

Early detection of Rotavirus Infection Among Calves in Kuwait Using Molecular Methods

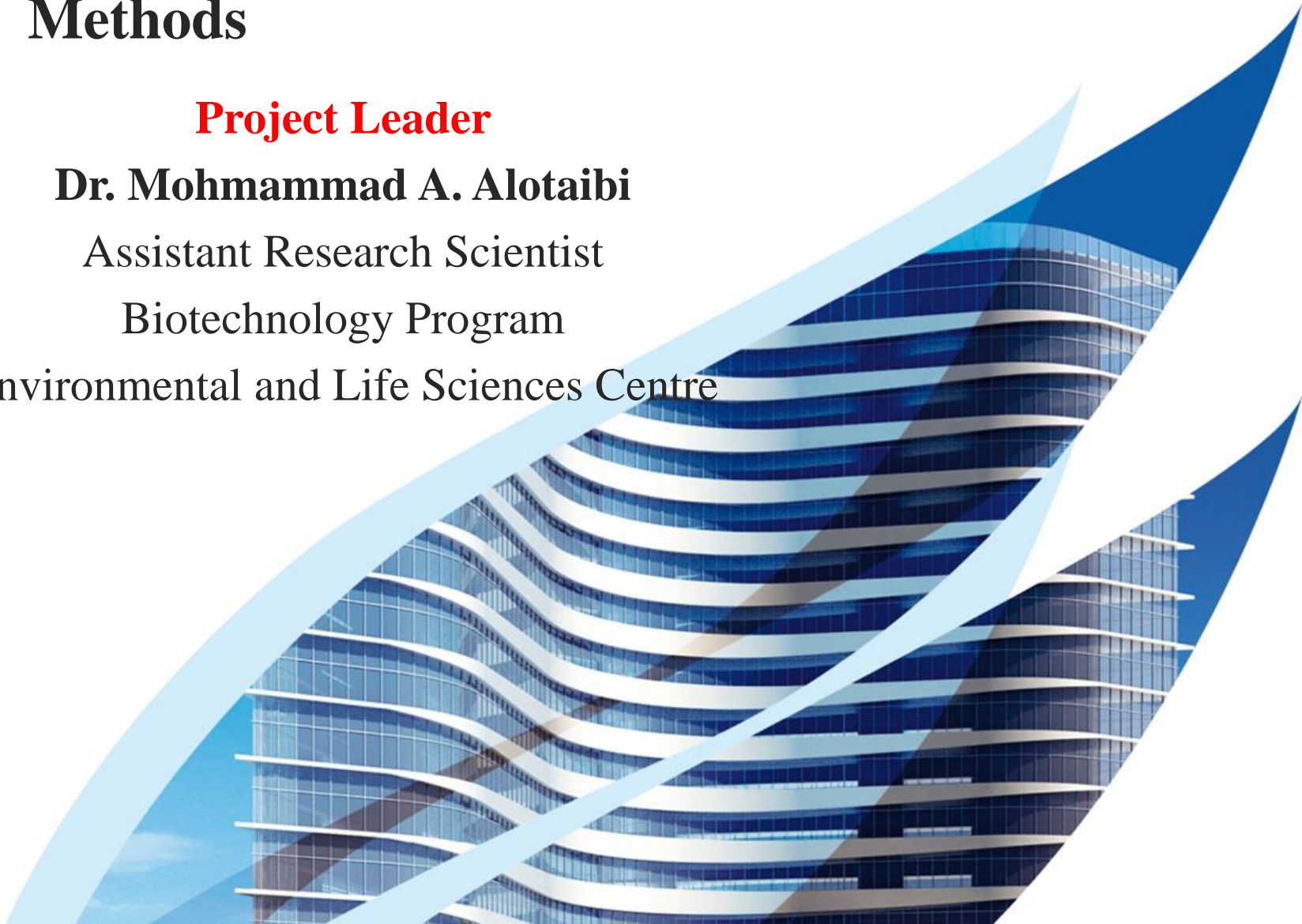
Project Leader

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Biotechnology Program

Environmental and Life Sciences Centre



Importance

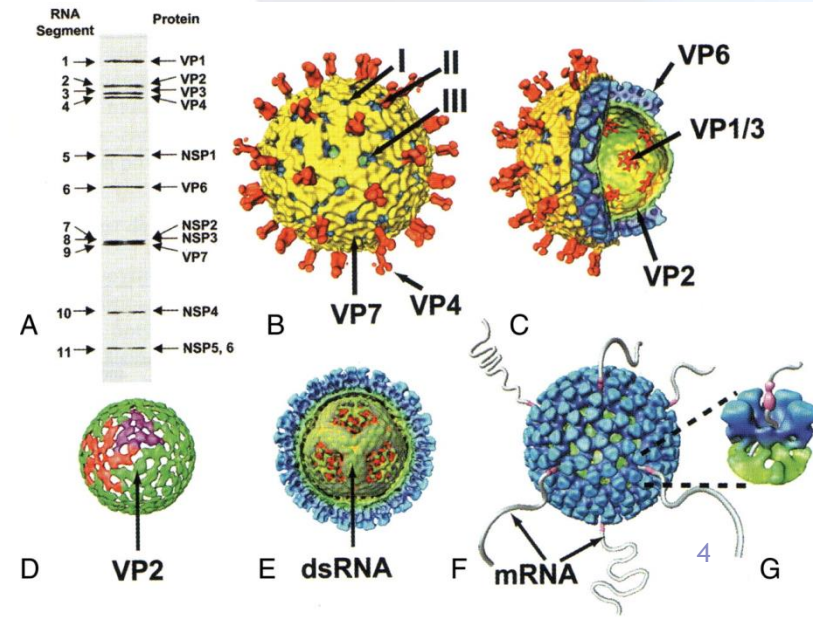
- The number of cattle Livestock in Kuwait is 27,000.
- It represent the main source of milk production in Kuwait.
- The annual milk produced in Kuwait 59,854 Ton.
- As a source of meat product.
- Food Security.

Calves pathogens

- Main pathogens that causes diarrhoea in calves include *Salmonella*, *E. coli* k99, Rotavirus, Coronavirus and *Cryptosporidia*.
- Calves mortality in some farms in Kuwait reaches 90%.

Rotavirus

- Discovered by Bishop *et al.* (1973) as non-bacterial cause of diarrhea.
- dsRNA with 11 fragments.
- There are 7 Groups (A-G).
- Groups A-C can be found in humans and animals.
- Groups E-G in animals.



Pathogenecity and Impact of Rotavirus

- It is considered the leading etiologic agent of gastrointestinal of acute diarrhoea, dehydration, acidosis leading to death.
- It causes mitochondrial swelling and shortening of intestinal villi.
- Incubation period is less than 48 hrs.
- First infection does not lead to immunity.
- Re-infection occur at any age.

Cont.

- Remains active for long time on surfaces.
- Washing with detergents does not remove it.
- Rotavirus is responsible for the hospitalization of 2.7million children annually in developed countries e.g. USA.
- More than 1 Million gastroenteritis cases were due to rotavirus B in china.
