MOLECULAR AND SEROLOGICAL METHODS APPLICATION FOR THE DIAGNOSIS OF AVIAN MYCOPLASMOSIS IN KUWAIT POULTRY INDUSTRIES

KISR
Kuwait Institute for Scientific Research

Presented by:

Ebtesam Al-Ali

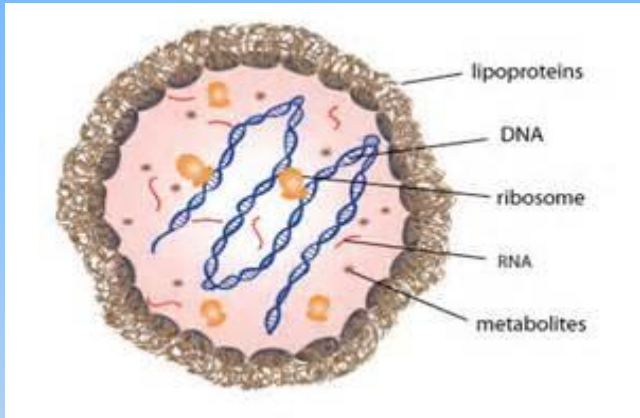
Introduction

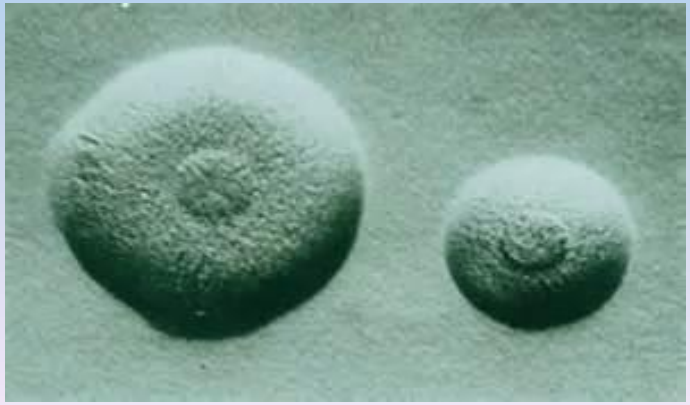
Mycoplasmosis in Chickens

Raising chickens, turkeys and other types of poultry entails undertaking the serious responsibility of disease prevention. Small poultry flocks are susceptible to a number of respiratory infections. Regardless of whether birds are raised for meat, egg, breeding or show purposes, respiratory infections results in decreased performance that leads to economical losses.

The chronic respiratory disease of chickens and infectious sinusitis of turkeys are called avian Mycoplasmosis; the causative agent for this disease is *Mycoplasma gallisepticum* (MG). *Mycoplasma* has a wide distribution in nature, they lack the cell wall and they include important pathogens of animals, plants and insects.

It is difficult to diagnose the Mycoplasma infections based on symptoms alone, for the diagnosis of this organism; we need a faster and more specific method because of the difficulty of culturing them in laboratory.

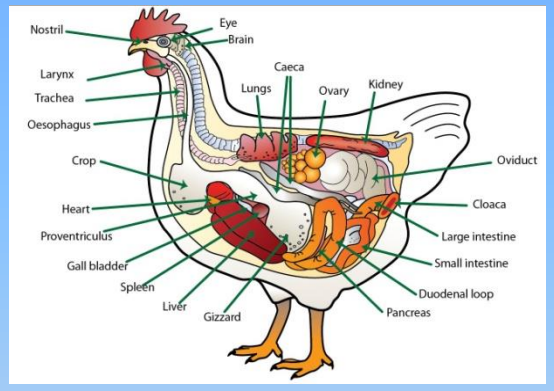




Mycoplasmas are very small prokaryotes with no cell walls, bounded by a plasma membrane only. They are resistant to antibiotics that affect cell wall synthesis. They are found in humans, many animal species, plants, and insects.

Mycoplasma pathogens cause respiratory and locomotory illness in chickens and other avian species.

Mycoplasmas is responsible not only for clinical diseases but also for economic losses such as: decreased weight gain, lowered feed conversion efficiency, reduced hatchability, and downgrading at slaughters.



