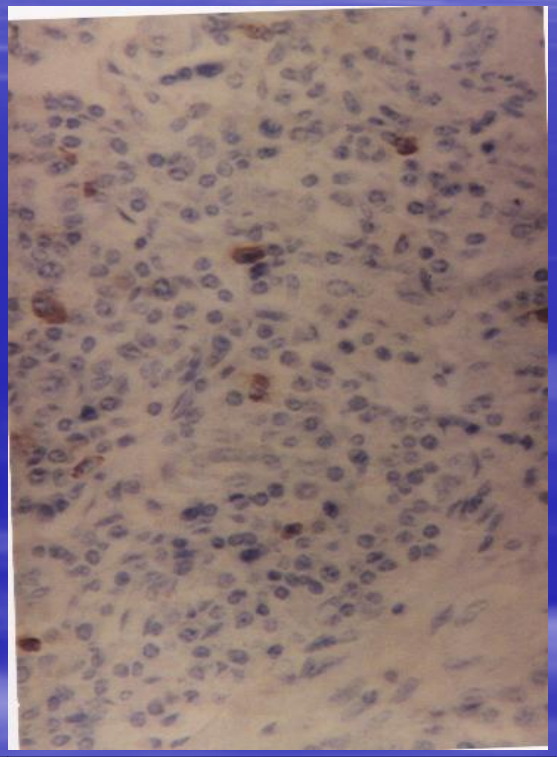


Fig.32 Mesenteric lymph node, cortex, many intra-cytoplasmic reaction, immunohistochemical stain, Mag. 205x

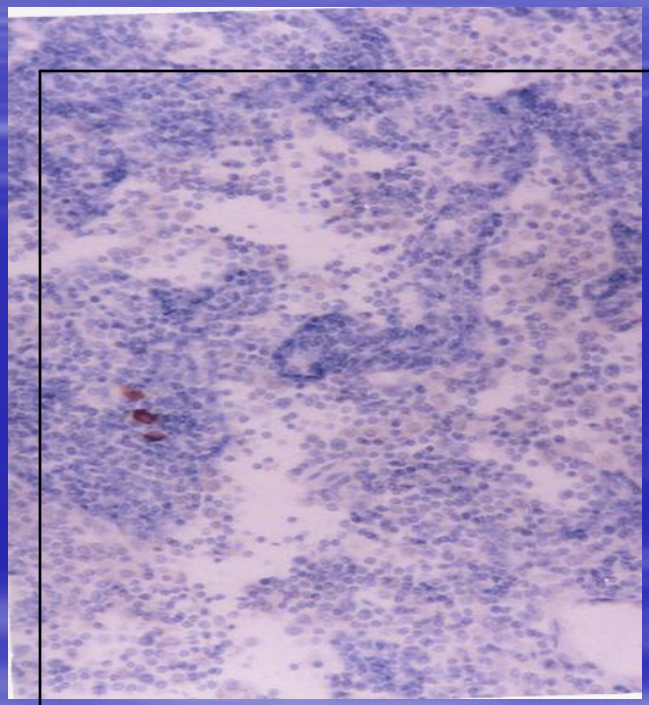


Fig.32a: Mesenteric lymph node, cortex, lymphoid follicle, intracytoplasmic reaction, immunohistochemical stain, Mag. 205x

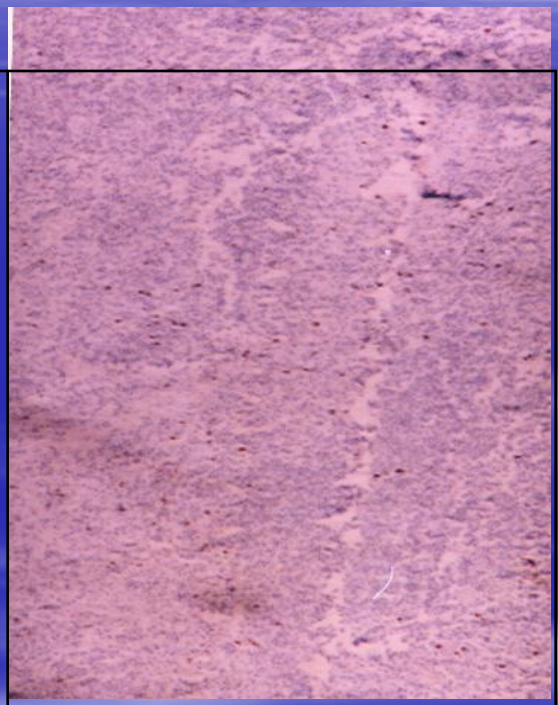


Fig. 34 : Mesenteric lymph node parenchyma many cell reaction, grade 4+ immunohistochemical stain, Mag. 82x

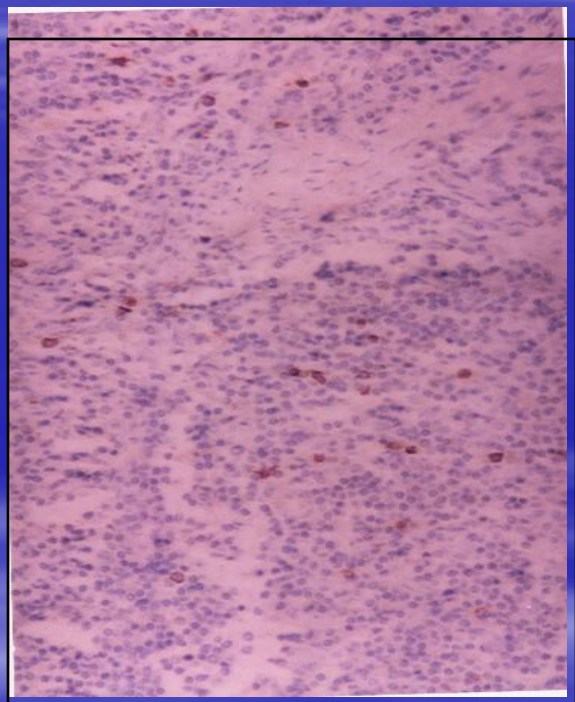


Fig.34a: Lymph node parenchyma many cell reaction, immunohistochemical stain . Mag.205x

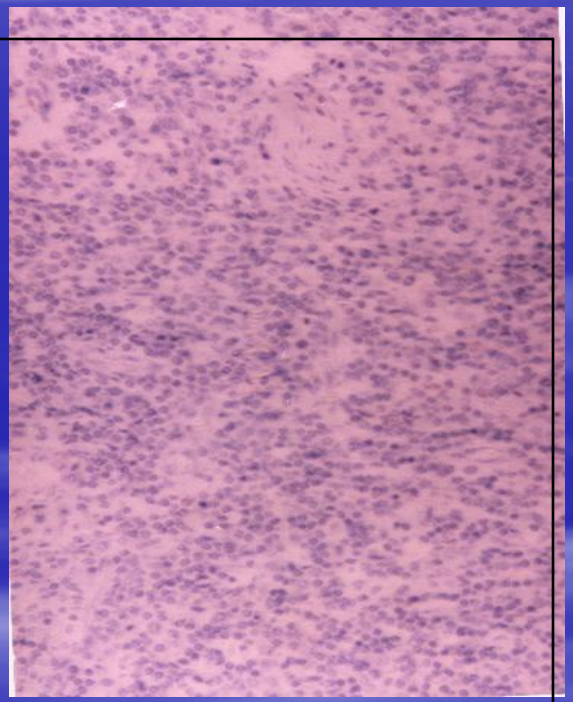


Fig. 34: control, Mag. 205x

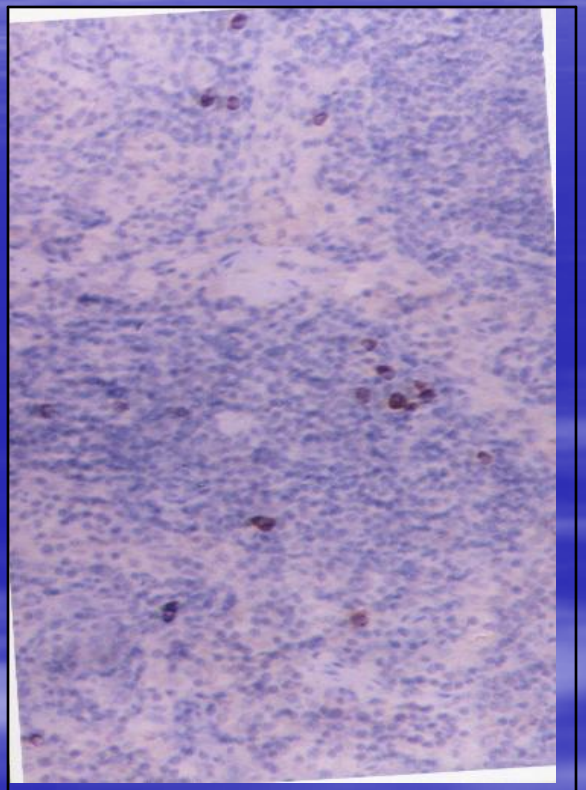


Fig. 32b: Mesenteric lymph node , cortex, many cell reaction, grade 3+ reaction immunohistochemical stain, Mag.205x

Culture

- Intestinal and lymph node scrapings were taken, prepared and cultured on Middle Brook 7H10 agar base media with and without mycobactin J , incubated at 37ºC for 16 weeks.
- Samples were examined starting from 8 weeks after inoculation of the media every week.
- There was no growth until 16 weeks, all the cultures were examined for the detection of growing bacteria.
- In few culture with mycobactin J, white spots scattered on the media were observed Figure 35.
- and from each of them smear was taken and stained by ZN stain Figure 35.
- Additionally suspected culture media were examined by ZN stain, except cultures without any growth and clean surface media.

Culture

- Out of 260 samples inoculated, 23 (9%) were positive for *Mycobacterium paratuberculosis* ,and (%91)237 of them were negative Table 13 .
- From the positive samples, 13% of them were from Irbid, 5% from Sweleh and 4% from Amman.
- From the positive samples one sample was from goats and 22 of them were from sheep.
- In some cases micro- fungal growth masked the stained smear from the culture, but only 9(5.6%) of the cultures were contaminated by mold growth and discarded. To verify the presence of the bacilli in the mixed stained smears immunihistochemical stain was conducted.

Culture

- The bacteria were stained brown in the contrast to others that took the counter stain (haematoxiline).
- When we traced the samples which were positive by culture, they were grade III and II by histopathological examination, and 1+ grade by immunohistochemical stain reaction.

Table.13 Culture results after 16 weeks incubaton peroid, from intestine And lmph node samples, 2002

Sites	No. of cultured samples	+ve (%)	-ve (%)
Amman	49	(2)4	(96)47
Sweleh	91	(5)5	(95)86
Irbid	120	(16)13	(87)104
Total	260	(23)9	(91)237

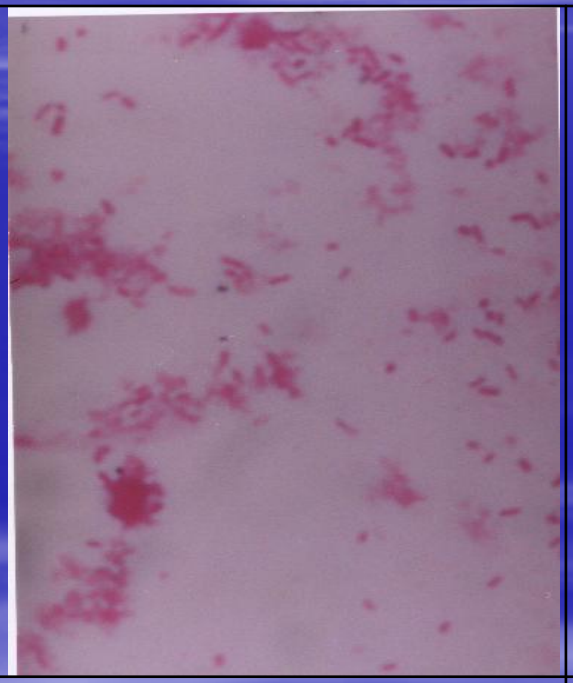


Fig. 37: clumps and dispersed form of *Mycobacterium paratuberculosis,* from culture, Mag.2050x

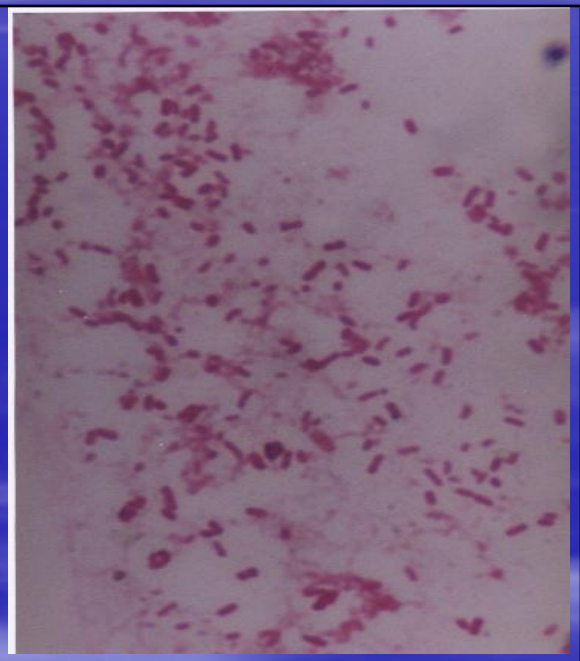


Fig. 37a: clumps and dispersed form of *Mycobacterium paratuberculosis,* from culture, Mag.2050x

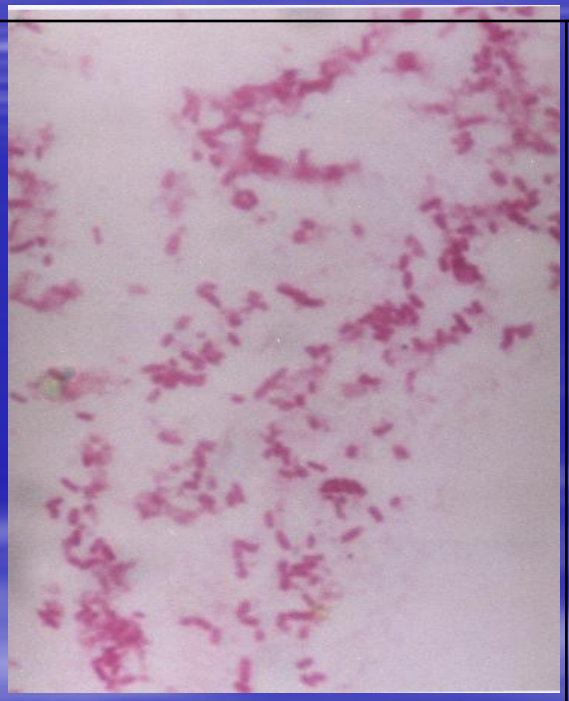


Fig. 37b: clumps and dispersed form of *Mycobacterium paratuberculosis* from culture, Mag.2050x

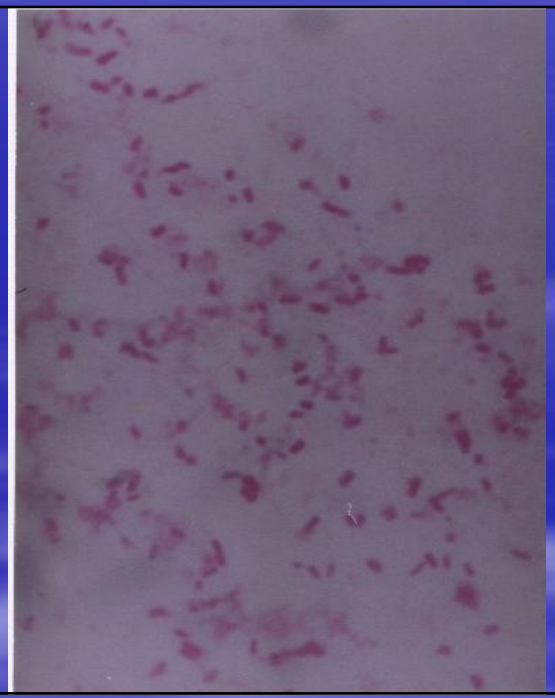


Fig. 37c: clumps and dispersed form of *Mycobacterium paratuberculosis* from culture, Mag.2050x

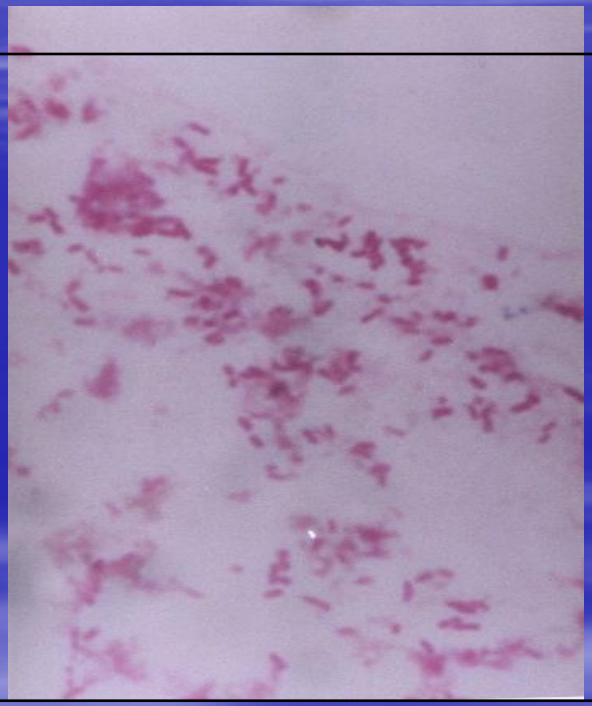


Fig. 37d: clumps and dispersed form of *Mycobacterium paratuberculosis* from culture, Mag.2050x

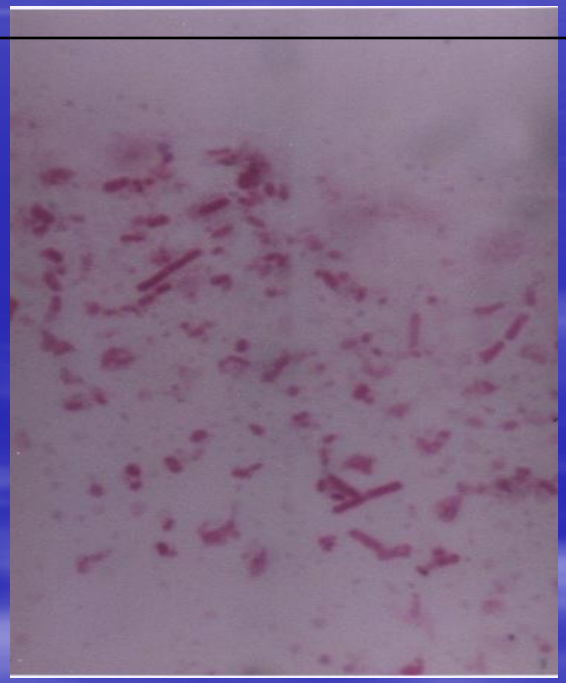


Fig. 37e: clumps and dispersed form of *Mycobacterium paratuberculosis* from culture, Mag.2050x

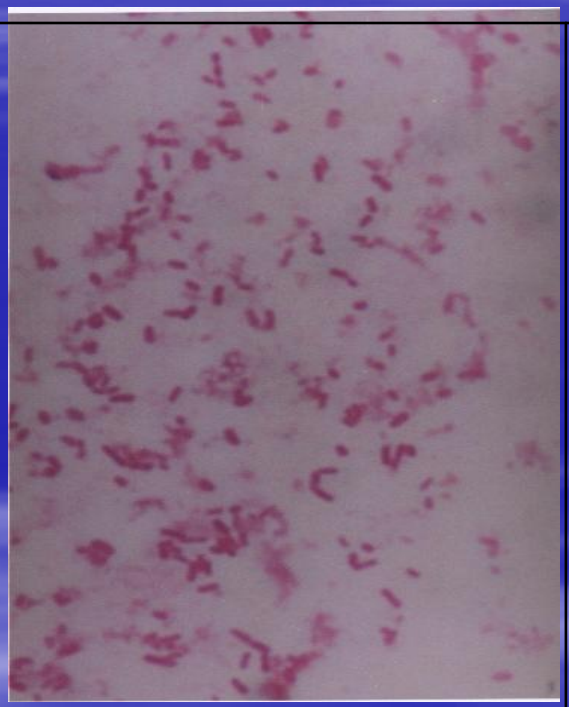


Fig. 37f: clumps and dispersed form of *Mycobacterium paratuberculosis* from culture, Mag.2050x

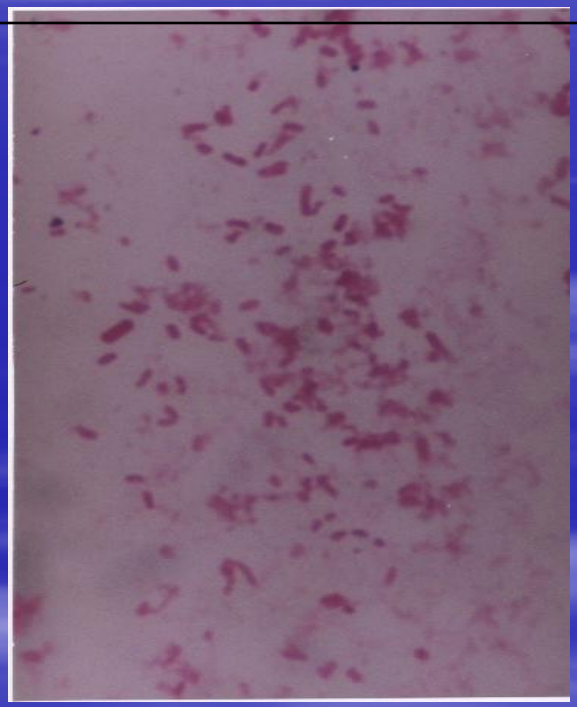


Fig. 37g: clumps and dispersed form of *Mycobacterium paratuberculosis* from culture, Mag.2050x

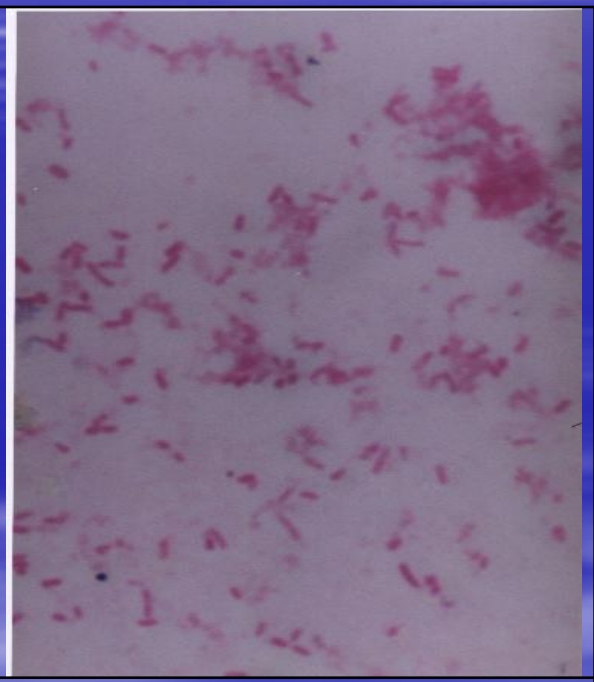


Fig. 37h: clumps and dispersed form of *Mycobacterium paratuberculosis* from culture, Mag.2050x

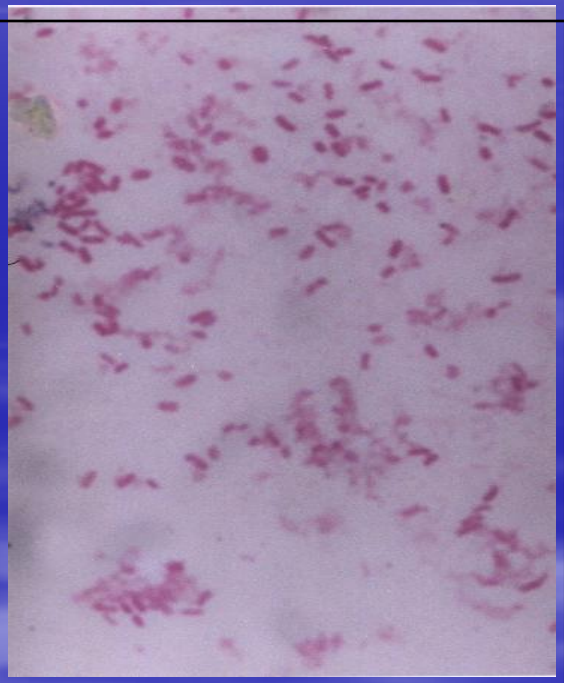


Fig. 37i: clumps and dispersed form of *Mycobacterium paratuberculosis* from culture, Mag.2050x

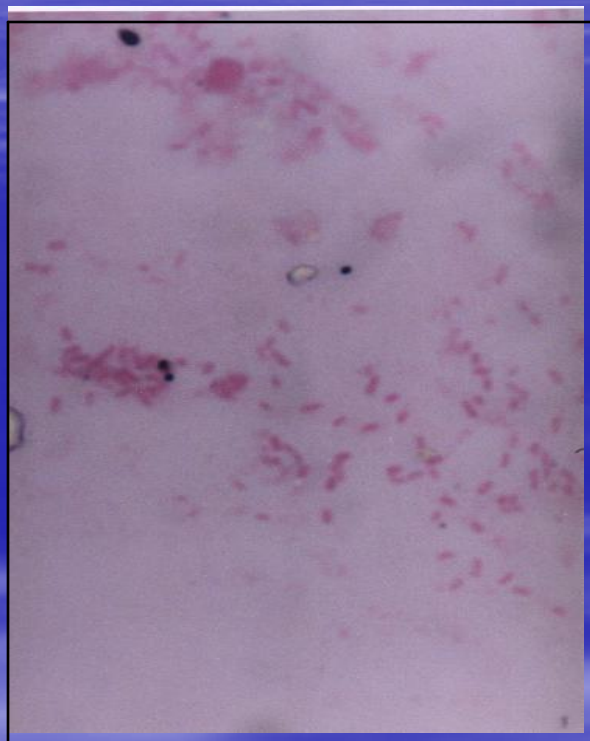


Fig. 37j: clumps and dispersed form of *Mycobacterium paratuberculosis* from culture, Mag.2050x

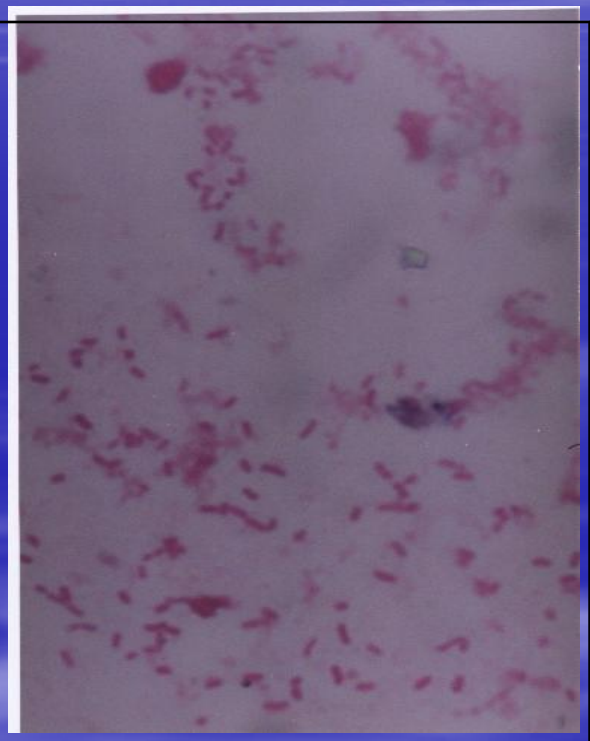


Fig. 37k: clumps and dispersed form of *Mycobacterium paratuberculosis* from culture, Mag.2050x

Fig.36: White rough small spots colony of *mycobacterium paratuberculosis*

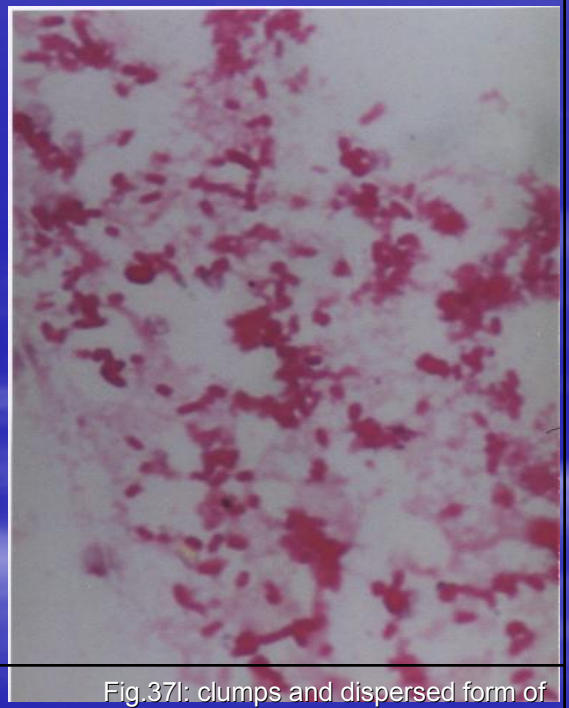


Fig.37l: clumps and dispersed form of *Mycobacterium paratuberculosis* from culture, Mag.2050x

Statistical Analysis results

- Samples that showed mild reaction (1+) by immunohistochemical stain were graded as III and II by histopathological examination .
- The number of samples in each grade were 36 and 47 samples respectively.
- Where as, samples graded 2+ and 3+ by immunohistochemical stain 10)samples for each grade), revealed the same grade by histopathlogical examination 10 samples from each grade.
- Although statistical analysis was done to see if both diagnostic tests are correlated with each other and it was found that the correlation was significant at 0.01 level (99% confidence interval) Table 15.
- The highest number of samples were graded as III by histopathological examination followed by II, while the highest number of samples showed 1+, by immunohistochemical stain , followed by 2+ Figure 21 and 28 respectively

Statistical Analysis results, cont.

- To evaluate the accuracy of the direct smear as a diagnostic method ,sensitivity and specificity analysis was carried out, considering the histopathological method as a reference in all cases and he others as screening tests.
- It was found that direct smear sensitivity and specificity was 31.5% and %50 respectively.
- The sensitivity and specificity for culture was 14.8% and 100% respectively Table 14 and figure 36 .

Statistical Analysis results, cont.

- Correlation and measure of agreement (Kappa) of histopathological and immunohistochemical staining was carried out to see the extent of relation between them.
- The results showed that there is no difference between these two diagnostic techniques Table 15.
- They agree 90.4% table 16. The correlation and the agreement were found between 1+ by immunohistochemical stain and grade II and III of histopathological lesion.
- Where as, the over all finding showed a significant difference between them Table 17 .

Table 14. Sensitivity and Specificity value for Direct smear, immunohistochemical stain and Culture

Type of test	Sensitivity %	Specificity %	Predictive value
Direct smear	31.5	50	95.8
Culture	14.8	100	100

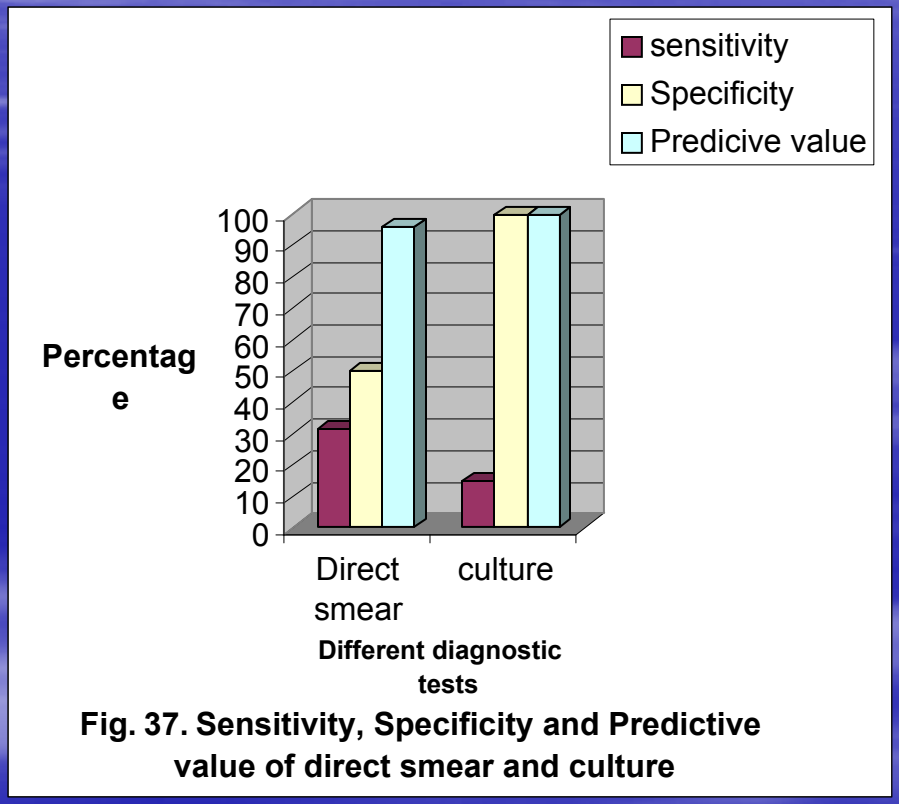


Fig. 37. Sensitivity, Specificity and Predictive value of direct smear and culture

Table 15.Chi-Square Tests for histopathological lesions and immunohistochemical stain grading

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.015	1	.904		
Continuity Correction	.000	1	1.000		
Likelihood Ratio	.015	1	.904		
Fisher's Exact Test		1		1.000	.539
Linear-by-Linear Association	.014	1	.904		
N of Valid Cases	162				

a Computed only for a 2x2 table

b 0 cells (.0%) have expected count less than 5. The minimum expected count is 11.29.

Correlations Table 15. Tests for histopathological lesions and immunohistochemical stain grading

			Immunohistochemical stain	Histopathological lesions
Spearman's rho	Immunohistochemical stain	Correlation Coefficient1	1.000	-.009
		Sig. (2-tailed)		.905
		N	162	162
	Histopathological lesions	Correlation Coefficient	-.009	1.000
		Sig. (2-tailed)	.905	
		N	162	162

Table.16. Measure Agreement of Histopathological lesions and immunohistochemical stain grading .Symmetric Measures

		Value	Asymp. Std. Error	Approx. T	Approx. Sig.
Measure of Agreement	Kappa	-.009	.071	-.120	.904
N of Valid Cases		162			

a Not assuming the null hypothesis.

b Using the asymptotic standard error assuming the null hypothesis.

Table 17.Correlations of Histopathological lesions and immunohistochemical stain results according to their grade ** .Correlation is significant at the .01 level (2-tailed

			Histopathological examination	Immunohistochemical stain
Spearman's rho	Histopathological examination	Correlation Coefficient	1.000	.303
		Sig. (2-tailed)		.000
		N	152	152
	Immunohistochemical stain	Correlation Coefficient	.303	1.000
		Sig. (2-tailed)	.000	
		N	152	152

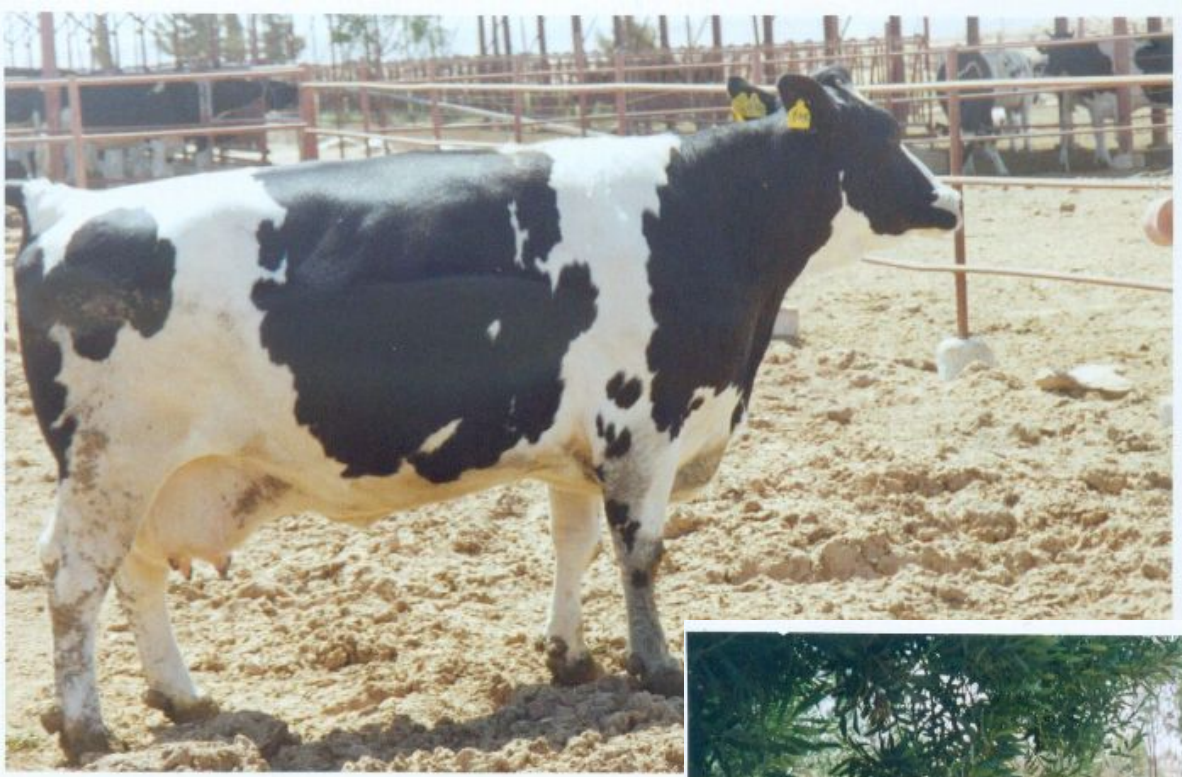
- When the crypts replacement by macrophages is exaggerated, the crypts remnant epithelial cells can confuse during section examination with epitheloid cell nests.
- In the present study the tissue section slides were examined carefully avoiding considering the presence of epitheloid cells around atrophied crypts.

Lecture #9

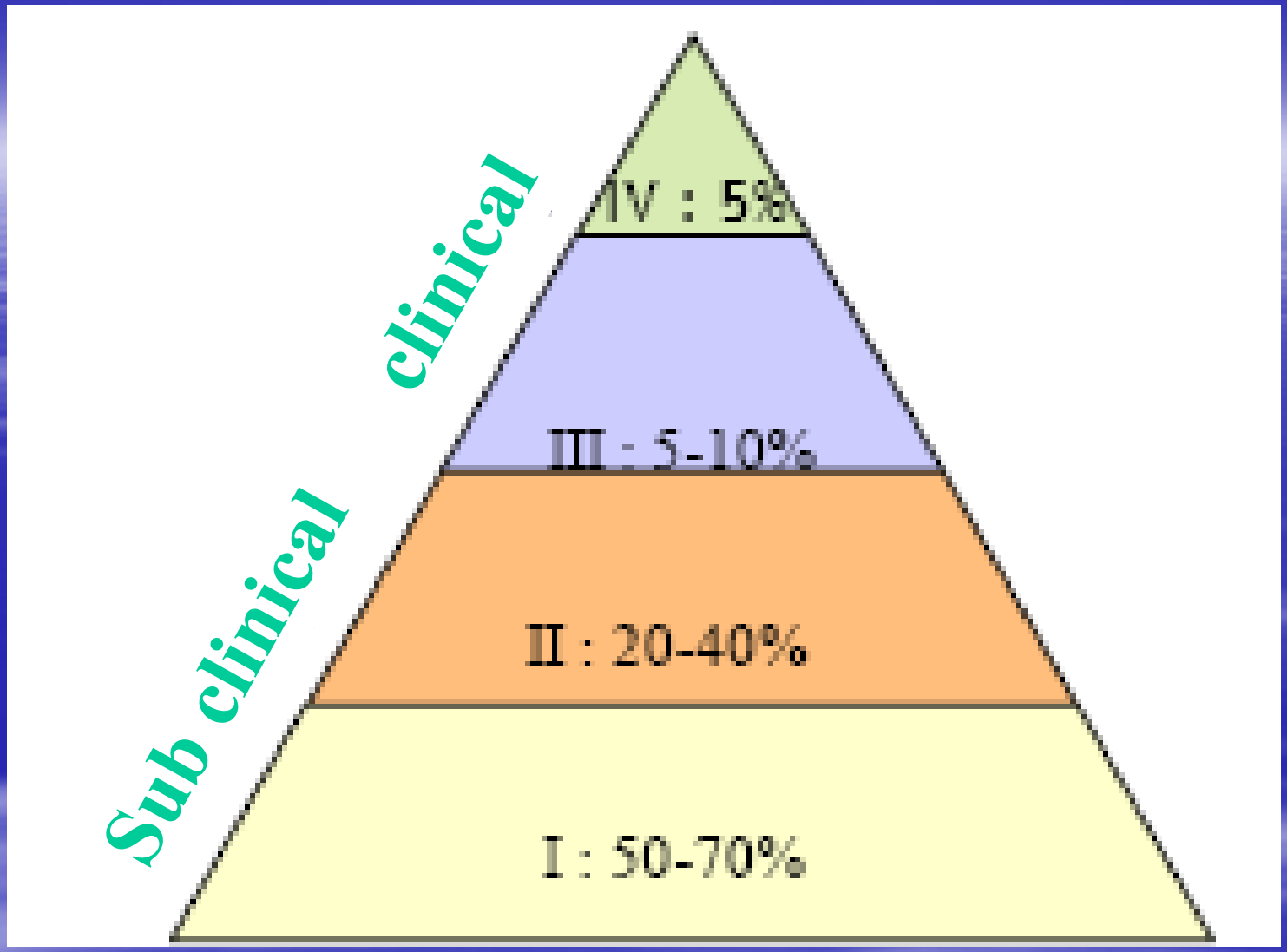
Johne's Disease in Cattle in Jordan

PRESENTED BY:

Professor Nabil Hailat DVM, PhD, Project coordinator. Faculty of Veterinary Medicine (FVM), Jordan University of Science and Technology (JUST)

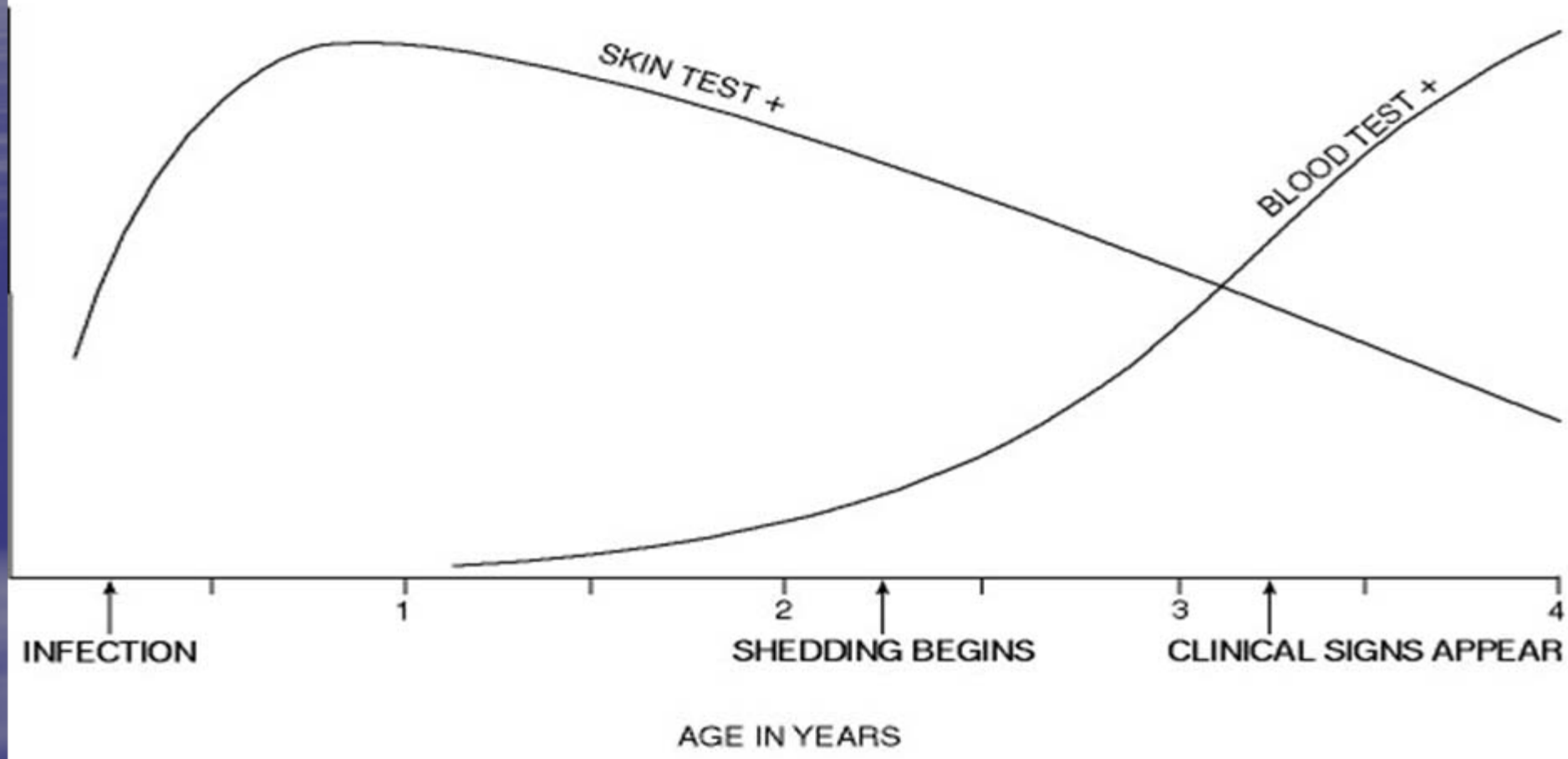


Investigation on the Prevalence and Pathological diagnosis of Paratuberculosis (Johne's disease) in Apparently Healthy Cattle in Central and Northern Jordan.



Proportional distribution of infected animals.

Johne's Disease
Hypothetical Course



http://www.vetmed.iastate.edu

JUSTIFICATION & RATIONAL

- Paratuberculosis is the third leading disease causing significant economical losses in animal industry world wide (Johne's watch).
- It is very prevalent in the Jordanian national Sheep flocks and goat herds.
- It is a silently spreading disease.
- It is reported that Paratuberculosis is a zoonotic disease related with Crohn’s disease (in Humans).