- It counts for 5% of all children deaths (WHO, 2012).

Rotavirus in Water

- It is present in raw and treated water.
- Some studies found that the virus present in 100% of the collected samples.
- It can be found in surface water.

Diagnosis methods

- Electron Microscopy (EM).
- ELISA (Enzyme linked-immunosorbent assay).
- Latex agglutination (LTA).
- RT-PCR (reverse transcriptase polymerase chain reaction).



- The project fall in the disease management in the Biotechnology program.
- A key role in the reduction of the morbidity and mortality of calves.
- Help in the reduction of the financial loss for livestock industry in Kuwait.

Project justification:

- There are no earlier studies for the detection of the virus using sensitive, specific and quick technique e.g. RT-PCR.
- Previous studies did not target rotavirus antigen specifically.
- Rotavirus type is still ambiguous.
- New techniques are required to inspect and type rotavirus groups in calves in Kuwait.
- The research output will be helpful in minimising both morbidity and mortality rates of calves in Kuwait and in controlling the spread of the disease.
- Reduction of viral spread between calves will reduce the expense of importing new dairy calves from abroad.

Objectives

- Adapt and develop molecular technique to be applied in Kuwait.
- To compare and determine the correlation between immunological (Latex agglutination) and the molecular (RT-PCR) methods.
- Provide a preventive and control measures for the spread of rotavirus for PAAF and farmers.