Objective

- **The objective of this work is to evaluate two commercial kits for the detection of Mycoplasma infection in chicken, a PCR diagnostic kit (VenorMGs) and an ELISA diagnostic kit (ProFLOK) in comparison to the traditional culture method.**

Methodology

Field Specimens

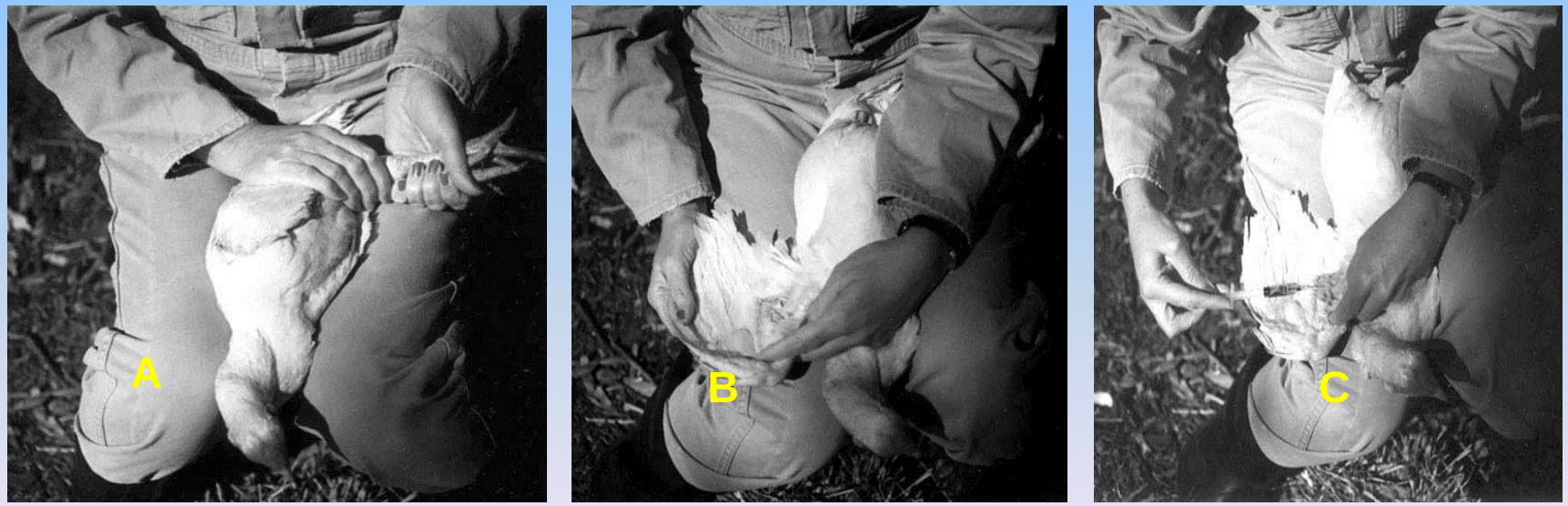
Endo-tracheal and choanal cleft swabs were collected from chicken suffering from respiratory symptoms and a symptomatic chickens.

Culture Methods

The standard *M. gallisepticum* strain ATCC 15302 and *M. synoviae* ATCC 25204 (American type culture collection, Atlanta, USA) was grown in Pleuro pneumonia like organisms (PPLO) broth and agar supplemented with Mycoplasma supplements (DIECO) and glucose (0.1%) plus phenol red (0.002%) to serve as an indicator for growth. The clinical specimens (10 ml) were inoculated on PPLO agar. The cultures were incubated at 37° C in an atmosphere of 5% CO2 and 95% air.

Sampling for ELISA

At least 30 or more sera per flock were randomly collected at standard time intervals. Serum sample storage (4°C for up to four days or -20°C for longer periods) were needed to provide reliable test results.



Blood Collection from chickens; a) & b) positioning the chicken (c) blood sample collection.

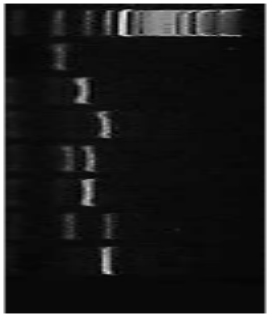
Sample preparation for PCR tests

Swabs from trachea and choanal cleft of a chicken were placed into a tube containing 0.5 ml PBS, washed gently, the excess liquid was squeezed from the swab by rolling the swab against the wall of the tubes, - heated for 15 minutes at 95°C, - the samples were stored on ice and test within 24 hours.