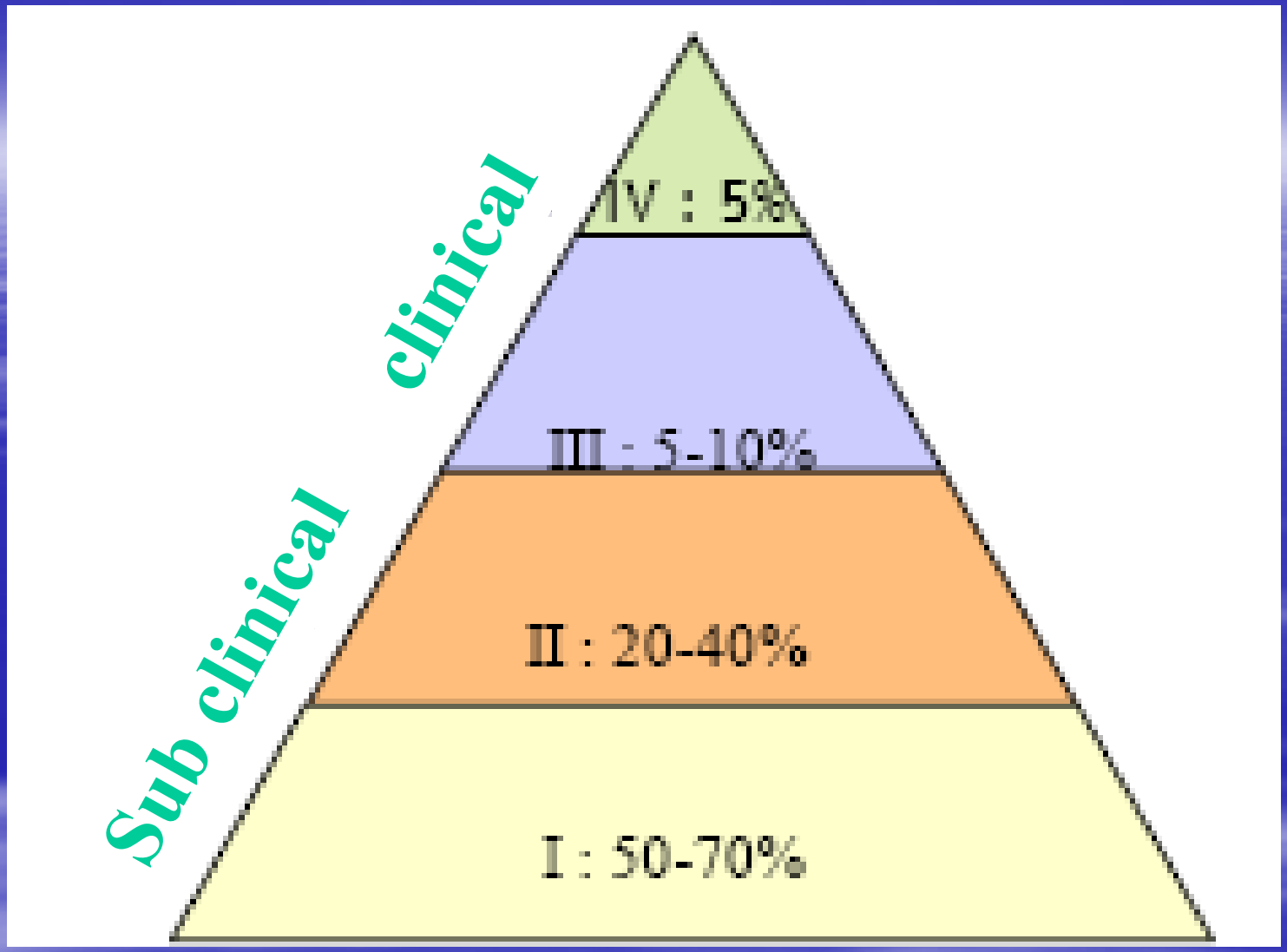
- The disease was not studied before in Arab world (except Egypt, 2005), however It is well studied in many other countries.
- It is seriously considered one of the Agroterrorism and bioterrorism issues (Robert Cohen).
- Could be used as a trade barrier in the WTO.

OBJECTIVES

- To investigate the occurrence of Johne's disease in apparently healthy cattle and camels in Jordan.
- To compare different diagnostic methods
- To estimate the economical impact of Paratuberculosis in cattle and camel industry in Jordan

CLINICAL SIGNS & STAGES OF THE DISEASE

- **Stage 1** Infected, Asymptomatic, Non shedders.
- **Stage 2** Asymptomatic, Shedders.
- **Stage 3** Symptomatic, Shedders with diarrhea.
- **Stage 4** Advanced clinical stage with:
  - Profuse, untreatable diarrhea.
  - Emaciation (weight loss).
  - Decreased milk production.
  - Bottle jaw.
  - Anemia, and infertility are the dominant late signs.
  - **Animals die in a cachectic state.**



Proportional distribution of infected animals.

DIAGNOSIS

- Identification of the causative agent
  - Fecal smear and acid-fast stain
- Detection of host response
  - Necropsy and Histopathology.
  - Immunohistochemistry.
  - Enzyme-linked immunosorbent assay (ELISA).

METHODS

Table 1. Distribution and type of collected sample (cattle).

Samples / Region	Ileum	MLNs	Serum
Irbid	120	120	NA
Ramtha	143	143	120
Total	**263**	**263**	**120**

Methods:

1- **Gross examination.**

2- **Tissue sections were subjected to:**

- Histopathological Examination.
- Ziehl-Neelsen stain (Acid Fast Stain).
- Immunohistochemistry (IHC).

3- **Serum samples were subjected to;**

- ELISA technique (serological test).

RESULTS

Gross examination:

- 19 out of 263 examined Intestine (Ileum) showed thickening of the mucosa with corrugation.
- Twenty of 263 cows were emaciated.
- Congestion was found in some cases.
- Enlargement of corresponding MLNs.
- Other pathological finding were also recorded.

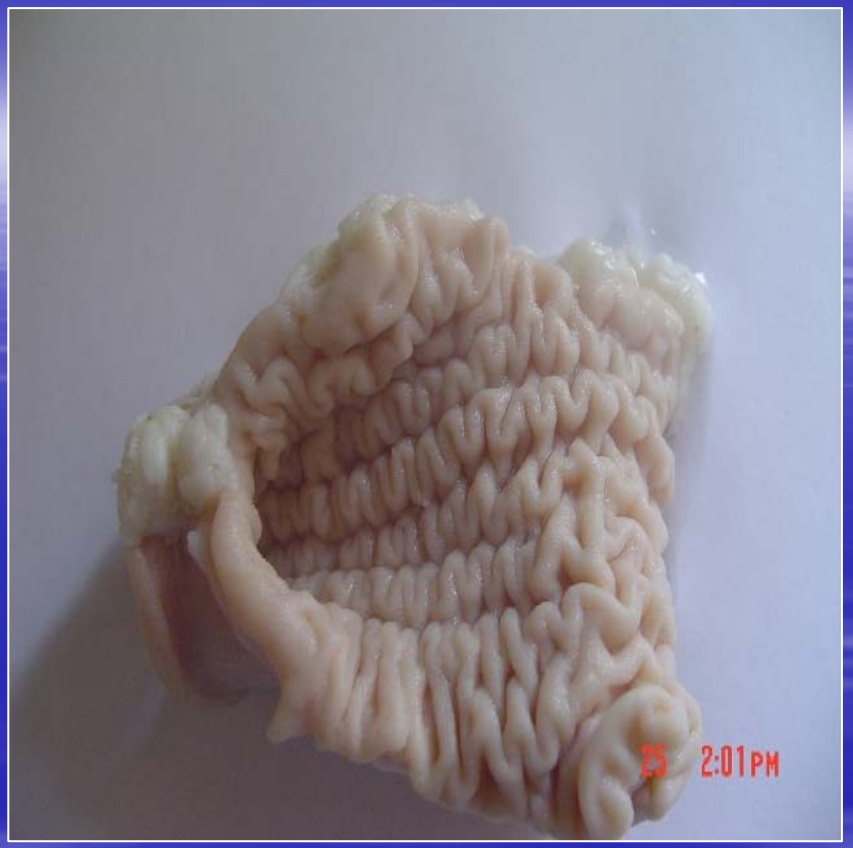


Fig 1&2: Cattle, intestine (Ileum), corrugation of the intestinal mucosa and thickening of the intestinal wall 6 folds normal size.

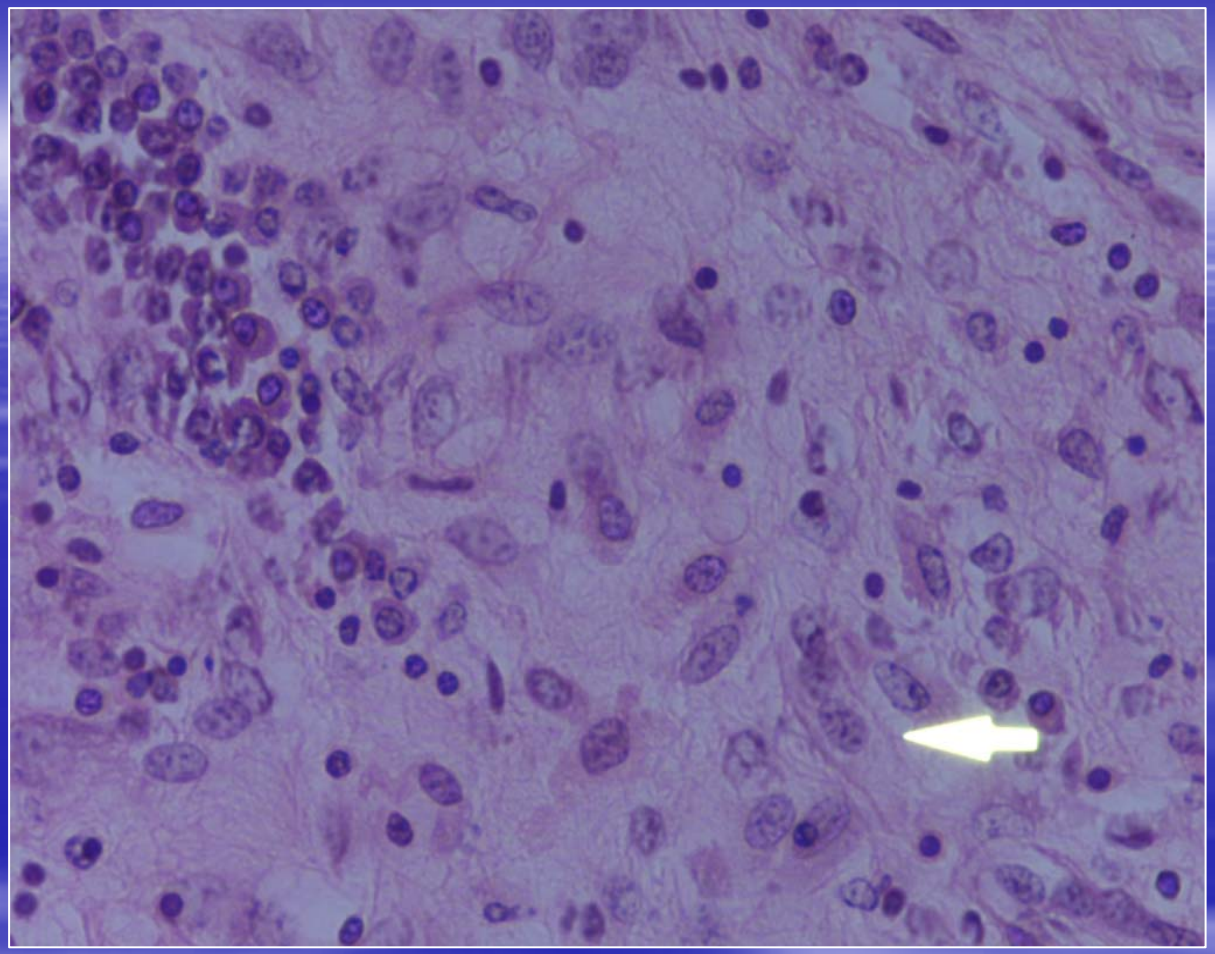
Table 3. Prevalence of subclinical Johne's disease in cattle using histopathology, immunohistochemistry (IHC), ELISA and Ziehl-Neelsen stain (ZN).

Technique / Region	Histopathology	IHC	ELISA	ZN
Irbid	65 %	65 %	NA	4 %
Ramtha	69 %	64 %	6 %	4 %
Total	**66 %**	**65 %**	**6 %**	**4 %**

Table 4. Prevalence of subclinical Johne's disease in cattle using Histopathology & Immunohistochemistry.

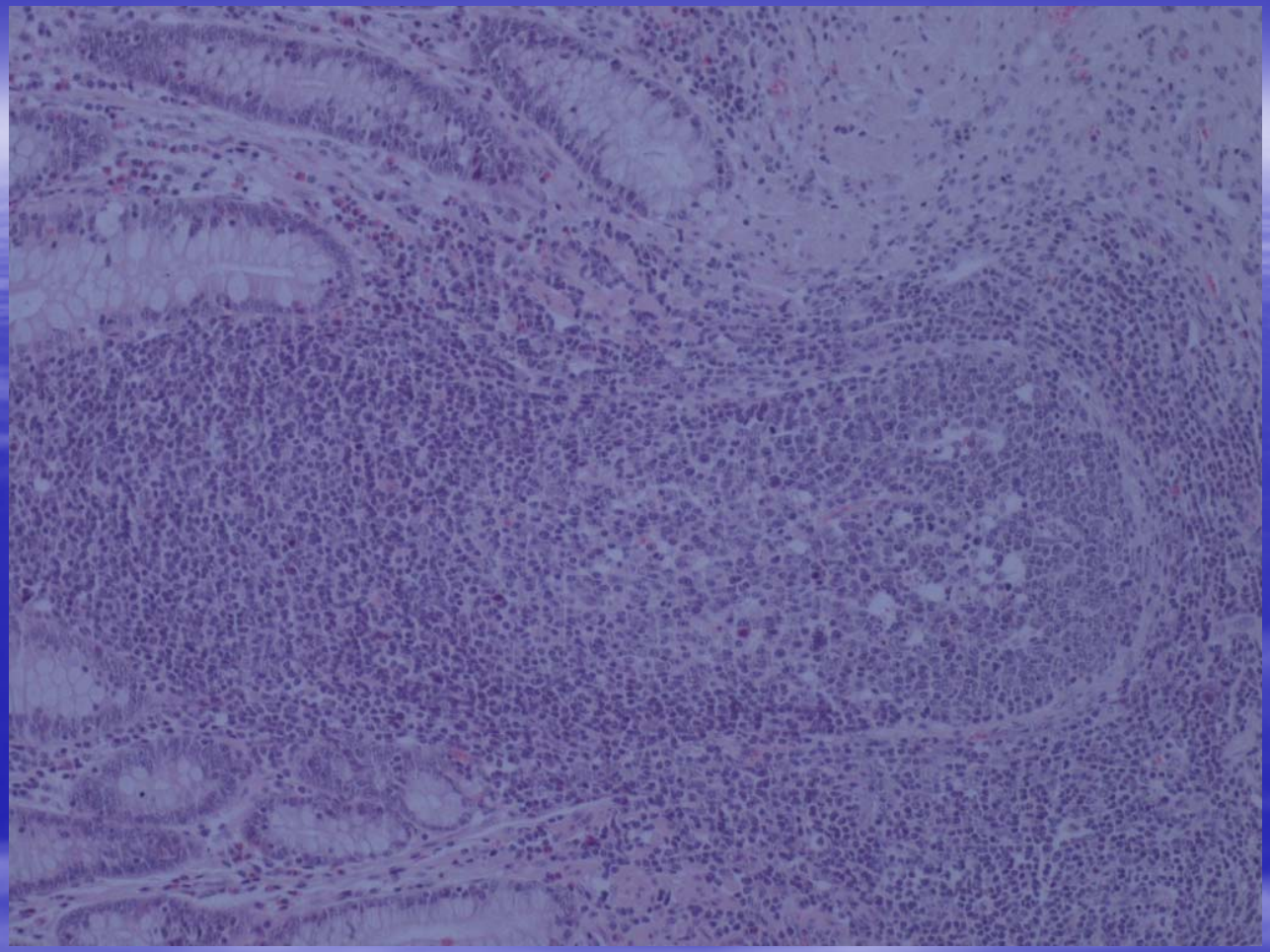
Technique / Tissue	Histopathology	Immunohistochemistry
Ileum	66 %	65 %
MLNs	25 %	61 %

- Histopathological examination showed different intestinal lesions represented mainly by mononuclear cell infiltration Lymphocytes, Macrophages, Epitheloid Cells and in advanced cases Multinucleated Giant Cells.
- Epitheloid cells were distributed either in nests or scattered between crypts (Ileum) and in different localizations in MLNs.



Cattle, intestine (Ileum), sheets of epitheloid cells with some macrophages and lymphocytes. H&E, X100

- Granulomatous enteritis was observed in sever cases
- Sever cellular infiltration resulted in thickening of intestinal mucosa.
- Lymphoid proliferation has resulted in projection of lymphoid tissue toward mucosa



Cattle, intestine (Ileum), lymphoid proliferation toward the mucosa. H&E, X1

Lesions other than previously listed were:

- Infiltration with Eosinophils
- Cryptitis
- Mast cells were also observed in some sections.

MLNs showed variable lesions consist in:

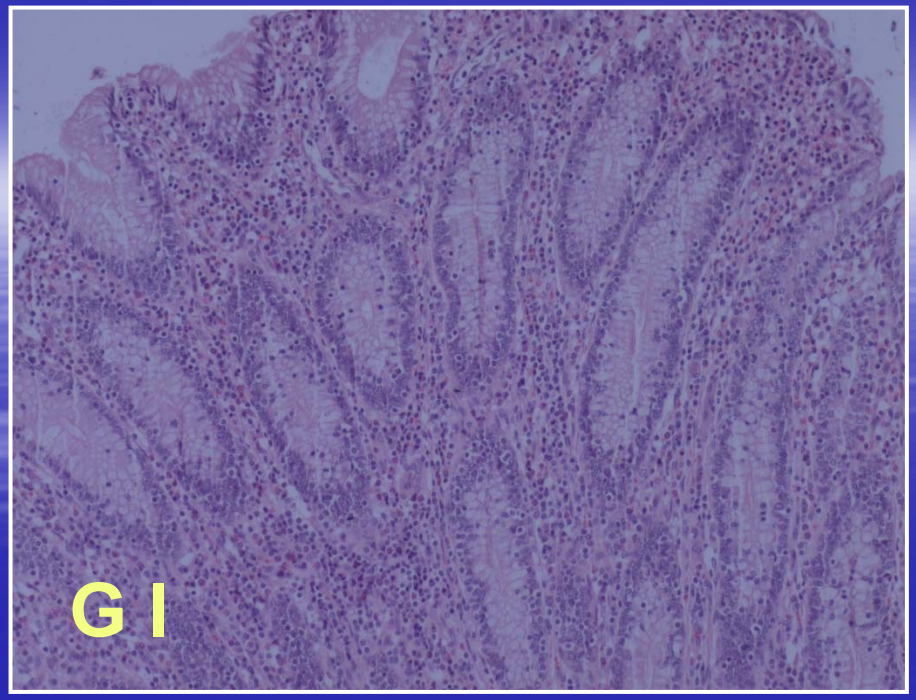
- Lymphoid hyperplasia
- Infiltration with epitheloid cells and macrophages

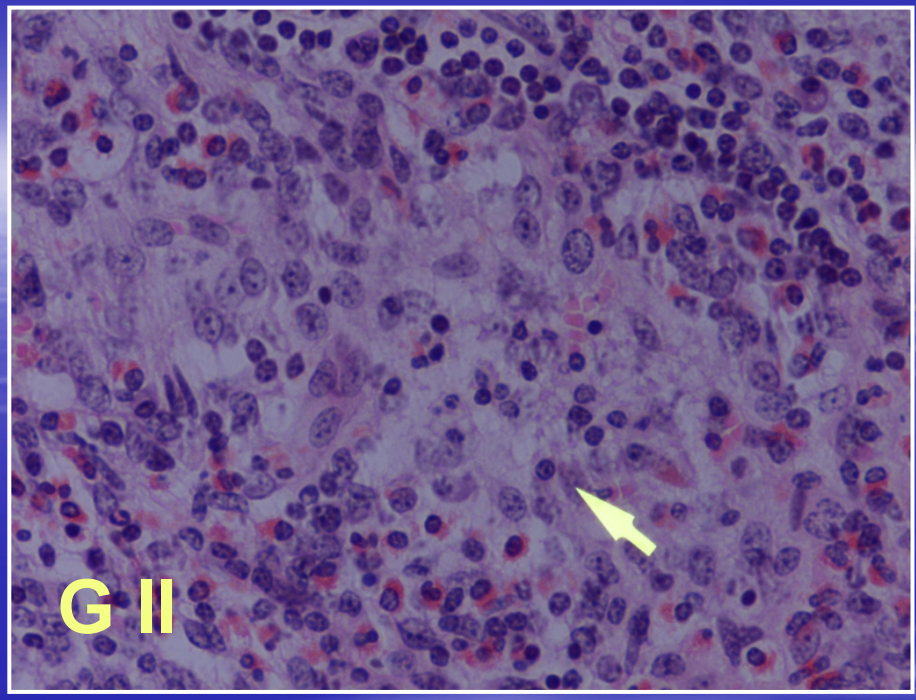
Table 5. Grade distribution of pathological lesions (Ileum) of subclinical Johne's disease in cattle using histopathological examination.

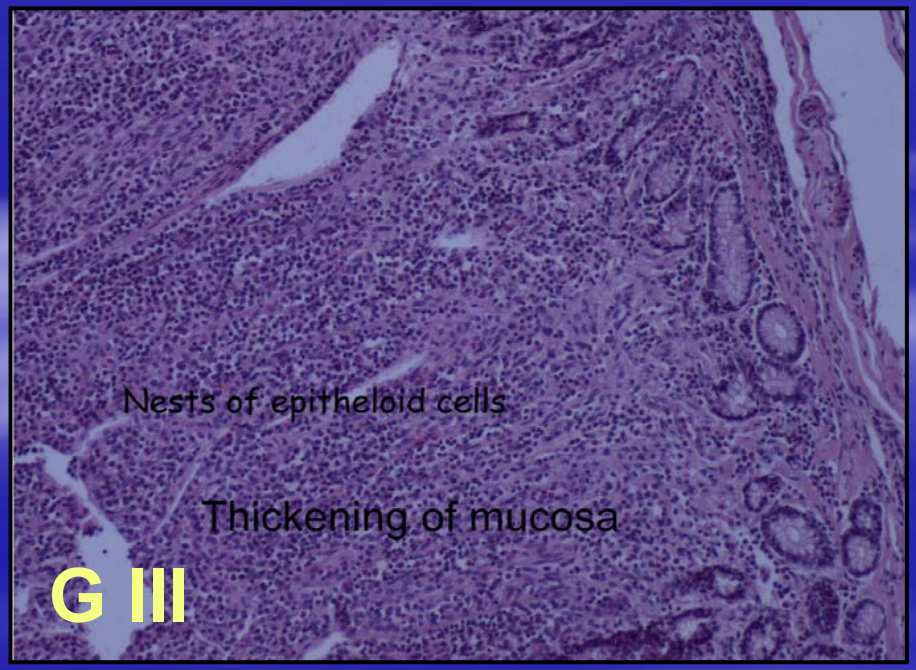
Grade(%) / Region	Grade I	Grade II	Grade III	Grade IV
Irbid	45 %	10 %	7 %	4 %
Ramtha	42 %	12 %	8 %	7 %
Total	**43 %**	**11 %**	**7 %**	**6 %**

Table. Grade distribution of pathological lesions (MLNs) of subclinical Johne's disease in cattle using histopathological examination.

Grade(%) / Region	Grade I	Grade II	Grade III	Grade IV
Irbid	12 %	8 %	7 %	2 %
Ramtha	7 %	7 %	6 %	3 %
Total	**9 %**	**8 %**	**6 %**	**2 %**


G I


G II


Nests of epitheloid cells
Thickening of mucosa
G III

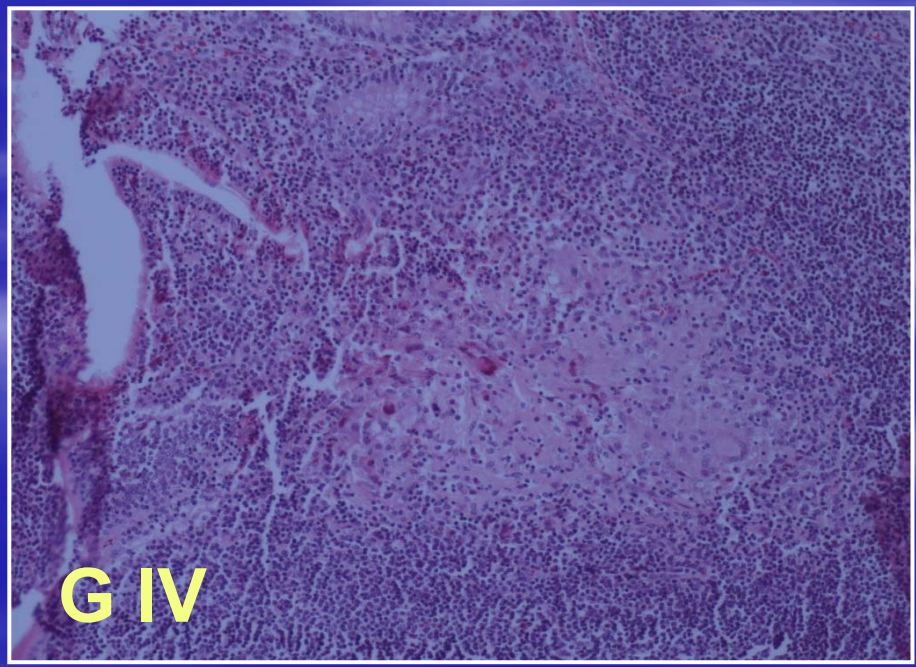
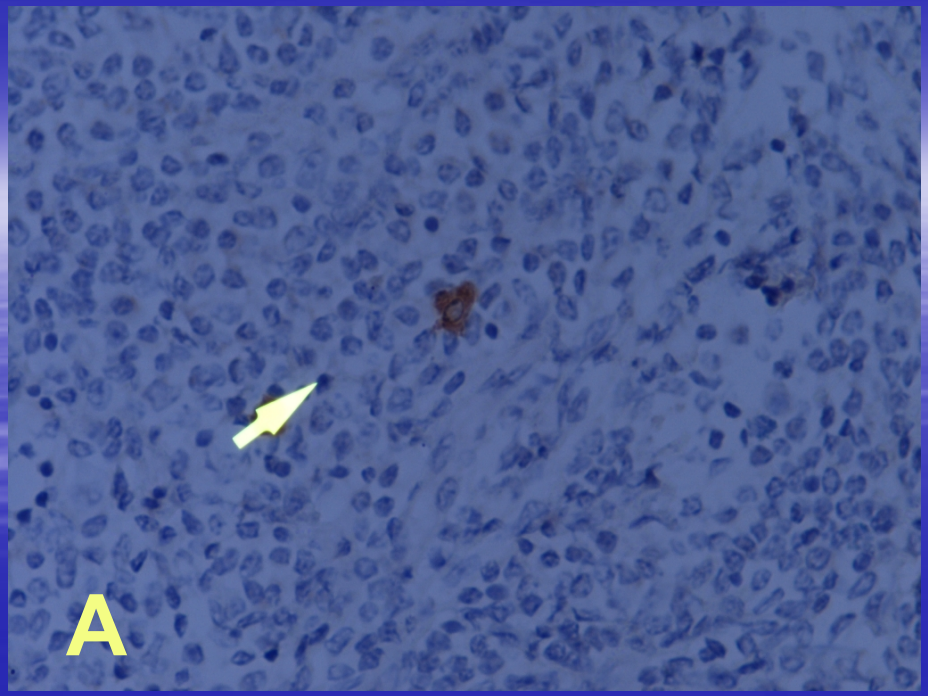

G IV

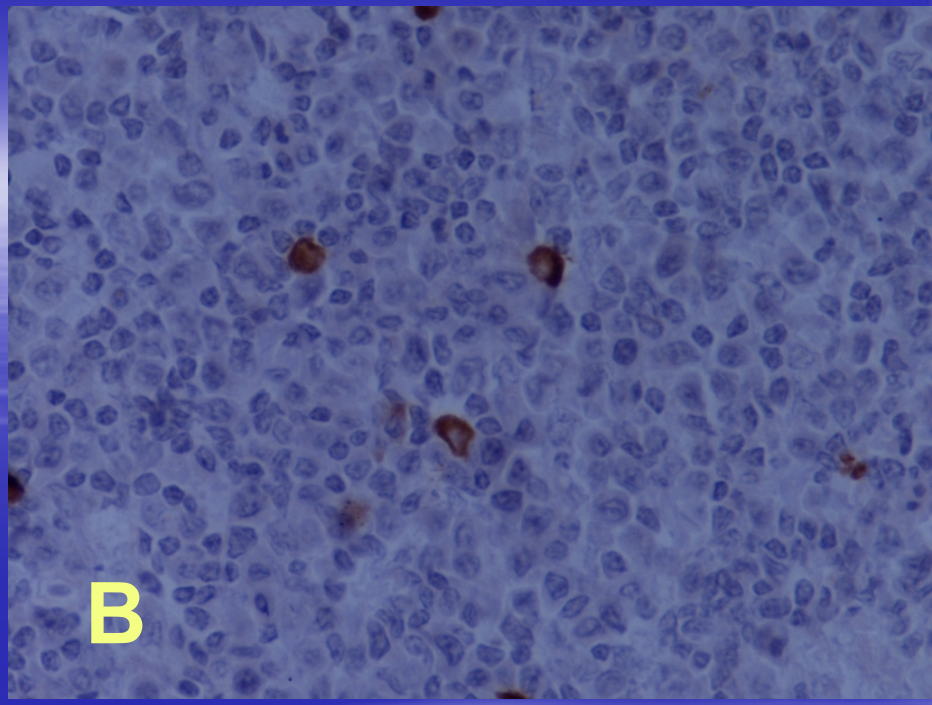
Table. Stage distribution of subclinical Johne's disease in cattle using Immunohistochemistry (Ileum).

Stage (%) / Region	Stage I	Stage II	Stage III
Irbid	30 %	30 %	5 %
Ramtha	32 %	32 %	0 %
Total	30 %	30 %	3 %

Table. Prevalence of subclinical Johne's disease in cattle using Histopathology (MLNs) : Staging approach.

Stage (%) / Region	Stage I	Stage II	Stage III
Irbid & Ramtha	**36 %**	**19 %**	**6 %**





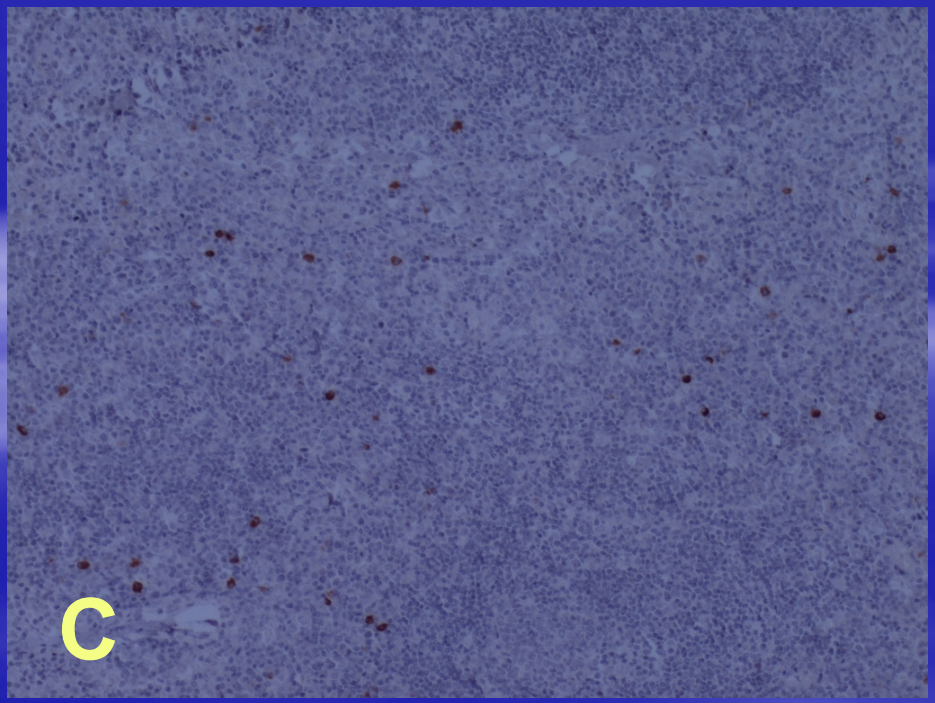


Plate 1 : A, stage I, B, stage II and C, stage III

Table. Prevalence of subclinical Johne's disease in cattle using ELISA technology.

Result / Region	Total number	Positive (%)	Negative (%)
Ramtha	100	6 (6%)	94 (94%)
Dhelil	84	0 (0%)	84 (100%)
Total	**184**	**6 (3%)**	**178 (97%)**

Table . Prevalence of subclinical Johne's disease in camel using histopathology and immunohistochemistry technique.

Technique / Tissue	Histopathology	Immunohistochemistry
Ileum	32 %	34 %
MLNs	17 %	30 %

Table. Prevalence of subclinical Johne's disease in camel using Histopathology (Ileum): Grading approach.

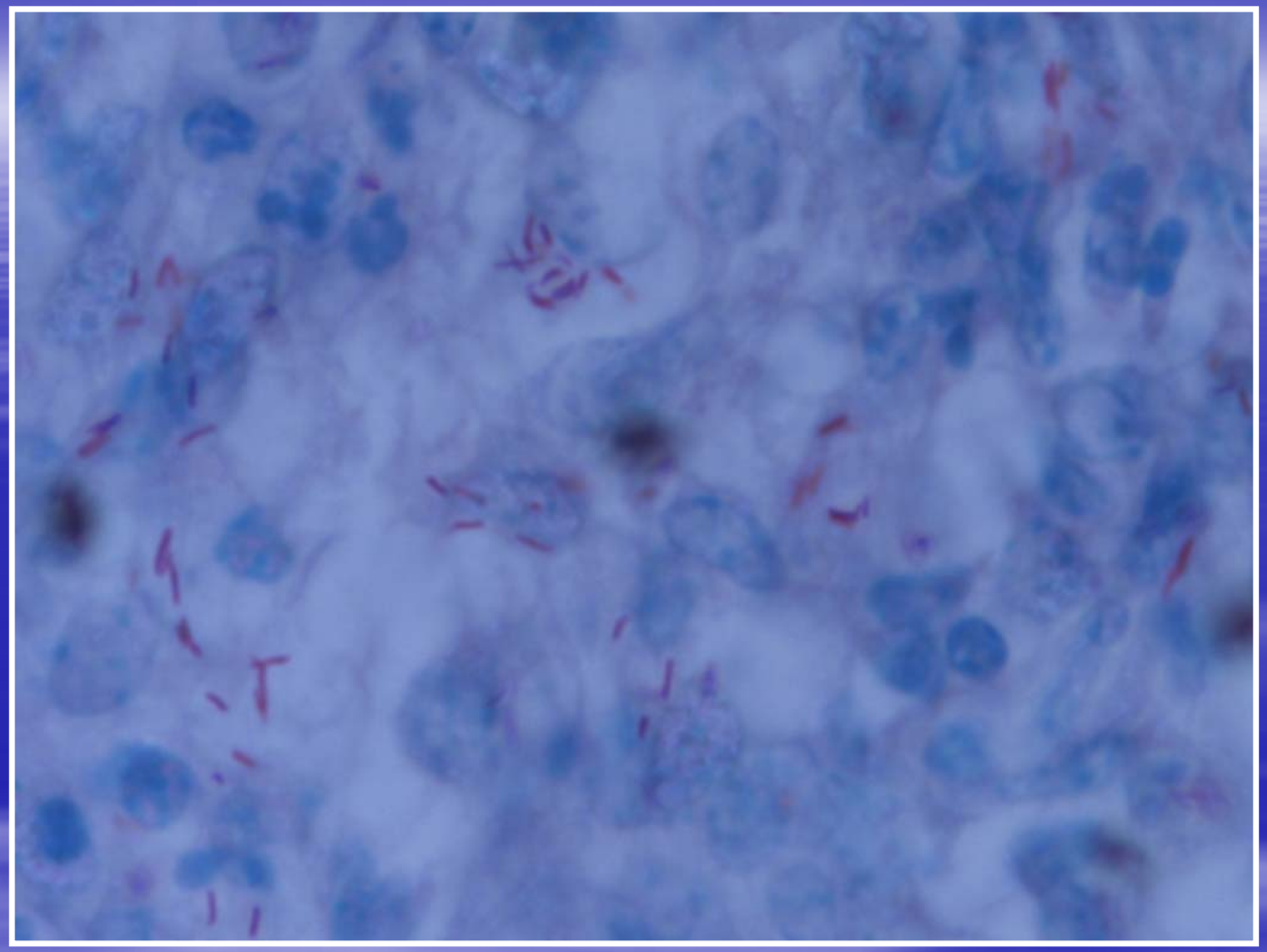
Grade (%) / Region	Grade I	Grade II	Grade III	Grade IV
Ramtha	18 %	7 %	7 %	0 %

Table. Prevalence of subclinical Johne's disease in camel using Immunohistochemistry (Ileum) : Staging approach.

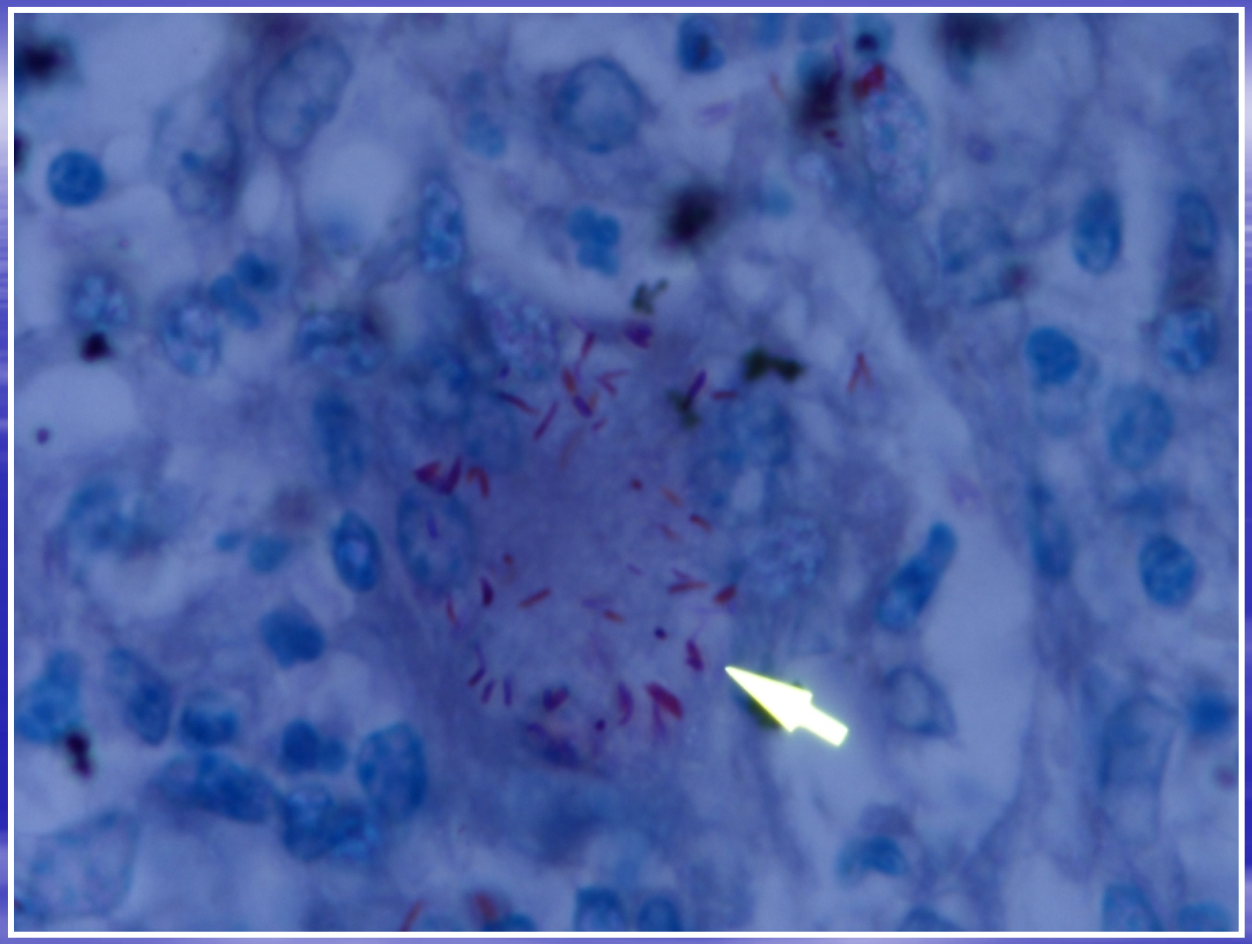
Stage (%) / Region	Stage I	Stage II	Stage III
Ramtha	**32 %**	**32 %**	**0 %**

Table: Ziehl-Neelsen results (cattle & camel).

Result / Region	Total number	Positive (%)	Negative (%)
Cattle	120	**4 (3%)**	116 (97%)
Camel	30	**0 (0%)**	30 (100%)



Cattle, intestine (Ileum), scattered Acid Fast Bacilli.
ZN, X100



Cattle, lymph node, aggregate of intra and extra-cellular (macrophages) acid fast bacilli. ZN, X100

CONCLUSION

- The disease occurs in 66% and 65% apparently healthy cows as they were examined by histopathology and IHC respectively. So the disease is very prevalent in Jordan.
- 66% apparently healthy cows showed lesions compatible with Johne's disease.
- Histopathology is compatible with IHC.

- Histopathological examination of MLNs has lower diagnostic value compared to ileum.
- The disease is also present but not prevalent in camels 32%.
- Where ELISA was proved to be a good screening test by others, we have a prevalence of only 6% this is due perhaps to the sample size, sampled animal age and lack of test replicates.

- Ziehl-Neelsen stain is an untrustable technique.
- Of great diagnostic value is histopathology.
- IHC is a laborious, expensive (7$ per head) and time consuming technique (8 hours per run).

What we need??

Depending upon our results and previous (sheep and goat) studies results, we believe that Johne's disease is very prevalent in Jordan and causes significant economical losses. This situation indicates that Jordan need an urgent specific prevention and control programs.


Thank you

Lecture #10

Algeria Paratuberculosis In *Algeria really unharmed by this pathology Is*

PRESENTED BY:

Dr. Ryad Bouzidc, Faculty of Veterinary Medicine

Is Algeria really unharmed by this pathology ?

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INTRODUCTION

Paratuberculosis is an infectious, enzootic, contagious, incurable, disease with a long period of incubation; it is caused by a slow growing, acid fast bacterium, ***Mycobacterium avium subspecies paratuberculosis***. The disease is world wide distributed especially in United Kingdom, Netherlands, Belgium, Scandinavian countries and France.

In Belgium, the seroprevalence of the infection (analyses by ELISA) cattle would be of 17,4 % [2], in Netherlands of 54,7 % [07].

In France [9], data collected during the last enquiries in relation with the paratuberculosis prevalence are partial

They don’t allow to quantify neither the clinic prevalence nor the infection prevalence

In the United States, 22 % of the 1008 dairy milky cattle tested belong to the twenty main milk-proceducing states have a prevalence of infected animals superior to 10 % [6], and 9 % of milk-feeding cattle would be touched by the infection

Algeria is officially declared unharmed of paratuberculosis and no real study has been realized up till now

The first part of this work will consist of a bibliographical reminder over this disease ,in the second ,will present some information on the algerian live-stock and the main pathologies declared by the

direction of the veterinary services of the ministry of agriculture

I.PRESENTATION OF THE DISEASE:

I.1 Clinical Signs:

In cattle the disease is characterized by intractable chronic diarrhea, emaciation, and hypoproteinemia in animal older than 19 months. In Small ruminants (sheep and goats) the clinical disease is similar to that observed in cattle except that diarrhea dose not occur. Animals lose weight and die after few weeks of chronic evolution. Paratuberculosis present the most chronic evolution from all bacterian diseases.Without any treatment

(The paratuberculosis present the most chronical evolution of all the bacterian diseases of the cattle)

I.2. Lesions:

Macroscopic lesions:

The gross lesions in Johne's disease is a chronic, segmental thickening of the caudal small intestine, cecum, and proximal colon. Affected segments have a corrugated mucosa that is focally ulcerated. Mesenteric lymph nodes are greatly enlarged,they appear lately ,during the clinical phase of the disease ,the lesions are only of a weak importance ,indeed absent

The local, and specific lesions interest the intestinal tract and the Imphatic system of drainage .

The initial localization of the infections process and the small distal intestine ,especially the terminal ileum and the ileo-caecal valvula

Microscopic lesions:

At microscopic examination, noncaseating granulomas consist of macrophages with foamy cytoplasm and large numbers of acid-fast organisms. In contrast, sheep, goats and deer can have a tuberculoid (caseating) granulomas in the intestines, lymphatics, and lymph nodes, sometimes with central mineralization. These lesions are composed of well-differentiated epithelioid ells in a whorled pattern and a variable number of Langhans-type giant cells. Organisms are few. Granulomas of either type occur in the regional lymph nodes.