Swab Sampling

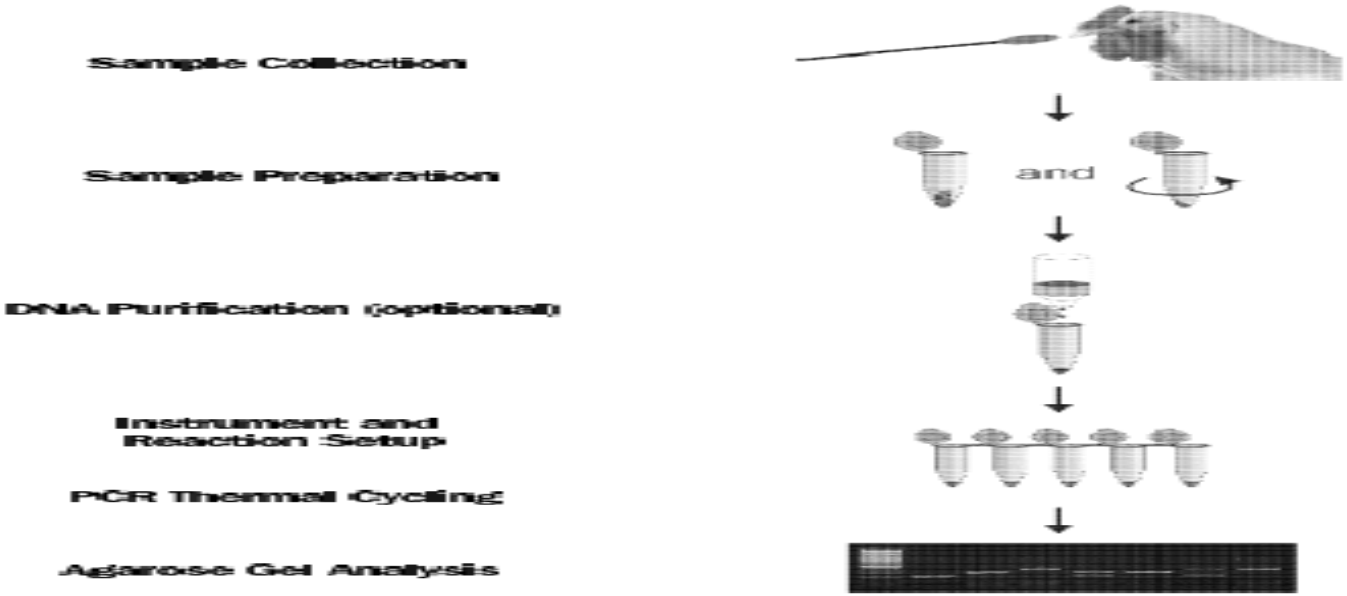
Blood Sample Collection from Chicken

Venor®MGS



100 bp DNA ladder
no template control
positive control *M. gallisepticum*
positive control *M. synoviae*
weak infection with *M. gallisepticum*
strong infection with *M. gallisepticum*
weak infection with *M. synoviae*
strong infection with *M. synoviae*
sample inhibiting PCR

Scheme of the protocol

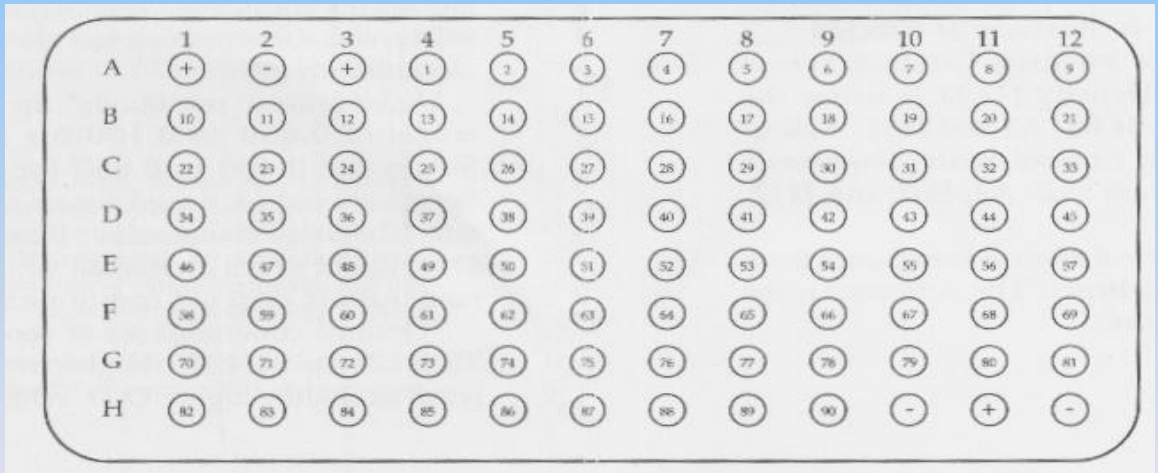


Agarose gel representation of result interpretation; b) Scheme of the protocol for PCR testing for Mycoplasma.