Much more precocious than the microscopic we observe them in the ,lesions would be intestine and the corresponding lymphatic knots The histological examination revel an important cells of the macrographical infiltration by the cells of de(type and some giant cells epithelioid [5,10[.)Langhans

3. Diagnostic Techniques :

❖ **Detection with Coproculture :**

❖ it is the most reliable techniques of confirmation of the excretion by the animals l'animal,the results are available 12 to 16 weeks after the reception of samples of feces by the laboratory

❖ **Detection with PCR :**

A polymerase chain reaction (PCR)-based assay was developed for detection of insertion sequence of Mycobacterium avium subsp paratuberculosis in animal feces. This technique assay included DNA extraction and PCR assay using commercially kits. It is susceptible, rapid but difficult and

expensive technique

❖ **Detection with ELISA :**

Put in evidence the specific apparent antibodies following immune reaction of the animal when contaminated,the reliability is less because the presence of anti-bodies over an infected animal by paratuberculosis is not systematic

No technique presents sufficient reliability

II. Bovin live-stock and the breeding system :

Bovin numbers are difficult to determine with regard to herd fluctuation of constant movements and the breeding mode of herd and according to the statistics of the direction of veterinary sevices et selon les [8]

The number of live-stock is as follows :

Sheep: 8 896 919

goats : 3 272 024

Cattle:1 434 770

Camels : 278 023

Sheep predominate with 80 percent of the total, over eighten million ewes. Goats are in second place with 13 percent of which does comprise half. The cattle herd is small, 1.5 - 1.6 million head of which 58 percent are milch cows. Agro-ecological zones differ in their livestock specialization. Cattle are mainly limited to the north of the country with some enclaves elsewhere. The steppe is the favourite zone for sheep and goat raising, over ninety percent of their total is there, causing serious over-exploitation of the herbage.
percent of cattle are in the North, of these 53 percent are to the east and 24 80 percent to the west with 23 percent in the centre

The main cattle breed, the Atlas Brown, has four sub-breeds:

1-the Guelmoise has a dark grey coat and is found in the forest zone,

2-the Cheurfa has a whitish coat and is found in the pre-forest zone,

3-the Chélifienne has a fawn coat,

4-the Sétifienne has a blackish coat and is adapted to hard conditions.

Exotic breeds are represented by: the Dutch Friesian, an excellent milker, is widespread in the coastal region and constitutes 66 percent of improved breeds; the French Friesian is also widespread and a good milker; the Pie Rouge de l'Est and the Montbéliarde are present in small numbers. These breeds, introduced to raise production, find themselves under ecological conditions very different from those of their countries of origin. Although they are imported for their high genetic potential, their performance decreases because of the strain on their metabolism in adapting to the local environment. Algeria's sheep herd is dominated by three breeds, well adapted to local conditions [1,3,4]

As for the raising mode,it is ditributed as follows :

II.1. raising in mountainuous zones :

Extensive system and in freedom,animals are left free during the first hours of the morning to be brought back to the far mat the end of the day

II.2. raising in interior plains :

Herds are in confinement in raising buildings,but also in grazing ground during all the year ,the pastures are sometimes restricted to bad qualities (rare and short grass) and constitute at less the essential of the nutrition added to concentrated heyIt exists a type of inter-mediate and sumi-extensive raising where the animals are set free during all the day and in the surroundings of the farm,are confined in a stable where we give them hey

II.3. Health state of live-stock

II.3.1. Main diseases of obligatory declaration in Algeria [9]

Sheep-Pox

three centres in 2006, six in 2005, against thirty four centres in) 2004), the wilayates (departements) the most affected are : Nâama, Tébessa et Djelfa

-

Rabies

This pathology continues to harm in our contry at an enzootic state (871 cases have been declared in 2006 against 907 in 2005)

Bovin brucellosis

An average of 1.00% (2006) against 0.72% (2005)

Caprine brucellosis

Infection percentage is of 5.37% (2006) against 6.03% registred in 2005.

Bovin tuberculosis

of 0.25% (2006) to 0.35% (2005) and 0.32% (2004) , besides, in 2006 over 136484 bovins at 20460 exploitations ,the lest of IDR has reveled 368 positive cases.

Other encountered diseases

Various infections and parasitical diseases affect our live-stock, allmixed species

Commentaries and conclusion

It is revealed that all the information available over the animal pathologies in Algeria are essentially drawn from the magazine (Santé Animale Mondiale of the Office International des Epizooties. They are official data communicated in the animal reports,transmitted the OIE (151 member contries)

The reality on the ground is some thing else, the pratician veterinarians often declare paratuberculosis symptoms.

This pathology is underestimated in Algeria because it doesn't provoke attrocious mortality cases similar to big epizooties as the bovin plague ,the bovin contagious peri-pneumonia or the charcoal ,it doesn't constitue a priority.
Unfortunately ,this period of inattention favours its implantation by active transmission herds

Generally the breeders sloughter their emaciated animals and less productive before the appearance of clinical signs of the disease before its diagnostics
The paratuberculosis is an important disease regarding its

economic impact over the productivity of the breedings

The precocious reform,the decreasing of milk production ,reduced fertility,the growing lateness and the increased mortalities are the main consequence of this disease

In addition the scientific community continues to search to know if it exists a relation between the disease of Crohn (a disease of the intestine that the pathology has a link with the paratuberculosis) in the human and MAP

A multitude of studies have been published over this possible link but up till now,they are insufficient to conclude that MAP causes the diseases of Crohn.

On the contrary this potential impact for the public health is more ans more mediatized and could become a subject of auxiety for the consumer

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Lecture #11

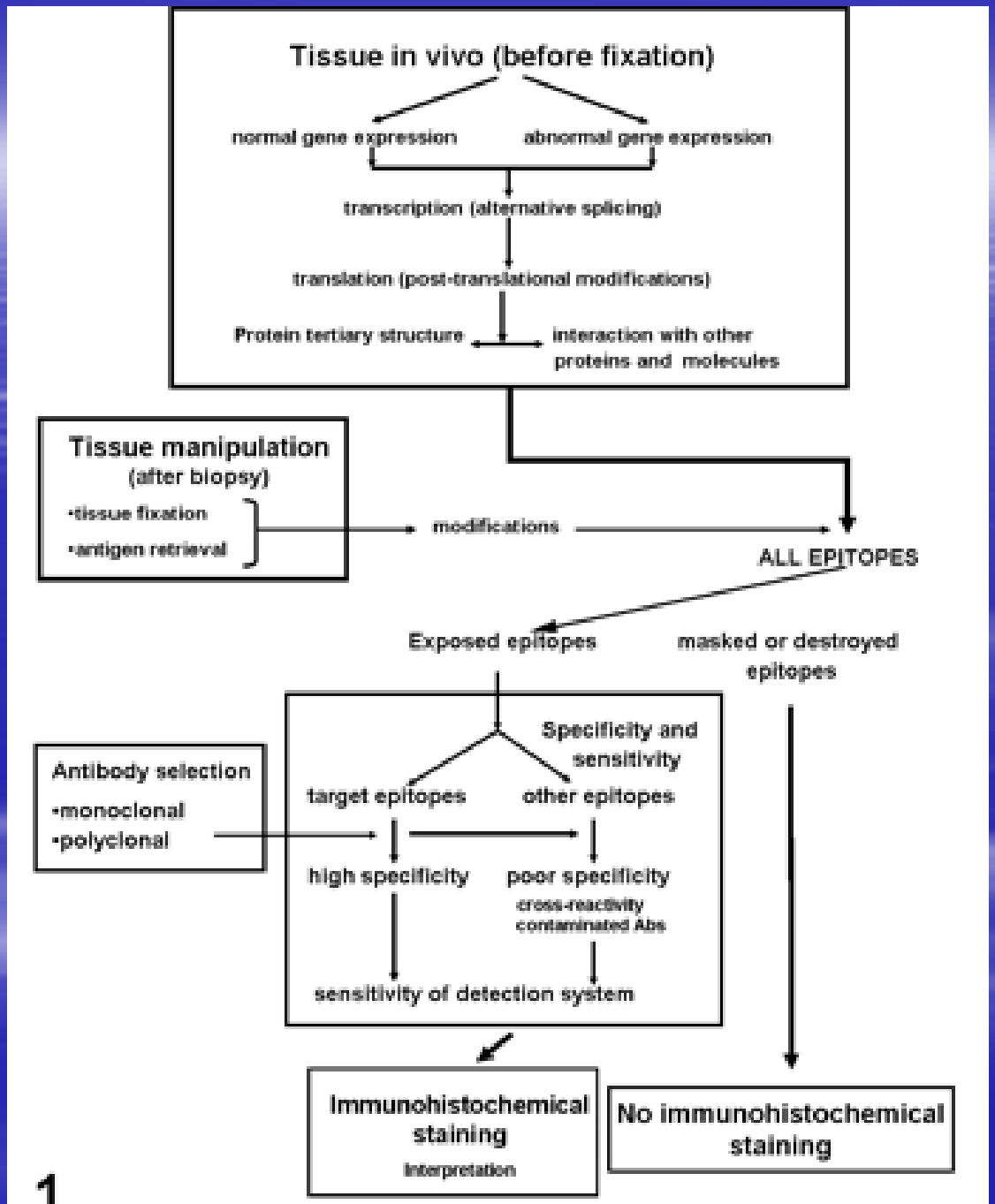
Principles of Pathology and Technical Aspects of Immunohistochemistry

PRESENTED BY:

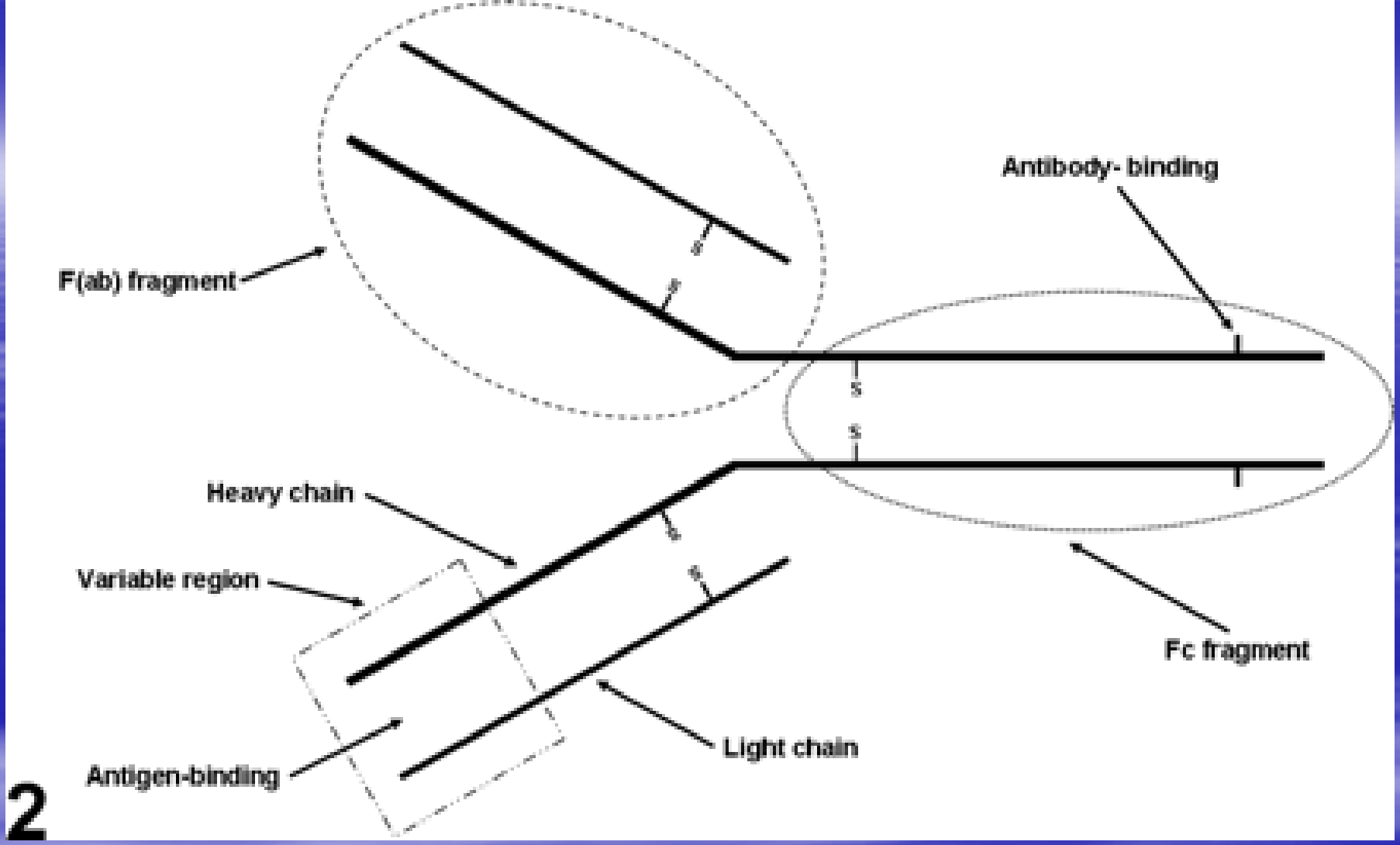
Professor Nabil Hailat DVM, PhD, Project coordinator.
Faculty of Veterinary Medicine (FVM), Jordan University of Science and Technology (JUST

Immunohistochemistry

- is an integral technique in many veterinary laboratories for diagnostic and research purposes.
- The basis of IHC is very simple and bridges three scientific disciplines: immunology, histology, and chemistry.
- This review will cover technical aspects of immuno-histochemistry, including those related to Abs and Ags, fixation, AR, detection methods, background, and troubleshooting.


Tissue in vivo (before fixation)
normal gene expression
abnormal gene expression
transcription (alternative splicing)
translation (post-translational modifications)
Protein tertiary structure
interaction with other proteins and molecules
Tissue manipulation
(after biopsy)
•tissue fixation
•antigen retrieval
modifications
ALL EPITOPES
Exposed epitopes
masked or destroyed epitopes
Specificity and sensitivity
target epitopes
other epitopes
Antibody selection
•monoclonal
•polyclonal
high specificity
poor specificity
cross-reactivity
contaminated Abs
sensitivity of detection system
Immunohistochemical staining
Interpretation
No immunohistochemical staining

1



2

- Immunohistochemistry is based on the binding **of** Abs to a specific Ag in tissue sections. The most common immunoglobulin (Ig) used in immunohistochemistry is IgG; IgM is less commonly used.

The nature of Ag-Ab interactions

- The bonds involved are weak (mostly hydrophobic and electrostatic) and not covalent.
- Hydrophobic bonds happen between macromolecules with surface tensions lower than that **of** water.
- Electrostatic interactions are caused by attractive forces between one or more ionized sides **of** the Ag determinant and oppositely charged ions on the Ab-active site.
- Van der Waals forces are weak electrostatic interactions between dipolar molecules or atoms

The affinity of the Ag-Ab reaction is important for practical reasons

1) high-affinity Abs will bind more Ag in a shorter incubation time than low-affinity Abs.

2) in general, the higher the affinity the more dilute the Ab solution can be

Selection of immunogens

- Two broad groups **of** immunogens exist:

1- Synthetic peptides (adv. &dis ad.)

2- purified proteins as immunogens avoids many **of** the problems generated by the use **of** synthetic peptides

Monoclonal and polyclonal Abs

- Polyclonal Abs have higher affinity and wide reactivity but lower specificity when compared with monoclonal Abs.

Tissue Microarrays

- The tissue microarray technology allows simultaneous examination **of** hundreds **of** samples on a single microscope slide

Fixation

1) adequately preserve cellular components, including soluble and structural proteins
2) prevent autolysis and displacement **of** cell constituents, including Ags and enzymes
3) stabilize cellular materials against deleterious effects **of** subsequent procedures
4) facilitate conventional staining and immunostaining

Fixatives types

1- cross-linking (non-coagulating) fixatives.

2- coagulating fixatives.

Formaldehyde and cross-linking fixatives

- Formaldehyde is the gold standard **of** fixatives for routine histology and immunohistochemistry
- Formaldehyde preserves mainly peptides and the general structure **of** cellular organelles.
- It is a good preservative **of** lipids if the fixative contains calcium

mechanism of fixation

- The basic mechanism of fixation with formaldehyde is the formation of addition products between the formalin and uncharged reactive amino groups (–NH or NH2), forming cross-links. Once the addition product (reactive hydroxy methyl compound) is formed, additional cross-linking will happen. Thus, in the presence of a second reactive hydrogen, the hydroxymethyl group will form a methylene bridge

- The final result **of** formaldehyde fixation is a pr**of**ound change in the conformation **of** macromolecules, which could make the recognition **of** proteins (Ags) by Abs impossible or, at best, difficult
- Overfixation can produce false negative results in IHC from excessive cross-linksز
- under-fixation can also produce unexpected results

Overfixation

- Long-term storage **of** formalin-fixed tissues in alcohol will stop the formation **of** additional cross-links and, therefore, will have a beneficial effect in Ag detection if these tissues are needed eventually for immunohistochemistry.
- Overfixation can also be partially corrected by soaking tissue in concentrated ammonia plus 20% chloral hydrate.

- the use **of** 10% buffered formalin will produce more cross-links than non-buffered formalin and therefore will have more deleterious effects for immunohistochemistry.
- The duration **of** fixation can alter immunohistochemica reactions, resulting in failure to detect an Ag, weak reaction, increased background, detection **of** the Ag in an unexpected cell compartment, or altered cross-reactivity.

Other fixatives

- Many **of** the formalin substitutes are coagulating fixatives that precipitate proteins by breaking hydrogen bonds in the absence **of** protein cross-linking. The typical non–cross-linking fixative is ethanol.

Ag Retrieval

- AR is particularly necessary when tissues are fixed in cross-linking fixatives.
- Approximately 85% **of** Ags fixed in formalin require some type **of** AR to optimize the immunoreaction

AR with enzymes

- Many enzymes have been used for this purpose, including trypsin, proteinase K, pronase, ficin, and pepsin.
- The PIER mechanism is probably digestion **of** proteins, but this cleavage is nonspecific and some Ags might be negatively affected by this treatment.
- The effect **of** PIER depends on the concentration and type **of** enzyme, incubation parameters (time, temperature, and pH), and the duration **of** fixation

disadvantages of PIER

- The disadvantages of PIER are the rather low number of Ags for which it is the optimal AR method, possible alteration of tissue morphology, and possible destruction of epitopes

Heat-induced epitope retrieval

- the chemical reactions between proteins and formalin can be reversed, at least in part, by high temperature or strong alkaline hydrolysis.
- Heating can unmask epitopes by hydrolysis **of** methylene cross-links.
- Tissue-bound calcium ions might be important in masking some Ags during fixation. Calcium chelating substances (e.g., EDTA) are sometimes more effective than citrate buffer in AR
- not all Ags benefit from AR, even after prolonged formalin fixation.

Miscellaneous AR methods

- Pretreatment with concentrated formic acid improves the signal in some IHC tests.
- Another AR method is incubation **of** slides in strong alkaline solution, urea, acid solutions, borohydride, and a solution **of** sucrose.

Detection Systems

- labels (reporter molecules) are attached to the primary, secondary, or tertiary Abs **of** a detection system to allow visualization **of** the immune reaction
- The most commonly used labels are enzymes (e.g., peroxidase, alkaline phosphatase, glucose oxidase).

Direct methods

- The reaction is a one-step process with a primary Ab conjugated with a reporter molecule.
- The method is quick but lacks sufficient sensitivity for the detection **of** most Ags in routinely processed tissues.

Indirect methods

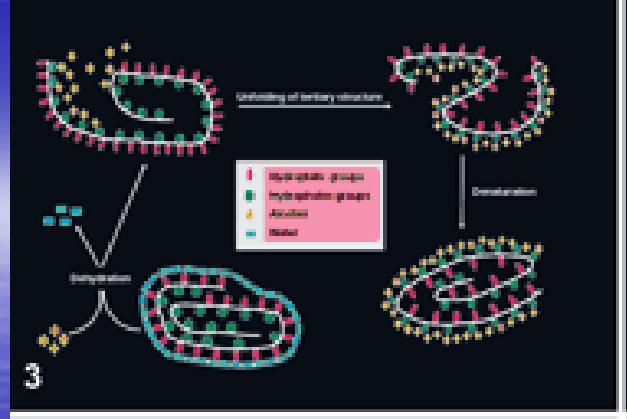
- *Avidin–biotin methods*:

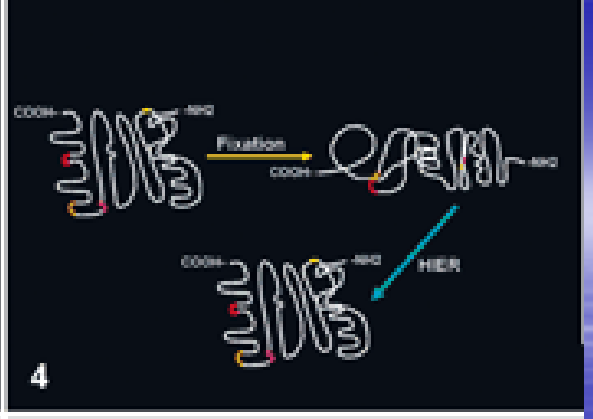
  - ABC immunohistochemical method.

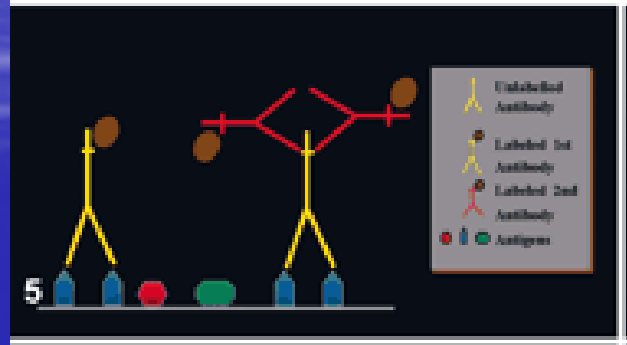
  - Labeled streptavidin (LSAB)–peroxidase method.

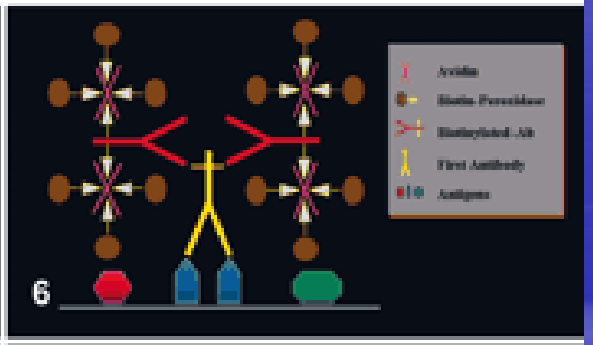
  - Peroxidase–antiperoxidase (PAP) method.

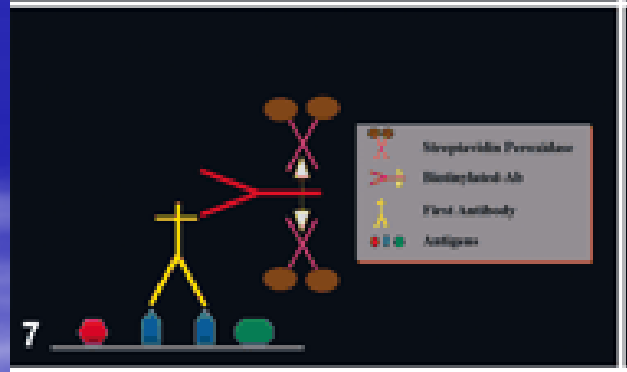
  - Two-step polymer-based immunoperoxidase. The secondary reagent has many molecules **of** label and secondary antigen attached to a polymer backbone.

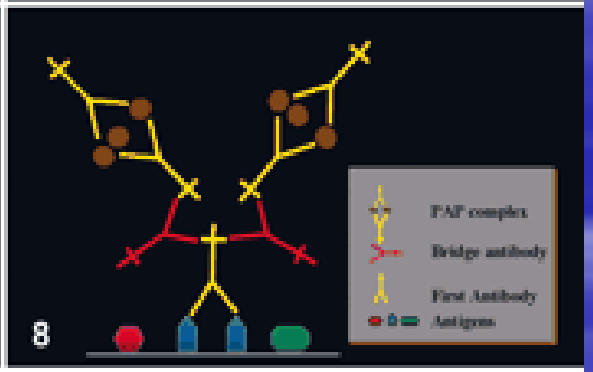

Unfolding of tertiary structure
Denaturation
Hydrophobic groups
Hydrophobic groups
Alcohol
Water
Dehydration
3

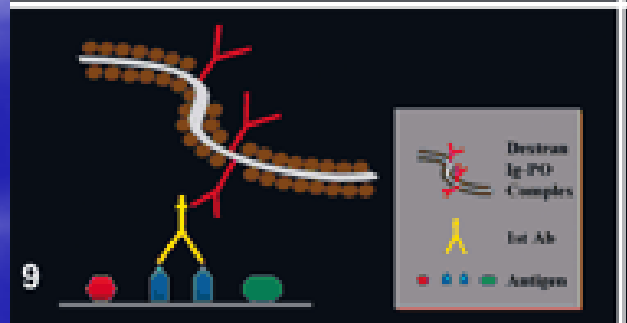

COOH-
-NH2
Fixation
COOH-
-NH2
COOH-
-NH2
HIER
4

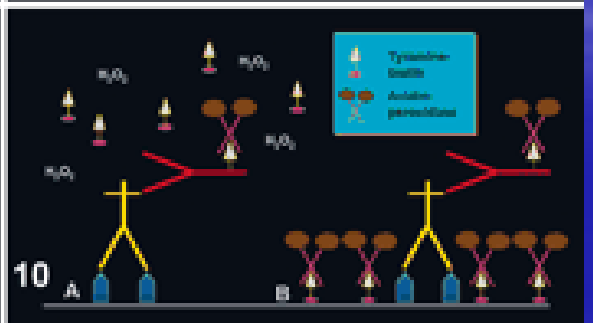

Unlabelled Antibody
Labeled 1st Antibody
Labeled 2nd Antibody
Antigens
5


Avidin
Biotin-Peroxidase
Biotinylated Ab
First Antibody
Antigens
6


Streptavidin Peroxidase
Biotinylated Ab
First Antibody
Antigens
7


PAP complex
Bridge antibody
First Antibody
Antigens
8


Dextran
Ig-PO
Complex
1st Ab
Antigen
9


Tyramine-
biotin
Avidin-
peroxidase
A
B
10

Polyvalent detection systems

- The main difference from monospecies detection systems is that the secondary reagent is a cocktail **of** Abs raised against Igs from different species.
- allowing one secondary reagent to be used for both polyclonal (e.g., from rabbit and goat) and monoclonal (e.g., from mouse) Abs.

# of Ag Increasing the sensitivity detection

- by increasing the incubation time **of** the primary Ab, or enhancing the intensity **of** the chromogen precipitate.

Causes of Background Staining in Immunhistochemistry

- **Background produced by hydrophobic interactions of proteins**:

-The increased hydrophobicity **of** proteins during fixation increases the background staining in immunohistochemical procedures.

-This background staining from overfixation can be remedied by postfixation with Bouin's, Zenker's, or B5 fixatives.

- the most common method to reduce background from hydrophobic interactions is the use **of** blocking proteins prior to incubation **of** the primary Ab

Background produced by ionic and electrostatic interactions

- Nonimmune binding **of** Igs to tissues or cells with negative charge (e.g., endothelium, collagen) can be blocked effectively by diluent buffers with high ionic strength.
- AR with 1% zinc sulfate, 0.01 M citrate (pH 6.0), or 0.01 M Tris (pH 9.0) can result in nonspecific nuclear staining

Endogenous peroxidase activity

- Enzyme activity naturally present in red blood cells, granulocytes and neurons can react with DAB to produce a brown product indistinguishable from specific immunostaining.
- In tissue sections with abundant hemorrhages or with acid hematin, a stronger (10%) solution **of** H_2O_2 might be needed to remove this endogenous activity, or a longer incubation in less concentrated solutions.
- Use **of** H_2O_2–methanol is not recommended for specimens in which cell surface Ags are to be detected

Endogenous alkaline phosphatase

- Two isoenzymes **of** AP in mammalian tissues can produce background staining with AP methods: intestinal and non-intestinal forms.
- The nonintestinal form is easily inhibited by 1 mM levamisol (L-tetramisole).
- The intestinal is**of**orm can be blocked with 1% acetic acid, but it can damage some Ags.

Avidin and biotin as sources of background

- The high ionic attraction **of** basic egg white avidin for oppositely charged cellular molecules such as nucleic acids, phospholipids, and the glycosaminoglycans in the cytoplasm **of** mast cells could result in nonspecific binding.
- Substituting avidin from egg white with streptavidin (from *Streptomyces avidinii*), which has a pI at a pH **of** 5.5–6.5, reduces significantly the nonspecific binding in IHC methods.
- Binding **of** avidin used in detection systems to endogenous biotin can produce strong background and needs to be inhibited.

- This binding can be suppressed with alkaline buffers, preincubation **of** tissue sections with unlabeled avidin and biotin, or incubation with nonfat dry milk .
- Some commercial kits containing 0.1% **of** streptavidin and 0.01% **of** biotin block this endogenous activity. This can interfere with interpretation **of** nuclear Ag staining (proliferation markers, herpes infections).

Free aldehydes

- False positive staining might result from the non-specific attachment **of** conjugated Abs to free aldehyde groups introduced by aldehyde-containing fixatives present in the tissue.
- prolonged fixation in formaldehyde can also produce free aldehydes.
- bolish by
- (sodium borohydride, ammonium chloride, ammonium carbonate buffer, lysine, glycine).

Fc receptors

- Fc receptors **of** mononuclear blood cells can bind to IgG **of** antisera.
- Nonspecific staining can also happen in paraffin sections because **of** attraction **of** the Fc portion **of** Igs to basic groups present in collagen fibers.
- The use **of** F(ab')2 fragments **of** the Igs instead **of** the whole Ig molecule eliminates nonspecific

Nonspecific Ag diffusion

- Diffusion **of** soluble proteins from their constituent cells and their nonspecific sequestration by other cells **of** different lineage or the cell interstitium is a common problem in thyroglobulin detection; it can be observed also with myoglobin, glial fibrillary acidic protein, and other cellular proteins.

Pigments

- Tissues with abundant melanin or ferrous pigment, such as hemosiderin, can reduce the signal-to-noise ratio **of** immunocytochemical reactions
- Giemsa stain.
- potassium permanganate to block melanin

Questions ??

Lecture #12

Infectious Bovine Keratoconjunctivitis
(Pinkeye – Contagious Ophthalmia- IBK)

PRESENTED BY:

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History of IBK

- It was first reported by Billings in Nebraska in 1988.
- The infectious nature of the disease was reported in England in 1897.
- IBK affects all breeds of cattle throughout the world and characterized by excessive lacrimation, conjunctivitis and keratitis.

History of IBK (Cont'd)

The losses of beef cattle industry in US are estimated to be $ 150 million annually (10 million calves and 3 million feedlot cattle are affected annually).

CAUSES

- ***Moraxella bovis***

Both of :

- Ultraviolet light.
- Infectious bovine rhinotracheitis virus.

Have been incriminated as predisposing factors.

- Ultraviolet irradiation and inoculation of *M. bovis* produce the disease experimentally in calves.

Incidence:

- Herford cattle and Herford crossbreds appear to have a much higher susceptibility.
- Increased risk of clinical disease for younger cattle and increased severity of the disease in calves are consistent findings.
- IBK has a high morbidity rate and a low mortality rate.
- IBK could occur throughout the year but most prevalent during the warmer months.

Clinical Signs:

Earliest signs :

- Epiphora.
- Blepharospasm.
- Photophobia.
- Conjunctival hyperamia and edema (bulbar).
- No corneal lesion at this time (Does not retain fluorescein stain).

Clinical Signs (Cont'd):

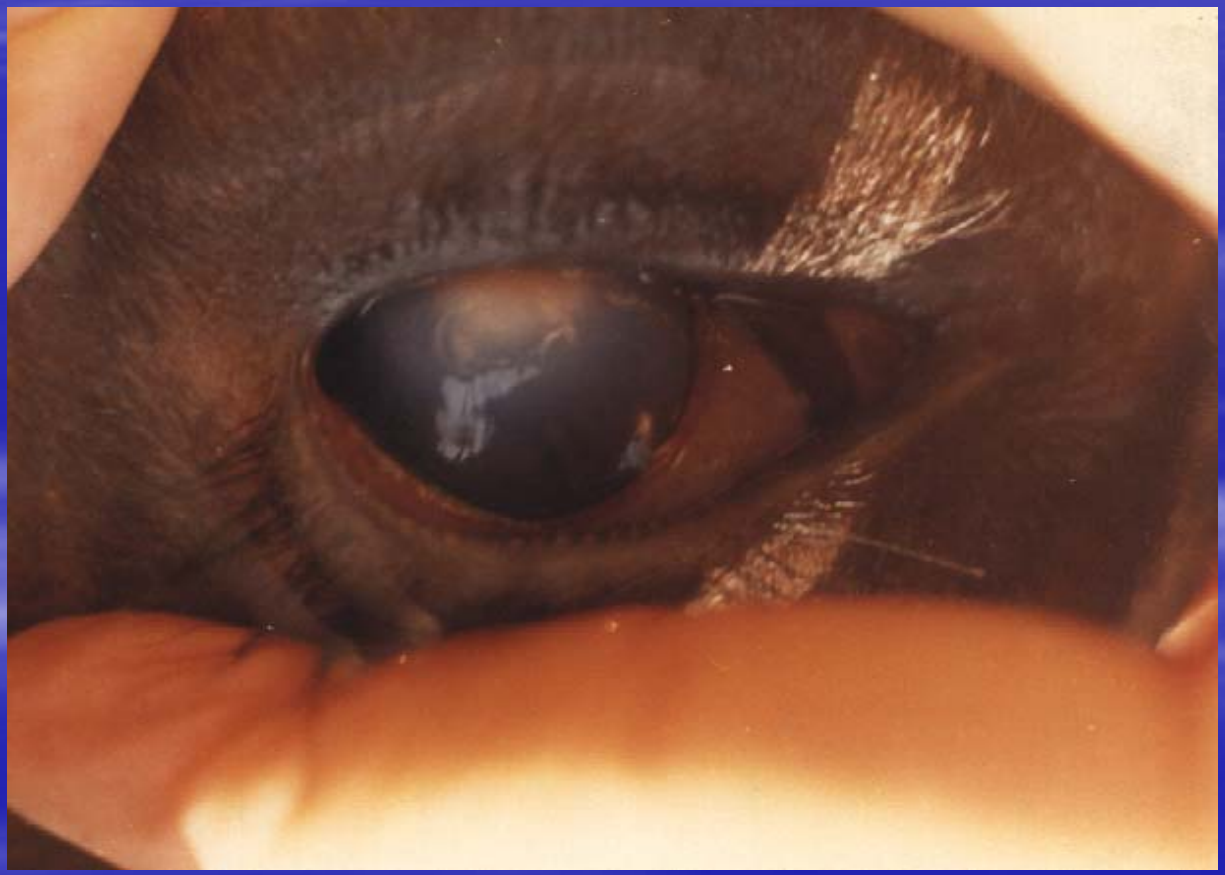
After 48 hours:

- Small Corneal abscess (at the center of the cornea) as a pale yellow/ white raised are.
- Decrease in the corneal integrity staining of the cornea with fluorescein.

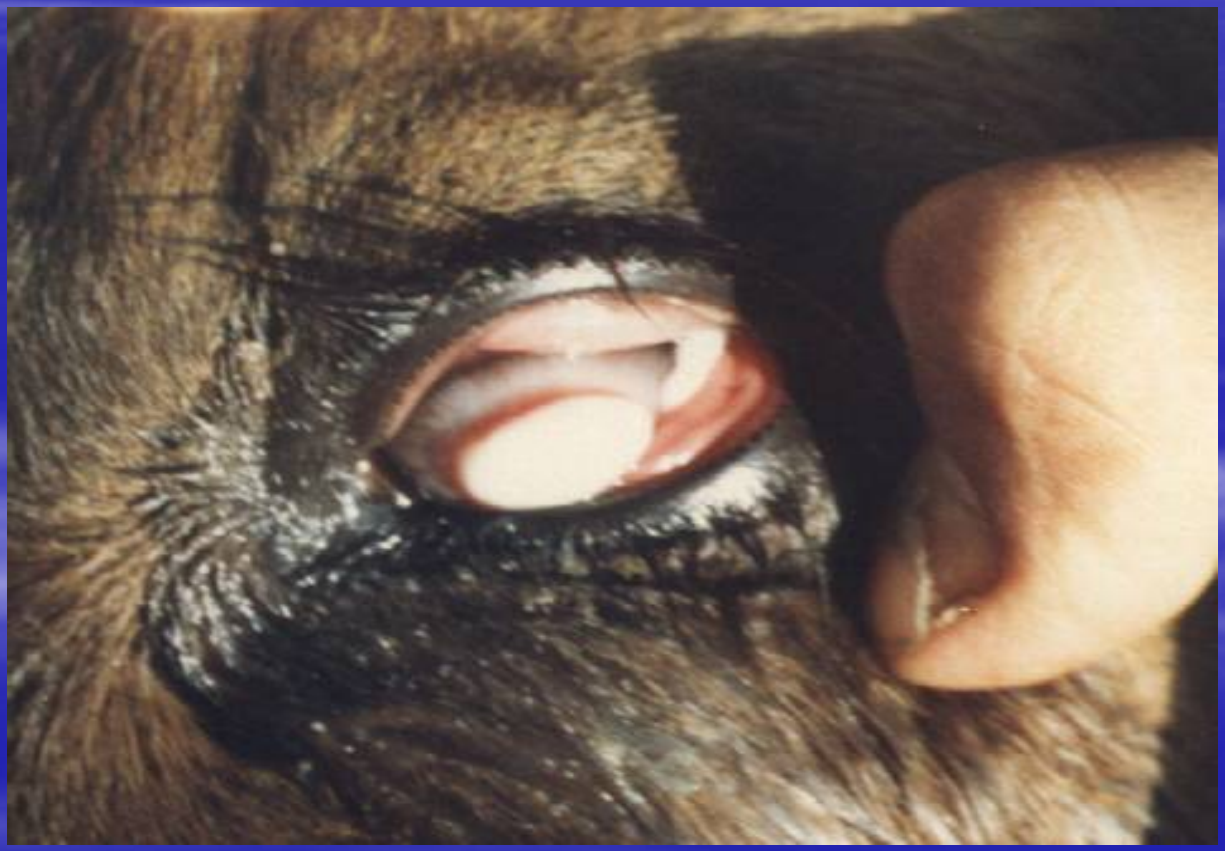
Clinical Signs (Cont'd):

- Within the next 24-48 hours, corneal opacity increase in size, or slove leaving shallow ulcer that could be stained with fluoresein.
- Mild to moderate aqous humor flare and irridocyclitis.
- Corneal opacity develops.
- Hypopyon.
- Corneal vascularization.

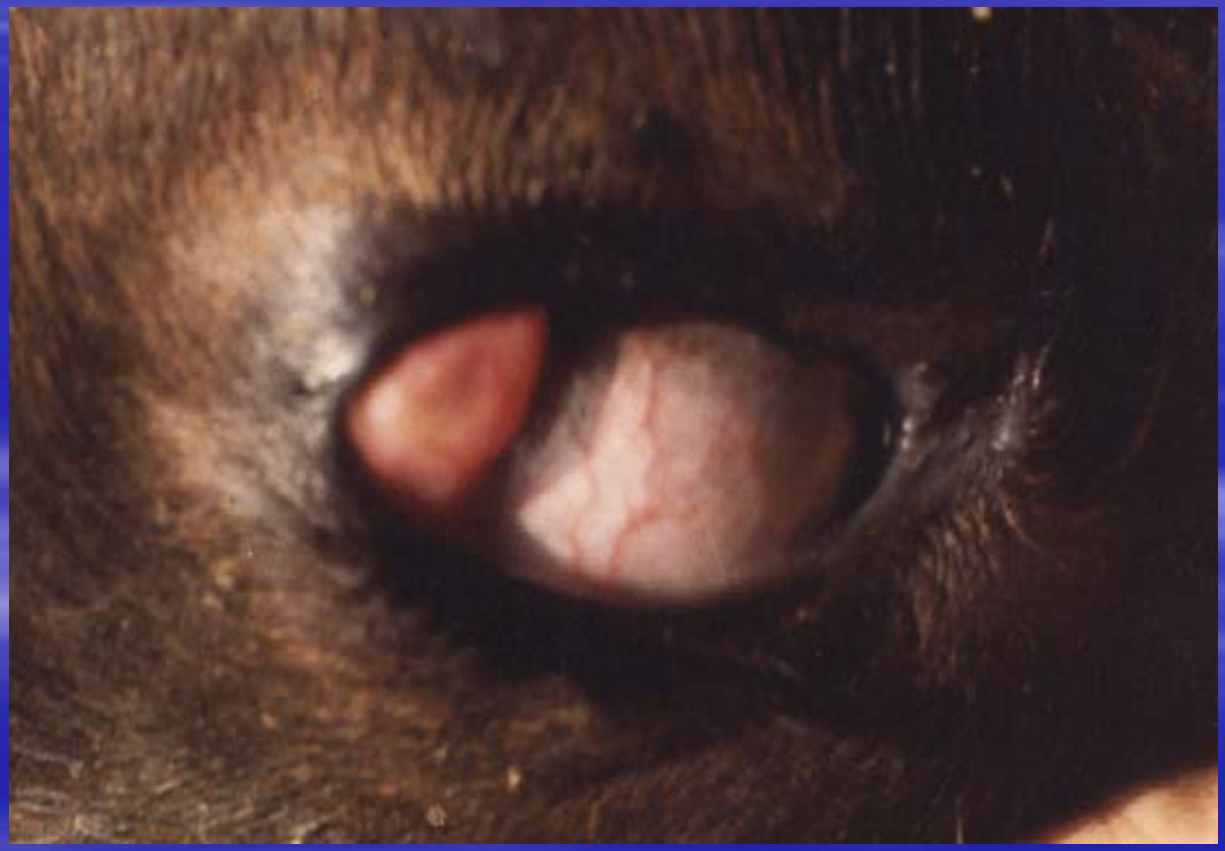
Clinical Sings (Cont'd)



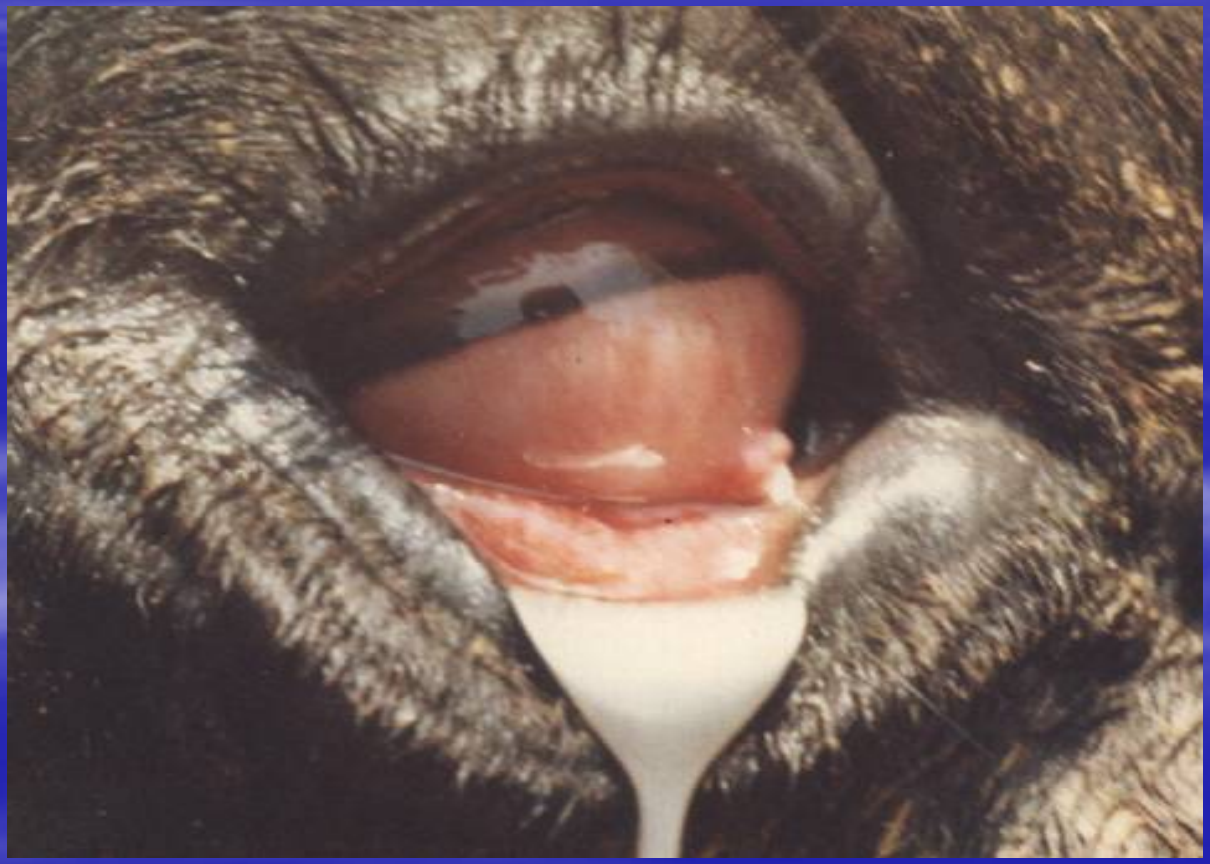
Clinical Sings (Cont'd)



Clinical Sings (Cont'd)



Clinical Sings (Cont'd)



Clinical Sings (Cont'd)

Differential Considerations

- **Malignant catarrhal fever**

  (generalized corneal opacity, Systemic ill).
- **Infectious bovine rhinotrachitis**

  (Conjunctivitis, peripheral corneal opacity,

  usually no ulceration- also have respiratory signs).
- **Any cause of keratitis or conjunctivitis as grass , trauma, etc.**

Treatment of IBK

- **Medicinal Treatment.**
- **Surgical Treatment.**

Treatment of IBK (Cont'd)

- **Medicinal Treatment:**

  - Moraxilla bovis is susceptible to most antibiotics and antiseptic solution.
  - Pencillin, ampicillin, oxytetracycline, neomycin & erthromycin have been recommended by various authors.

Treatment of IBK (Cont'd)

- Equally important as the choice of antibiotic is the route of therapy:

Topical (Highest level – Frequent application).

Subconjunctival administration:
(more prolonged level - bulbar conjunctiva)

Parenteral administration: more prolonged level

Treatment of IBK (Cont'd)

Suggested protocol

- Parenteral long acting Oxytetracycline (20mg/kg), Treatment of contact animals will reduce the incidence of new cases).

- Subconjunctival administration of procaine penicillin G or kanamycin (100 mg).

- Steroid application (Subconjunctival injection of 1: 1 combination of antibiotic and steroid).

- Topical Atropine 1-2% several times a day (Cycloplagia).

- Protection from sunlight and dust.

Treatment of IBK (Continue)

- **Surgical treatment:**

  Nictitating Membrane Flap

  (Deep # perforated corneal ulcers).

- The flap provides a mechanical support to the diseased cornea to seal the deep ulcers (temp. – metabolism).
- Local analgesia.
- The membrane is sutured to the dorsolateral bulbar conjunctiva of the upper eyelid).
- Use chromic catgut (No.1), tension device (polyethylene tubing, buttons.

Treatment of IBK (Continue)

- **Surgical treatment:**

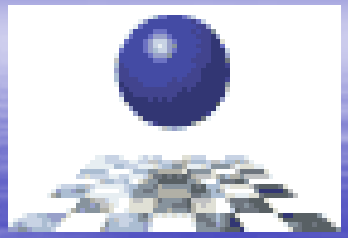
  Temporary complete tarrsorraphy

  (extensive cornel lesions).

- Four to six interrupted horizontal mattress sutures.
- The sutures should not penetrate the full thickness of the eyelids.
- The sutures should be positioned at the level of the opening of the mebiomian glands.

Vaccination- Control

- Partial protection results from vaccination
- Vaccinated cattle showed reduced incidence of IBK and reduced severity of ocular lesion.
- Some commercially available vaccine as BovEye (*Norden*) , Piliguard (*Schering*).
- Routes of administration either subcut or subconjunctival. (Studies showed that subcutaneous vaccination offered better results than that subconjunctivally).



GOOD LUCK

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QUESTIONS

Lecture #13

بسم الله الرحمن الرحيم

جامعة بنها
كلية الطب البيطرى
المركز الجامعي لأمراض الطيور والأرانب

Benha University
Faculty of veterinary Medicine

أنفلونزا الطيور

vian INFLUENZA

PRESENTED BY:

Prof Dr Amal Abdel Naser, Faculty of Veterinary Medicine.
Benha University,Egypt

- عرف هذا المرض في عام 1878 كمرض حاد وفوق الحاد يصيب الطيور الداجنة وكان آنذاك يسمى بطاعون الطيور .
- وفي عام 1930 عزل الفيروس المسبب لهذا المرض لأول مرة وفي عام 1955 تأكد العلماء من أن هذا الفيروس له علاقة بالفيروسات التي تسبب الأنفلونزا في الثدييات .

- فيروسات الأنفلونزا عبارة عن ثلاثة أنواع هم A,B&C
- النوع A يصيب الانسان والخنازير والخيول والطيوروبعض الثدييات المائية.
- النوع B والنوع C يصيب الانسان فقط.

فيروسات النوع الأول أو A قسمت من حيث الضراوة إلى:

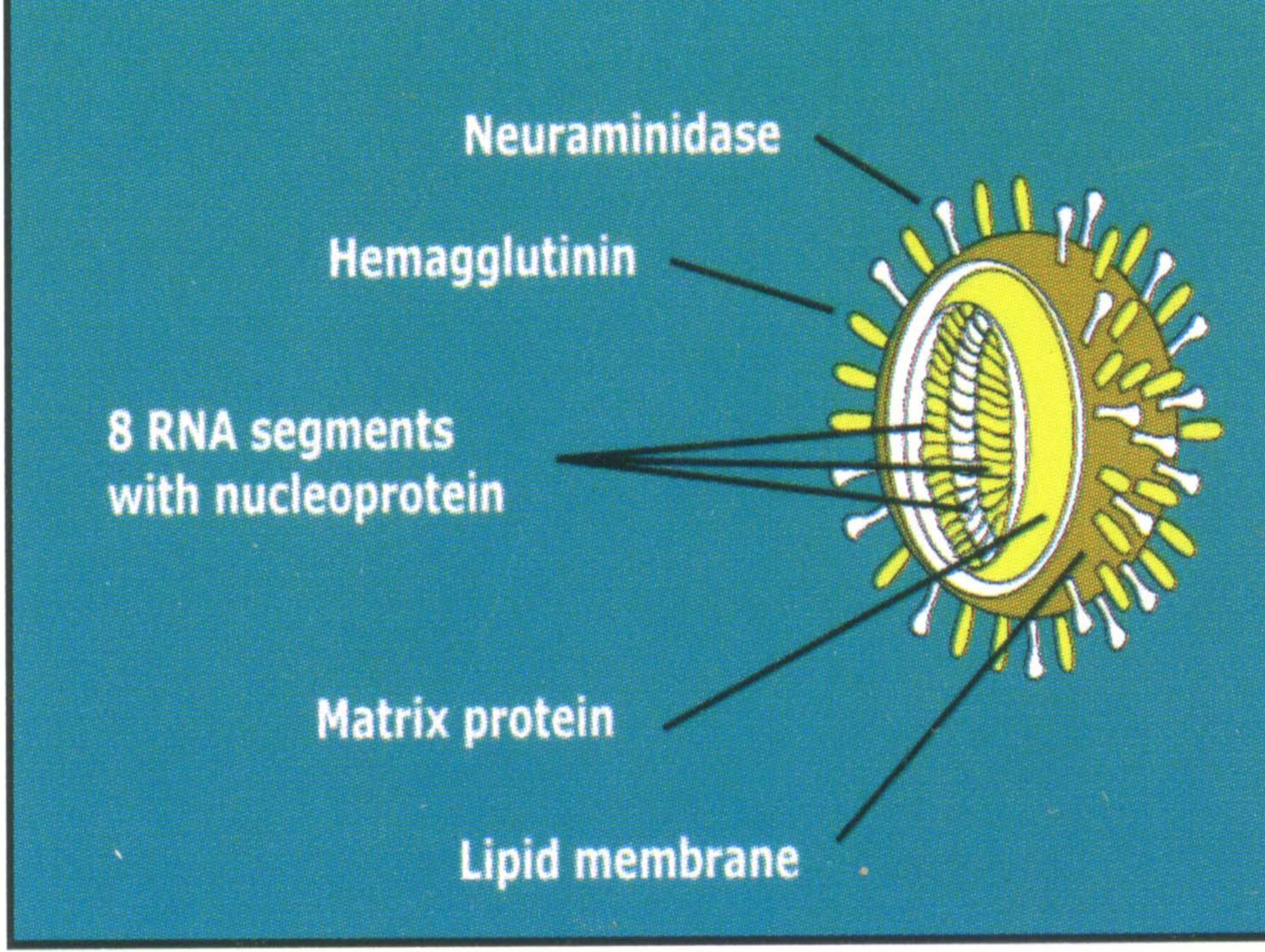
- عترات شديدة الضراوة
- **Highly Pathogenic Avian InfluenzaViruses**
- عترات ضعيفة الضراوة
- **Low Pathogenic Avian Influenza Viruses**

- يسبب المرض فيروسات من عائلة الأرثو ميكزوفيريدي وهى مغلفة RNA ، سطح الفيروس مغطى بنوعين من النتوءات البروتينية H&N . يوجد 16 أنتيجين من نوعH و 9 أنتيجين من نوع N.

- وجد أن الطيور المائية والطيور البرية المهاجرة تكون مستودع لفيروسات الأنفلونزا شديدة الضراوة ولا تسبب لها أي أعراض إكلينيكية .

•ولكن هذه الفيروسات إذا انتقلت للدواجن التي تربي تحت نظام التربية المكثفة لأغراض تجارية مثل الدجاج والرومي تسبب لها مرض أنفلونزا الطيور شديد الضراوة.
ويستمر إفراز الفيروس من هذه الطيور بعد شفائها من المرض لمدة ثلاث إلى أربع أسابيع وكذلك تنتقل هذه الفيروسات ميكانيكيا .


INFLUENZA VIRUS PARTICLES


Neuraminidase
Hemagglutinin
8 RNA segments
with nucleoprotein
Matrix protein
Lipid membrane

• الأوبئة في الإنسان

•الوباء الأول

• ظهر في عام 1918-1919 وسمي بالأنفلونزا الاسبانية (A/H1N1) .

•ونتيجة لهذا الوباء توفي أكثر من نصف مليون شخص في الولايات المتحدة الأمريكية ومن 20-50 مليون شخص توفوا في جميع أنحاء العالم .

• معظم الوفيات كانت في خلال الأيام الأولى لظهور المرض والباقي توفوا نتيجة لمضاعفات المرض ، نصف الذين توفوا تقريبا كانوا من صغار السن والبالغين الأصحاء .

•الوباء الثانى

• ظهر في عامي 1957-1958 وسمي بالأنفلونزا الاسيوية (A/H2N2) ظهر في الصين في فبراير 1957 ثم انتقل إلى الولايات المتحدة الأمريكية في يونيو من نفس العام وتسببت في وفاة 70 شخص.

الوباء الثالث

- ظهر في عام 1968-1969 وسمي بأنفلونزا هونج كونج (A/H3N3) وبداية ظهر في هونج كونج في مطلع 1968 ثم انتقل إلى الولايات المتحدة الأمريكية في نهاية العام ولازال منتشر إلى الآن.

ملحوظة : يوجد الفيروس و ينتشر بين الناس لعدة سنوات .

•أنفلونزا الطيور وانتقالها للإنسان

•أول مرة ثبت فيها أن فيروسات أنفلونزا الطيور يمكن انتقالها مباشرة من الطيور المصابة إلي الإنسان كان في هونج كونج في عام 1997 .

•أنفلونزا هونج كونج 1997:- ثبت بالدليل القاطع أن الفيروس انتقل من الطيور إلي الإنسان وتم احتجاز 18 حالة مصابة في المستشفي توفي منهم 6 حالات .

- والفيروس كان من نوع (A/H5N1) .تم إعدام 1.5 مليون دجاجة لكي يتخلصوا من مصدر الفيروس وجد أن الانتقال من شخص إلي آخر نادر الحدوث .

- وفي عام 1999 أيضا في هونج كونج أصيب طفلين بأنفلونزا الطيور (A/H9N2) وشفي الطفلين .

- وفي عام 2003 اكتشفت حالتين من أنفلونزا الطيور (A/H5N1) في شخصين من هونج كونج سافروا إلي الصين أحدهما شفي والأخر توفي.

- وأيضا في هولندا من نفس العام ظهرت حالات أنفلونزا الطيور (A/H7N7) بين الأشخاص الذين يعملون في مزارع الدواجن وعائلاتهم في أثناء وباء أنفلونزا الطيور في هذه المزارع توفي علي إثرها شخص واحد والأعراض كانت عبارة عن أعراض نفسية مع إصابة في العين .

- وأيضا في نفس العام تم احتجاز طفل في المستشفي في هونج كونج مصاب بأنفلونزا الطيور (A/H9N2) ولكنه شفي .

•الأعراض فى الإنسان

•تتراوح بين أعراض مميزة للإنفلونزا مثل الكحة آلام في الحلق رئوي وأعراض والعضلات إلي عدوي في العين والتهاب تنفسية حادة فشل في معظم أجهزة الجسم وقلة في خلايا الدم البيضاء.

• وبالتشريح بعد الوفاة للأشخاص الذين توفوا من المرض وجد أن الصفة الغالبة هي Reactive Hemophoagocytic Syndrome مع تنكرز في الرئة والكلي واستنفاد اللمف .

الأعراض فى الطيور:-

- تعتمد على عدة عوامل أهمها سلالة الفيروس وعمر ونوع الطائر والحالة المناعية له والمراض المصاحبة ونقص التغذية والعوامل البيئية(الغبار- الأمونيا- البرد)

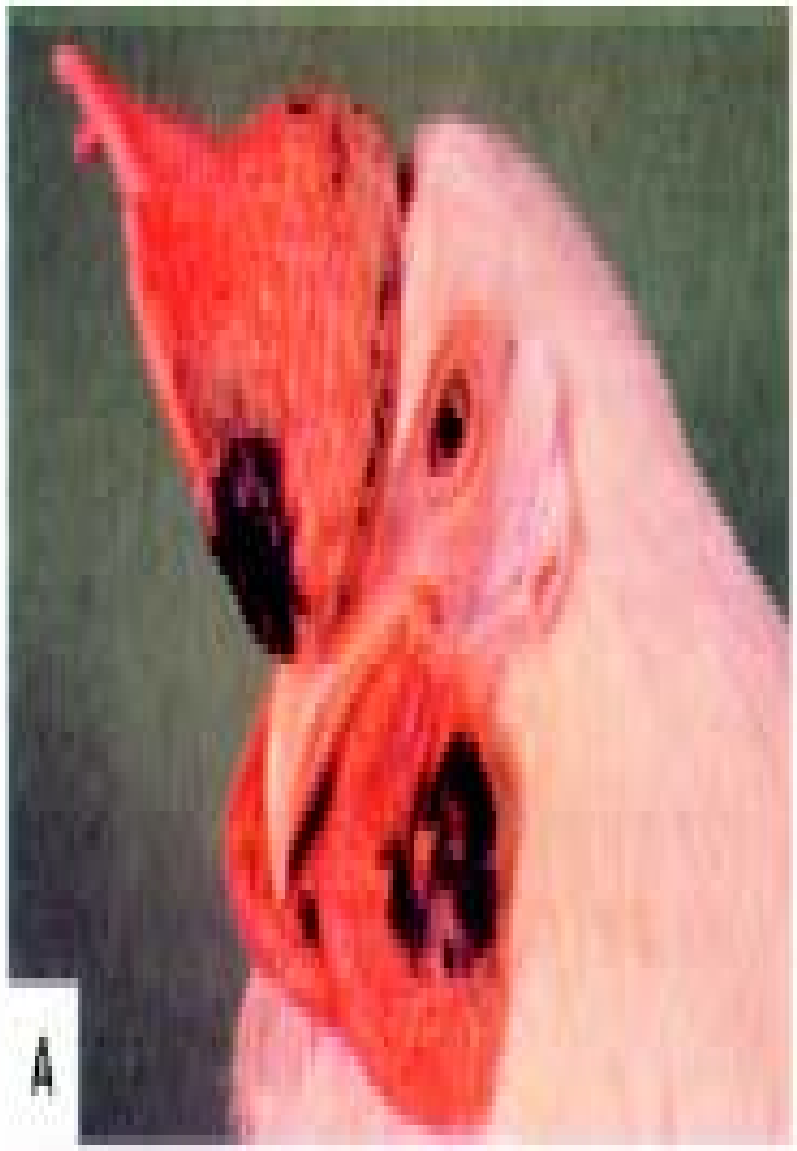
Broilar Breeder,HPLA, congestion&cyanosis of the comb and wattles.

caged layers,HPAI,prostration and reluctant to move in preagonic phase.

28-day-old turkey affected by LPAI, caseous deposits in the infraorbital sinuses

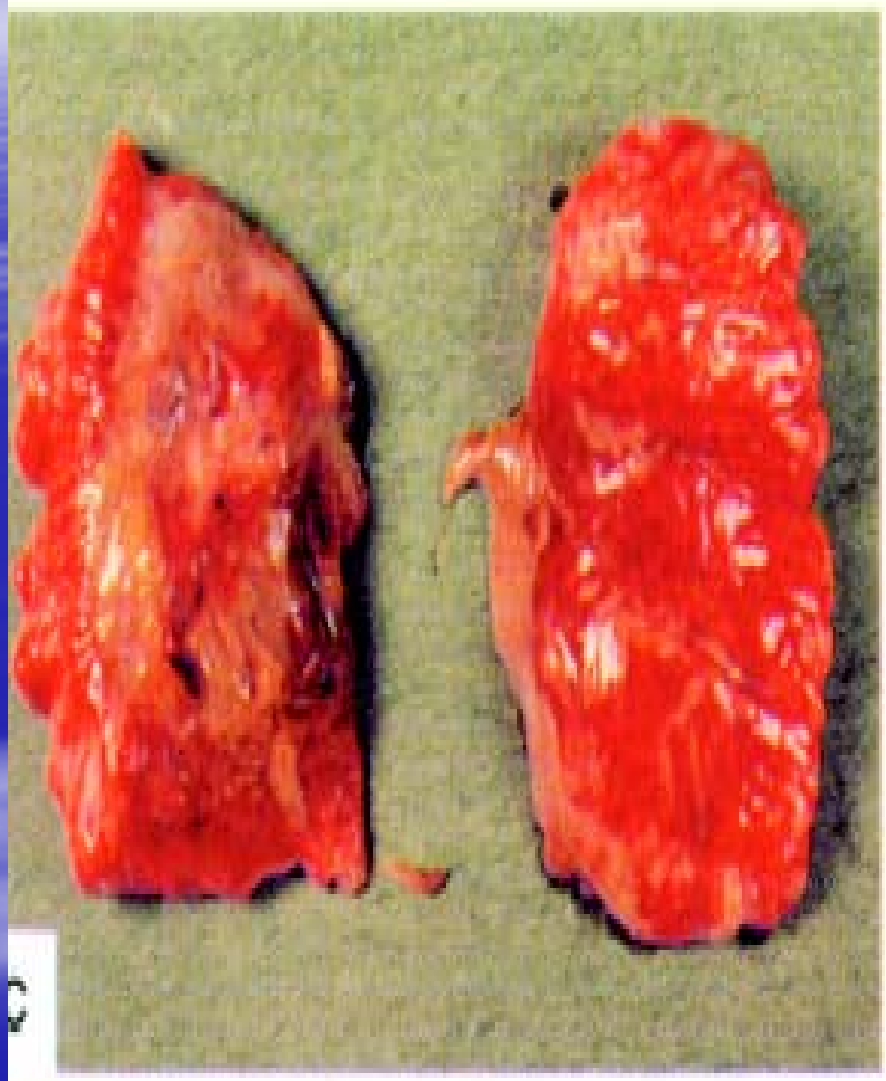
28-day-old poults affected by LPAI, severe conjunctivitis and swelling of infraorbital sinuses, note also general depression and ruffled feathers.

Hemorrhages and mutifocal necrosis of comb and wattles 7 days post infection



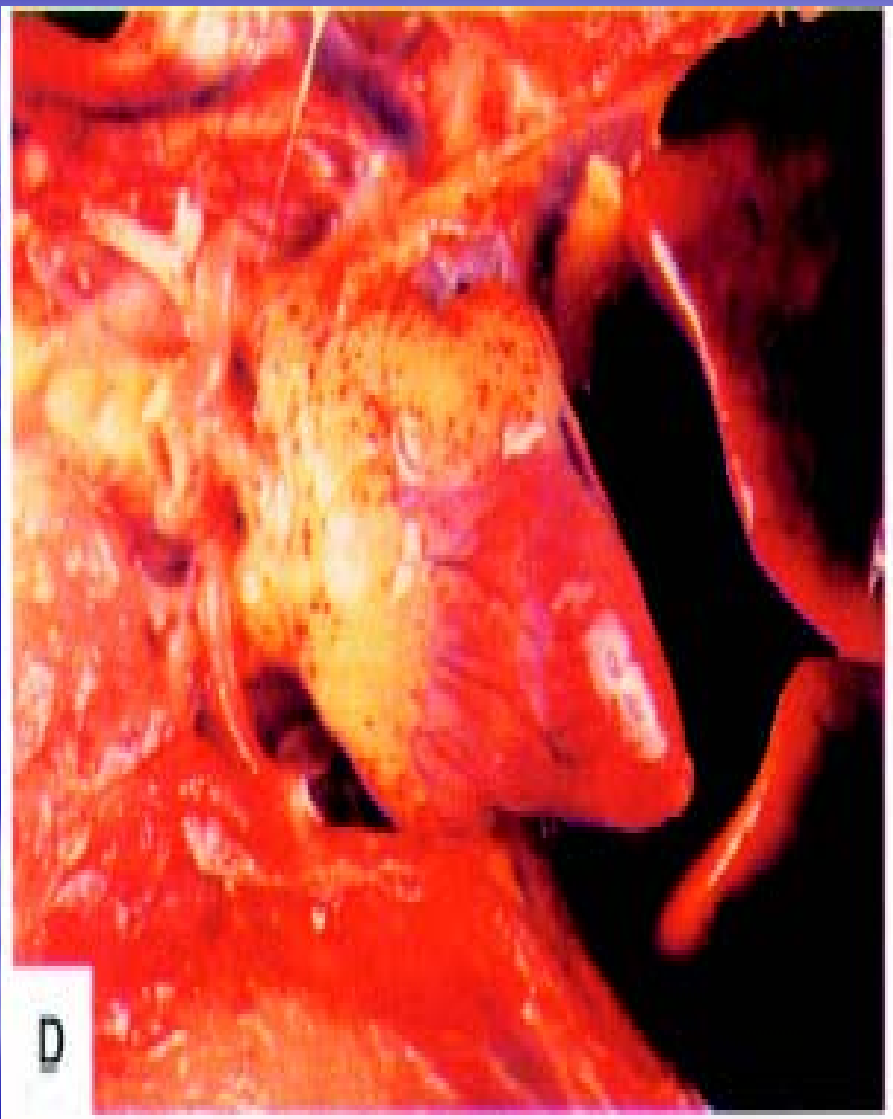
Lesions in adult WL . chickens, 47–59 weeks of age, exposed to HP A/chicken/NJ/12508/86 (H5N2) derivative influenza virus by the intranasal/intratracheal routes. A. Multifocal necrosis and hemorrhage of comb and wattles 7 days post infection (DPI). (USDA—Brugh)

Severe edema, necrosis, and hemorrhage of comb and wattles, 7 DPI. (USDA—Brugh).

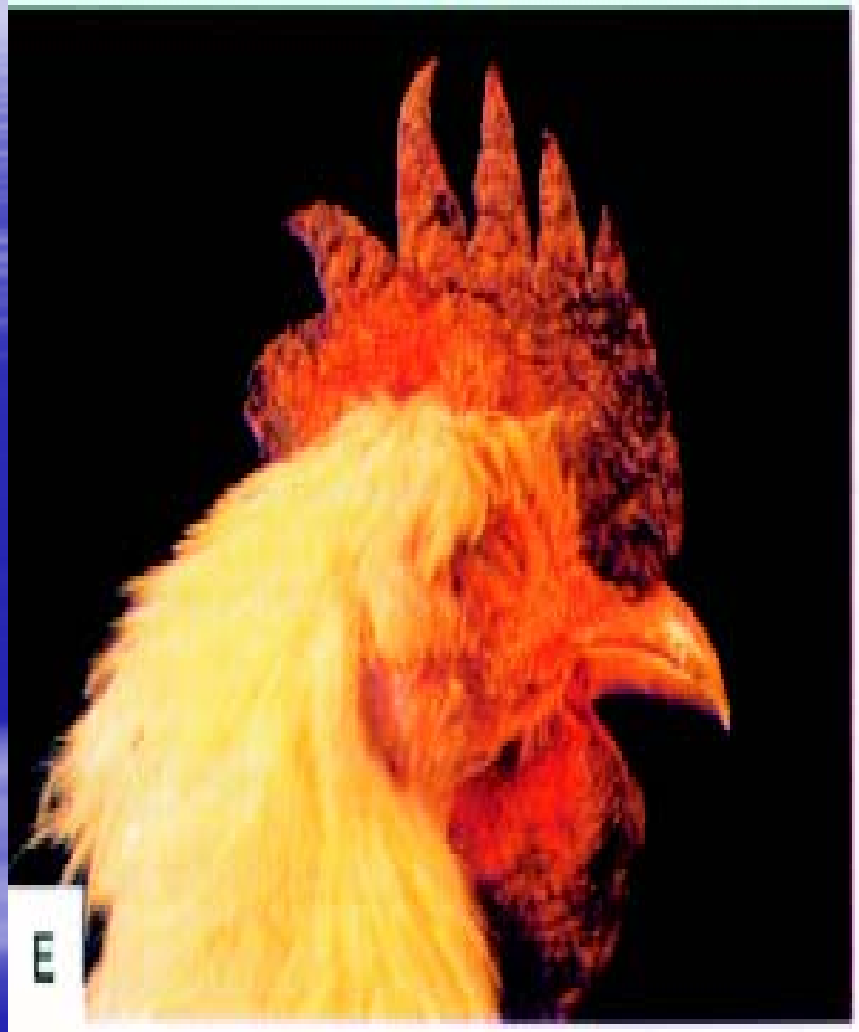


Bilateral ventral medial pneumonia with edema, 3 DPI.

(USDA—Brugh)



D. Petechial hemorrhages in epicardial fat, 4 DPI. (USDA—Brugh)



E. Severe necrosis of

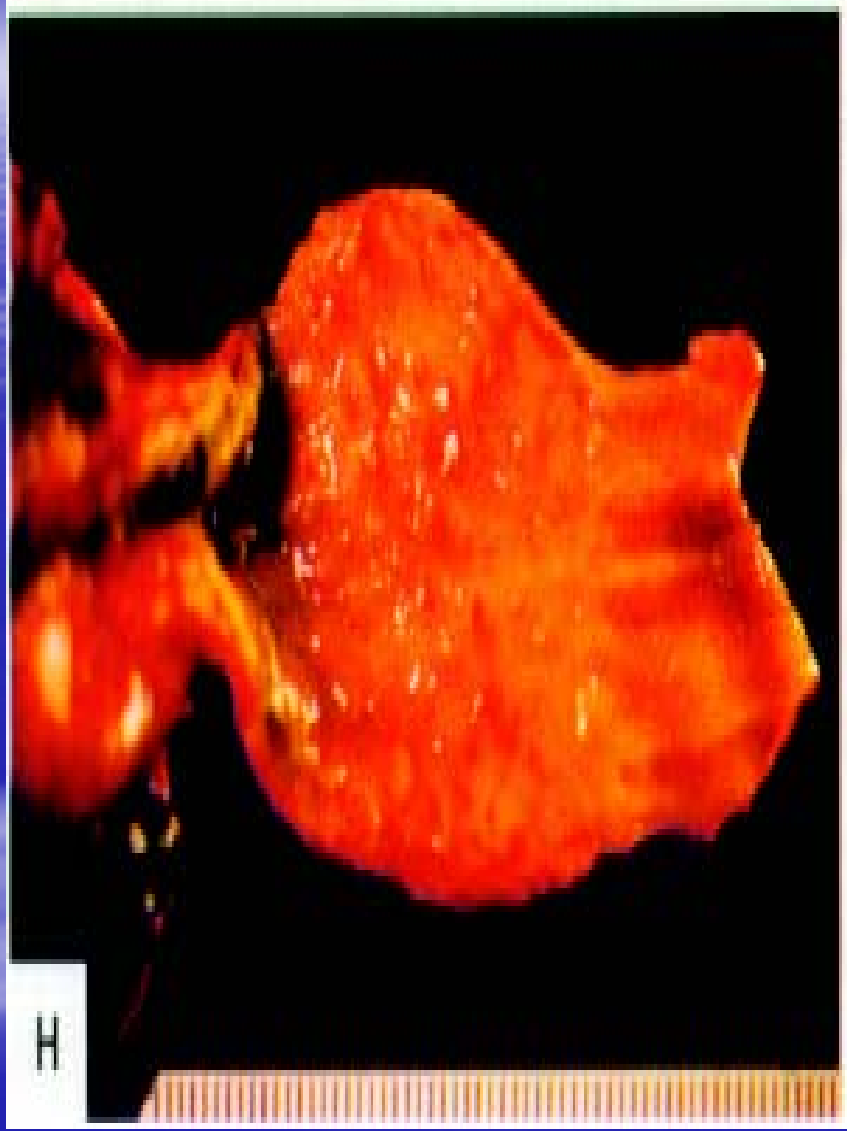
comb and wattles, 12-week-old WL, IN exposure, 4 DPI. (USDA—Swayne)

F. Severe edema and necrosis of comb and wattles,

week-old WL, IN - exposure, 4 DPI. (USDA—Swayne)

G. Severe subcutaneous hemorrhages of leg shanks, 4-weekold

WR, IV exposure, 4 DPI. (USDA—Swayne)



H. Petechial hemorrhages around the ducts of the proventricular glandular region, 16-week-old WL, IN exposure, 4 DPI. (USDA—Swayne).

•المرض ضعيف الضراوة

- **Low Pathogenic Avian Virus (LPAI) Influenza**

•**في العدوي الغير مصاحبة بأي إجهاد** : إنخفاض في إنتاج البيض أو التوقف نهائيا عن إنتاجه مع نقص 20% في نسبة الفقس ،أعراض تنفسية ،قلة في سحب العلف ،هبوط ،التهاب في الجيوب الأنفية ، معدل نفوق منخفض يتزايد مع الوقت ويصل إلي القمة عند اليوم السادس والسابع من بداية الأعراض .

- **فى العدوى المصاحبة للإجهاد** :نري أن الأعراض السابقة تزيد حدة معدل النفوق يمكن أن يصل من 60 إلي 70 % . البط والطيور المائية تكون مقاومة للمرض حتي عند الإصابة بالعترات شديدة الضراوة التي تصيب الدجاج هذه الطيور تكون حاملة أو مستودع للفيروس ولكن في حالة الإجهاد نري فيها التهاب في الجفون ،أعراض تنفسية ،زيادة في النفوق.

الصفة التشريحية فى الطيور :

- تعكس الأعراض الإكلينيكية وهي إما أن تكون غائبة أي صفات مرضية على الطائر النافق في الطور فوق الحاد . أو نري أنزفة واحتقان في القناة التنفسية والأحشاء الداخلية في الطور الحاد . أو التهاب في القناة التنفسية مع إفرازات صديدية إلي متجبنة في الجيوب الأنفية في الطور الضعيف .

- هذه الأعراض الإكلينيكية والصفات التشريحية لا يعتد بها ولابد من عزل الفيروس أو التعرف علي الأجسام المضادة الخاصة به لكي يتم تشخيص المرض.

- **عزل الفيروس :**

- في أجنة الطيور التأكد من الفيروس بواسطة اختبار تلزن الدم (HA) ثم اختبار مانع تلزن الدم (HI) أو باختبار البلمرة المتسلسل (PCR)

التحكم في المرض في الطيور Control in Birds

- يجب أن تكون علي مستوي دولي وهذا يحتاج إلي تشريعات مشددة لكي نحمى صناعة الدواجن وكذلك الإنسان من هذا المرض وخصوصا العترات شديدة الضراوة .

- وذلك بمنع التجارة في الدواجن ومنتجاتها مع الدول التى بها هذه العترات وكذلك ذبح كل الطيور الموجودة في المزارع والأسواق فى أماكنها مثلما حدث في هونج كونج 1997 .

- في ايرلندا عام 1984/1983 تم ذبح 270 ألف بطة ليست عليها أية أعراض إكلينيكية ولكن عزل منها الفيروسات شديدة الضراوة وبهذه الطريقة تم التخلص من المرض بسرعة وأيضا في نفس السنة تم ذبح 17 مليون طائر في أمريكا عندما انتشرت هذه العترات في بنسلفانيا وفرجينيا .

- بعد التخلص من كل الطيور يجب حرق أو دفن الفرشة في أماكنها وعدم نقلها ثم ينظف المكان جيدا ويطهر جيدا ويترك خالى لمدة أسبوعين ثم بعد ذلك يمكن استقبال طيور جديدة .

رعاية الطيور :

1. - يجب أن تكون الطيور من نوع واحد وعمر واحد.

2- يجب أن تكون المزارع بعيدة عن تجمع الطيور المائية أو البرية ولا تكون قريبة من بعضها البعض

3- منع الطيور البرية ما أمكن من الدخول إلي المزارع .

4- يجب أن تكون حركة الإنسان بين المزارع محدودة .

5- تطهير الأجهزة والأدوات المختلفة وكذلك التطهير الدوري لكل الأماكن التى تتعامل مع الطيور سواء كان بالبيع أو الذبح.

6- لا بد من تغيير ملابس العاملين في المناطق الموبوءة وكذلك أخذ الإجراءات الصحية السليمة عند الدخول إلى مزارع الدواجن.

7- يجب أن توضع جميع الطيور التي تظهر عليها أعراض تنفسية حادة تحت الحجر البيطري إلى أن تأتي نتيجة التشخيص المعملي.

8- يجب إجراء الاختبارات السيرولوجية على الطيور التي تعاني من العترات ضعيفة الضراوة أو الطيور المشكوك في أنها تحمل هذه العترات . وإذا ثبت تواجد هذه العترات لا بد من التخلص من كل الطيور لأن هذه العترات ضعيفة الضراوة ممكن أن تتحول إلى عترات شديدة الضراوة (Mutation)

التحصين في الطيور:

- توجد عدة لقاحات تجارية تستخدم في البلدان التي تعاني من هذا المرض مثل إيطاليا والولايات المتحدة والشرق الأقصى.

- هذه اللقاحات إما أن تكون حية أو ميته أو لقاحات مصاحبة وهذه اللقاحات منعت الأعراض الإكلينيكية والصفات التشريحية ولكن بعضها لم يمنع تكاثر الفيروس وإفرازه عند إجراء اختبار.

- **علاج أنفلونزا الإنسان:**

•الأدوية التي تستخدم لعلاج الفيروسات الخاصة بالإنسان يمكن أن تستخدم لعلاج الأشخاص الذين يعانون من أنفلونزا الطيور .

•ولكن هذه الفيروسات الخاصة بأنفلونزا الطيور نادرا ما تصيب الإنسان وممكن أن تكون هذه الأدوية غير فعالة لأن الإنسان لا يوجد لديه مناعة كافية ضد هذه الفيروسات.كذلك ممكن استخدام هذه الأدوية المثبطة لإنزيم النيورامينيديز .

•الإجراءات التى يجب اتخاذها فى حالة إصابة البشر بوباء انفلونزا الطيور فى المستقبل لا قدر الله

1-زيادة وعي العامة وخصوصا الأشخاص الذين يتعاملون مع الدواجن أو منتجاتها.

2- محاصرة المكان المصاب ومنع دخول أو خروج أي طيور حية أو نافقة أو الأشياء المتعلقة بها وكذلك منع حركة أي من البشر المتواجدين في المكان مع لبس الأقنعة الواقية والالتزام بتعليمات الصحة العامة.

3-التخلص من كل الطيور النافقة أو المصابة أو المخالطة لها والمجاروة لها وكذلك الفرشة والعلف وأطباق البيض... إلخ. إما عن طريق الدفن في حفر 2م عرض × 2م عمق ويوضع فوقها هيدروكسيد الكالسيوم ثم طبقة من الأرض حوالي 50 سم ثم محاولة أخذ عينة كل أسبوع لعزل الفيروس (Cannula) أو بالحرق أماالعلف ممكن تعريضه للتعفير

4-تطهير العنابر المصابة وتطهير المفقسات وحجرات تخزين البيض وتعبئته ونقالات البيض و مصانع منتجات البيض والشاحنات التي تنقل الطيور الحية والبيض والعلف كذلك غسيل وتطهير الجدران والأرضيات والأقفاص (المعالجة بالحرارة) والسقايات والعلافات وكذلك مخازن المياه وصوامع العلف.

الوضع الحالي :-

- آسيا هي القارة التي تعاني الآن من أنفلونزا الطيور أيضا عانت فيتنام من موت 63 شخص من أنفلونزا الطيور آخرهم كان في أكتوبر الماضي.
- أما الصين أغلقت كل أسواق الدواجن (168 سوق) وكذلك أسواق طيور الزينة (المنزلية) ومنعت بالقانون تربية الدجاج في المناطق الريفية وطلبت من المواطنين تربية الحمام في الأقفاص وكذلك تحصين كل الحيوانات بما فيها الحيوانات المنزلية ضد أنفلونزا الطيور ومرض الحمي القلاعية وهددت بأن الذي يمتنع عن تنفيذ ذلك ممكن أن يتعرض لإحدى العقوبتين الحجز أو الغرامة المالية.

- وفي الصين نفقت حوالي 9 آلاف دجاجة وتم التخلص من 369 ألف دجاجة في قطر 3 كيلومتر في مقاطعة (Liaoning) .
- كذلك أغلقت الصين أي مداخل تؤدي إلى أماكن الوباء لم ترصد أي حالة أنفلونزا طيور بين البشر في الصين للآن .
- كذلك أمرت السلطات في اليابان بالتخلص من 180 ألف دجاجة بعدما وجدت الأجسام المضادة بها.كما صرح الاتحاد الأوربي بان عترة H5N1 التي قتلت 60 شخص في آسيا وجدت في كرواتيا.

- هناك 41 حالة تم التأكد من إصابتها بأنفلونزا الطيور في أوبئة 2005/2004 تتراوح أعمارهم من 2-58 سنه، متوسط الأعمار في تايلاند وفيتنام 14 عام وفي كمبوديا 22 عام.
- حوالي 89 % من الوفيات في تايلاند كانت بين الأطفال أقل من 15 عام وعلل هذا بأن الأطفال يكونون على مقربة من الأرض يزحفون أو يمشون بلا أحذية في أرض بها زرق الدواجن وممكن أن يضعوا أيديهم الملوثة في فمهم علاوة على ان حالات الوفاة كانت فقط في المناطق الريفية المكتظة بالسكان والذين يعيشون جنبا إلى جنب مع الدواجن وحيوانات المزرعة الأخرى.

- الفيروس قليل الضراوة الذي يصيب الدجاج بدون أعراض ممكن أكل بيضه طالما تم تعريضه للحرارة الشديدة ويكون الصفار جامدا وكذلك الدجاج أمن طالما أنه يطهى جيدا والبيض يمكن أن يكون ملوث من الخارج (قشرة البيض) بالإفرازات المليئة بالفيروس (التنفسية والزرق).

المؤتمرات:-

1- المؤتمـر الاقتصـادي التعاونــي لآسيا والباسيفيك

Asia Pasific Economic Corporation (APEC)

في 31 أكتوبر تم الاتفاق على برامج لوقف حالات انفلونزا الطيور ومنع عبورها من دولة لأخرى وكذلك شراء وتخزين الأدوية المضادة للفيروسات . Tamiflu and Relenza . كما ركز زعماء هذه البلاد على الاستعداد وليس الذعر.

2- أيضا في نفس اليوم اجتمع ممثلو 53 دولة أفريقية في رواندا وركزوا على محاربة انتقال أمراض الحيوان عن طريق الحدود وبالذات أنفلونزا الطيور.

3- اجتمع في 7 نوفمبر 2005 في جنيف اكثر من 300 عالم وخبير في الصحة العامة والبيطريين ومسئولين من الحكومات لكي يتشاركوا فيما تعلموه وكذلك في التخطيط لما يجب عمله في المستقبل.


THANKS
FOR
YOUR ATTENTION

Contneu Lectuer #13

PREVENTION & CONTROL OF AVIAN INFLEUNZA

- Prevention
- Separation of infected birds & their secretions & excretions from susceptible ones by preventing the movement of personell , vehicles & equipments ….etc).
- Recovered flocks should be isolated from any other flocks or birds , once a flock is infected it should be considered a potential source of virus for life.

Reduce contact between wild birds & domestic sp.on open range

- LPM --- reservoir of AI virus
- Swine--- source of viruses transmmited mechanically or by infected people.
- Staff must be trained on lab. Tests necessary to monitor field infection in a vaccinated population, identify areas of failure & drift strains.

PRVENTION

- Education of the attendants regarding the introduction of viruses, their spread & how they can prevent such events.
- In HPAI---- quarantine, slaughter, disposal & clean-up.
- Control of AI infection in the animal reservoir.
- Also prevention & control of HPAI outbreaks are the most important steps tp prevent outbreaks of HPAI.

DETECTION OF THE DISEASE

- Must be prompt and complete is essential to the management of the pandemic potential---------- RESPONSIBLE RESPONSE

VACCINATION

- Inactivated influenza virus vaccines, effective in controlling clinical signs & mortality.
- Inactivated homologous vaccines, detection of field exposure only through sentinel birds (H5N1 vaccine against H5N1 field virus). Infected sentinel may be identified by clinical signs or serology.

Inactivated hetrologous vaccine

- Detection of field exposure by detection of antibodies to the N of field virus (H5N2 vaccine against H5N1 field virus). Vaccinated exposed birds will have antibodies to the N of the vaccine strains & of the field virus which is a marker of infection.

Recombinant influenza virus vaccines

- Recombinant adenovirus-AIV H5
- Recombinant NDV – AI- H7 vaccine
- Recombinant fowl pox- AI H5 vaccine
- Salmonella vector- a virulent deleted mutants, licensed for use as live vaccine in USA; vector for AI genes.
- Administred by i.n, slc and in ovo .

ADVANTAGES OF VACCINATION

- Enable the DIVA (differentiation between infected & vaccinated birds) concept.
- Increases resistance to field challenges.
- Reduce shedding of virus.
- Reduce transmission of virus.
- May not prevent infection.

IMPORTANT

- Vaccination & monitoring result in eradication plus appropriate management.

- Monitoring of the situation is essential to detect viral circulation & identify drifted strains

HUMAN INFECTION

- Direct handling of sick or dead birds or its secretions or excretions.
- Possible exposure –aerosols, direct hand to mouth & consumption of raw products mainly in young age.
- Types of poultry – village or LPM.

Lecture#14

Avian Influenza Surveillance and Diagnostics

PRESENTED BY:

Dr. Saed. Gharaibeh, FVM, JUST

Avian Influenza Surveillance and Diagnostics

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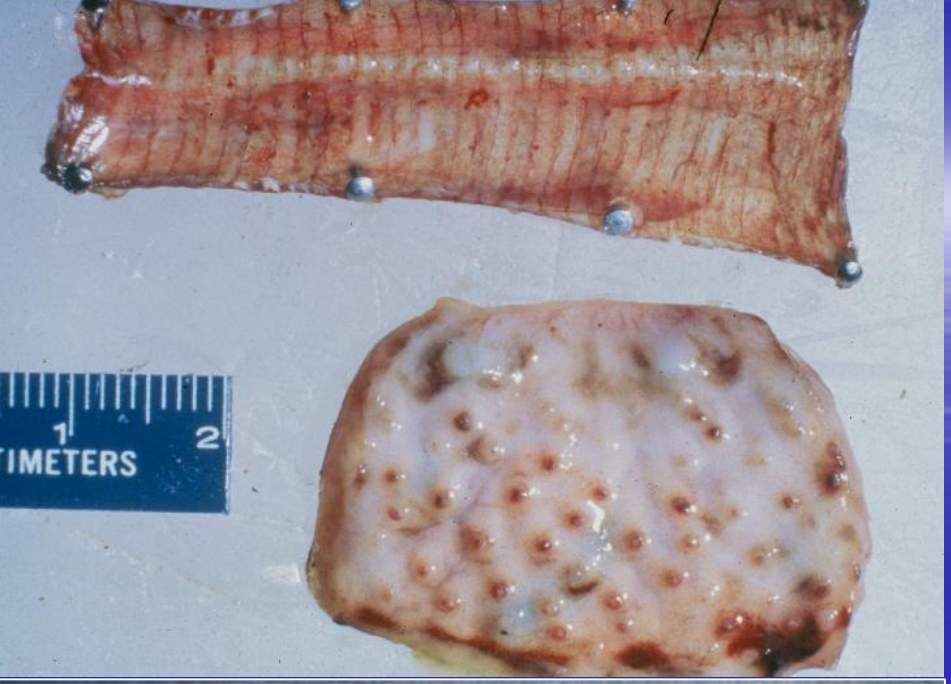
Surveillance Strategies

- The frequency of active surveillance should be at least every 6 months. Surveillance should be composed of random and targeted approaches using virological, serological and clinical methods.

- Random surveillance is conducted using serological tests. Positive serological results should be followed up with virological methods.