Manual Processing of ELISA Data

The plate was read using an ELISA plate reader set at 405- 410 nm. The average Positive Control Serum absorbance was calculated. Using the absorbance values of wells Al, A3 and H 11.

The average Normal Control Serum absorbance was calculated using values obtained from wells A2, HI 0 and H12. Both the averages were recorded.



Uncoated 96 well microtitre plate showing the serum dilution setup

The average normal control absorbance was subtracted from the average positive absorbance. The difference is the Corrected Positive Control.

The sample to positive (Sp) ratio was calculated by subtracting the average normal control absorbance from each sample absorbance.

The difference is divided by the Corrected Positive Control. The following equation format was used:

$$SP = \frac{(\text{Sample Absorbance}) - (\text{Average Normal Control Absorbance})}{\text{Corrected Positive Control Absorbance}}$$

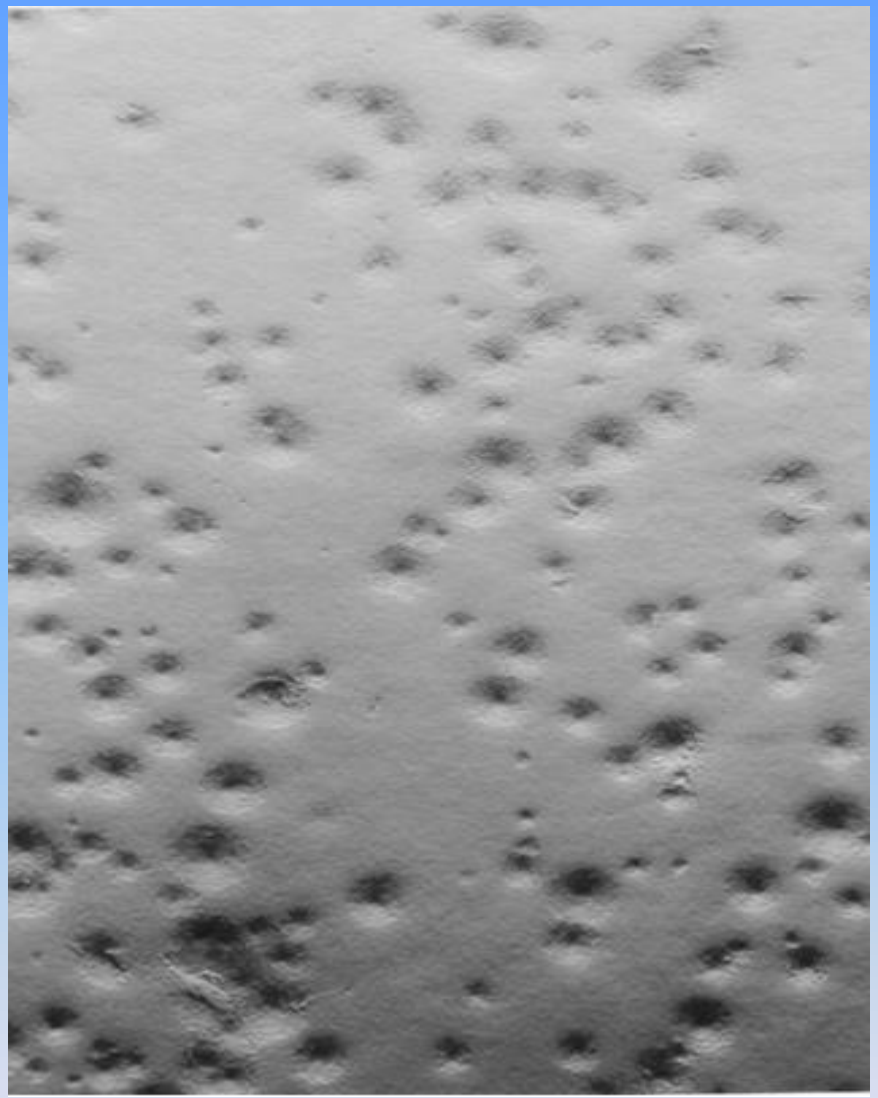
Results

Comparison between Broiler and Layer Flocks

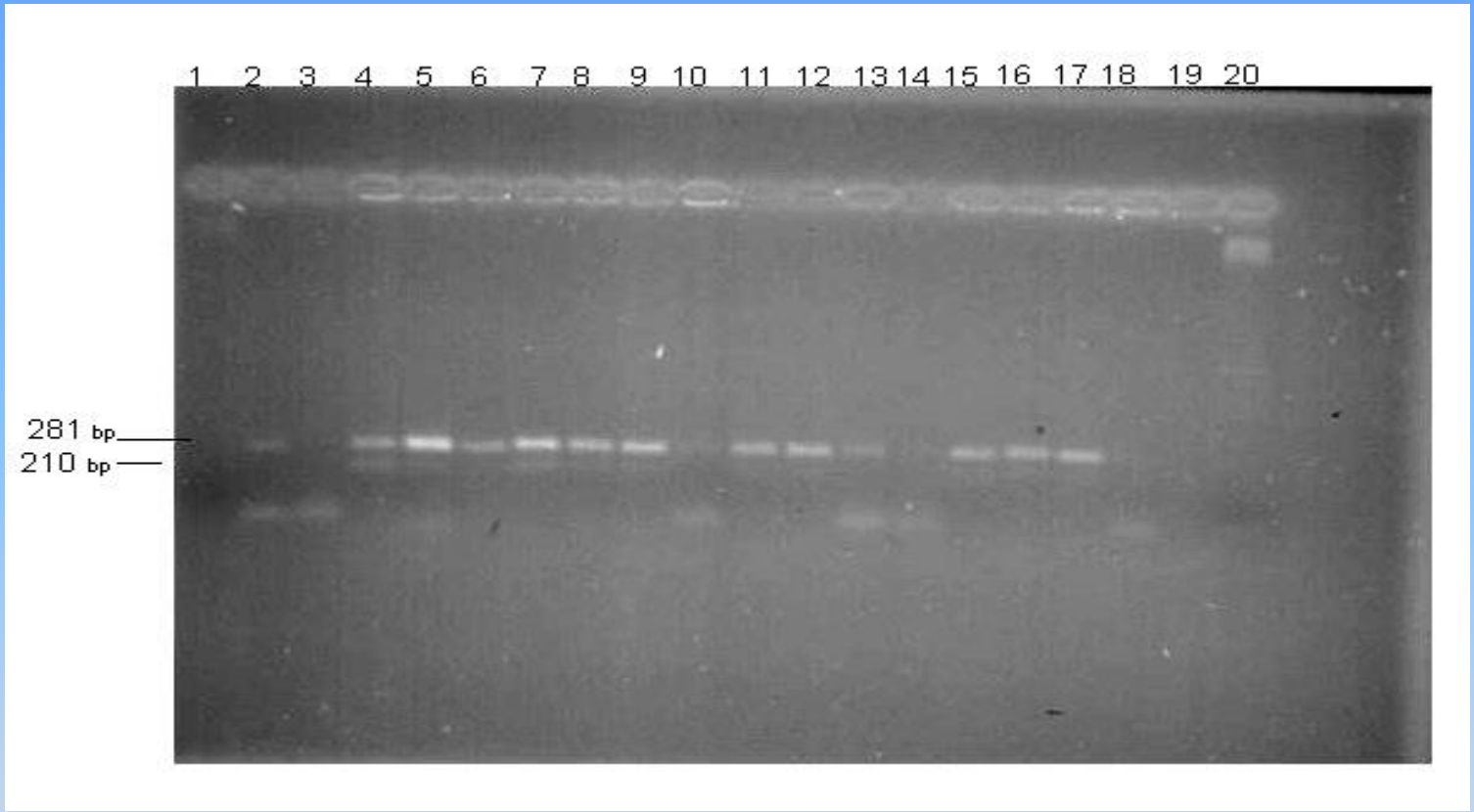
Chicken Type positive	Total Tested	Diagnostic Method Used			Total
		Culture	PCR	ELISA	
Broiler	24	2 (8%)	14 (58%)	10 (42%)	14 (58%)
Layer	26	5(19%)	15 (59%)	14 (54%)	16 (62%)

Comparison between the Testing Method in Relation to the Source of Sample

Testing Method	Total, Positive	Total Tested	Percentage (%)
PCR			
Tracheal Swab	17	30	56.6%
Choanal Cleft swab	12	20	60%
Culture			
Tracheal swab	6	30	20%
Choanal Cleft Swab	1	20	5%