Types of Surveillance

- Clinical surveillance (detection of clinical signs)


1
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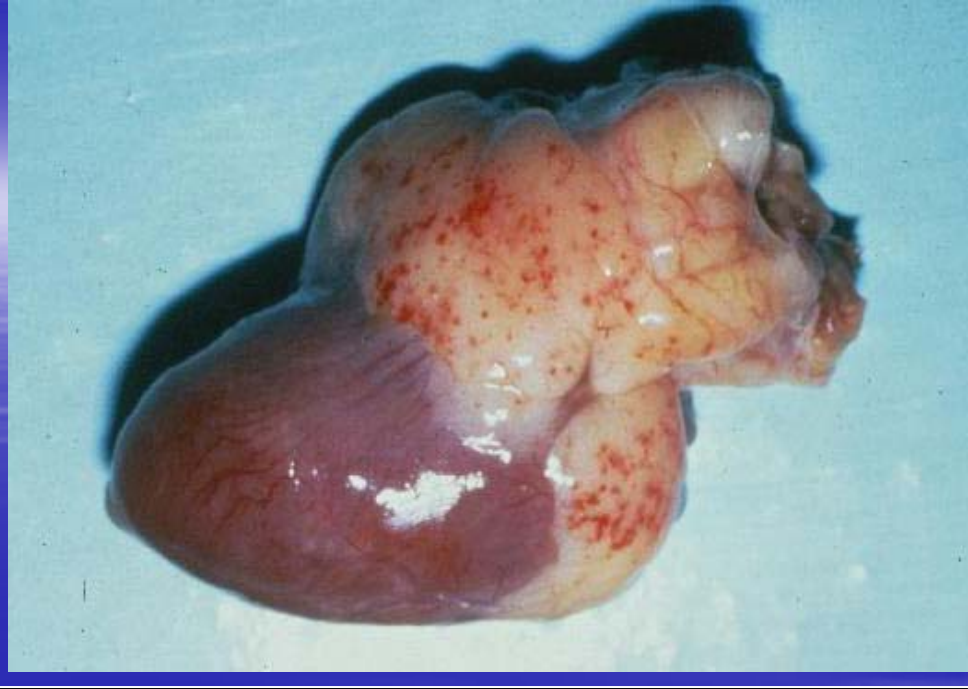
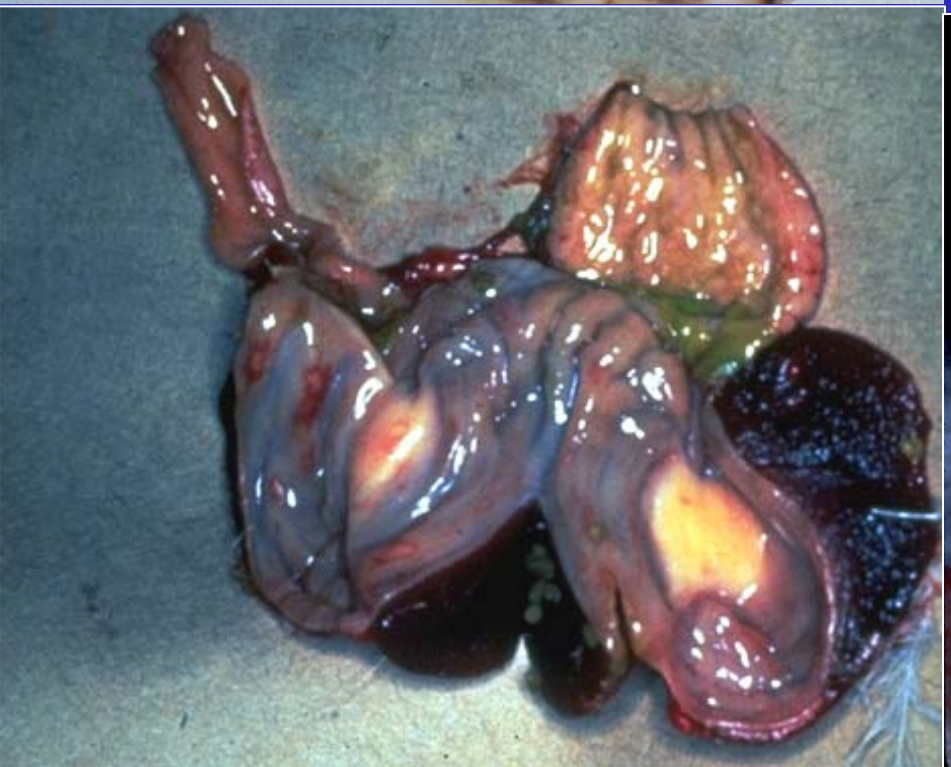
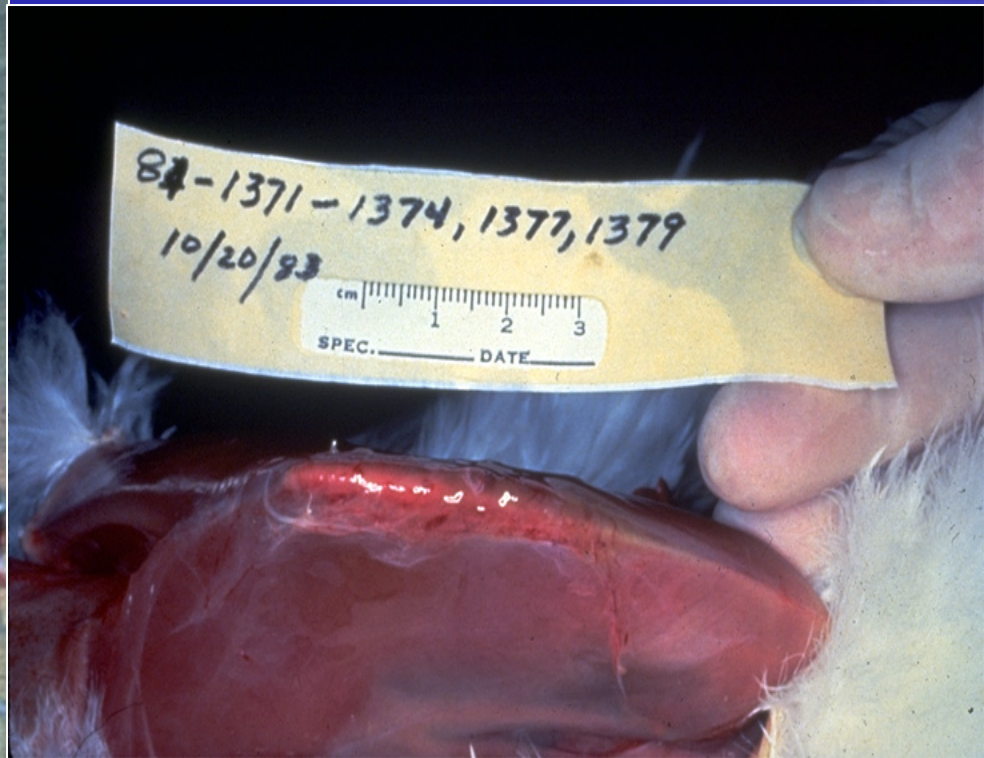
TIMETERS






84-1371-1374, 1377, 1379
10/20/83
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Types of Surveillance

- Virological surveillance:
  - to monitor at risk populations
  - to confirm clinically suspect cases
  - to follow up positive serological results
  - to test ‘normal’ daily mortality, to ensure early detection of infection in the face of vaccination

Types of Surveillance

- Serological surveillance: Positive antibody test results can have four possible causes:
  - natural infection
  - vaccination
  - maternal antibodies derived from a vaccinated or infected parent flock
  - positive results due to the lack of specificity of the test.

Types of Surveillance

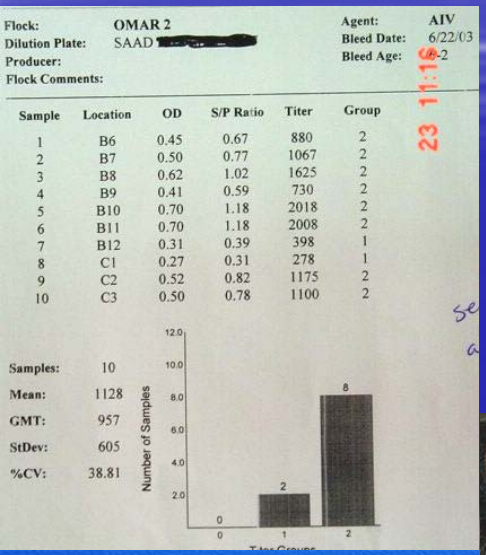
- In the case of vaccinated populations, the surveillance strategy should be based on virological and/or serological methods and clinical surveillance. It may be appropriate to use sentinel birds for this purpose.

Handling an Outbreak

- Diagnosis: Identification of the agent
  - Serology
  - Antigen Capture

    Molecular

    Determining the pathogenicity



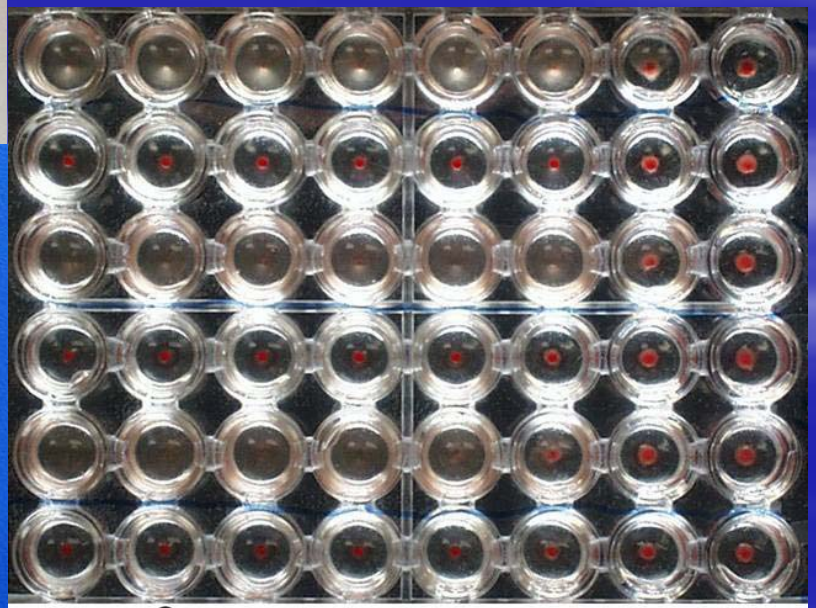
Flock: OMAR 2
Dilution Plate: SAAD [redacted]
Producer:
Flock Comments:

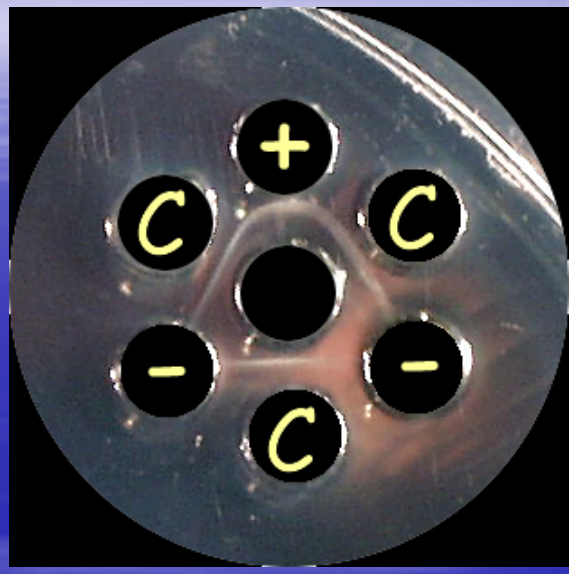
Agent: AIV
Bleed Date: 6/22/03
Bleed Age: [redacted]-2

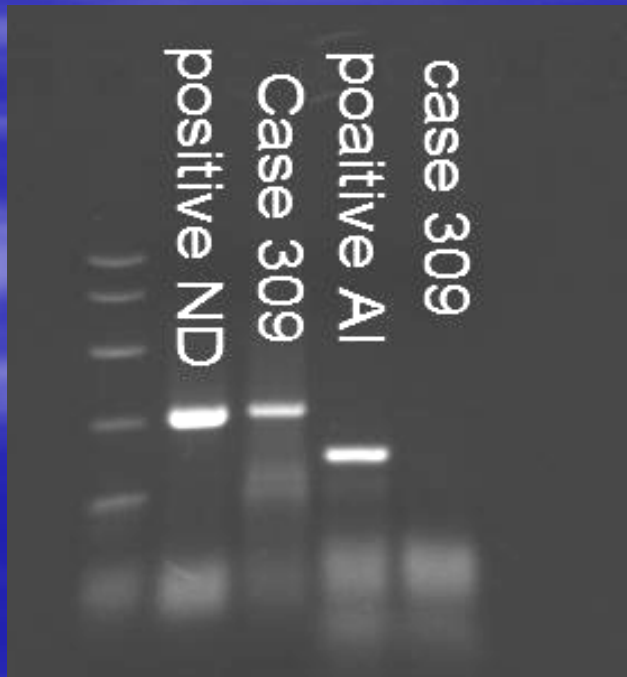
Sample	Location	OD	S/P Ratio	Titer	Group
1	B6	0.45	0.67	880	2
2	B7	0.50	0.77	1067	2
3	B8	0.62	1.02	1625	2
4	B9	0.41	0.59	730	2
5	B10	0.70	1.18	2018	2
6	B11	0.70	1.18	2008	2
7	B12	0.31	0.39	398	1
8	C1	0.27	0.31	278	1
9	C2	0.52	0.82	1175	2
10	C3	0.50	0.78	1100	2

23 11:1[redacted]

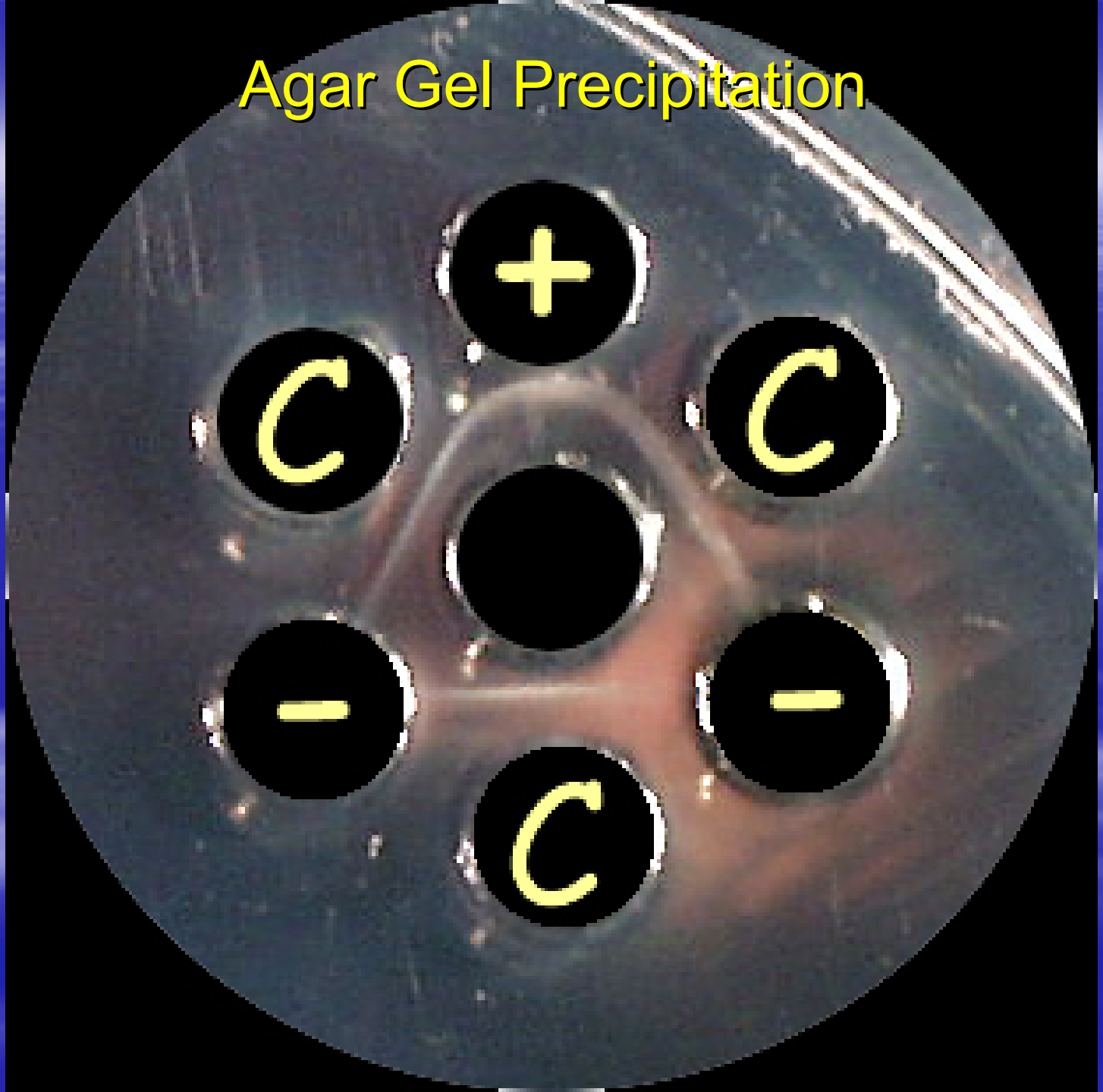
Samples: 10
Mean: 1128
GMT: 957
StDev: 605
%CV: 38.81







Agar Gel Precipitation



ELISA Readings

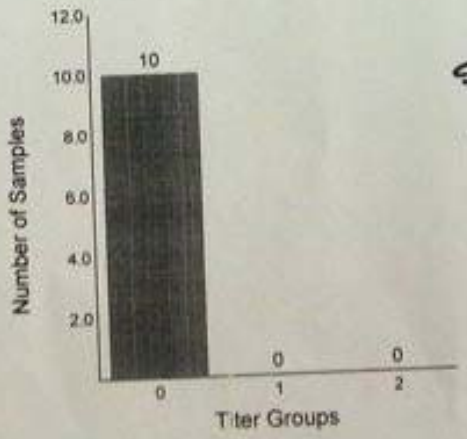
Negative Flock

Flock: OMAR 1
Dilution Plate: SAAD [redacted]
Producer:
Flock Comments:

Agent: AIV
Bleed Date: 6/8/03
Bleed Age: 4-2

Sample	Location	OD	S/P Ratio	Titer	Group
1	A8	0.17	0.11	0	0
2	A9	0.22	0.21	0	0
3	A10	0.18	0.13	0	0
4	A11	0.19	0.16	0	0
5	A12	0.26	0.28	0	0
6	B1	0.17	0.11	0	0
7	B2	0.19	0.16	0	0
8	B3	0.26	0.29	0	0
9	B4	0.24	0.26	0	0
10	B5	0.21	0.20	0	0

Samples: 10
Mean: 0
GMT: 0
StDev: 0
%CV: 35.93



serum

Positive Flock

Flock: OMAR 2
Dilution Plate: SAAD [redacted]
Producer:
Flock Comments:

Agent: AIV
Bleed Date: 6/22/03
Bleed Age: [illegible]-2

23 11:16

Sample	Location	OD	S/P Ratio	Titer	Group
1	B6	0.45	0.67	880	2
2	B7	0.50	0.77	1067	2
3	B8	0.62	1.02	1625	2
4	B9	0.41	0.59	730	2
5	B10	0.70	1.18	2018	2
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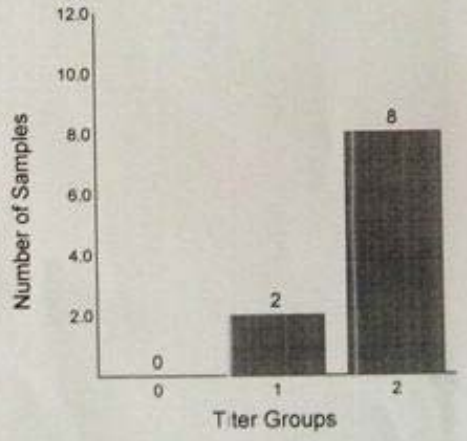
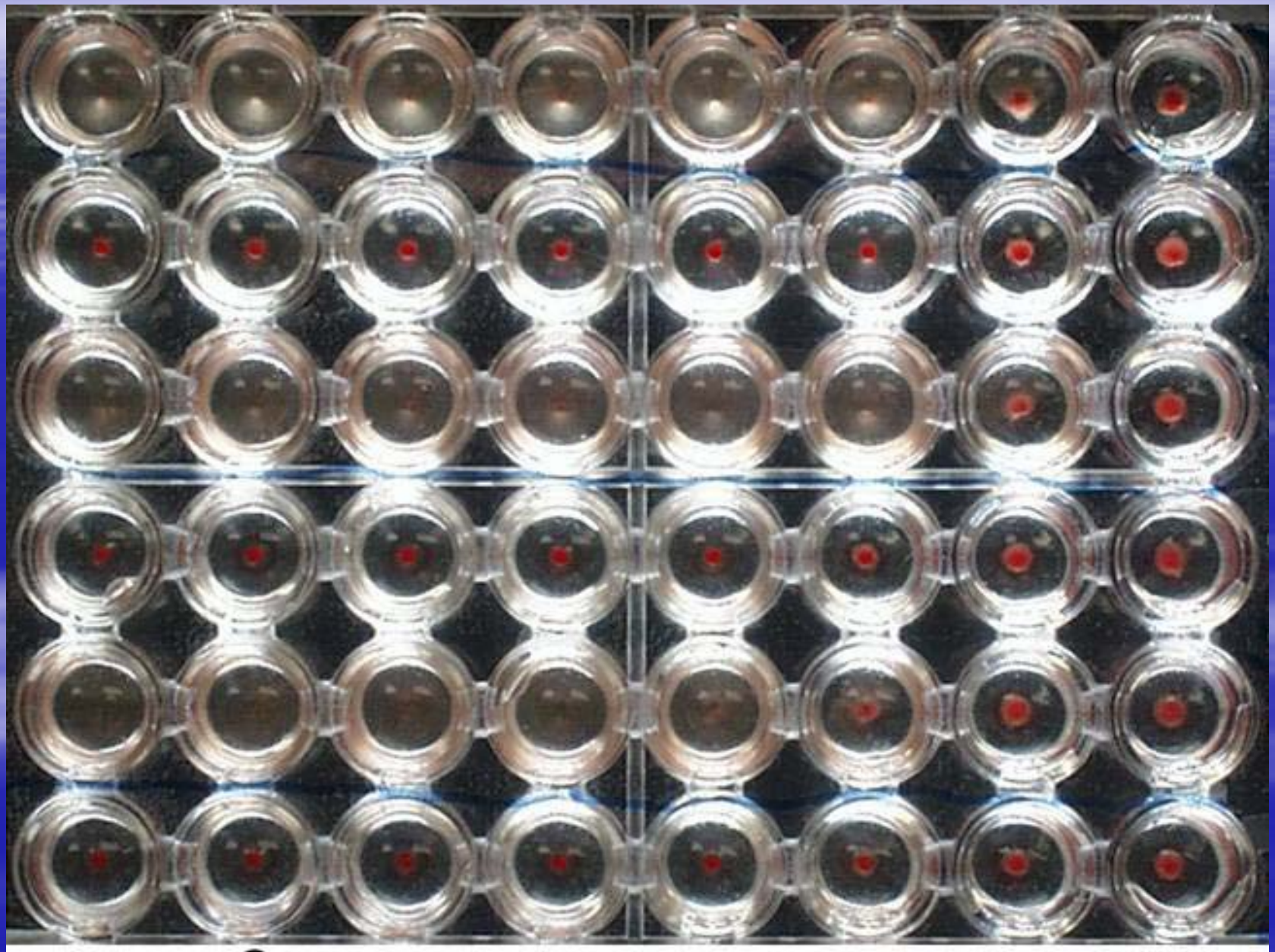
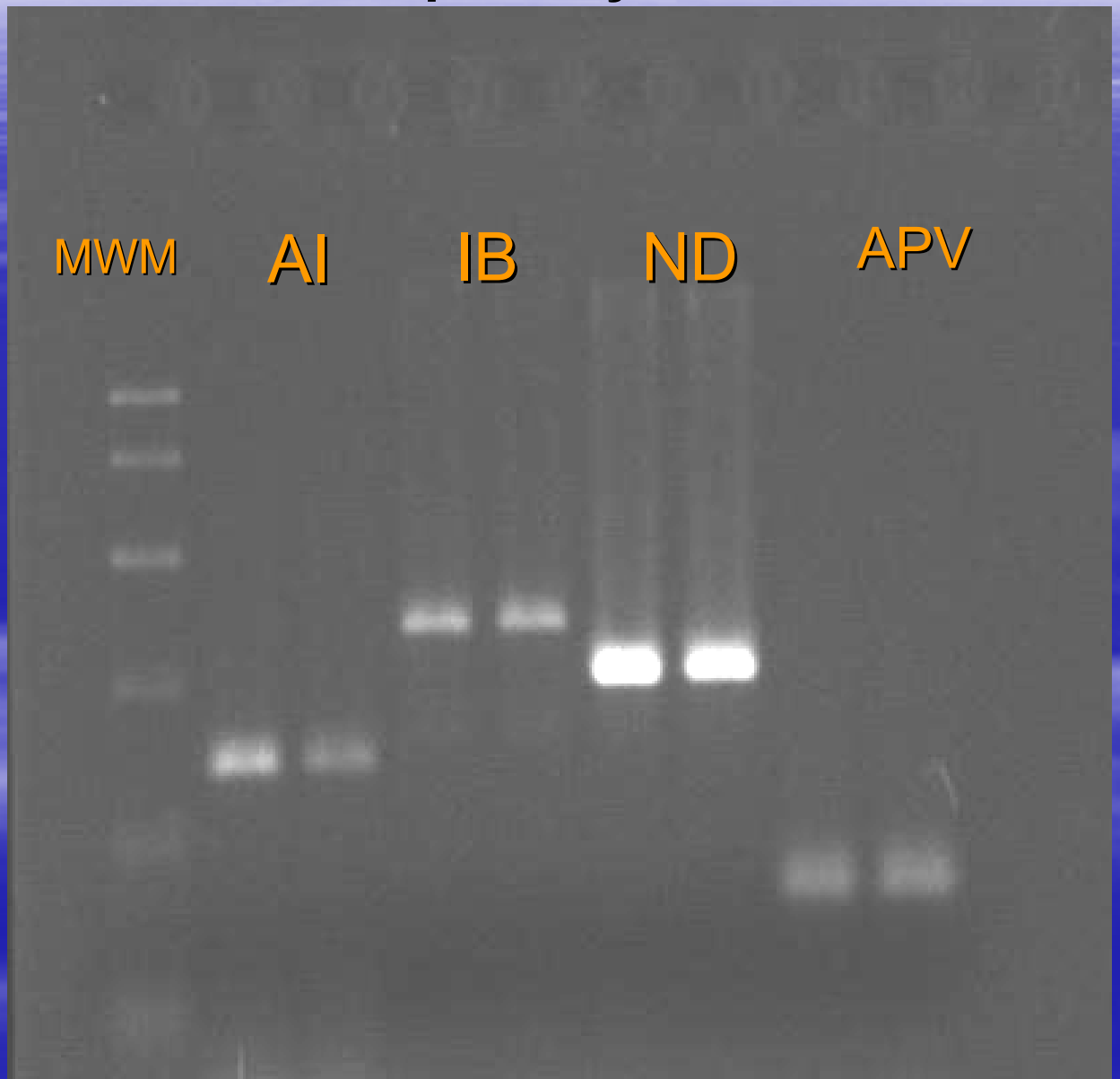


Hemagglutination Inhibition



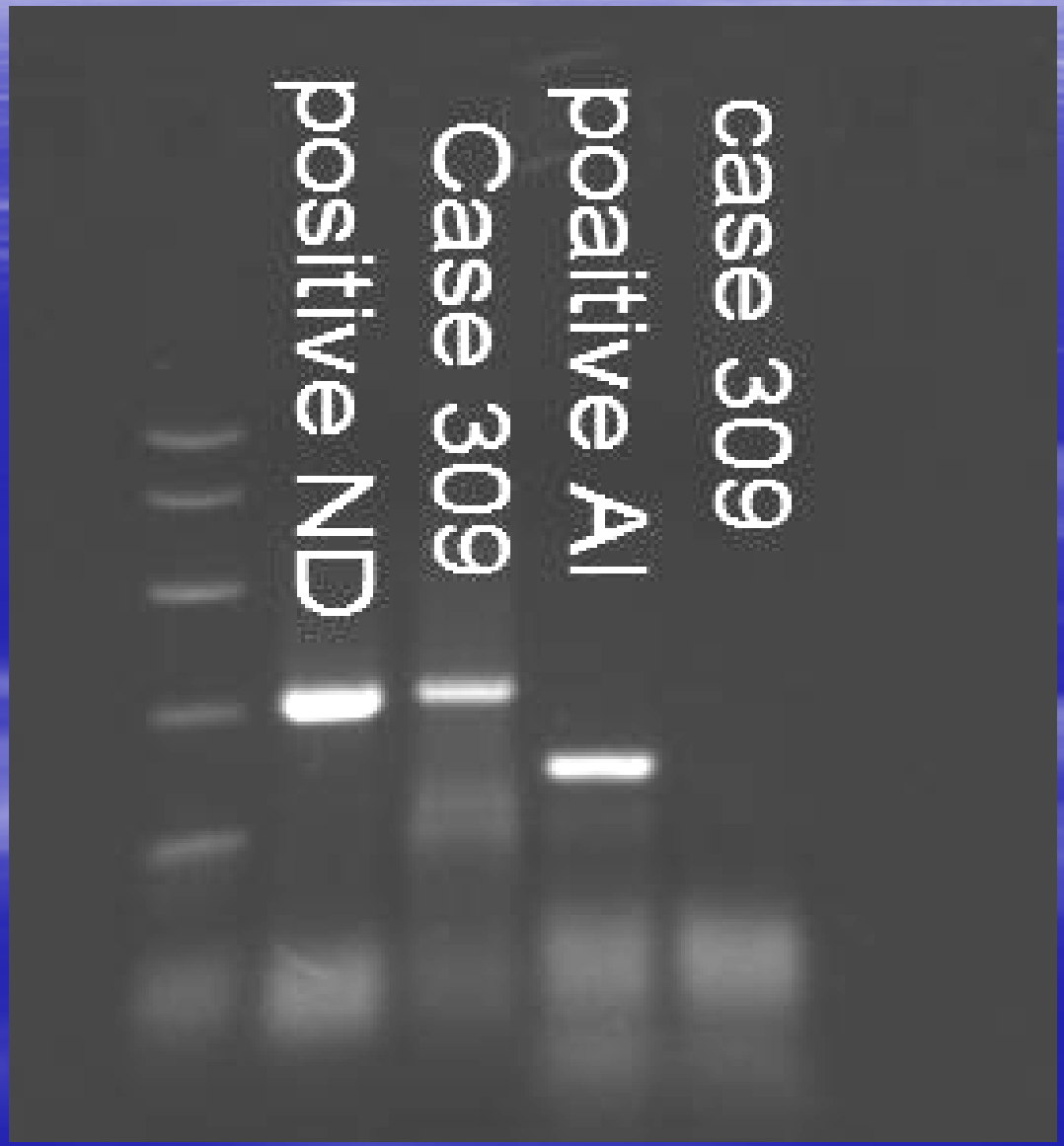
RT-PCR Diagnostics

Chicken Respiratory Disease viruses



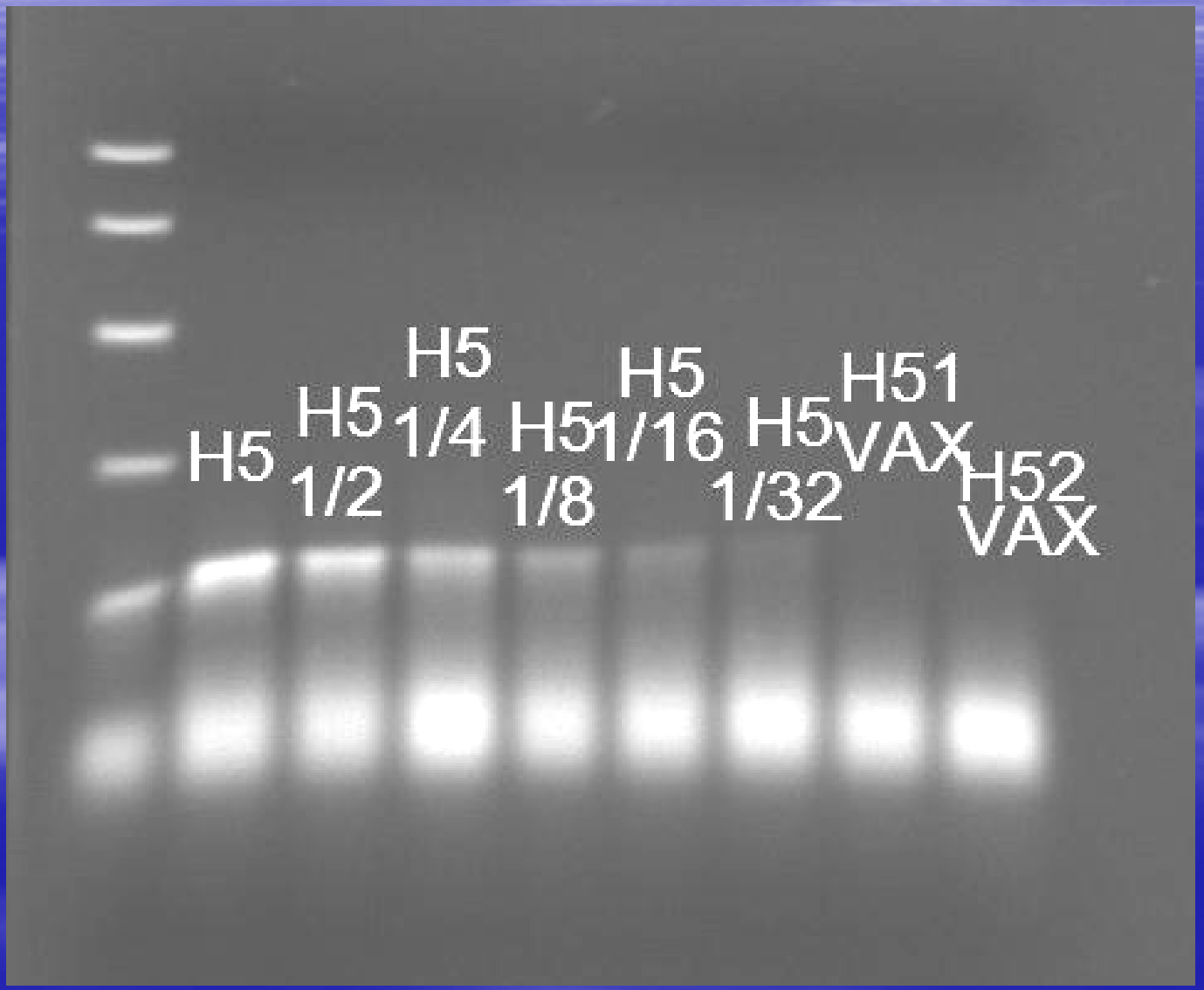
RT-PCR Diagnostics

Differentiating ND from AI



RT-PCR Diagnostics

Subtyping of AI



Handling an Outbreak

- Presence of contingency plan
- Zoning and Compartmentalization: 3 & 10 km radius zone guidelines are dissolving and each country is defining its own compartments.

Handling an Outbreak

- Culling
- Strategic vaccination

Handling an Outbreak

- Decontamination, disposal, and control of wild life

Handling an Outbreak

7. Food Safety

- Conventional cooking (temperatures at or above 70°C in all parts of a food item) will inactivate the H5N1 virus. Properly cooked poultry meat is therefore safe to consume.
- The H5N1 virus, if present in poultry meat, is not killed by refrigeration or freezing.
- Home slaughtering and preparation of sick or dead poultry for food is hazardous: this practice must be stopped.
- Eggs can contain H5N1 virus both on the outside (shell) and the inside (whites and yolk). Eggs from areas with H5N1 outbreaks in poultry should not be consumed raw or partially cooked (runny yolk); uncooked eggs should not be used in foods that will not be cooked, baked or heat-treated in other ways.
- There is no epidemiological following evidence to indicate that people have been infected with the H5N1 virus consumption of properly cooked poultry or eggs.
- The greatest risk of exposure to the virus is through the handling and slaughter of live infected poultry. Good hygiene practices are essential during slaughter and post- slaughter handling to prevent exposure via raw poultry meat or cross contamination from poultry to other foods, food preparation surfaces or equipment

Handling an Outbreak

7. Food Safety

- Conventional cooking (temperatures at or above 70°C in all parts of a food item) will inactivate the H5N1 virus. Properly cooked poultry meat is therefore safe to consume.

- The H5N1 virus, if present in poultry meat, is not killed by refrigeration or freezing.

- Home slaughtering and preparation of sick or dead poultry for food is hazardous: this practice must be stopped.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Handling an Outbreak

8. Import and export consideration.
9. Compensation

Vaccine / Industry / Politics

- The use of vaccine to aid in the control of AI is a political issue and different people have a different say on this.
- In some countries financial constrain preclude stamping out policy.
- In some countries, export markets are not an issue to prevent vaccination.
- In some countries, stamping out attempt may be unsuccessful.
- "With the ubiquitous nature of AI in wild birds it may be vaccination the most feasible tool to soften the sting of AI" Beard 1981

Vaccine / Industry / Politics

- "Field results have not shown vaccine to increase the risk of undetected infection; in fact, field experience has shown that vaccination greatly enhances a control program." Halvorson, 2002, Avian Pathology
- There is no way a vaccinated flock can be a greater threat to disease control than a non-vaccinated flock that breaks with AI. Halvorson, 2002, Avian Pathology
- Epidemiological observations have shown that serologically positive birds are not associated with AI transmission. Kradel, 1992
- Should the government set the rules when no indemnity is paid?

رجلان من فرق مكافحة أنفلونزا الطيور في الأردن يحاولان الامساك بدجاجة في بلدة كفرنجة شمال البلاد. «رويترز»

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Lecture #15

Avian Influenza

PRESENTED BY:

Dr. Mohamoud. Al-Natour FVM, JUST,

Avian Influenza

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Dept. of Pathology and Animal Health
Faculty of Veterinary Medicine
Jordan University of Science and Technology
(JUST)
Irbid – Jordan

Our objectives at JUST
Promote Preparedness against HPAI

- Provide update information about the disease
- Public Awareness
- Training Programs; Diagnostics, Preventive and Control measures
- Provide technical support and consultations
- Research

A.I. History

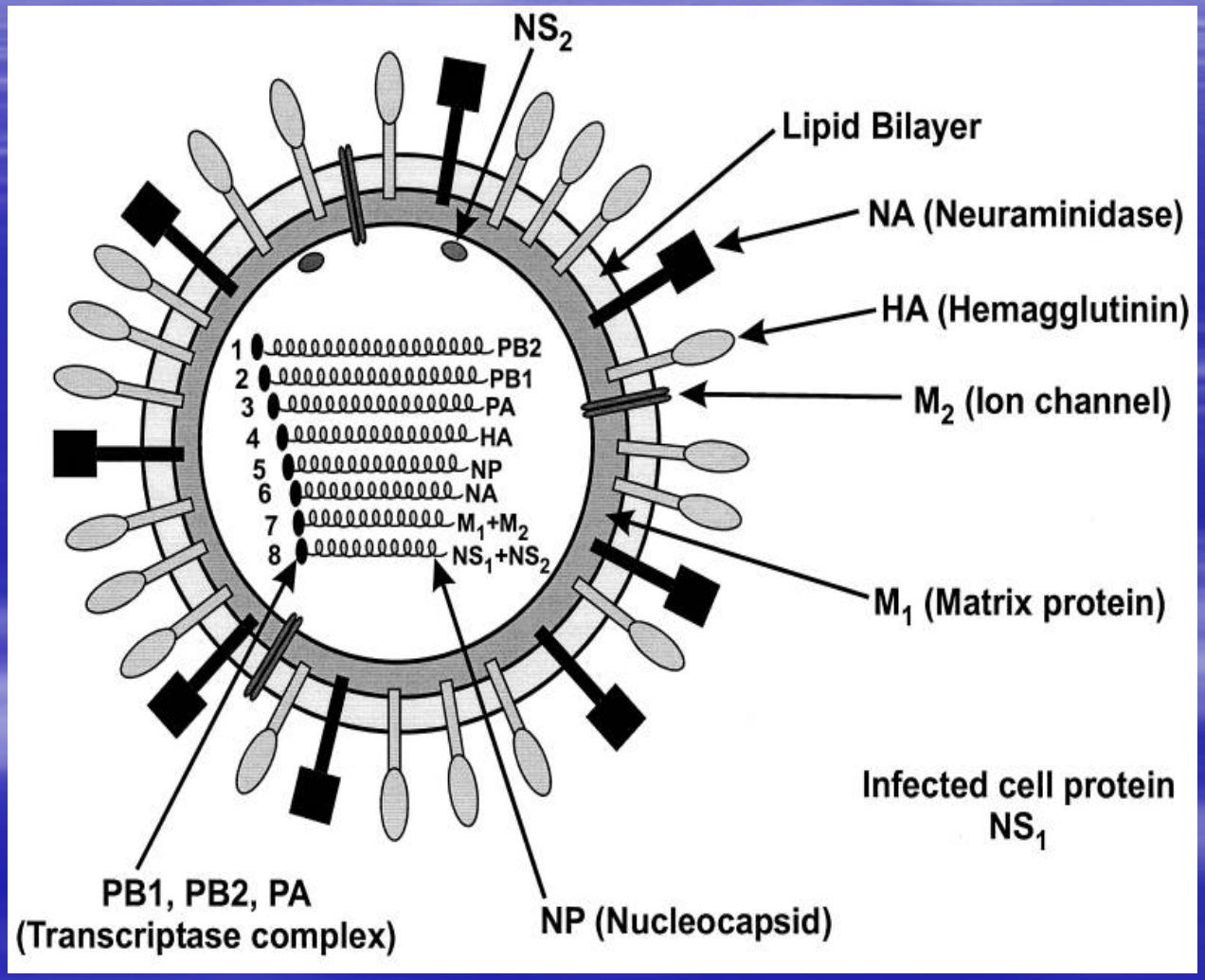
- 1878 fowl plague was described (Italy)
- 1901 fowl plague is caused by a virus
- 1955 it is type A influenza virus
- 1970 AGP test introduced
- 1972 waterfowl is a reservoir
- 1979 virulence and hemagglutinin cleavability was established
- 1997 direct transmission of H5 AIV from bird to humans

Orthomyxoviridae

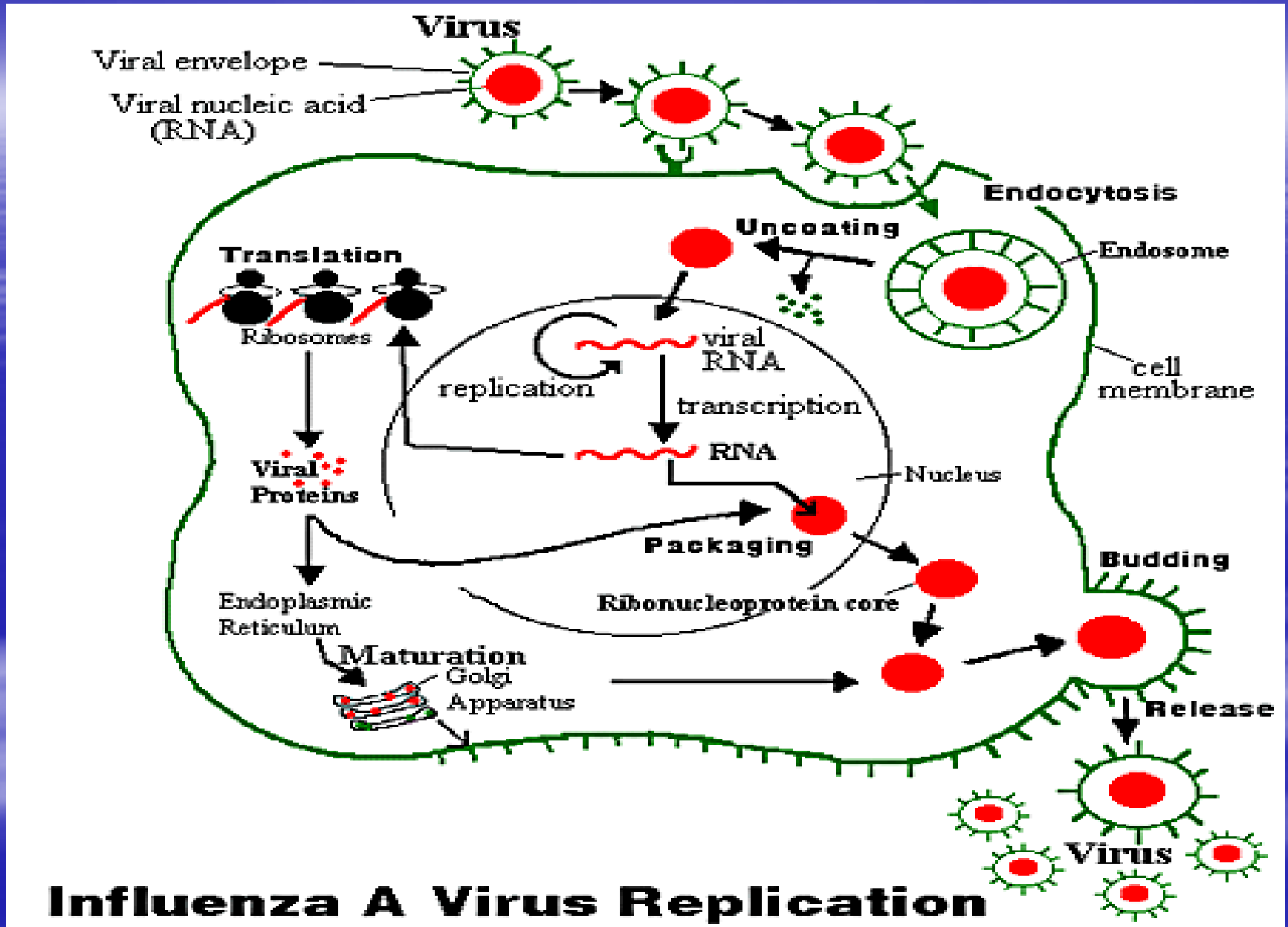
- Influenza virus A: Humans, Horses, Cats, Pigs, Birds, Marine mammals
- Influenza virus B: Humans
- Influenza virus C: Humans, Pigs

Orthomyxoviridae

- A.I. Virus



- Single stranded RNA –ve
- Segmented: 8 genes cod for 10 proteins
- Two glycoprotein surface projection:
- - Haemagglutinin (HA): H1-H16
- - Neuraminidase (NA): N1-N9
- Enveloped (20% lipid): Sensitive to heat, dryness and normal disinfectants
- Antigenic types A, B, C
- Pathogenicity var



Influenza A Virus Replication

Factors that sustain epizootics/epidemics

- Antigenic drift: Minor antigenic change in the HA and or NA {Point mutation in the gene coding for HA / NA}
- Reassortment and antigenic shift: Major antigenic change in the HA and or NA
- Segment reassortment: when cell is infected with 2 different influenza viruses
- Short term immunity
- Cross species transfer

Type A Influenza Surface Antigens
Surface Antigen Subtype

- **Heamagglutinine** (HA):1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and16
- Human: 1, 2, 3.
- Equine: 3 and 7
- Swine: 1 and 3
- Avian: 1-16

- **Neuraminidase** (NA): 1, 2, 3, 4, 5, 6, 7, 8 and 9
- Human: 1 and 2
- Equine: 7 and 8
- Swine: 1 and 2
- Avian: 1- 9

Nomenclature

- A/equine/Saskatoon/1/90(H3N8)
- Group: A
- Species: equine
- Location: Saskatoon
- Isolate number: 1
- Year: 1990
- Serotype of HA and NA: H3 and N8
- Examples:
- A/equine/Prague/1/56(H7N7)
- A/fowl/Hong Kong/1/98(H5N1)
- A/swine/Lincoln/1/86(H1N1)
- A/chicken/Jordan/1/05(H9N2)

Spread of infection among flocks

- Handling of infected birds
- Practical contact among flocks
- Loading of the birds before slaughtering
- Staff (veterinaries, vaccination team, technicians, etc.)