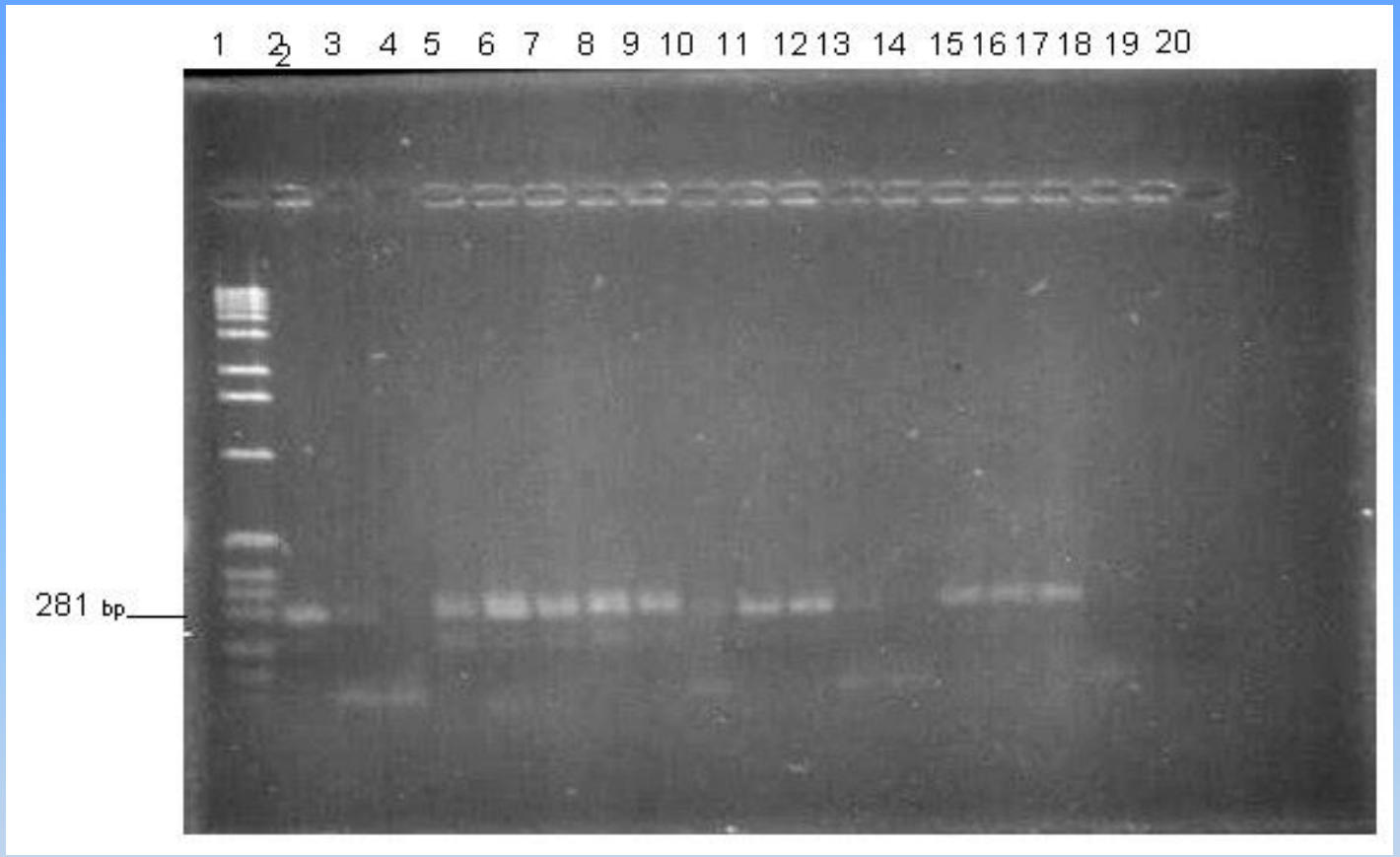


Compound light microscope image of *Mycoplasma gallisebticum* colonies on PPLO Agar Medium