Key properties of type A influenza virus

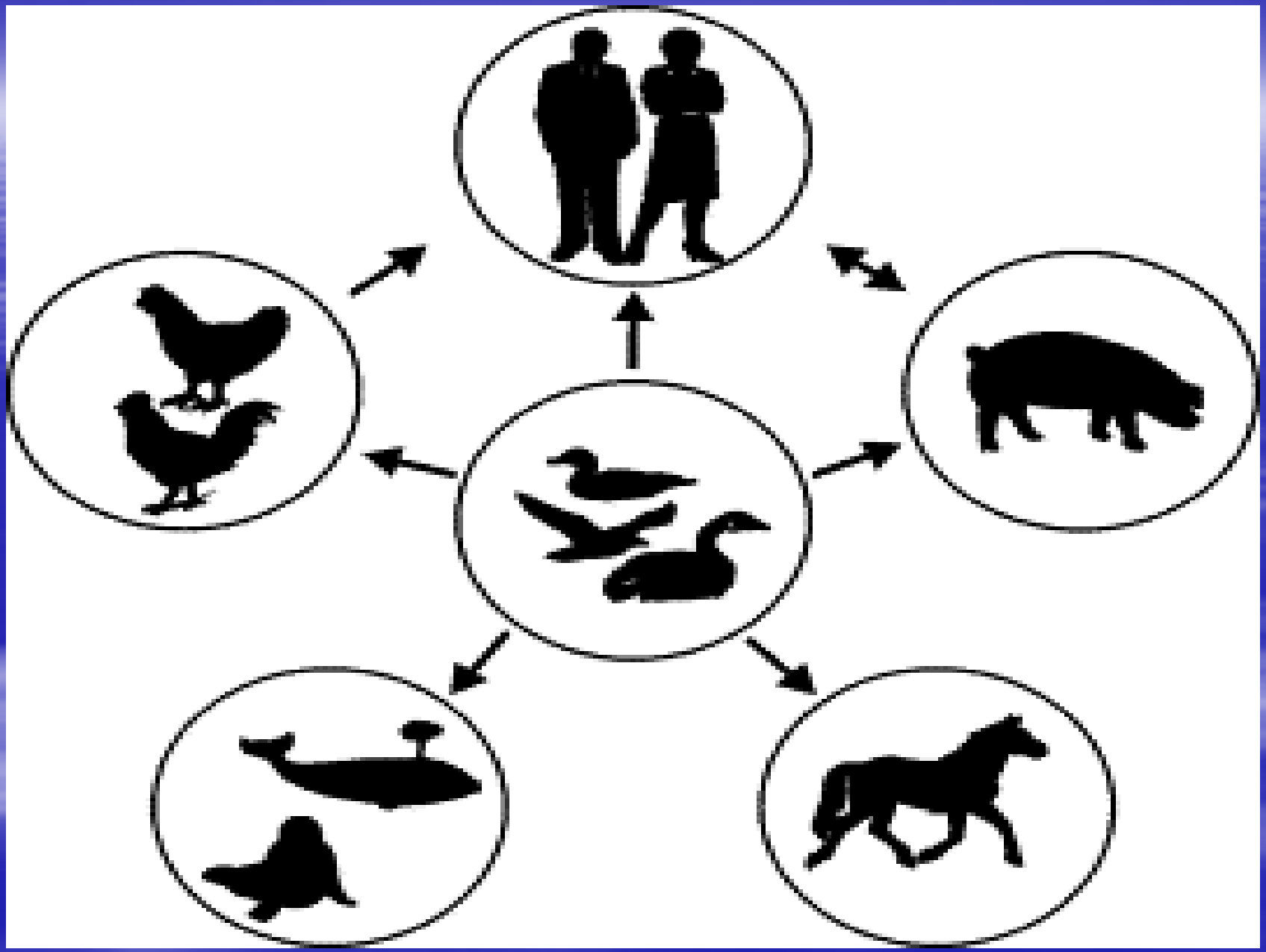
- Multiple serotypes
- Wide spectrum of pathogenicity
- Wide host range
- Global, Turkeys > Chicken
- International trade & of economic significance
- HPAI subtypes H5, H7
- Incubation period: 3-14 days
- Now AI is a zoonotic: Since 1997 Hong Kong virus

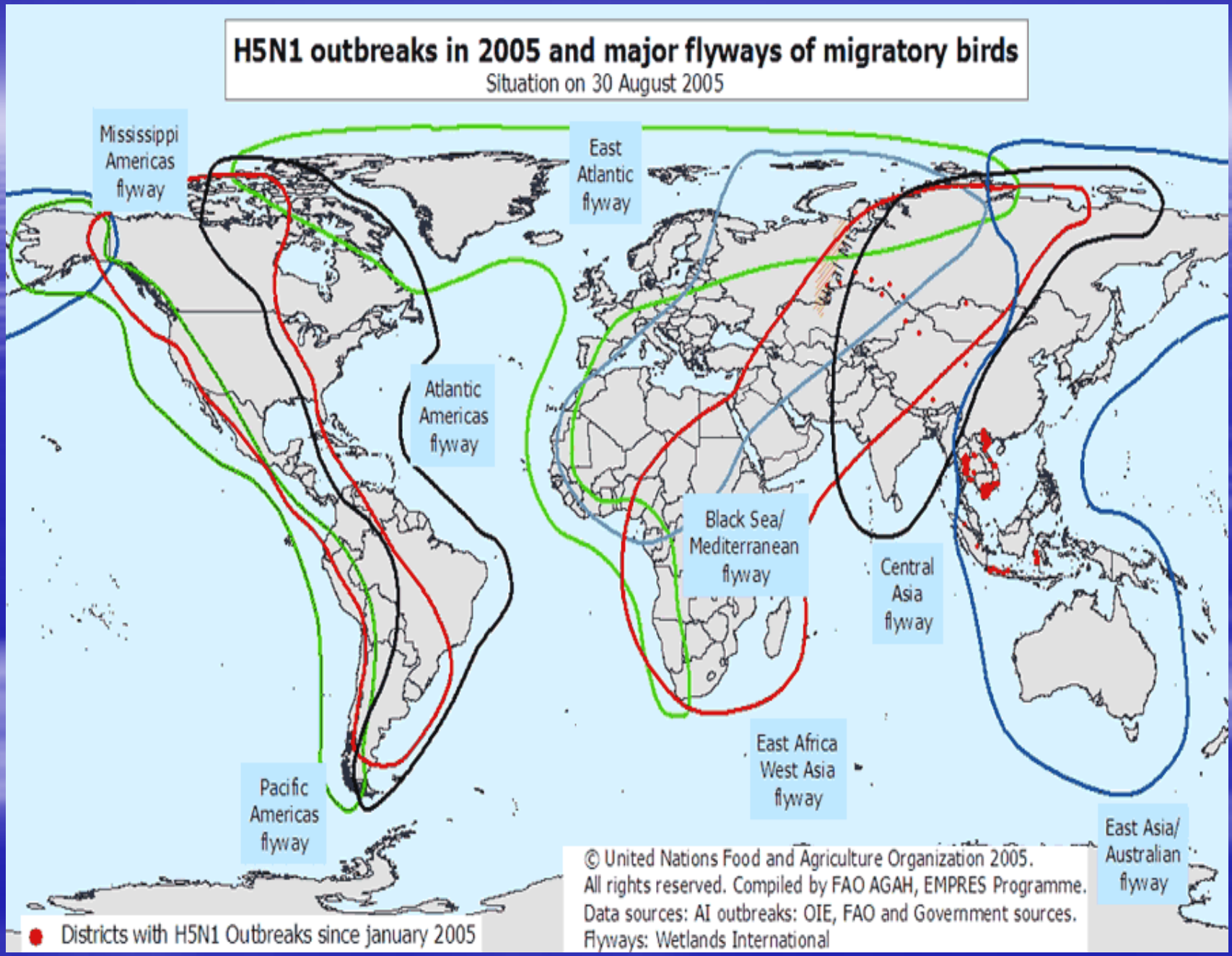
Evolution and Spread of flu viruses

- From aquatic birds to Poultry, Pigs, Horses
- From poultry to Pigs and Humans

Influenza H5N1: expanded host range?

- Domestic poultry
- Wild birds
  - infected
  - reservoir
- Humans
- Swine (China)
- Cats? (Netherlands)




H5N1 outbreaks in 2005 and major flyways of migratory birds
Situation on 30 August 2005
Mississippi Americas flyway
East Atlantic flyway
Atlantic Americas flyway
Black Sea/ Mediterranean flyway
Central Asia flyway
East Africa West Asia flyway
Pacific Americas flyway
East Asia/ Australian flyway
Districts with H5N1 Outbreaks since january 2005
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Data sources: AI outbreaks: OIE, FAO and Government sources.
Flyways: Wetlands International

HPAI – H5N1, 2003-2007

Countries reporting H5N1 in wild birds or poultry (updated 27/07/07)

Countries in Blod have reported outbreaks for 2007

Afghanistan, Albania, Austria, Azerbaijan, **Bangladesh,** Bosnia and Herzegovina, Bulgaria, Burkina Faso, **Cambodia**, Cameroon, **China**, Côte d'Ivoire, Croatia, **Czech Republic**, Denmark, Djibouti, Egypt, **France**, Georgia, **Germany, Ghana**, Greece, **Hong Kong (SARPRC), Hungary, India**, Indonesia, Iraq, Iran, Israel, Italy, **Japan**, Jordan, Kazakhstan, **Kuwait, Laos**, **Malaysia**, Mongolia, **Myanmar,** Niger, Nigeria, Palestine, **Pakistan**, Poland, **Republic of Korea**, Romania, **Russia, Saudi Arabia,** Serbia and Montenegro, Slovakia, Slovenia, Spain, Sudan, Sweden, Switzerland, **Thailand**, **Togo, Turkey**, Ukraine, **United Kingdom**, **Vietnam** (Total: 60)

Current situation of AI in Jordan

- 71% overall sero-prevalence of AI among broiler-breeder flocks in Jordan.

Reference: **Al-Natour**, M. Q. Abo-Shehada, M. N. Prev. Vet. Med. 70:45-50 (2005) LPAI (H9N2) up to 2005?

- 22 H9N2 viruses identified from 41 flocks in Jordan, A.A. of the HA cleavage site suggests LPAI H9N2 circulating in the Jordanian poultry industry.

Reference: **M. Q. Al-Natour**, N. M. Amarin, H. M. Al-Maaitah, I. Capua

Cont.

- Phylogenetic analysis of partial sequence of all 8 gene segments of Jordanian AIV (H9N2) showed all isolates closely related to each other and to other H9N2 in ME. Homology of M gene of Jo isolates and 2 Human isolates A/HK/1073/99, A/HK/1074/99 suggest a common origin for this gene.

Refernce: Monne, I., Cattoli, G., Mazzacan, E., Amarin, N.M, Al Maaiteh, H.M, **Al-Natour, M. Q.,** Capua, I., **2007**. **Genetic Comparison of H9N2 AI Viruses Isolated in Jordan in 2003.** *Avian Diseases.* 51, No.s1: 451-454.

- H5N1 confirmed in Jordan 18/03/06

Problem: virus can mutate and we are at high risk of a global pandemic

  - Two mutations of LPAI virus could make it highly pathogenic
  - Many more mutations needed to infect people

Original source of the virus

- Waterfowl (ducks, geese, shorebirds)
- Live bird markets
- Quail
- Pigs

How does the virus spread?

- From the birds
  - Saliva
  - Nasal secretions
  - Feces (shit)
- Spread in organic matter
  - litter, feces
  - Can live for 1 week to 3 months
- Spread by:
  - People: shoes, clothes, nasal passages
- Vehicles: especially in organic matter

Clinical signs (AI)

- Mainly respiratory signs in LAPI, decreased egg production, also egg shell quality
- HPAI, usually in chickens, swelling of head, comb turn blue, hemorrhage on shanks, - very high mortality and morbidity, respiratory, nervous and enteric can be involved (Similar to exotic ND)

How does the LPAI virus affect poultry?

- Chickens:
  - May have no signs of disease (+ve serology)
  - Decreased egg production (7-10 days 5-30%)
  - Poor egg shell quality
- Turkeys:
  - Respiratory signs
    - Snicking (coughing)
    - Mucous in the trachea
    - Decreased food and water intake
- Plus decreased egg production and shell quality

Lesions (AI)

- LPAI- respiratory and in laying bird reproductive also involved (ovarian atresia)
- HPAI- cyanosis of the head, ulceration of comb, red skin (all due to vascular damage), similar GI lesions to VVND, also severe respiratory lesions

Control (AI)

- Avoid contact with wild birds and live bird market
- Strict biosecurity
- Routine monitoring of blood in problematic areas
- Report outbreaks to authorities both LPAI and HPAI
- Vaccination only in the face of an outbreak might be a choice (only killed)- but not for H5 or H7
- No live vaccine –virus is mutagenic
- Recombinant vaccines

How do we control AI? Major Activities

- Quarantine
- Depopulation
- In-house composting
- Increased biosecurity
- Surveillance
- Rapid diagnosis (RRT-PCR)

General tips to help prevent spread of avian influenza

- Avoid going near poultry houses on farms
- Visit only one poultry farm per day
- Stay away from backyard poultry, live bird markets and waterfowl

Team Work and Cooperation

- Poultry industry (lead role)
- Diagnostic Labs (JUST & MOA Lab)
- Cooperative Extension
- Allied industries
- State police

Final Thoughts

- AI virus:
  - does not care about politics
  - does not recognize state lines
  - will spread if given the slightest opportunity
- The best weapon against AI or any emergency disease is BIOSECURITY!

Lecture #16

Genetic comparison Of H9N2 AI Viruses Isolated In Jordan in 2003

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Genetic comparison Of H9N2 AI Viruses Isolated

PRESENTED BY:

Dr. Nadim Amarin, Boehringer Ingelihieum

Introduction

In Poultry:

- LPAI H9N2 reported in the ME poultry since 1998, now the ME is endemic.
- In Jordan since 2000 we detected positive sera by ELISA.
- In 2003 we isolated more than 20 viruses.

In Human

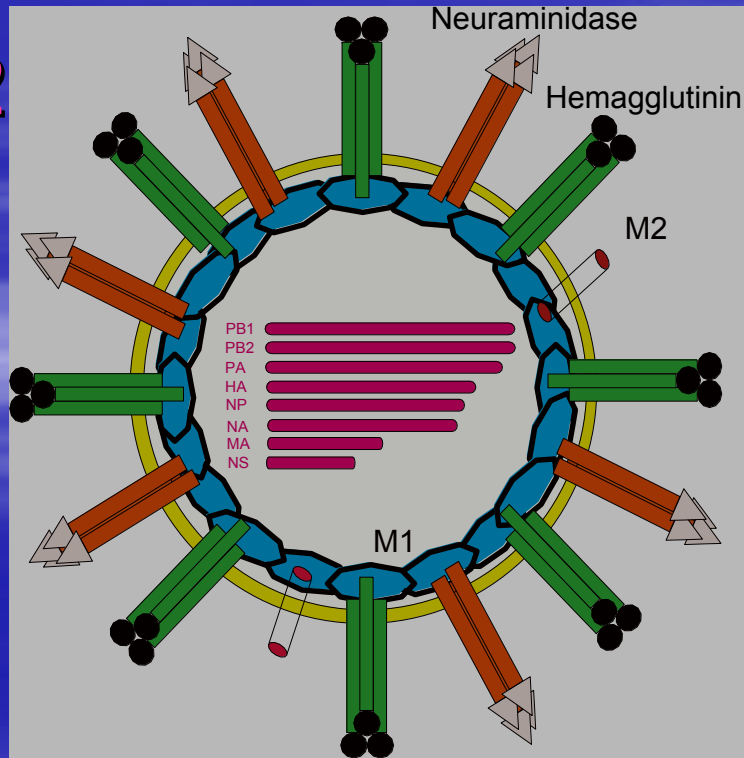
- Since 1999 H9N2 subtype sporadically introduced into human population in China causing flu-like illness, resulting in concerns about their implications in human health.

The aim of this study

- Improve our understanding on the evolution of the H9N2 subtype.
- Relationship between the Jordanian H9N2 and other H9N2 viruses.
- between Jordanian H9N2 Relationship viruses and influenza A viruses that caused human infections, such as H9N2 (Hong Kong and China), H7N7 (Netherlands) and H5N1 (Hong Kong and Thailand).

Morphology of AIV

- Single-stranded, RNA.
- Segmented genome: Reassortment
  - 8 segments, 1 or 2 proteins per segment
  - Encodes 10 proteins.
- 5: NP, 6: NA, 7: M1 and M2
  8: NS1 and NS2



Origins of H5N1 HPAI Influenza in Asia

Goose/Guangdong/1/96 H5N1

H5

H6N1

N1

Quail/Hong Kong/G1/97 H9N2

NP, MA, NS,
PB1, PB2, PA

????????

CK/Hong Kong/220/97

Hong Kong/156/97

Spackman

Materials and Methods:

H9N2 viruses		
Number	Species	Year
1453	Broiler	2003
554	Broiler	2003
1567	Broiler	2003
802	Duck	2003
1540	Broiler	2003
1409	Broiler	2003
1529	Broiler	2003
1408	Broiler	2003

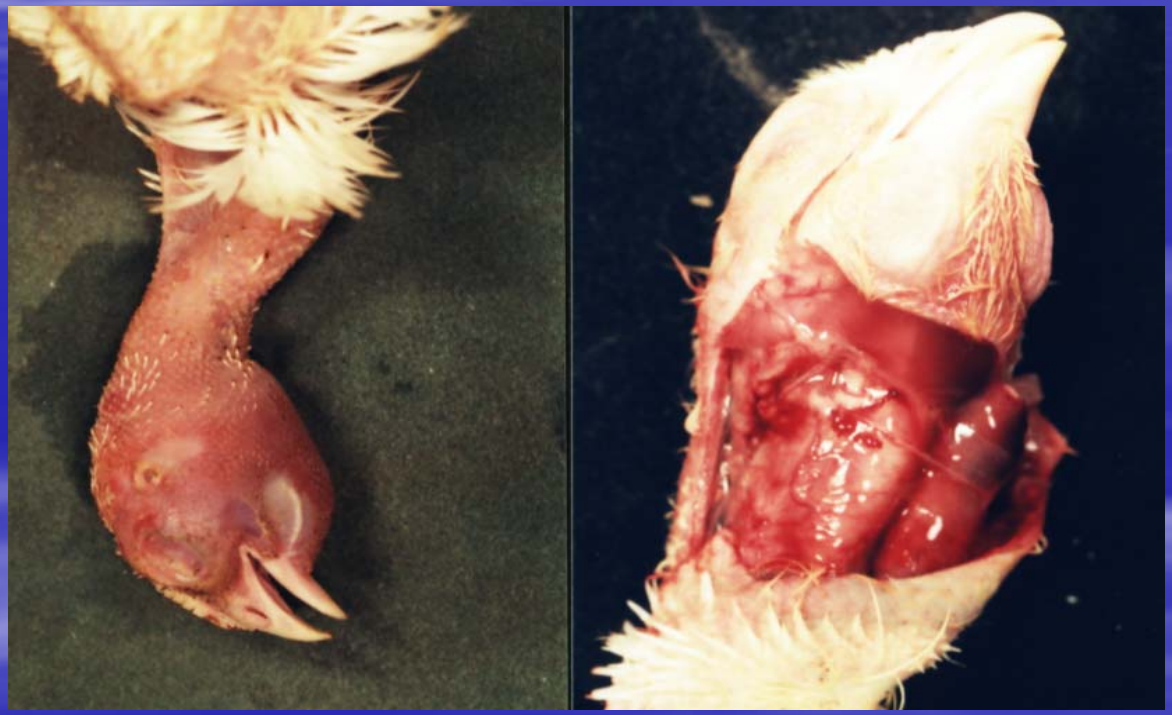
All viruses were grown in 9 to 10 day old embryonated fowls' SPF eggs. Subtype of the viruses was determined by standard haemagglutination inhibition and neuraminidase inhibition tests.

Clinical signs

Gasping

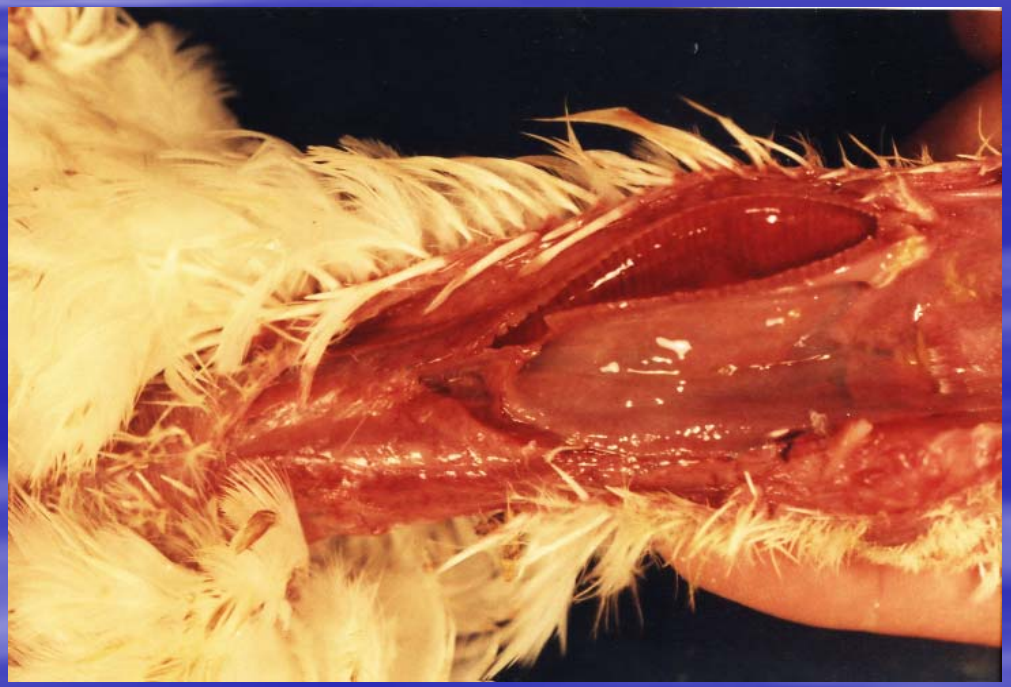
Nasal discharge

Sinusitis



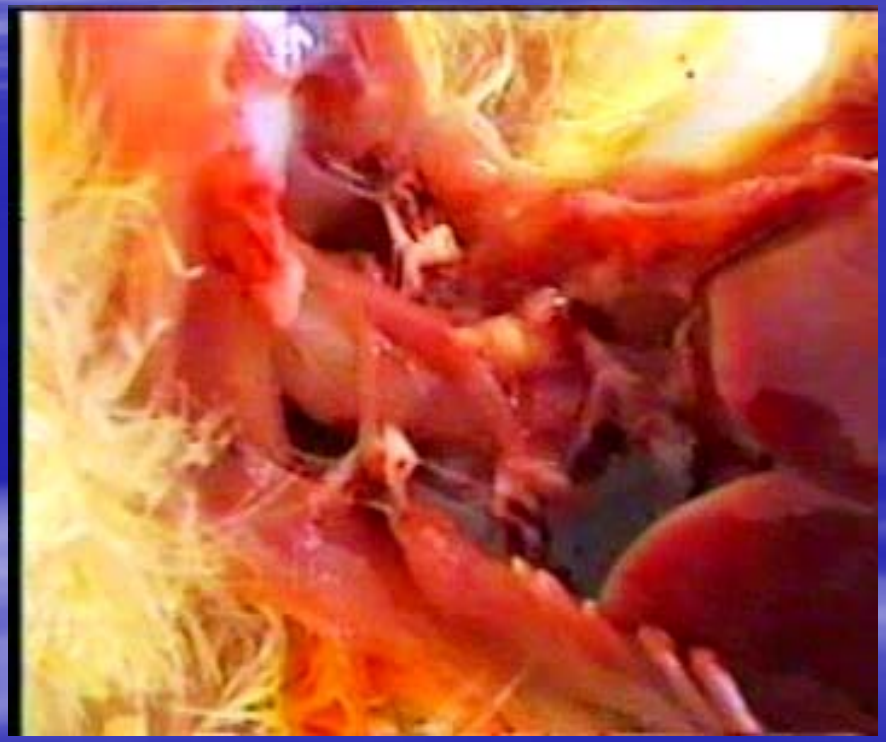
Lesions

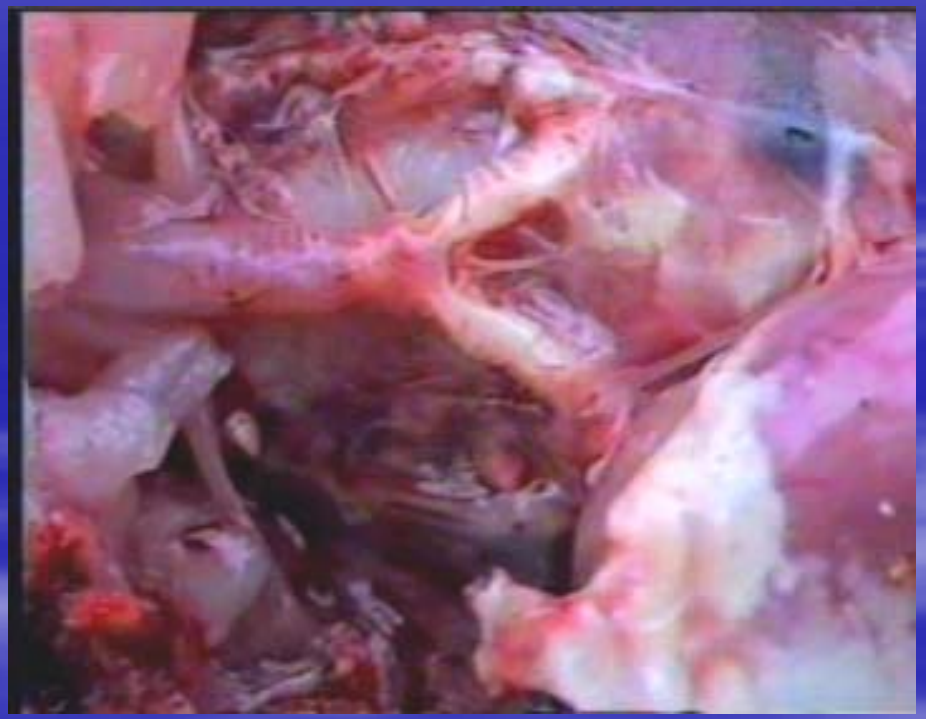
Tracheal exudates



Serous and Caseous exudates in the Trachea

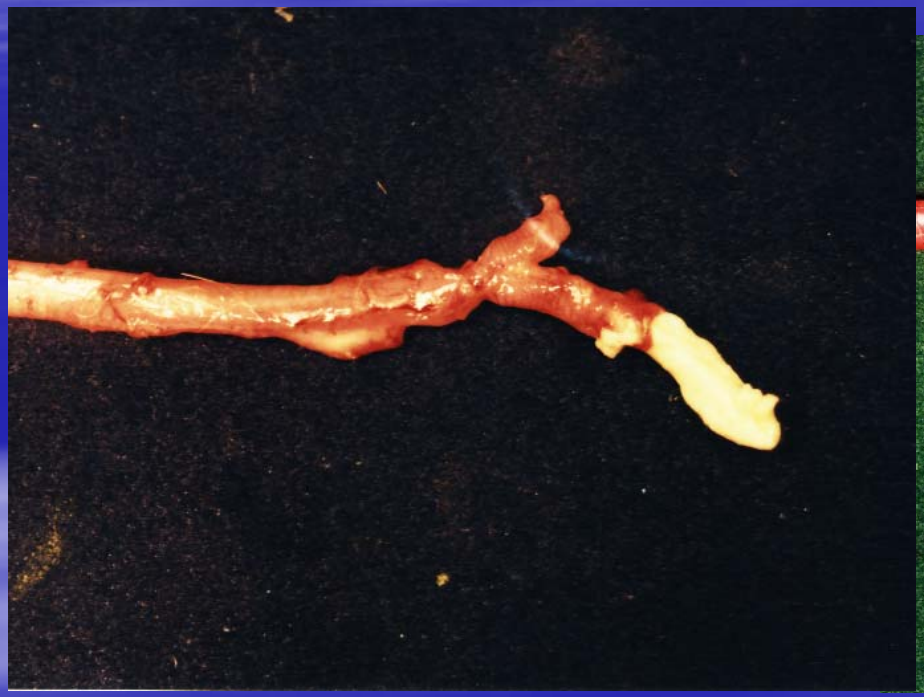
Lesions

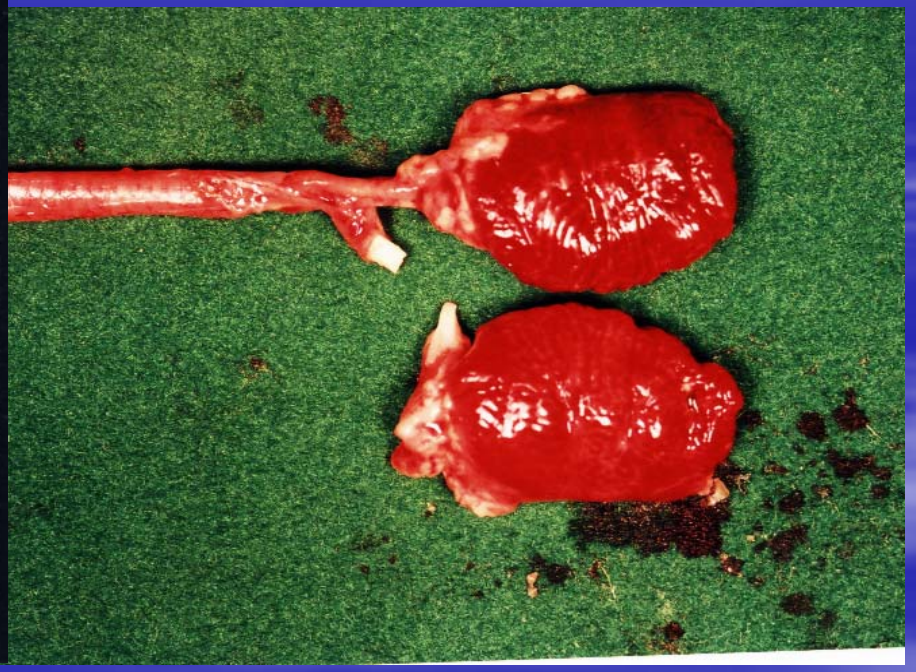




Caseous plug in the Bronchi

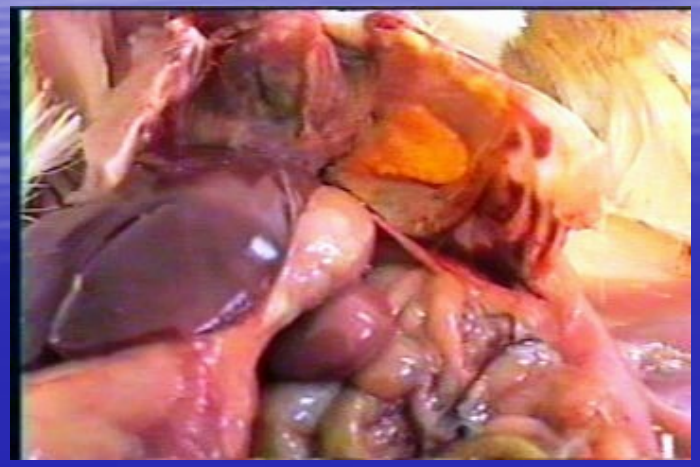
Lesions

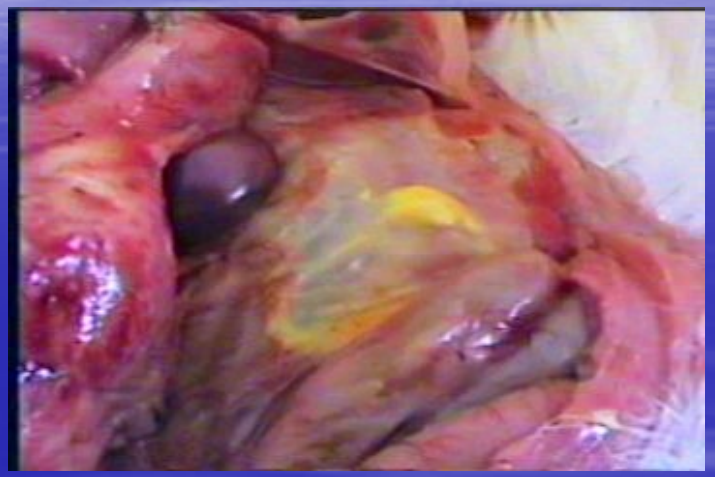




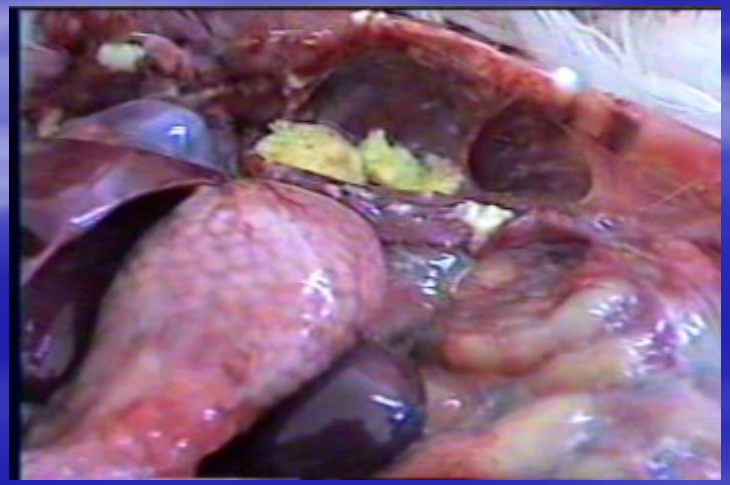
Caseous plug in the Bronchi and inside the lungs

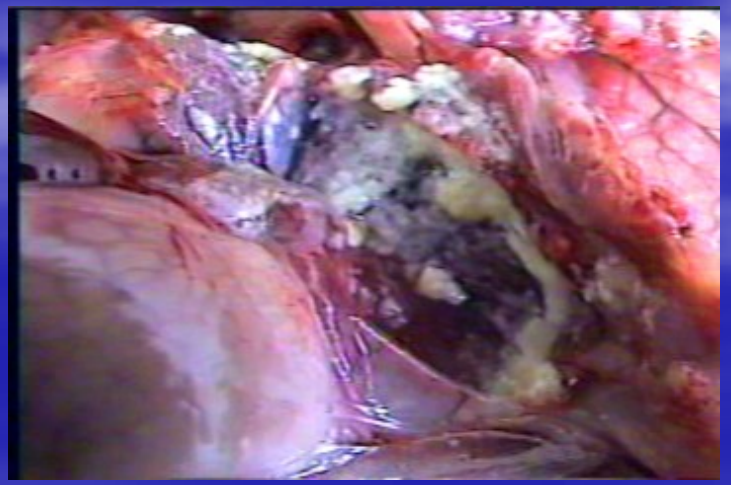
Lesions



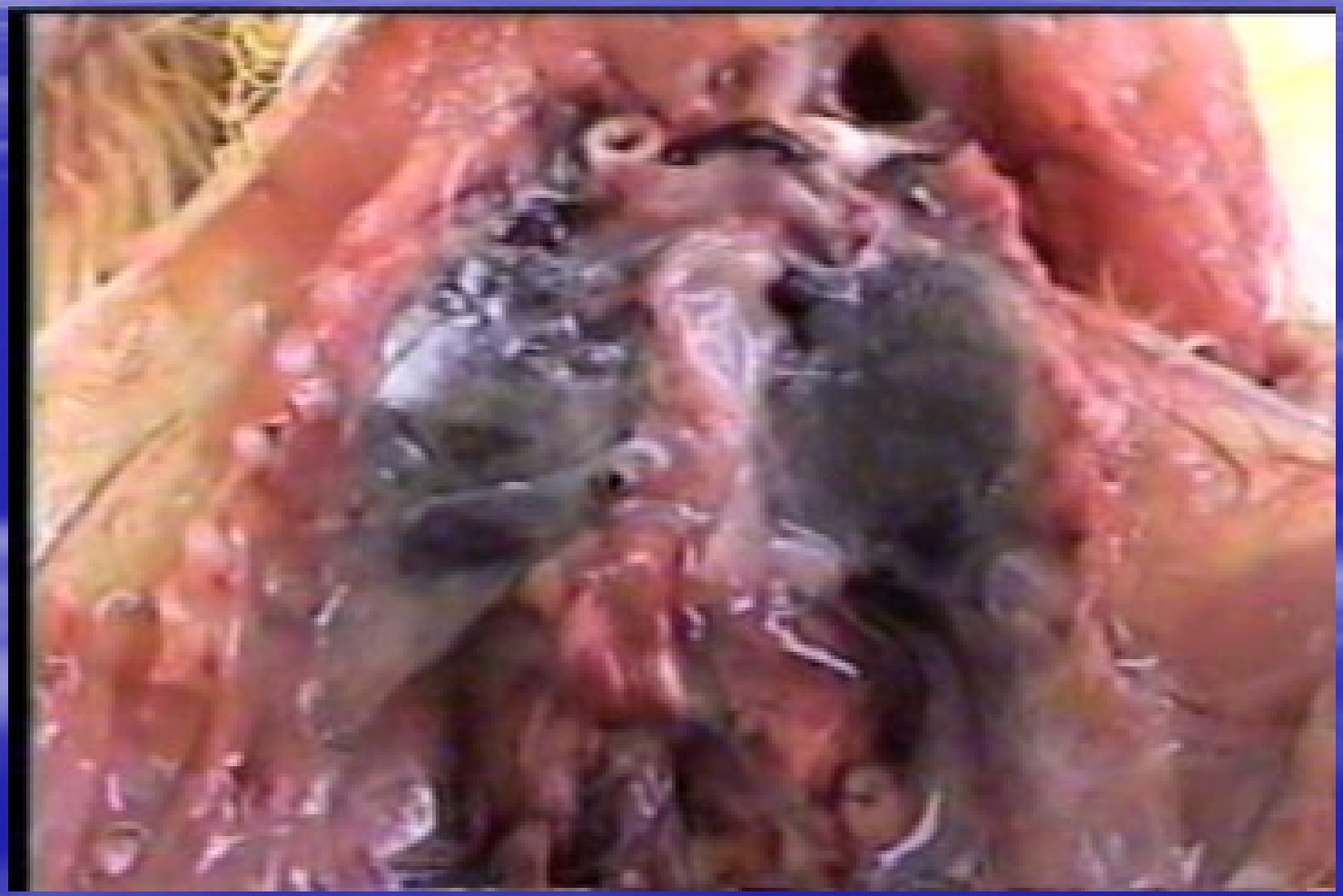


Cloudy or containing caseous exudates

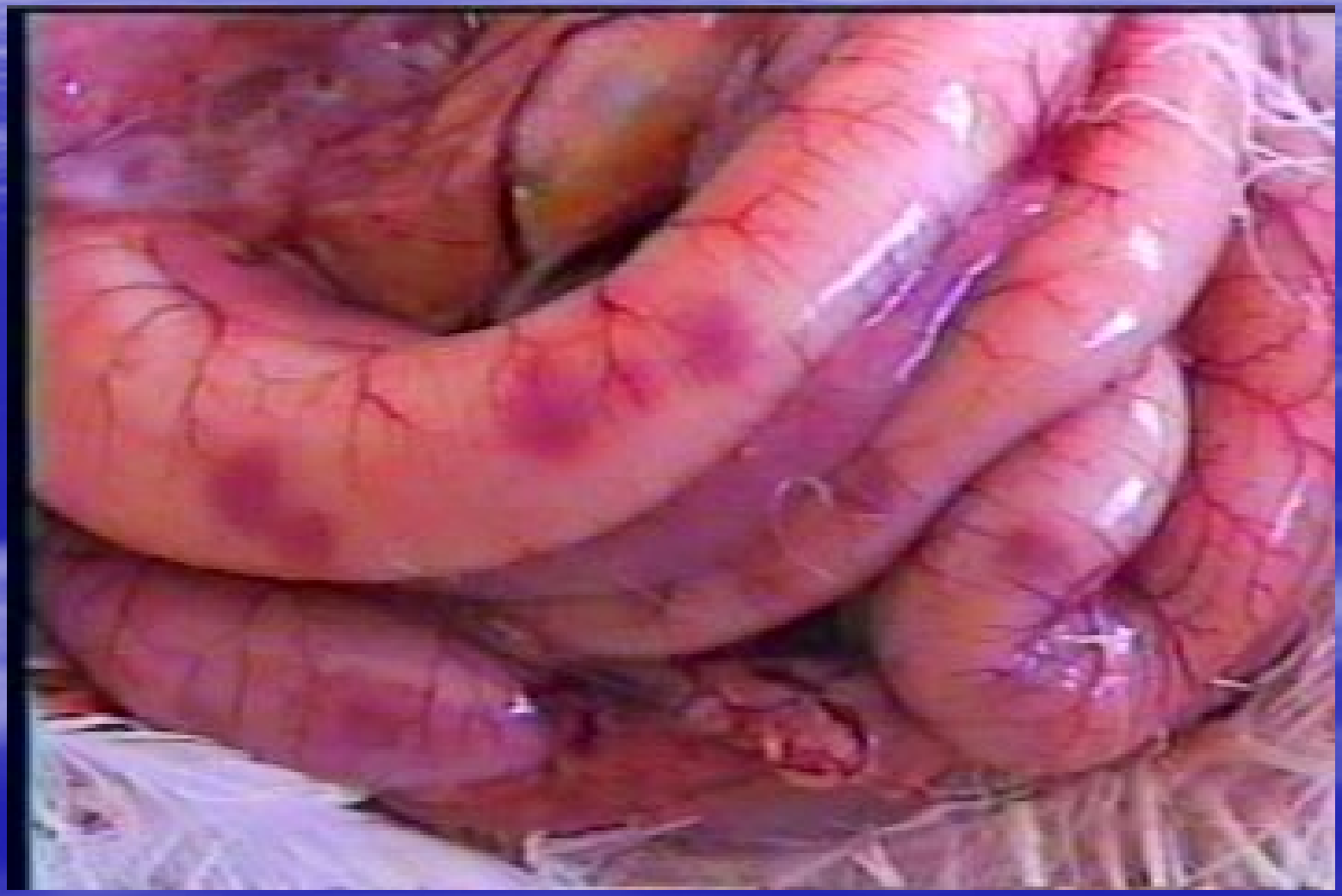




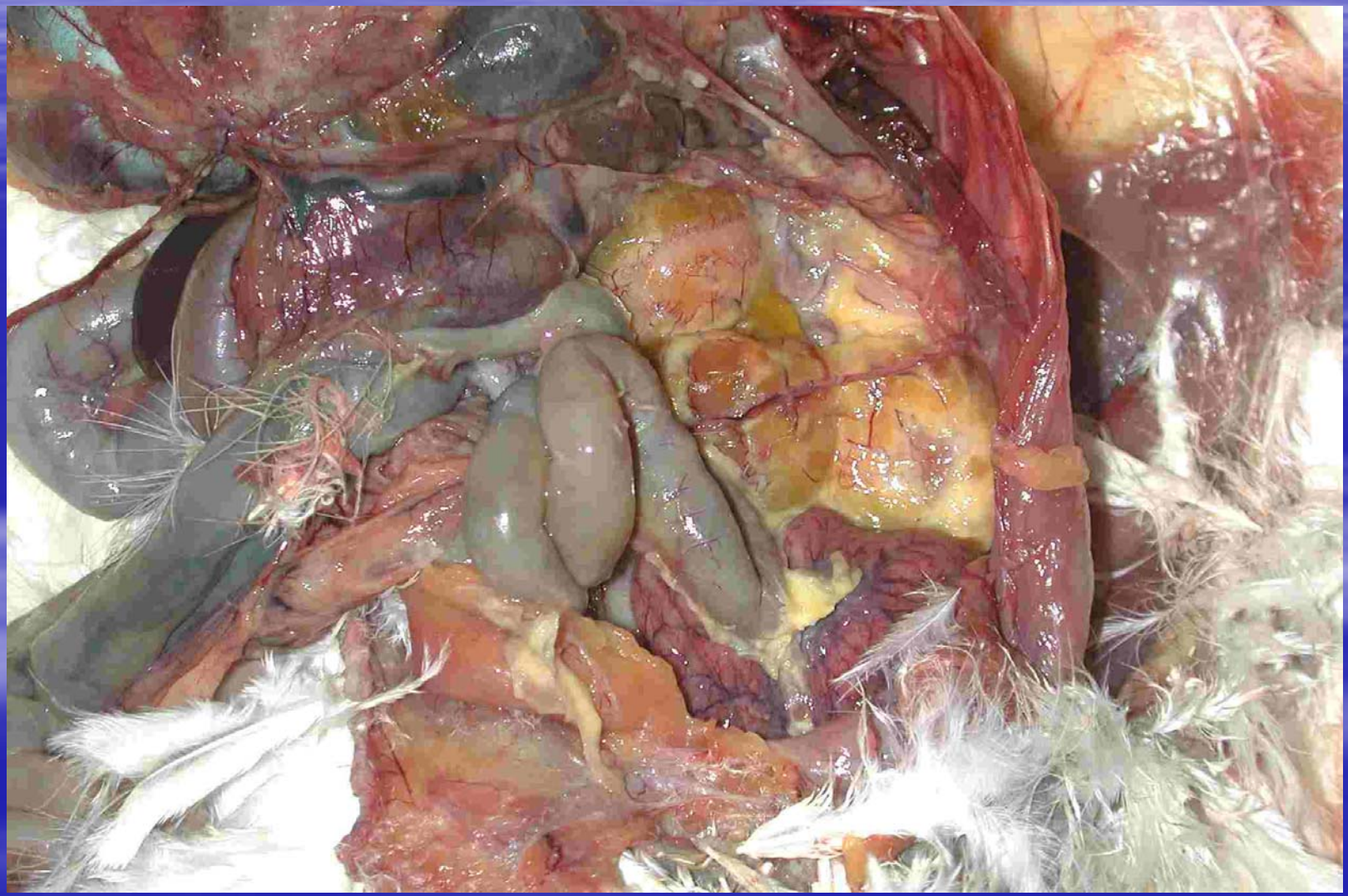
Pneumonia



Enteritis



Follicles



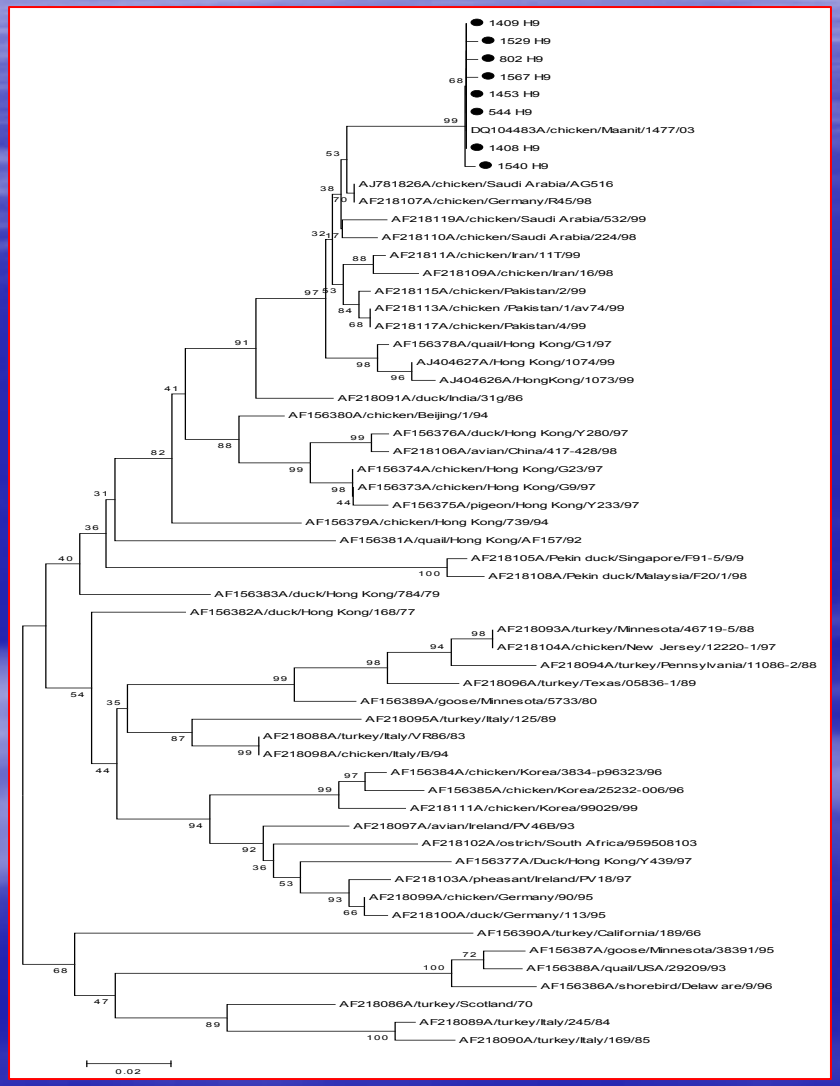
H9N2 Results

Species	Age	# of Birds	3 days Mor.	Parasites	Bacterias	PM
Layer	35 weeks	40000	30	-	E.coli	-
Breeder	44 weeks	40000	19	-	E.coli	Infl. Ovary
Broiler	43 days	7000	75	coccidia	E.coli	Trachitis
Duck	Variable	10000	9	Trichomonas	E.coli	ND??
BackYard	Variable	50	6	-	-	-
Broiler	42	7000	1300	Coccidia	Staph	CRD, Enteritis
Broiler	37	7000	585	coccidia	E.coli	

Materials and Methods

- Viral RNA was extracted from the allantoic fluid using the High Pure RNA Isolation Kit
- Amplification of the 8 viral genes was carried out by one-step RT-PCR using gene-specific primers
- The PCR products were purified with High Pure PCR product purification kit and then subjected to electrophoresis in a 2% agarose gel
- PCR products were sequenced using ABI PRISM BigDye Terminator V3.1 Cycle Sequencing kit
- Phylogenetic analysis was carried out using Clustal W software in the MEGA 3 programme

Results

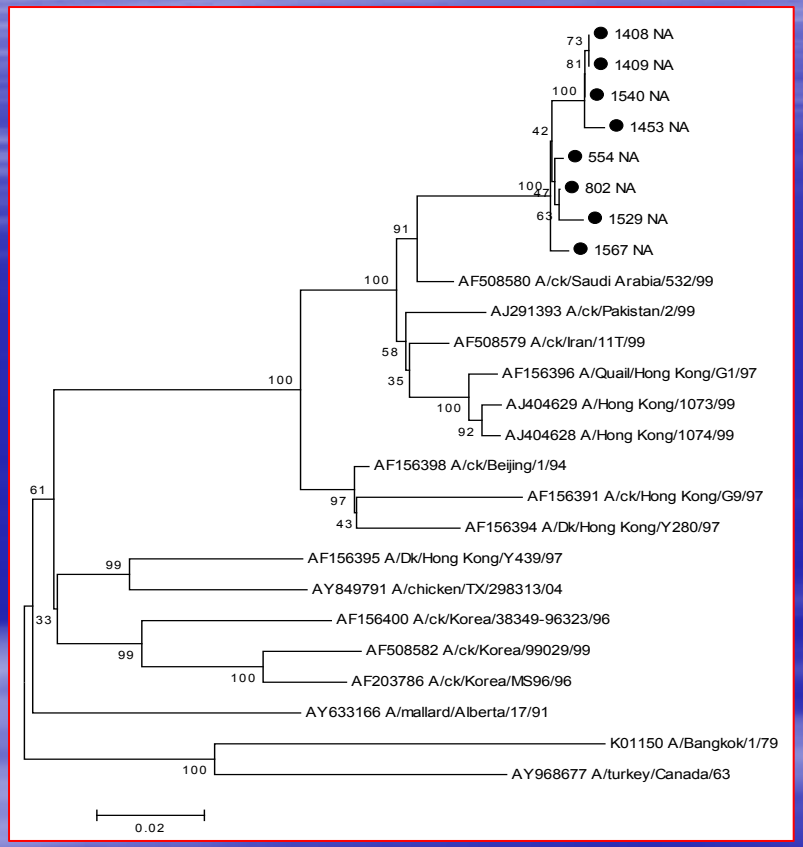


- phylogenetic relationship between the HA genes of Jordanian H9N2 viruses and the HA genes of viruses of H9 subtype isolated worldwide since 1970s.