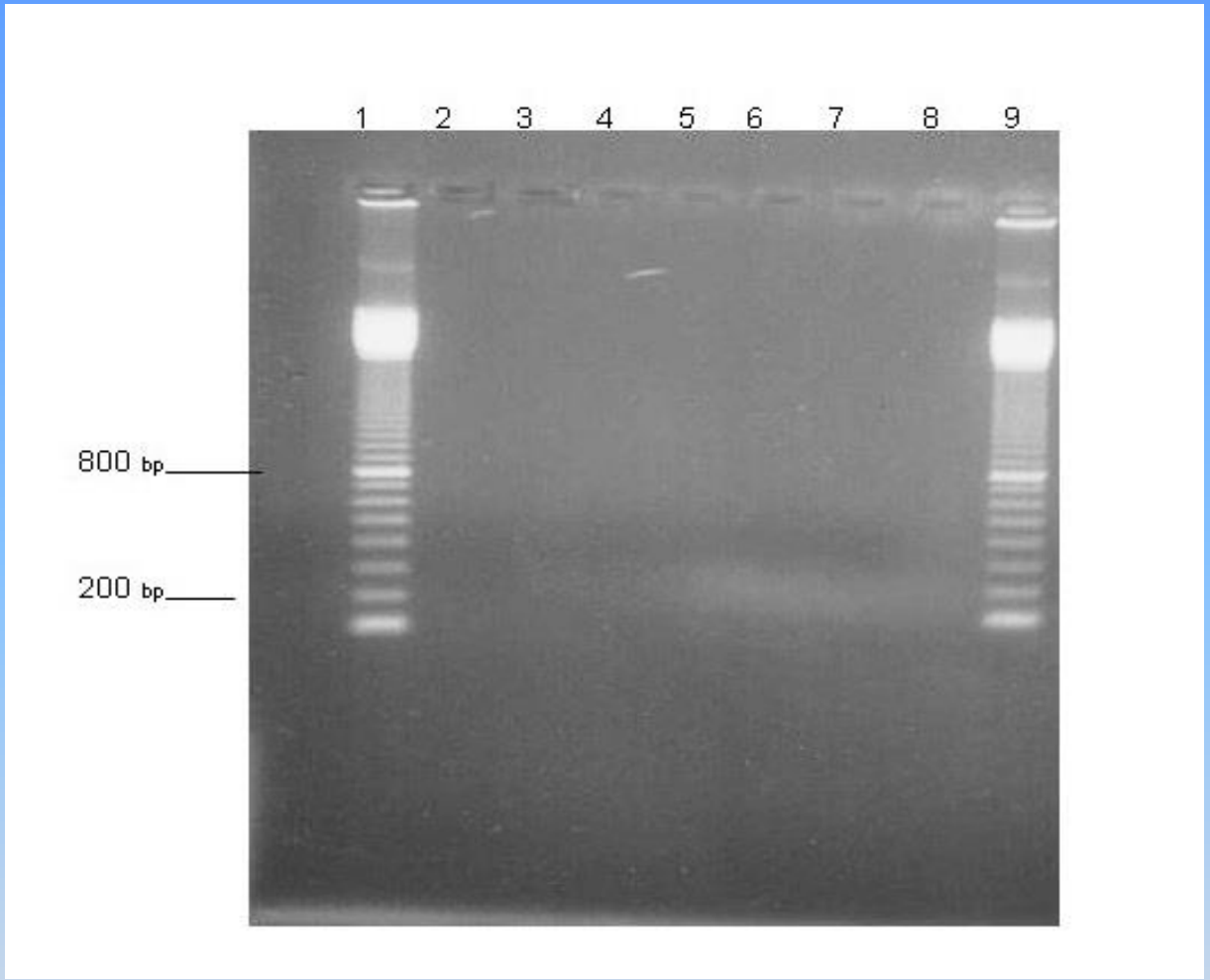
- Lane 1: DNA LCB ladder.
- Lane 2: Positive control.
- Lane 3: Negative control.
- Lane 4-19: Swab samples.
- Lane 20: DNA ladder.

PCR amplification of tracheal swab samples for chicken.



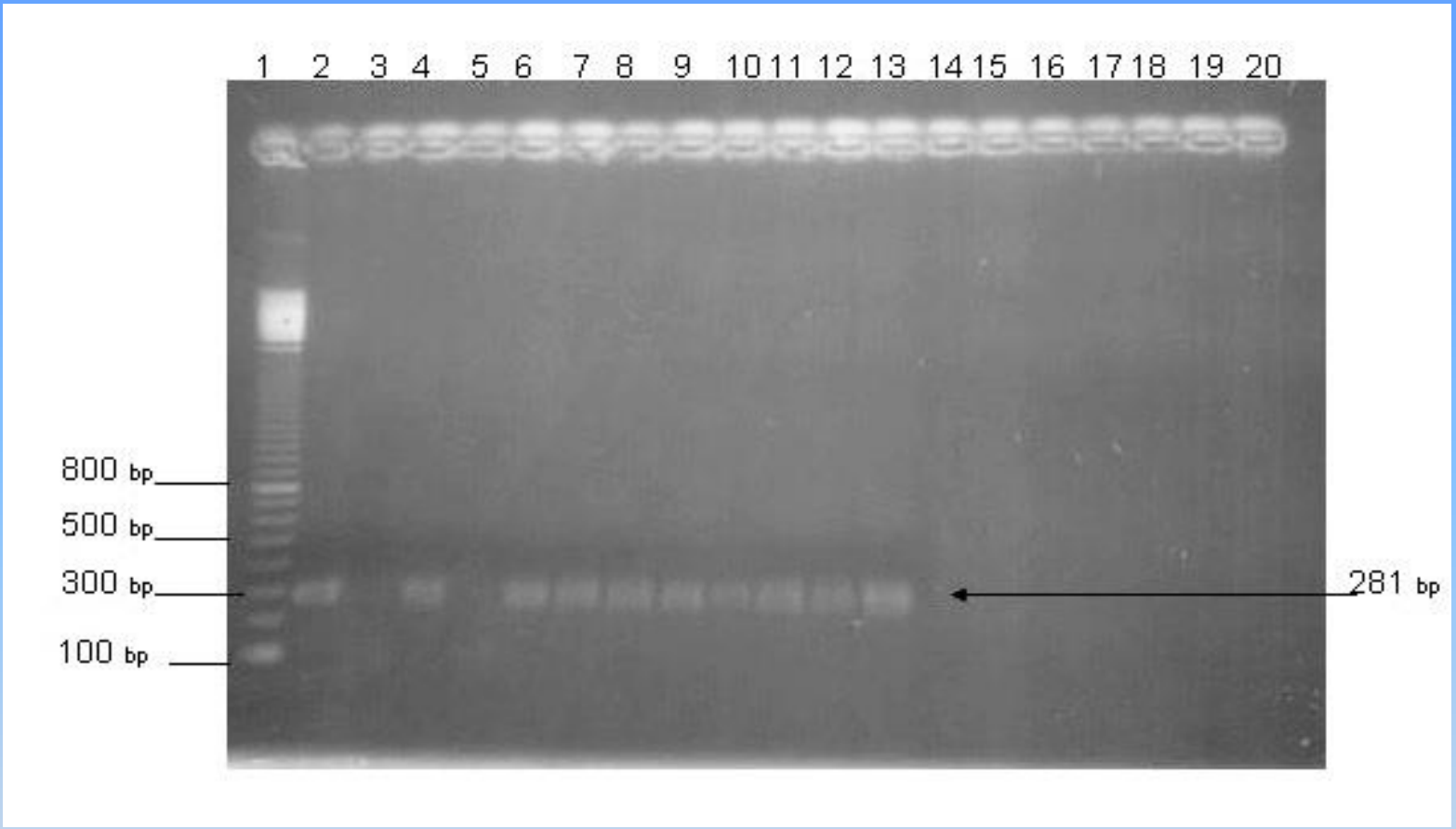
- Lane 1: DNA LCB ladder.
- Lane 2: Positive control.
- Lane 3: Negative control.
- Lane 19-20: Swab sample

PCR amplification of choanal cleft swab samples for chicken.



- Lane 1 & 9: DNA LCB ladder
- Lane 2 – 8 : Swab samples

PCR amplification of choanal cleft swab sample.



- Lane 1: DNA LCB ladder.
- Lane 2: Positive Control.
- Lane 3: Negative Control.
- Lane 4-13: swab samples.

PCR amplification of tracheal swab samples.

Conclusion

Precondition for successful production of commercial poultry free from MG includes establishment of monitoring program for MG and separation of MG infected and free flocks at the top of the production pyramid and complete separation of eggs from these flocks in the hatcheries. In addition, the impact of biosecurity has to be underlined, for this reason a very sensitive and selective methods (ELISA and PCR) of diagnosis are important.

Recommendations

Methods of testing for avian mycoplasmosis can help if used in a wide range surveillance program to establish a baseline data concerning the predominance of this disease in poultry industries in Kuwait. Such information is a prerequisite for future regional and international collaboration to identify the source of pathological agents, leading to the control of their spread among the farms in the country.

Thank You