Results

- According to a previous study (Banks *et al*., 2000), all the Jordanian isolates were placed in lineage 3, which consists of viruses isolated in Middle East, a German isolate and some Far Eastern isolates, including A/quail//Hong Kong/G1/97 strain which is closely related to the H9 gene of viruses that caused human infections, A/HK/1073/99 and A/HK/1074/99. These 2 viruses shared 95,2%-94,6% homology with HA genes of Jordanian isolates.
- The HA genes of the Jordanian viruses were more distantly related to the HA genes of other strains isolated in Hong Kong, as A/ck/Hk/G9/97 (90% homology) and A/dk/Hk/Y439/97 (80% homology).
- The percentage of homology with the Middle East strains, A/ck/Saudi Arabia/532/99, A/ck/Iran/11T/99 and A/ck/Pakistan/2/99, varied between 95,8% and 96,7%.
- The amino acid sequences at the site of cleavage to HA1 and HA2 of the Jordanian isolates were: PARSSR*G. This sequence is identical to those of the human viruses (A/HK/1073/99 and A/HK/1074/99) and it is typical of low pathogenicity viruses for the absence of multibasic amino acid.

Results



- Concerning the other surface protein gene, NA, the Jordanian strains showed very high homology to each other (99,0%-100%).
- All the Jordanian isolates showed the highest percentage of homology with A/ck/Saudi Arabia/532/99 strain (96,3%-97,1%).

		Percentage homology				
VIRUS	GENE	H5N1 A/HK/156/97	H9N2 A/HK/1073/99	H9N2 A/HK/1074/99	H9N2 A/ck/PK/2/199	H7N7 A/Netherlands/219/03
A/ck/Jordan/1540/03	HA	---	94,6%	95,2%	96,1%	---
	NA	---	94,8%	94,8%	95,2%	---
	PB1	90,2%	88,9%	88,3%	87,6%	92,1%
	PB2	88,1%	88,6%	87,8%	87,5%	88,1%
	M	98,5%	98,2%	98,0%	97,8%	91,5%
	PA	85,6%	84,4%	84,1%	84,7%	91,4%
	NP	90,2%	91,0%	91,3%	91,0%	89,6%
	NS	88,5%	87,2%	87,4%	86,9%	91,8%

genetic relationships between A/ck/Jordan/1540/03 and H9N2, H5N1 and H7N7 viruses isolated from human

Results

- Absence of multi basic amino acid sequence at HA cleavage site of the Jordanian isolates: PARSSR*GLF.
- All isolates are closely related to each other and to other H9N2 strains from ME.
- Nucleotide sequences of HA, NA, PB1, PB2 , PA and NP genes of Jordanian isolates are genetically distantly related with H9N2 isolated from human A/HK/1073/99 and A/HK/1047/99.
- The H9N2 viruses are closely related to the Pakistan strain H9N2 A/ck/PK/2/199.
- M gene is closely related to the corresponding gene of the H5N1 in HK (98.5) and the two H9N2 isolated from human A/HK/1073/99 strain and A/HK/1074/99 (98% - 98,2).

Conclusions

- The importance of the geographical parameters is strengthened by the similarity between genes of Jordanian isolates and all genes of A/Saudi Arabia/532/99 strain and Pakistan strain.
- Phylogenetic comparisons of nucleotide sequences of HA, NA, PB1, PB2, PA and NP genes of Jordanian isolates with corresponding genes of A/HK/1073/99 and A/HK/1074/99 strains showed that these Jordanian genes are distantly related with human isolates.
- Regarding the M gene, the homology observed in this study between the Jordanian and other Middle-Eastern isolates and the H9 and H5 viruses from Pakistan and China respectively might suggest a common origin for this gene.

Recommendations

- H9N2 is existed in several countries in Asia and ME.
- Regional collaboration in sequencing all the H9N2 isolates.

Acknowledgment

- Dr. Ilaria Capua
- Ministry of Agriculture in Jordan
- Dr. Abdel Hafez Zahdeh
- Dr. Nemer Al Natsheh

Thank You for your attention

شكرا لاستماعكم

Merci pour votre attention

Enhanced Diagnostic Capacity and Control Measures of Some Transboundary Animal Diseases with Emphasis on Pathology and Epidemiology of Johne's Disease/Paratuberculosis in Jordan, Egypt and Algeria

Paratuberculosis (Johne's disease) in Cattle

Definition: Johne's disease; an infectious, incurable, chronically progressive granulomatous enteritis (especially of jejunum and ileum) which affects domestic and exotic ruminants including dairy and beef cattle, sheep, goats, cervids and camelids

Is it in Jordan: Yes and in Egypt, Saudi Arabia, and in Morocco.
Is it found in milk: Yes?
Is it found in semen: Yes?

Cause: It is caused by Mycobacterium avium subsp. Paratuberculosis.

Does it cause disease in Humans: not confirmed nor disproved. The agent was isolated from patients with Crohn's.

Microorganism Characterization:

1. It is aerobic and grows at a temperature of 37ºC.
2. It has a rather wide growth temperature range, from 28ºC to 43ºC.
3. It has an acid-fast staining properties and its dependency on exogenous mycobactin for growth.
4. UV light had minimal effect on M. paratuberculosis.
5. Mycobacteria are resistance to acidic environments (as low as pH 2.5).
6. Survive in the acidic, humid environments of peat bogs and decomposed feces.
7. MAP can be isolated from ruminant feces, soil, and contaminated feed on farms with paratuberculosis infected ruminants.
8. Mycobacteria have a very thick cell wall and can survive in the environment for long time (a year).

Clinical Signs:
The disease is characterized by profuse, therapy-resistant intermittent diarrhea with high level of bacterial shedding, emaciation (weight loss), and decreased milk production
Diffuse edema, anemia, and infertility are the dominant late signs. And eventual death at cachectic state.

Gross and Microscopic Pathology: thickening, corrugation and edema of the ileum and its mesenteric lymph nodes associated with chronic granulomatous enteritis.

Diagnosis:
Based on clinical signs and laboratory testing:
There are five commonly used diagnostic methods:

1. Culture
2. Direct smear from intestinal mucosa and lymph node parenchyma scrapping.
3. Histopathology
4. Immunohistochemistry
5. PCR

Prevention and Control:

Why the interest in Johne's disease:

1. Apparent increase in the global prevalence of Johne's disease.
2. The increasing economic costs and potential trade implications. Worldwide, Johne's disease causes great losses for milk producers. The on-farm losses due to Johne's disease include (1) reduced milk production, (2) lower slaughter value of infected cows, (3) sub-optimal culling and (4) diagnosis and treatment costs.
3. Johne's disease has received increasing attention because of concern (not confirmed nor disproved) over the potential role of Map in some cases of Crohn's disease in humans.

Is it hard to control Johne's disease?
Yes, why?

1. The long subclinical phase (long incubation period)
2. Not sensitive enough Diagnostic test to detect animals in the subclinical phase of the disease.
3. Once an infected animal develops clinical signs, it is often hard to distinguish them from clinical signs of other common ruminant diseases .
4. The current vaccines have not yet shown to be effective enough

Control:

1. Test and Cull Cows
2. Cull off-spring of test-positive cows
3. Avoid or eliminate infection using AI from known disease free bulls
4. Correct herd / Environmental management: clean water, health feeding and grazing, avoid overcrowding.
5. Calf Management – clean colostrums

مشروع تحسين القدرات التشخيصية وإجراءات السيطرة على الأمراض العابرة للحدود مع التركيز على مرض نظير السل

مرض نظير السل في الأبقار

تعريف: مرض نظير السل هو مرض معدي لا يعالج، مزمن يصيب الأمعاء،و يصيب المجترات البرية و المستأنسة و التي تتظمن الأبقار و الأغنام و الماعز و الجمال .

هل هو موجود في الأردن ؟ نعم و يوجد في مصر و السعودية و المغرب.
هل هو موجود في الحليب ؟ نعم.
هل هو موجود في السائل المنوي ؟ نعم.

المسبب : بكتيريا **Mycobacterium.avium subsp paratuberculosis (MAP)**
هل يسبب المرض في الإنسان ؟ غير مؤكد و لكن تم عزل البكتيريا المسببة لمرض نظير السل في المصابين بمرض **Crohn's**.

خصائص البكتيريا:

1. هوائيه و تنمو على درجة حرارة 37 درجة سيلوسية.
2. لها درجة حرارة للنمو واسعة من 28-43 درجة سيلوسية .
3. تصبغ بصبغة **Acid Fast Stain** وتعتمد على **Mycobactin** في نموها.
4. تتاثر بشكل قليل بالأشعة فوق البنفسجية.
5. البكتيريا مضادة للبيئة الحمضية (**pH 2.5**).
6. تعيش في الوسط الحمضي و البيئة الرطبة و مستنقعات المياه و البراز.
7. يمكن عزل البكتيريا من براز المجترات و التربة و الغذاء الملوث في المزارع التي بها حيوانات مصابة.
8. البكتيريا لها جدار خلوي سميك و لذلك تعيش في البيئة لوقت طويل.

الأعراض المرضية

يتميز المرض بإسهالات شديدة و لا تستجيب للعلاج مع إفراز عدد كبير من البكتيريا، هزال و نقصان الوزن مع إنخفاض في إنتاج الحليب، تجمع السوائل و فقر دم و عدم الخصوبة وتلاحظ في المراحل النهائية للمرض و الموت المحتوم.

التشريح المرضي و النسيج المرضي

يصبح جدار الأمعاء سميك مع تعرج بطانة الأمعاء ووذمه في الأمعاء(**Ileum**) و العقد اللمفاوية(**Mesenteric Lymph node**) مع إلتهاب مزمن.

طرق التشخيص :
بالإعتماد على الأعراض المرضية و الفحوصات المخبرية يوجد 5 طرق:

1. الزراعة البكتيرية.
2. مسحة من بطانة الأمعاء و العقد اللمفاوية.
3. النسيج المرضي (**Histopathology**).
4. **Immunohistochemistry**.
5. **PCR.**

السيطرة و منع إنتشار المرض

لماذا الإهتمام بمرض نظير السل ؟

1. زيادة إنتشار المرض.
2. زيادة الخسائر الإقتصادية و التأثير على التجارة الدولية على مستوى العالم، لأن مرض نظير السل يسبب خسارات في إنتاج الحليب و يؤثر على إقتصاد المزرعة من خلال 1) إنخفاض مستوى إنتاج الحليب 2) إنخفاض قيمة الذبيحة 3) ذبح الحيوانات مبكرا 4) تكاليف التشخيص و العلاج.
3. زيادة الإهتمام بمرض نظير السل لعدم و ضوح علاقته مع مرض **Crohn's** .

هل يصعب السيطرة على مرض نظير السل ؟

نعم, لماذا؟

1. فترة حضانة المرض طويلة.
2. الفحوصات التشخيصية غير حساسة كفاية لتشخيص الحيوانات المصابة و التي لايظهر عليها أعراض مرضية.
3. صعوبة التميز بين الحيوانات المصابة بمرض نظير السل والأمراض الأخرى التي تتشابة بالأعراض.
4. عدم وجود لقاح فعال.

السيطرة على المرض

1. فحص و عزل الحيوانات المصابة .
2. عزل المواليد من الحيوانات المصابة.
3. تجنب انتشار المرض عن طريق الإخصاب الصناعي.
4. إدارة القطيع و البيئة\ من خلال توفر بيئة صحية و غذاء صحي غير ملوث بالبكتيريا و تجنب حشد عدد كبير من الحيوانات.
5. تغذية العجول بحليب معقم.

Enhanced Diagnostic Capacity and Control Measures of Some Transboundary Animal Diseases with Emphasis on Pathology and Epidemiology of Johne's Disease/Paratuberculosis in Jordan, Egypt and Algeria

Paratuberculosis (Johne's disease) in Sheep and Goat

Definition: Johne's disease; an infectious, incurable, chronically progressive granulomatous enteritis (especially of jejunum and ileum) which affects domestic and exotic ruminants including dairy and beef cattle, sheep, goats, cervids and camelids.

The disease is most common in cattle, sheep and goats, clinically affected animals are usually 2 years of age or older.

Is it in Jordan: Yes and in Egypt, Saudi Arabia, and in Morocco.
Is it found in milk: Yes?
Is it found in semen: Yes?

Cause: It is caused by *Mycobacterium.avium* subsp. *Paratuberculosis*. (MAP)

Does it cause disease in Humans: not confirmed nor disproved.The agent was isolated from patients with Crohn's.

Microorganism Characterization:

1. It is aerobic and grows at a temperature of 37ºC.
2. It has a rather wide growth temperature range, from 28ºC to 43ºC.
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5. Mycobacteria are resistance to acidic environments (as low as pH 2.5).
6. Survive in the acidic, humid environments of peat bogs and decomposed feces.
7. MAP can be isolated from ruminant feces, soil, and contaminated feed on farms with paratuberculosis infected ruminants.
8. Mycobacteria have a very thick cell wall and can survive in the environment for long time (a year).

Clinical Signs:

1. Chronic granulomatous degenerative enteritis that causes intermittent but persistent diarrhea, progressive weight loss, and eventually death.
2. The disease is untreatable and slowly, the typical manifestation of Johne's disease is profuse diarrhea passed effortlessly.
3. Emaciation is progressive and ultimately fatal, but the appetite is retained, and animals remain bright until the terminal stages.

4. Johne's disease in sheep and goats is comparable to that in cattle, occurring in adults and characterized by chronic wasting. The feces are soft, but there is usually no diarrhea, except intermittently in the stages progressive.

Gross and Microscopic Pathology: Thickening, in some cases (corrugation) and edema of the ileum and its mesenteric lymph nodes associated with chronic granulomatous enteritis.

Diagnosis:
Based on clinical signs and laboratory testing:
There are five commonly used diagnostic methods:

1. Histopathology
2. Culture
3. Direct smear from intestinal mucosa and lymph node parenchyma scrapping.
4. Immunohistochemistry
5. PCR

Prevention and Control
Eradication of the disease and estimation of the prevalence rate in an area are difficult due to

- The lack of reliable diagnostic method,
- The long incubation period of the disease and the failure to report cases.

Attempted control methods include culling suspected heavy shedders and careful manure management to prevent oral contamination of lambs.

Suggested manure management strategies include:
1) Avoiding fecal build-up in lambing areas.
2) Avoiding contamination of replacement rearing areas by adult manure.
3) Use of deep bedding and clean pens for young lambs.
4) Keeping ewe fleeces and udders "free" of fecal contamination by shearing or crutching ewes before lambing.
5) Artificial rearing of lambs, including feeding colostrums from a source known to be Johne's free.
6) Avoiding contamination of mangers and feed with boots, shovels, wheel barrow tires, and other cleaning equipment.

Ewes showing weight loss prior to lambing may be suspected of infection and shedding large numbers of organisms.
Their offspring are more likely to be infected and should not be kept for replacement.

Control of infected flocks grazing pasture may be impossible but suggested control measures include
1) Identifying and removing heavily shedding animals from pasture,
2) Weaning young lambs early and placing them on clean pasture without adult contact,
3) Avoiding commingling with other animal species of unknown Johne's disease status,
4) Keeping water sources free of contamination.

مشروع تحسين القدرات التشخيصية وإجراءات السيطرة على الأمراض العابرة للحدود مع التركيز على مرض نظير السل

مرض نظير السل في الأغنام و الماعز

تعريف: مرض نظير السل هو مرض معدي لا يعالج، مزمن يصيب الأمعاء، ويصيب المجترات البرية و المستأنسة و التي تتظمن الأبقار و الأغنام و الماعز و الجمال .
المرض منتشر في الأبقار و الأغنام و الماعز و الحيوانات المصابة أكثر من سنتين.

هل هو موجود في الأردن ؟ نعم و يوجد في مصر و السعودية و المغرب.
هل هو موجود في الحليب ؟ نعم.
هل هو موجود في السائل المنوي ؟ نعم.

المسبب : بكتيريا **Mycobacterium.avium subsp paratuberculosis (MAP)**
هل يسبب المرض في الإنسان ؟ غير مؤكد و لكن تم عزل البكتيريا المسببة لمرض نظير السل في المصابين بمرض **Crohn's**.

خصائص البكتيريا:

9. هوائيه و تنمو على درجة حرارة 37 درجة سيلوسية.
10. لها درجة حرارة للنمو واسعة من 28-43 درجة سيلوسية .
11. تصبغ بصبغة **Acid Fast Stain** وتعتمد على **Mycobactin** في نموها.
12. تتأثر بشكل قليل بالأشعة فوق البنفسجية .
13. البكتيريا مضادة للبيئة الحمضية (**pH 2.5**).
14. تعيش في الوسط الحمضي و البيئة الرطبة و مستنقعات المياه و البراز.
15. يمكن عزل البكتيريا من براز المجترات و التربة والغذاء الملوث في المزارع التي بها حيوانات مصابة.
16. البكتيريا لها جدار خلوي سميك و لذلك تعيش في البيئة لوقت طويل.

الأعراض المرضية

1. إلتهاب مزمن بالأمعاء و الذي يؤدي الى إسهالات و نقصان في الوزن وعدم نقصان في شهية الحيوان و هزال عام ثم الموت المحتوم.
2. لا يوجد علاج لهذا المرض .
3. مرض نظير السل في الأغنام و الماعز مقارنة بالأبقار يحدث في الحيوانات الناضجة و يتميز بهزال مزمن و بكون البراز لين و لا يوجد عادةً إسهال و لكن تحدث إسهالات متقطعة في نهاية المرض.

التشريح المرضي و النسيج المرضي
يصبح جدار الأمعاء سميك و في بعض الحالات تعرج في بطانة الأمعاءووذمه في الأمعاء(**Ileum**) و العقد اللمفاوية(**Mesenteric Lymph node**) مع إلتهاب مزمن للأمعاء.

طرق التشخيص :
بالإعتماد على الأعراض المرضية و الفحوصات المخبرية يوجد 5 طرق:

6. الزراعة البكتيرية.

7. مسحة من بطانة الأمعاء و العقد اللمفاوية.
8. النسيج المرضي (**Histopathology**).
9. **Immunohistochemistry**.
10. **PCR**.

السيطرة و منع إنتشار المرض
صعوبة السيطرة و التخلص من مرض نظير السل و الحد من إنتشارة بسبب:

1. فترة حضانة المرض طويلة.
2. الفحوصات التشخيصية غير حساسة كفاية لتشخيص الحيوانات المصابة و التي لايظهر عليها أعراض مرضية.

من طرق السيطرة على المرض:التخلص من الحيوانات الناشرة للبكتيريا و التخلص من برازها لمنع إنتشار المرض من خلال الأكل.

طرق مقترحة للسيطرة على إنتشار المرض من خلال البراز:

1. تجنب تراكم البراز في مناطق الولادة .
2. تجنب تلوث أماكن تربية المواليد من الحيوانات المصابة.
3. استخدام أرضية سميكة و تنظيف مناطق المواليد.
4. المحافظة على صوف و ضرع الأمهات نظيف من التلوث بالبراز عن طريق قص الصوف قبل الولادة.
5. التربية الإصطناعية للمواليد و إعطائها حليب خالي من التلوث بالبكتيريا .
6. تجنب تلوث العمال و الغذاء عن طريق أدوات المزرعة.
7. الحيوانات التي تظهر فقدان وزن قبل الولادة قد تكون أكثر إصابة و تفرز عدد كبير من البكتيريا.
8. المواليد التي يحتمل إصابتها بجب عدم الاحتفاض بها.

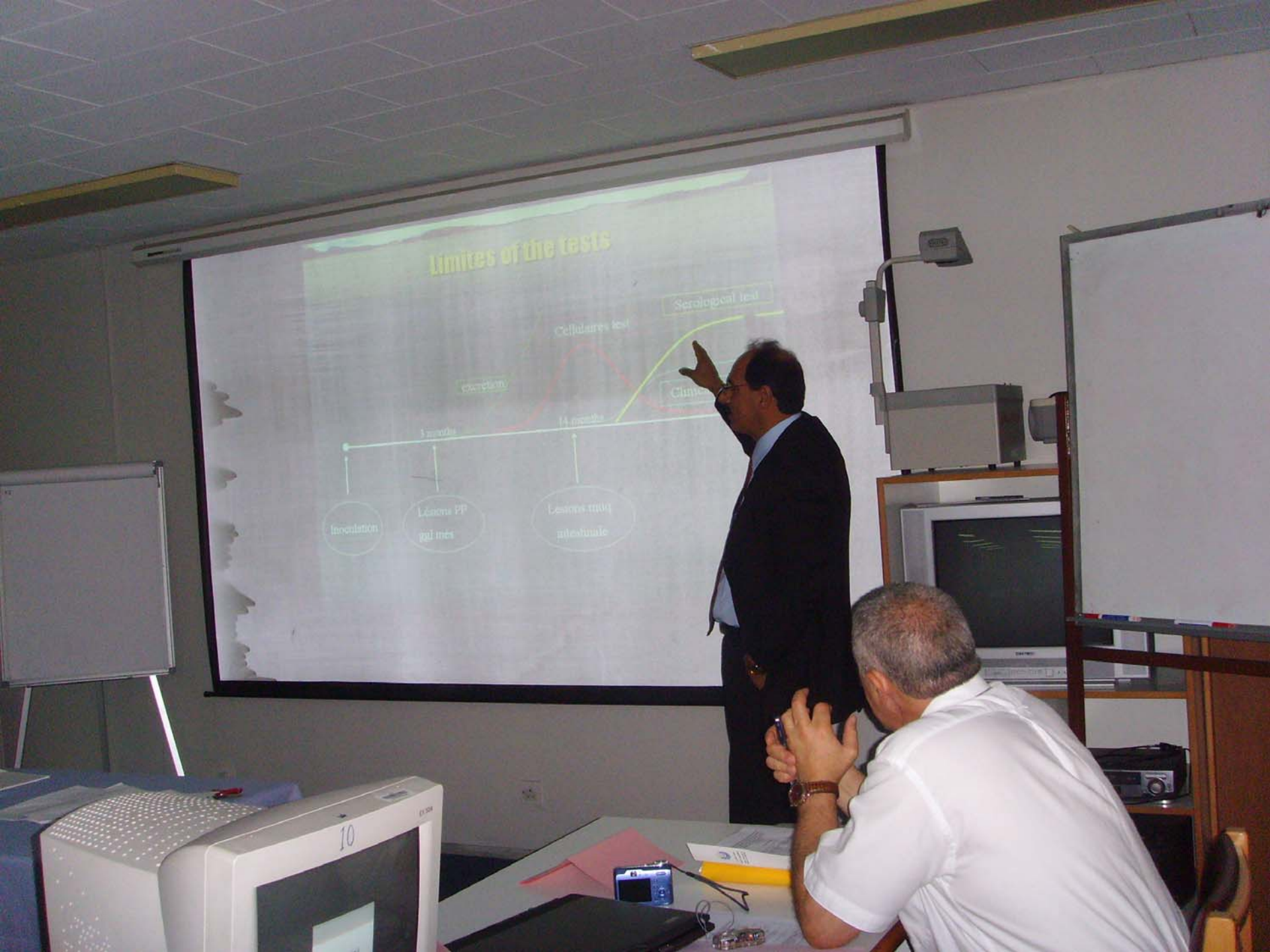
طرق مقترحة للسيطرة على إنتشار المرض في مناطق رعي الحيوانات المصابة

1. التشخيص والتخلص من الحيوانات المصابة في مناطق الرعي.
2. فطم الرضع و وضعهم في مناطق رعي نظيفة ،بعيداً عن الحيوانات المصابة.
3. تجنب مخالطة الحيوانات مع حيوانات أخرى غير معروف خلوها من المرض.
4. تجنب تلوث مصادر المياه.


7 10:27 AM


Orthomyxoviridae
A.I. Virus
Single stranded RNA -ve
Segmented: 8 genes cod for 10 proteins
Two glycoprotein surface projection:
Haemagglutinin (HA): H1-H16
Neuraminidase (NA): N1-N9
Enveloped (20% lipid): Sensitive to heat, dryness and normal disinfectants
Antigenic types A, B, C
Pathogenicity vary


Paratuberculosis diagnosis
1 Clinical signs together with the signalment
In bovine, intermittent diarrhea, emaciation and hypoproteinemia in animals older than 19 months
In small ruminants, the clinical disease is similar to that observed in bovine except that diarrhea does not occur

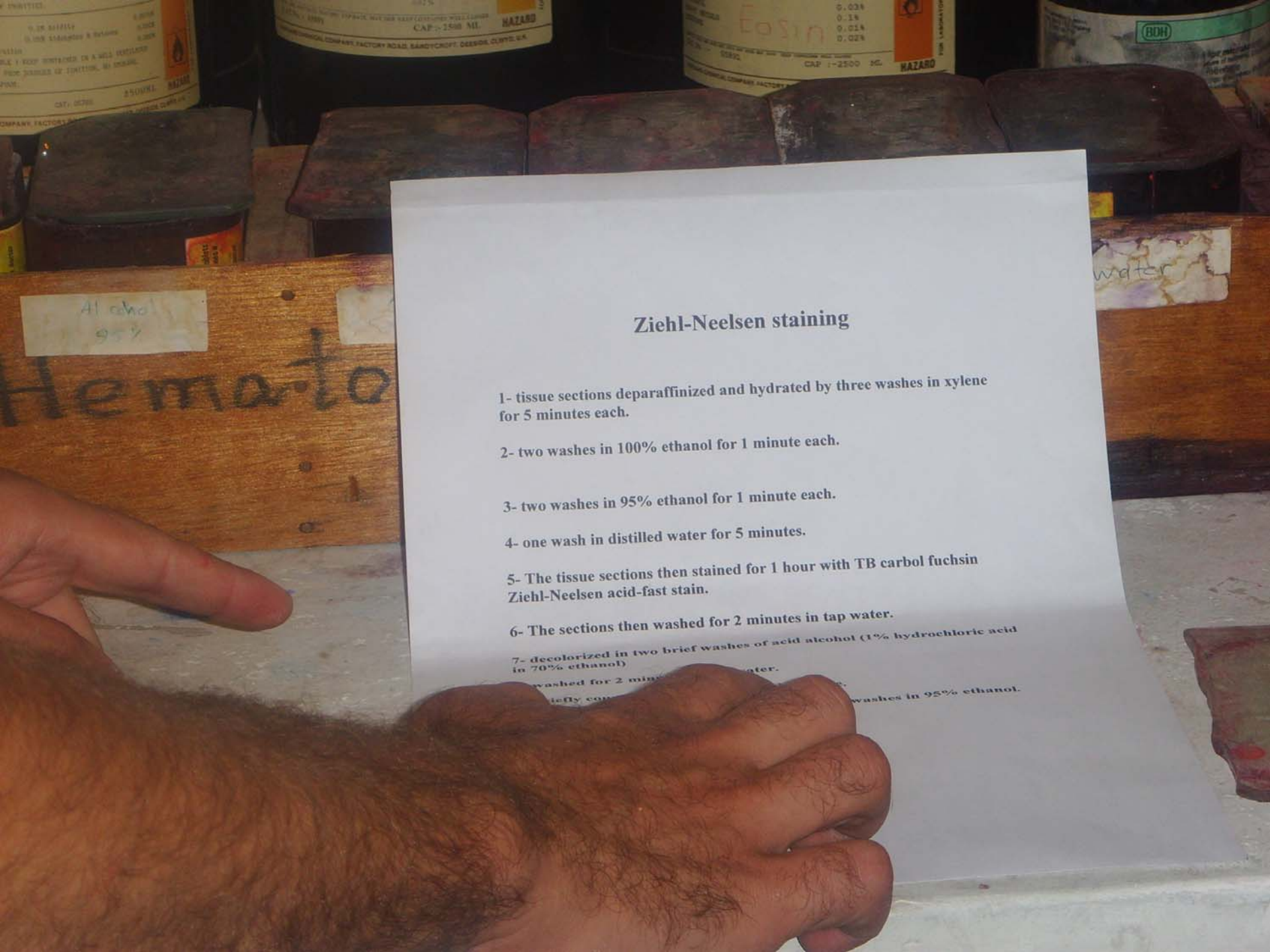

Limites of the tests
Sérologocal test
Cellulaires test
Clinic
3 months
14 months
Inoculation


Crohn's Disease
Etiology and Pathogenesis
Khaled Jadallah, MD


What causes Johne's disease ?


Paratuberculosis diagnosis
Clinical signs together with the signalment
In bovine, intermittent diarrhea, emaciation and hypoproteinemia in animals older than 19 months
In small ruminants, the clinical disease is similar to that observed in bovine except that diarrhea does not occur


How can you prevent your animals from getting Johne's disease?


Ziehl-Neelsen staining
1- tissue sections deparaffinized and hydrated by three washes in xylene for 5 minutes each.
2- two washes in 100% ethanol for 1 minute each.
3- two washes in 95% ethanol for 1 minute each.
4- one wash in distilled water for 5 minutes.
5- The tissue sections then stained for 1 hour with TB carbol fuchsin Ziehl-Neelsen acid-fast stain.
6- The sections then washed for 2 minutes in tap water.
7- decolorized in two brief washes of acid alcohol (1% hydrochloric acid in 70% ethanol)
washes in 95% ethanol.
Alcohol
Hemato
Eosin
water
BDH
HAZARD

CERTIFICATE شهادة

The Hashemite Kingdom of Jordan
Jordan University of Science & Technology
The Consultative Center for Science & Technology
Irbid - P.O. Box 3030 - Jordan

المملكة الأردنية الهاشمية
جامعة العلوم والتكنولوجيا الأردنية
المركز الاستشاري للعلوم والتكنولوجيا
إربد - ص.ب ٣٠٣٠ - الأردن

This is to certify that Prof. Hussam EL- Attar has completed لقد أتمّ

A training course in "Enhanced Diagnostic Capacity and Control Measures of Some Transboundary Animal Disease with Emphasis on Pathology and Epidemiology of John's Disease/ Paratuberculosis in Jordan, Egypt and Algeria" الدورة التدريبية بعنوان

Which is equivalent to ـــــ Training hours ساعة تدريبية ـــــ والتي تعادل

And was held during the period August 7- 9, 2007 وعقدت خلال الفترة ما بين

Director of the Consultative Center for Science & Technology مدير المركز الاستشاري للعلوم والتكنولوجيا

Prof. Khalil I. Ereifej الاستاذ الدكتور

Jordan University of Science & Technology
The Consultative Center for Science and Technology
Irbid - Jordan


Orthomyxoviridae
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Neuraminidase (NA): N1-N9
Enveloped (20% lipid): Sensitive to heat, dryness and normal disinfectants
Antigenic types A, B, C
Pathogenicity vary


Treatment of IBK (Cont'd)
- Equally important as the choice of antibiotic is
the route of ther
Topical (Highest
plication).
Subconjunctiva

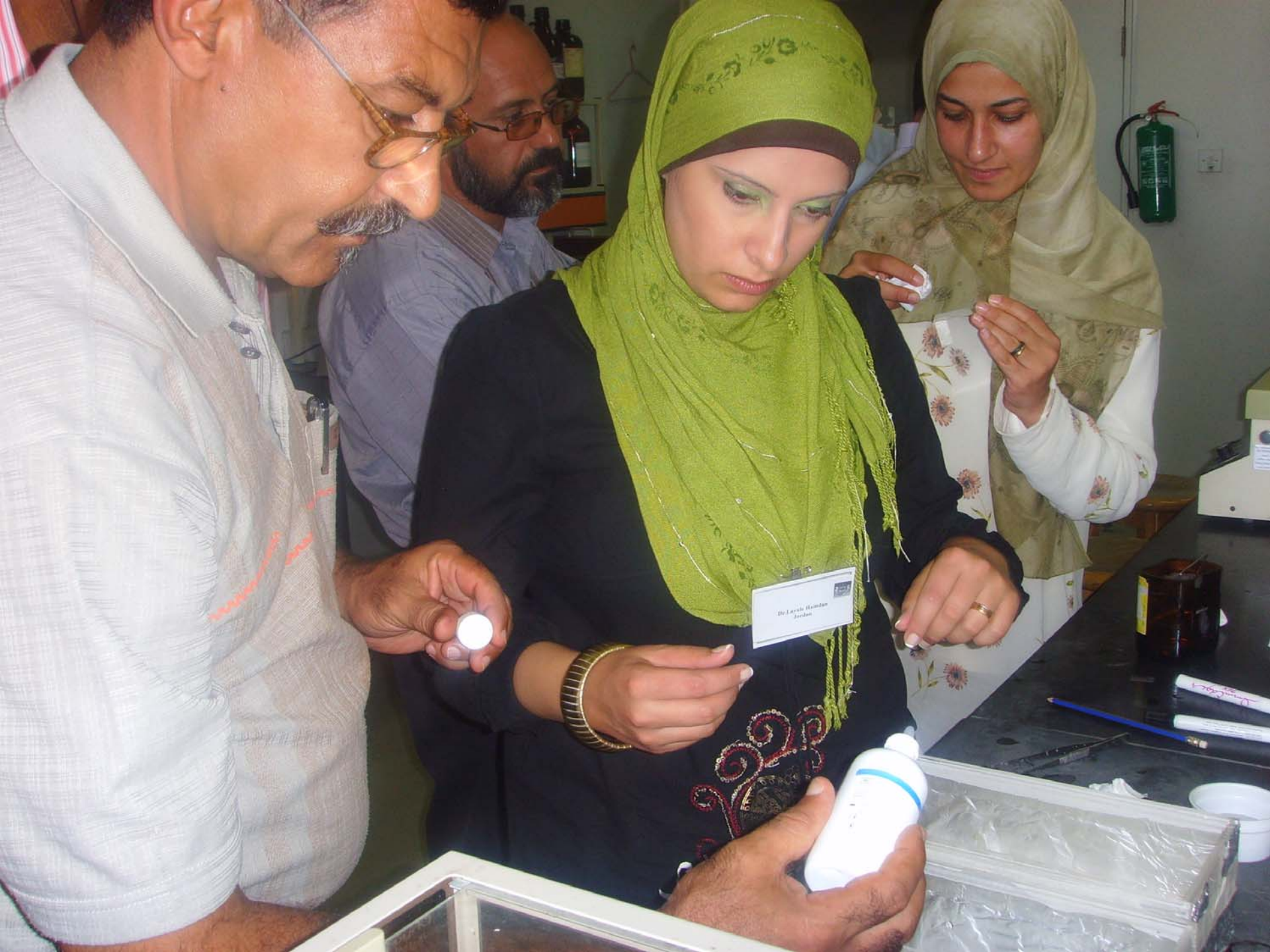

Regional
LATEX MED
EXAMINATION G
POWDERED
Disposable
Non-Sterile
Ambidextro


SILVER STAR


SONY
LEICA
9 11:31 AM


500
SOLUBLE


SOLUBLE

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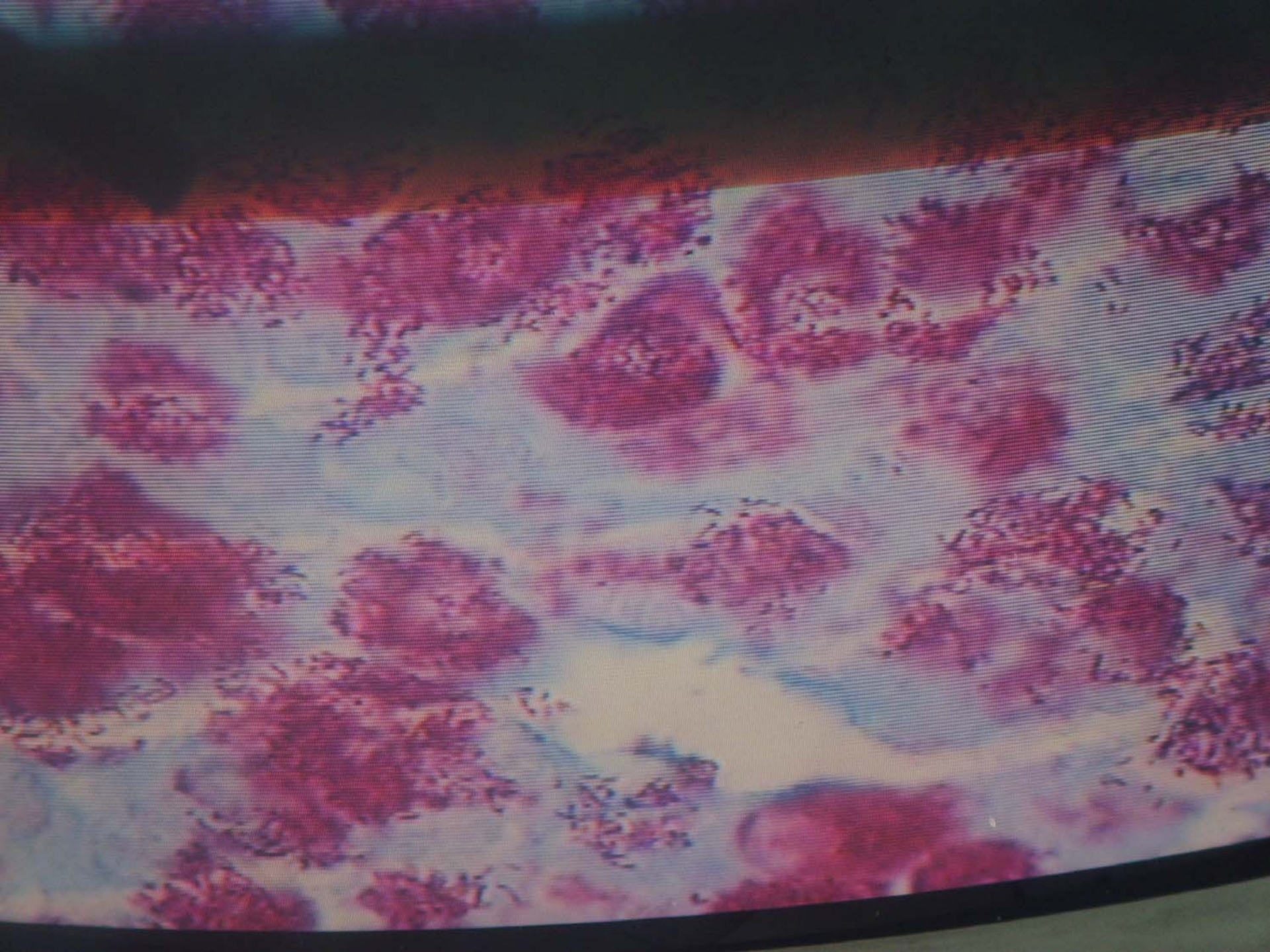



START
END OF CYCLE
IN - NORMAL
OUT - RAISE ROTOR
DELAY IN OPERATION
Leica
LEICA ST4040
30 10:03

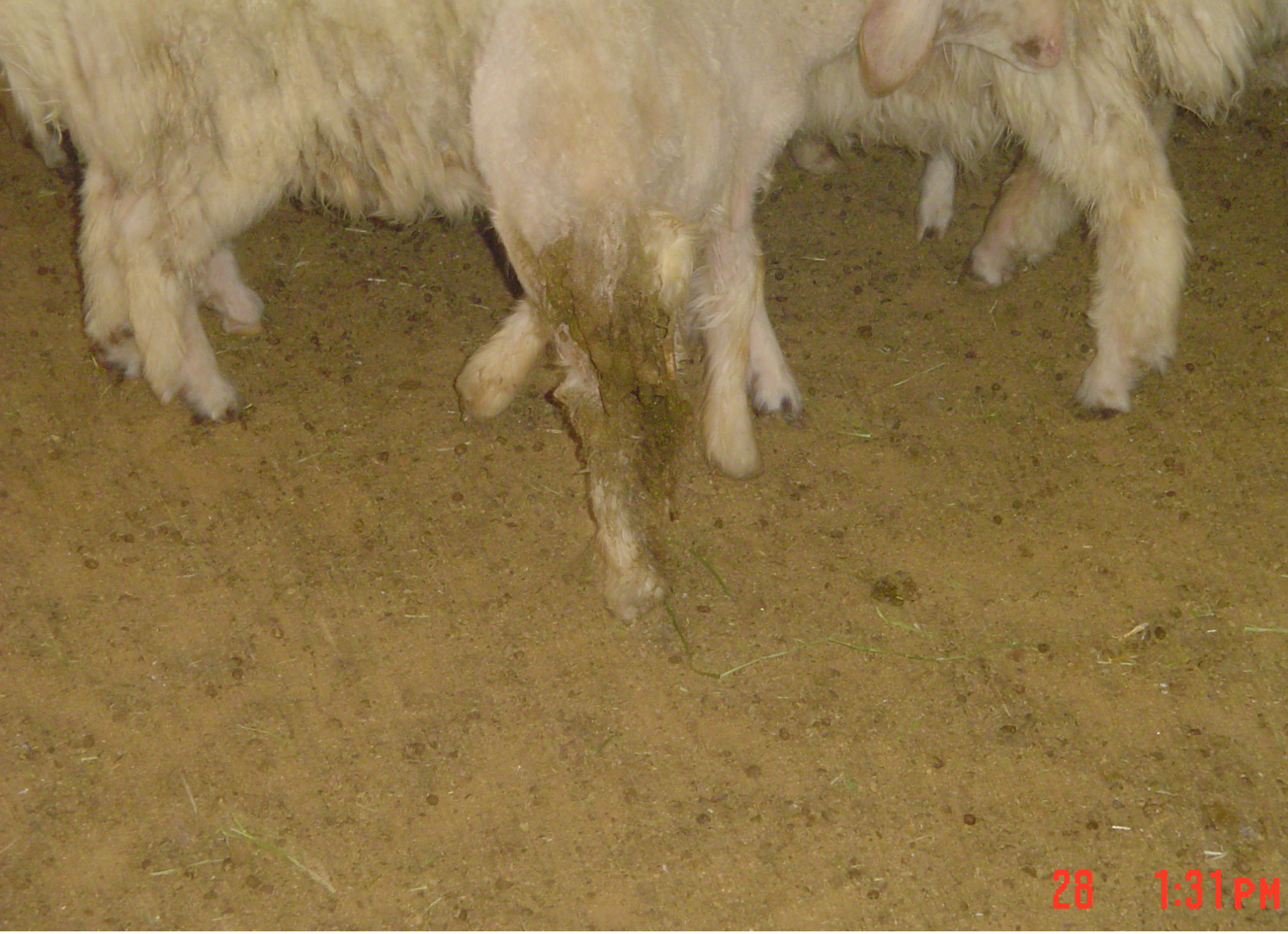

7|B
30 10:05 AM


30 10:06 A

وزارة الزراعة

محطة الفجيج الزراعية لتربية وتحسين

أغنام العواسي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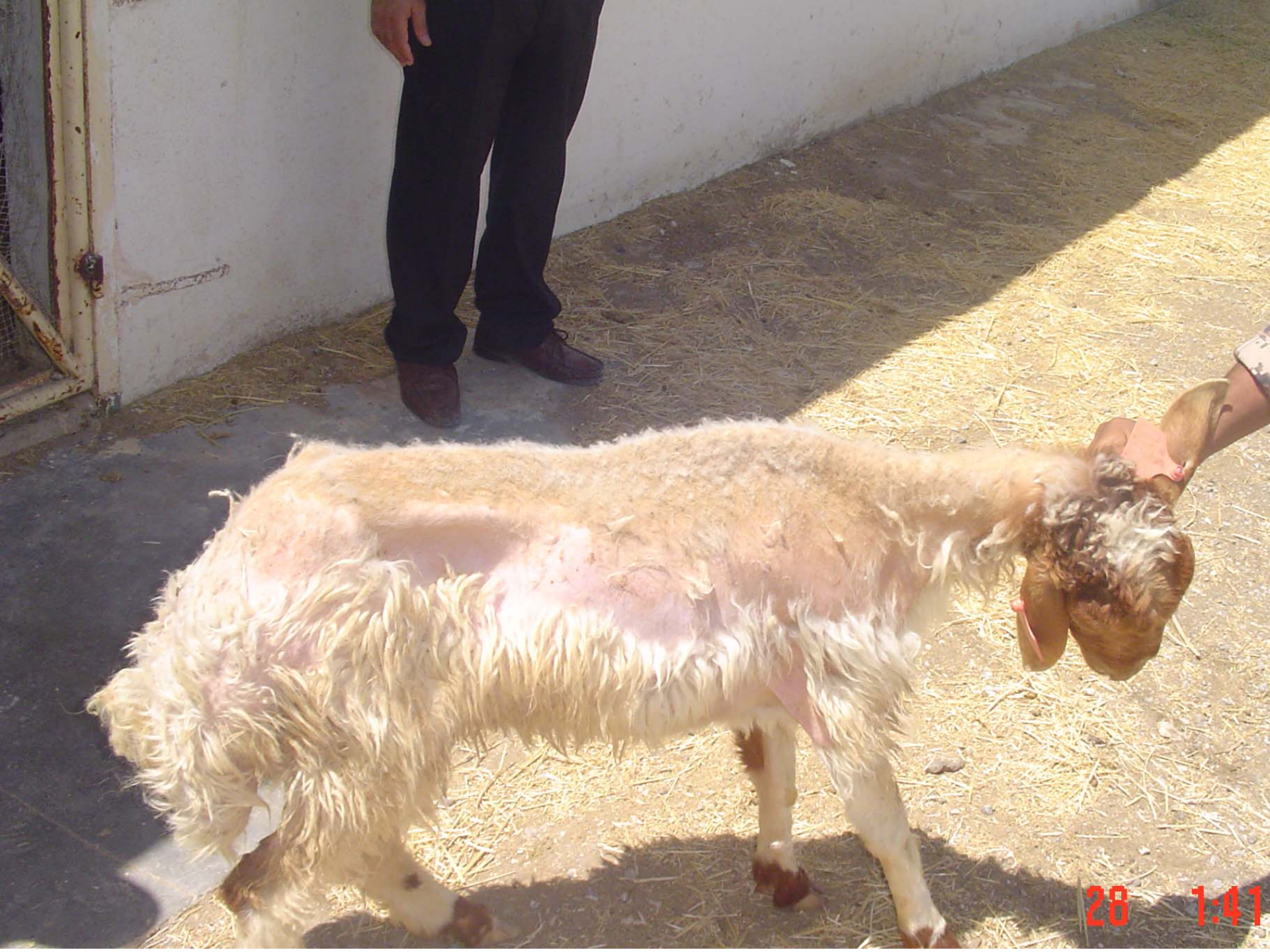
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28 1:33 P


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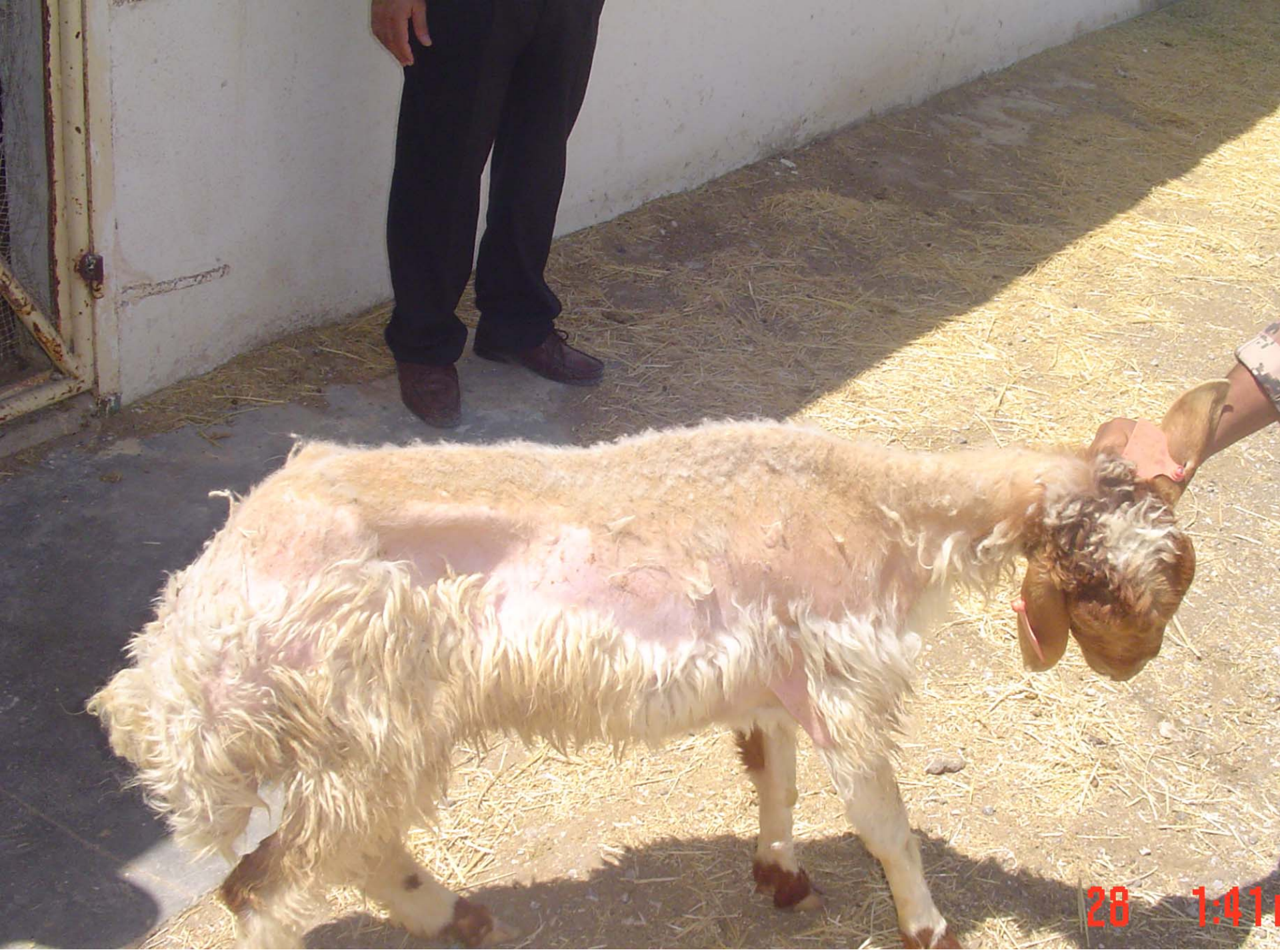
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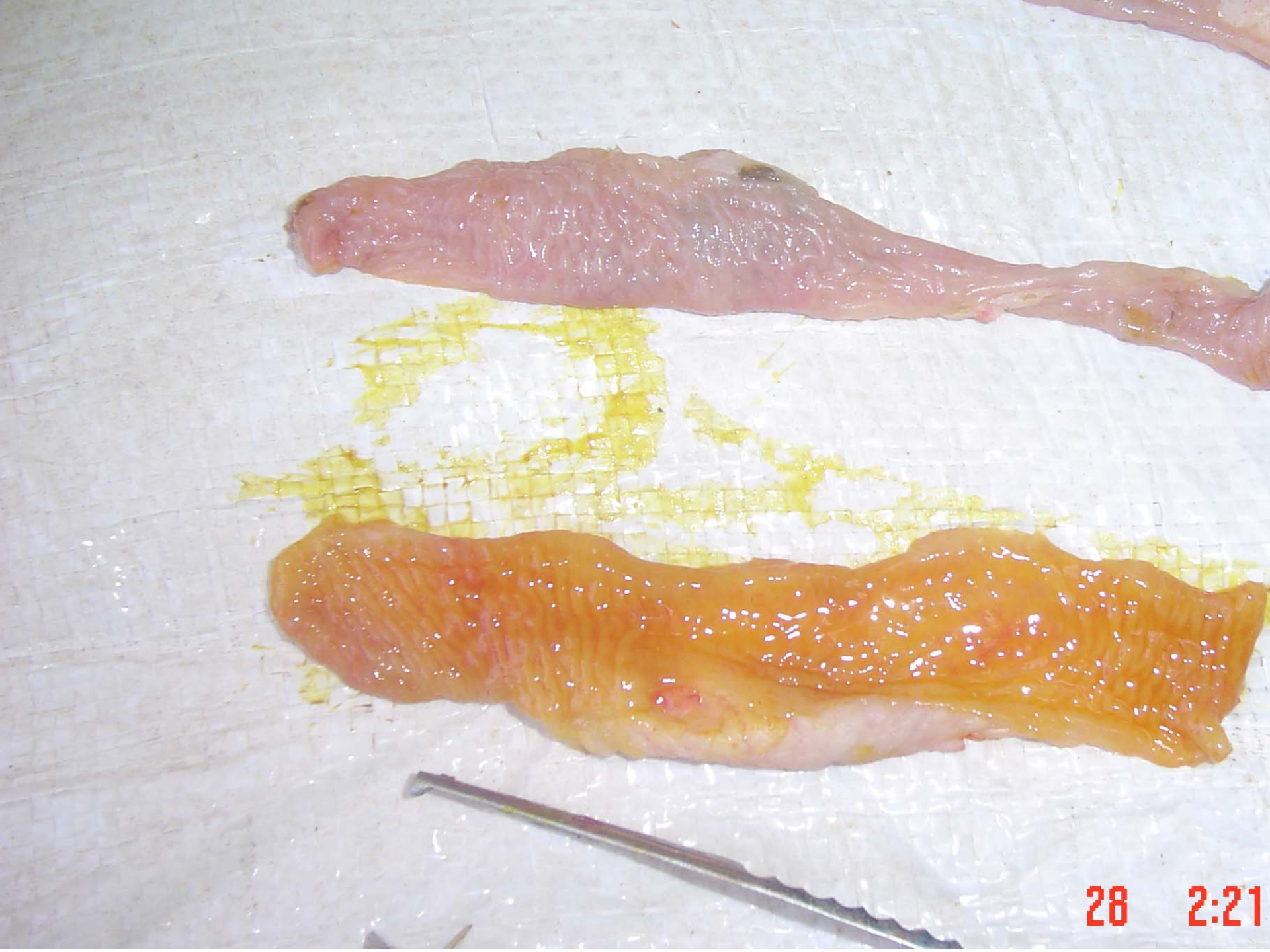
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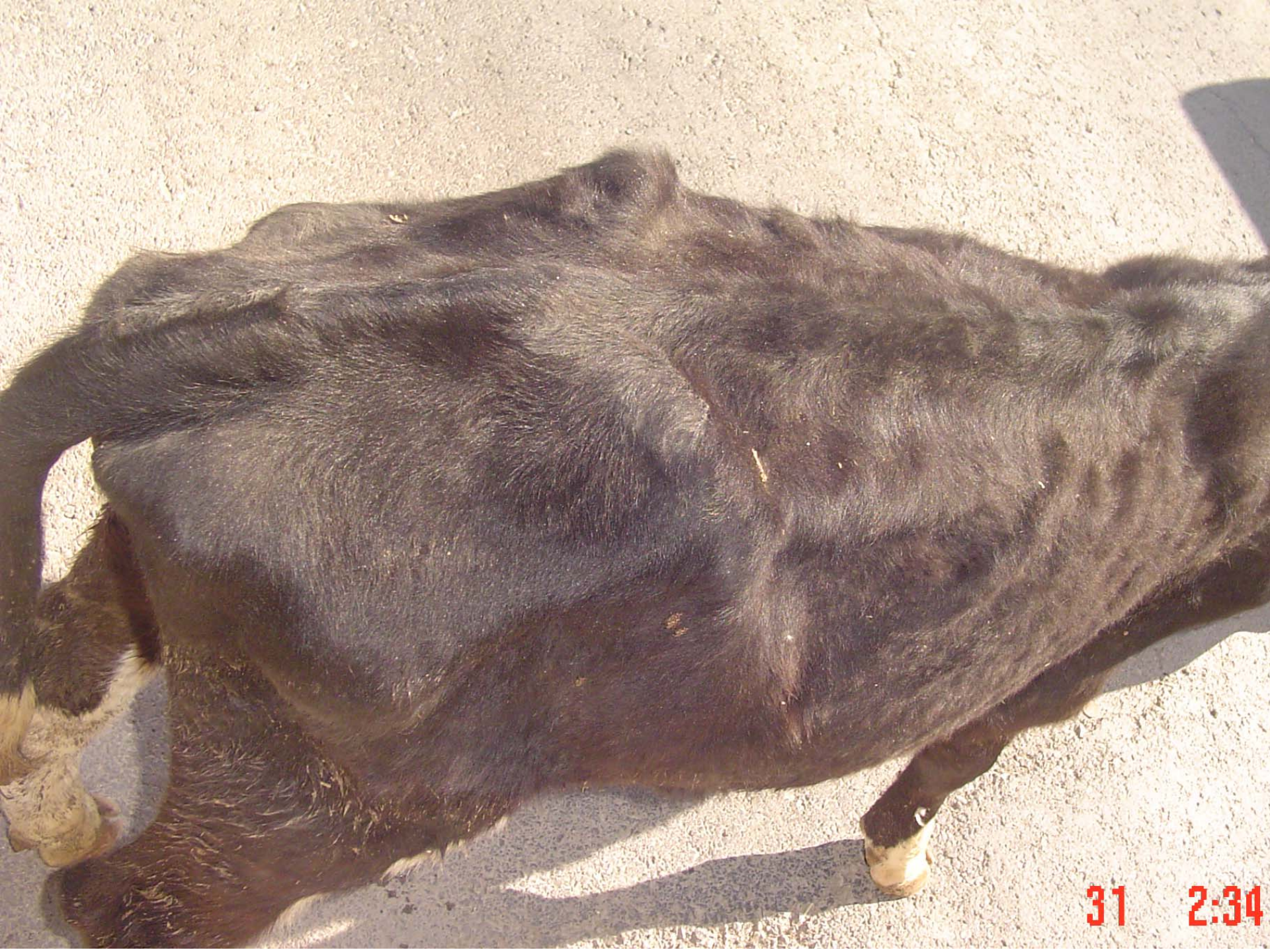
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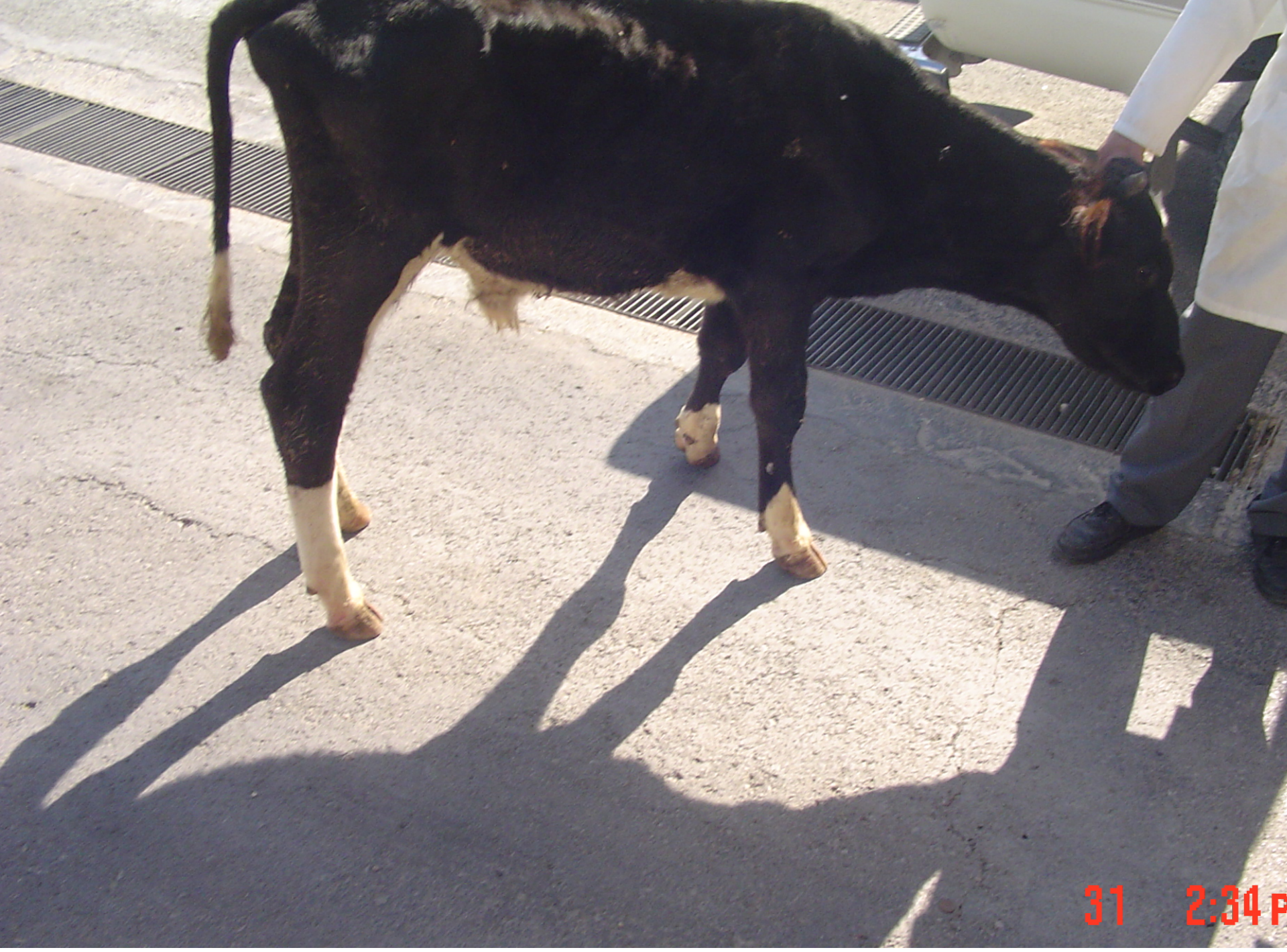
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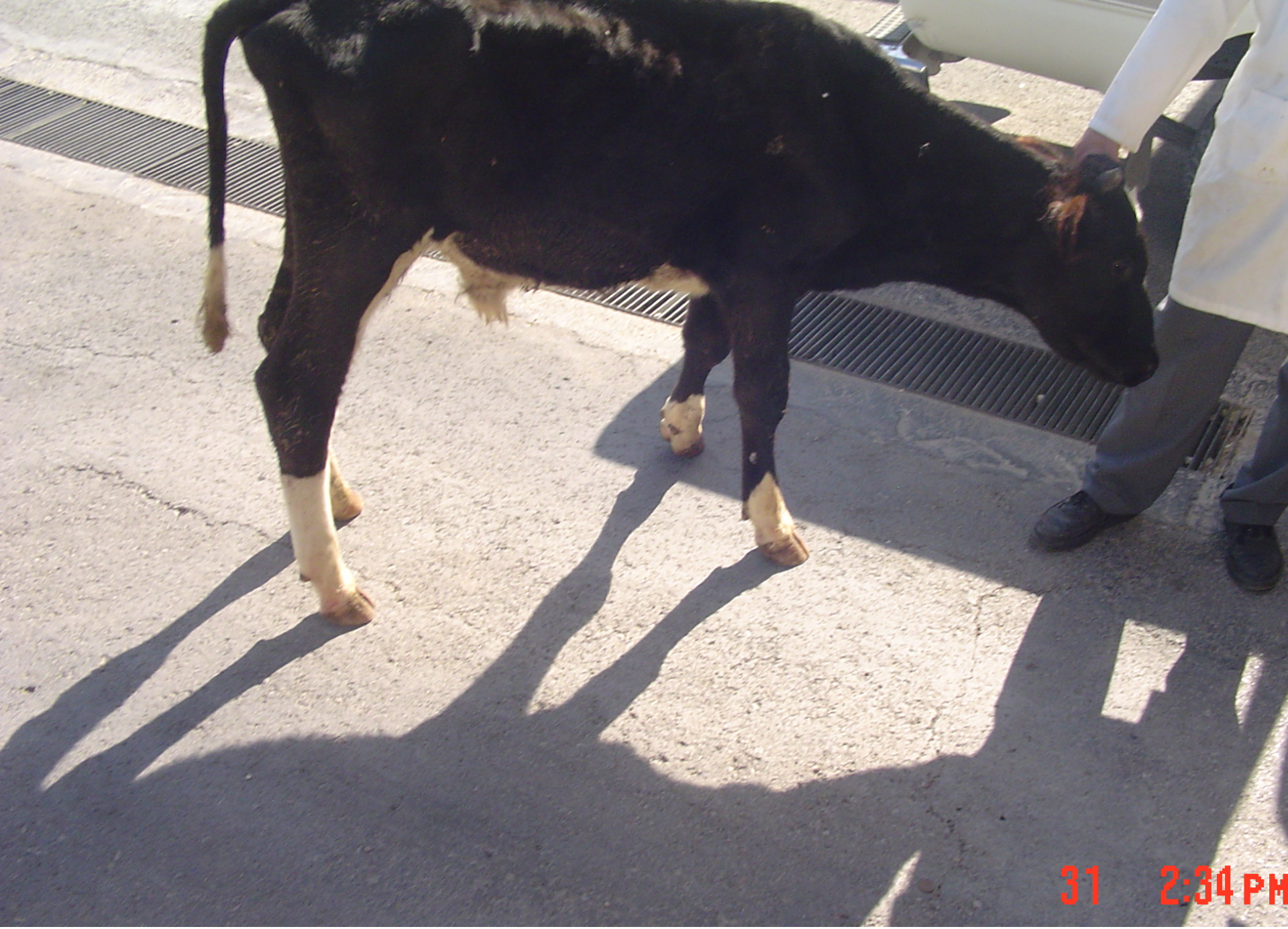
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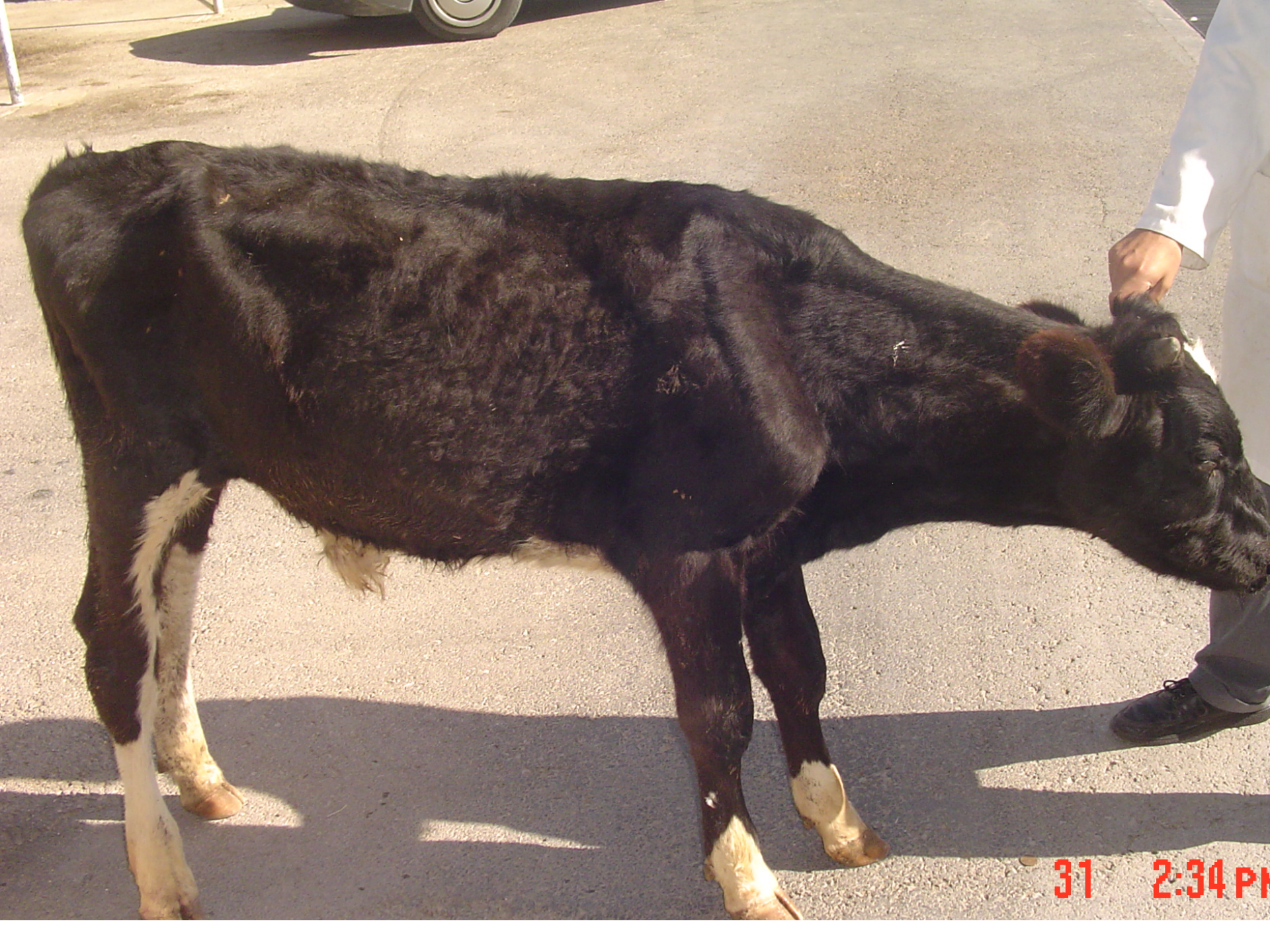
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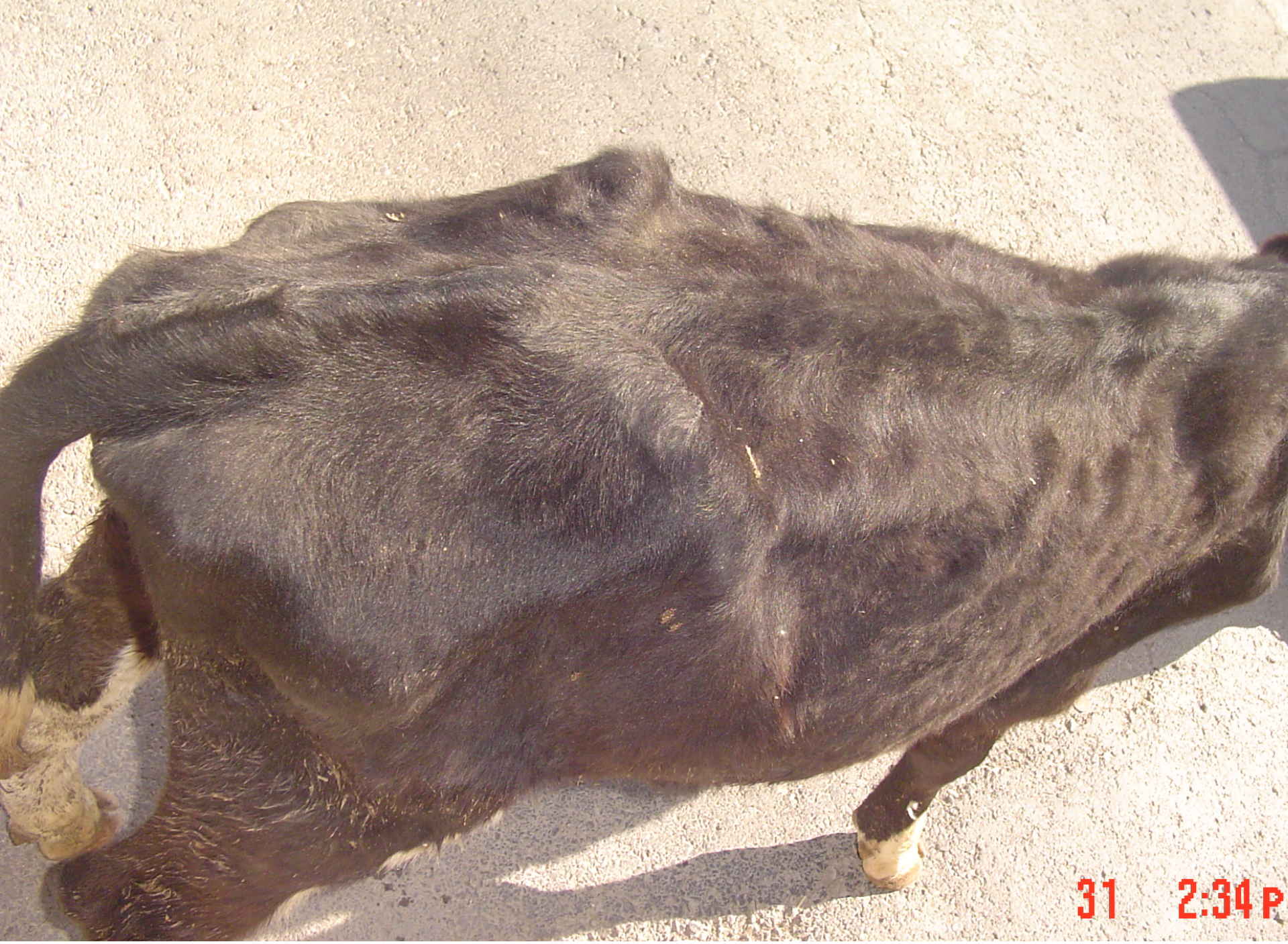
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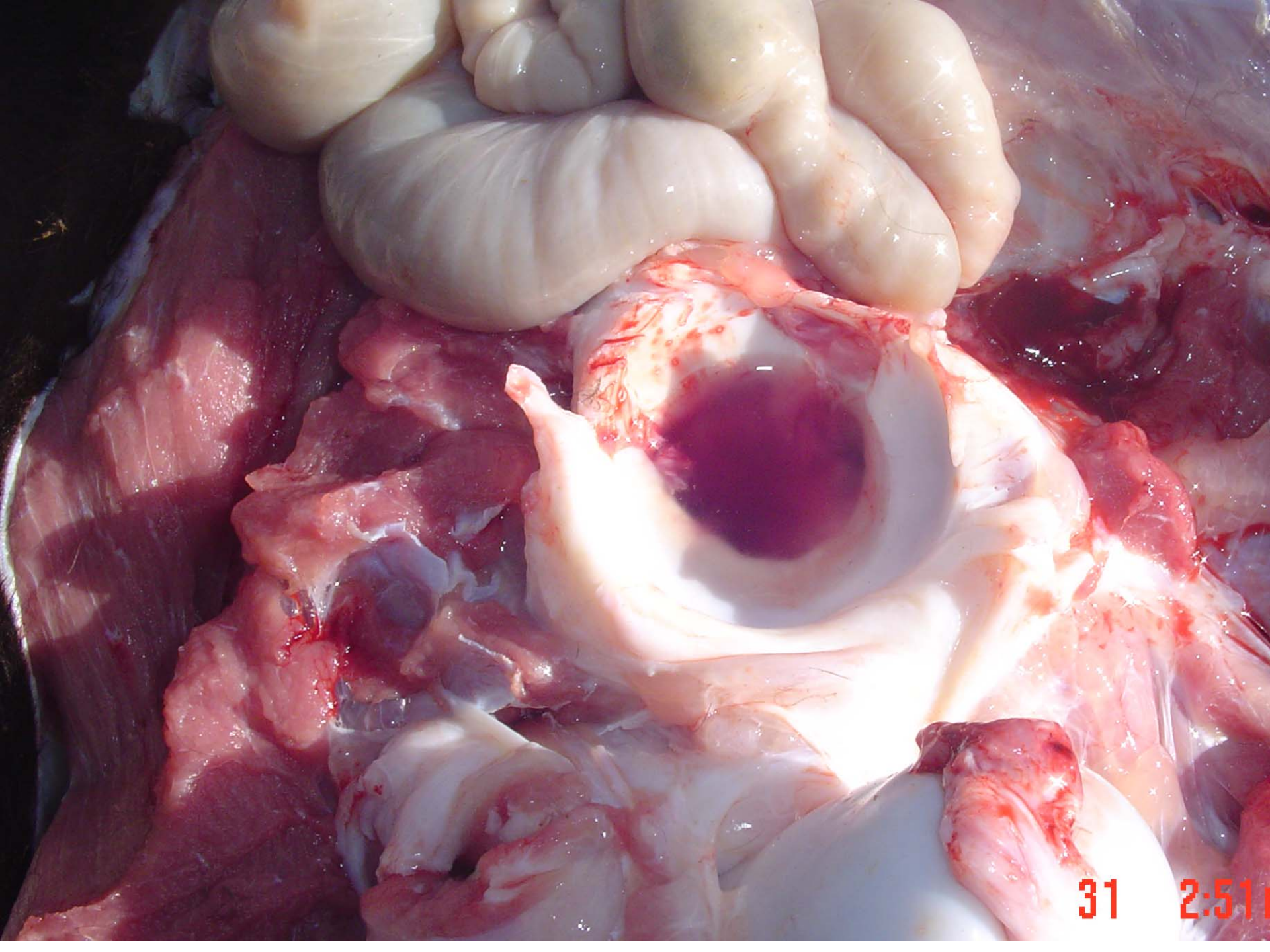
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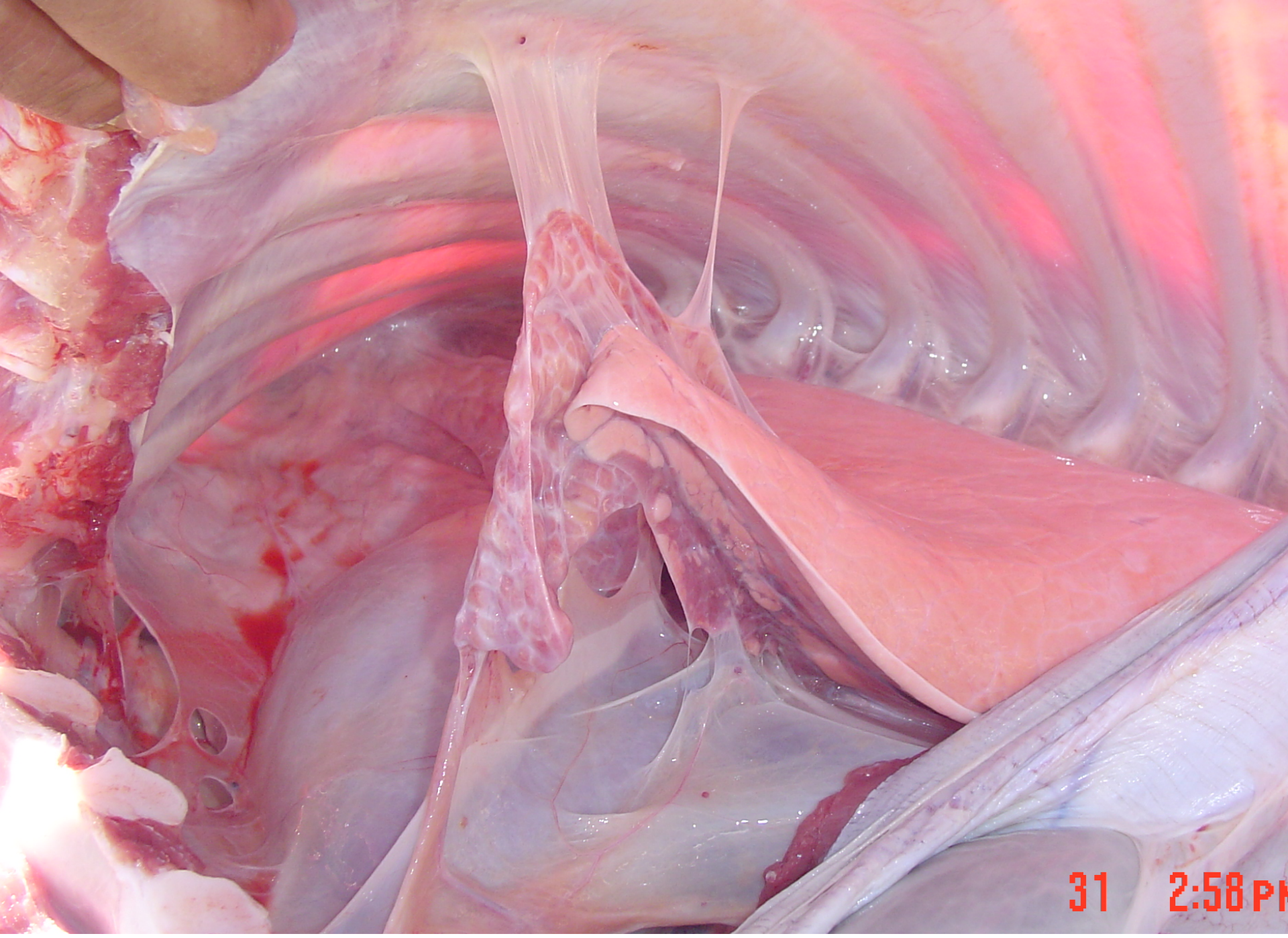
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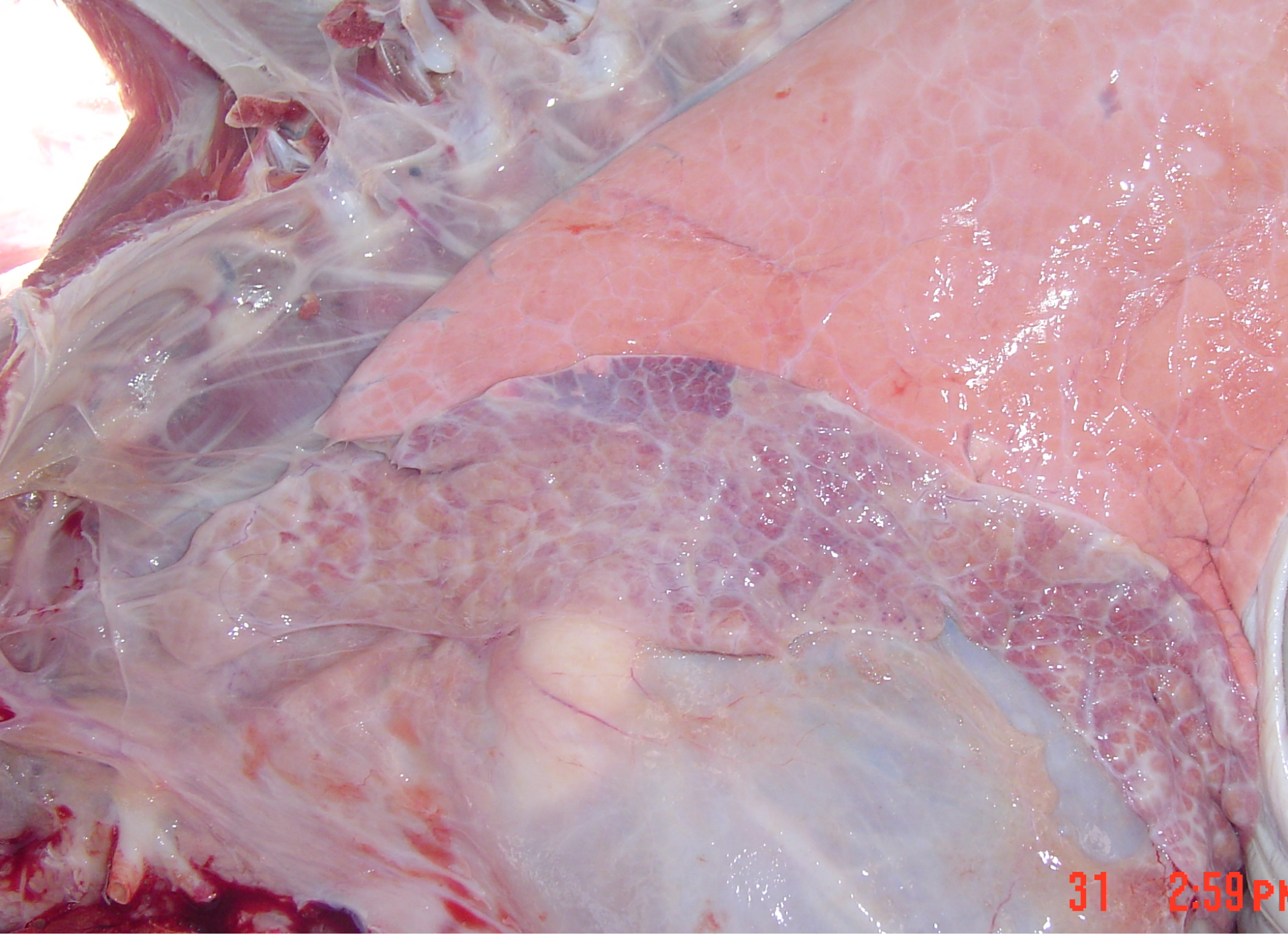
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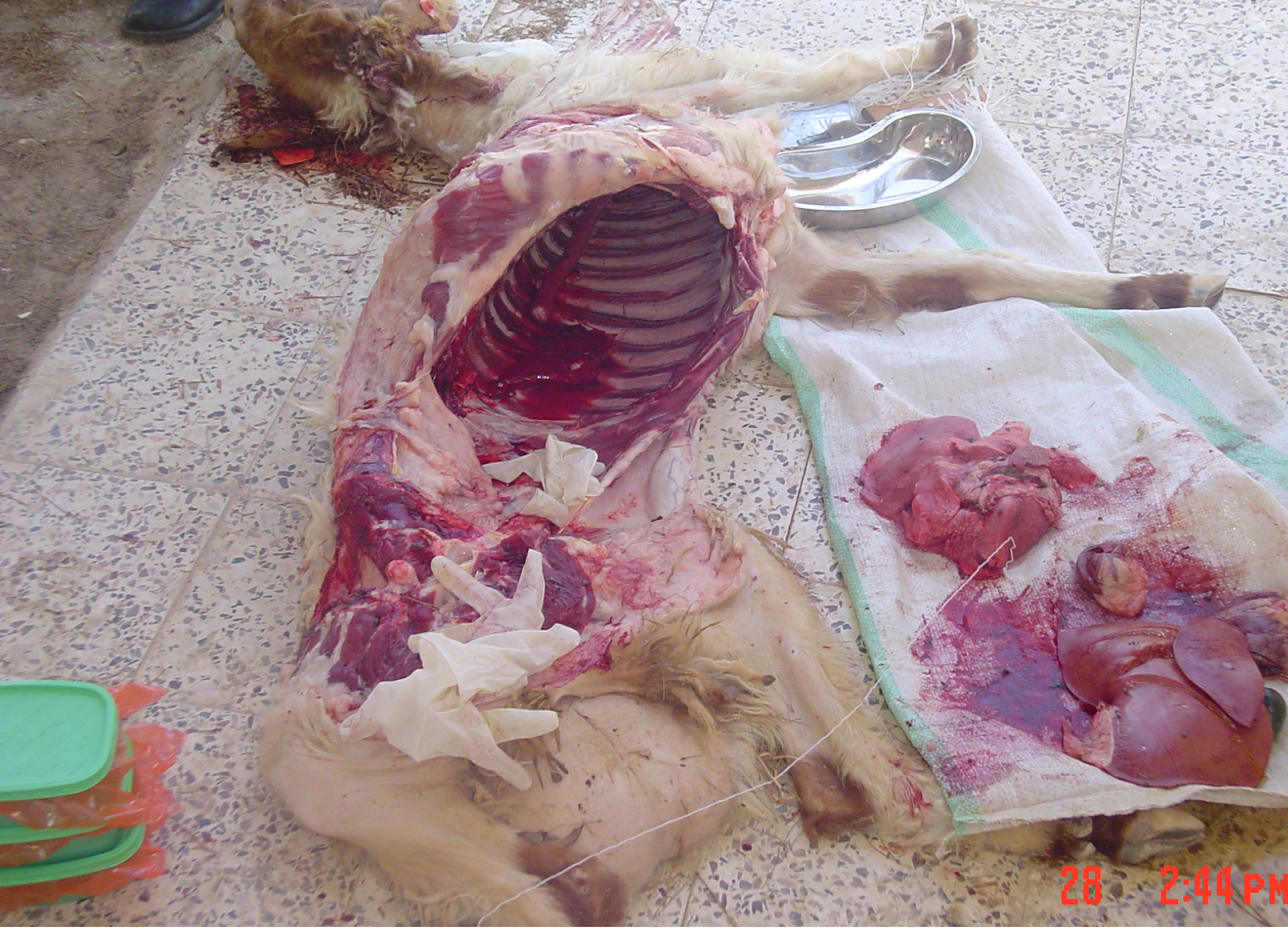
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