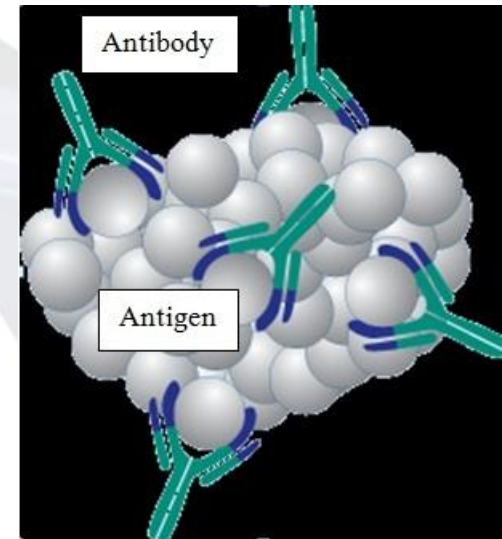
Expected outputs

- Developed RT-PCR diagnosis method for detecting rotavirus among calves.
- Establishment of an in house technical capability to handle molecular aspects of rotavirus infection.
- Developed control method measures for rotavirus infection to minimize the annual financial loss.
- Kuwaiti national professionals trained and skilled in areas of molecular diagnosis techniques.
- To build a scientific collaboration with PAAF which will reflect positively on the livestock sector in Kuwait.

Methodology

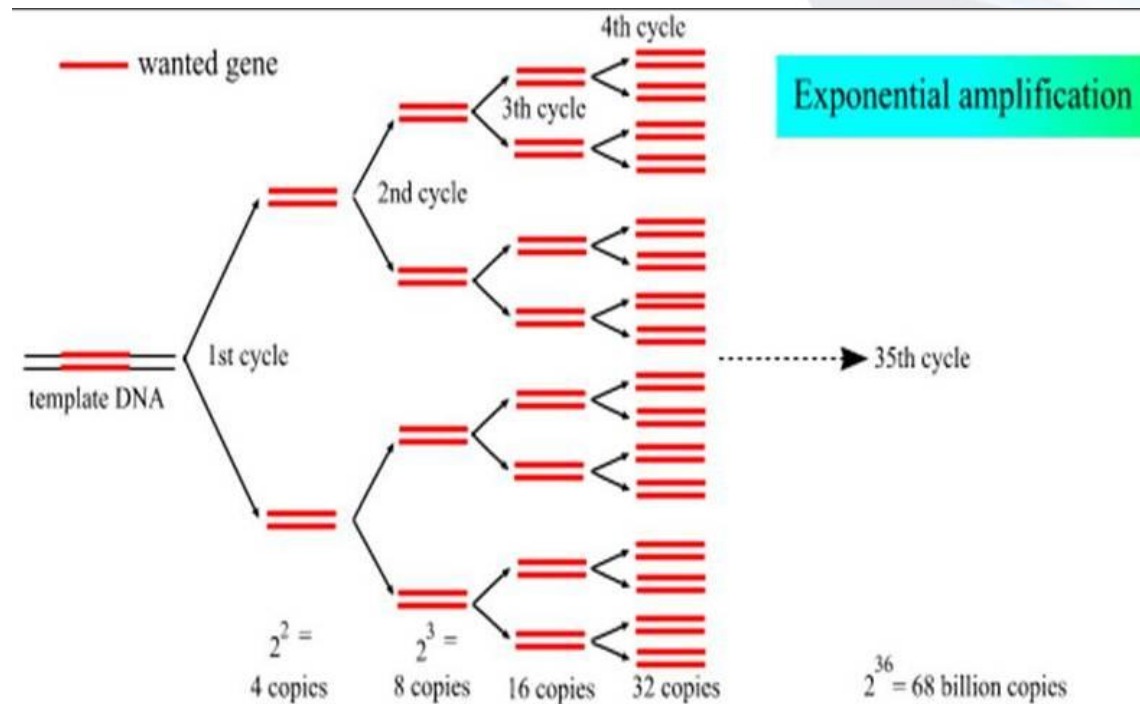
Latex agglutination

- This technique directed towards rotavirus antigen.



RT-PCR.

- Reverse transcribe viral dsRNA to cDNA.
- Amplification of cDNA to numerous copies of dsDNA.



Comparison of methods

- The comparison will determine the difference of both methods in;
 1. Sensitivity.
 2. Specificity.

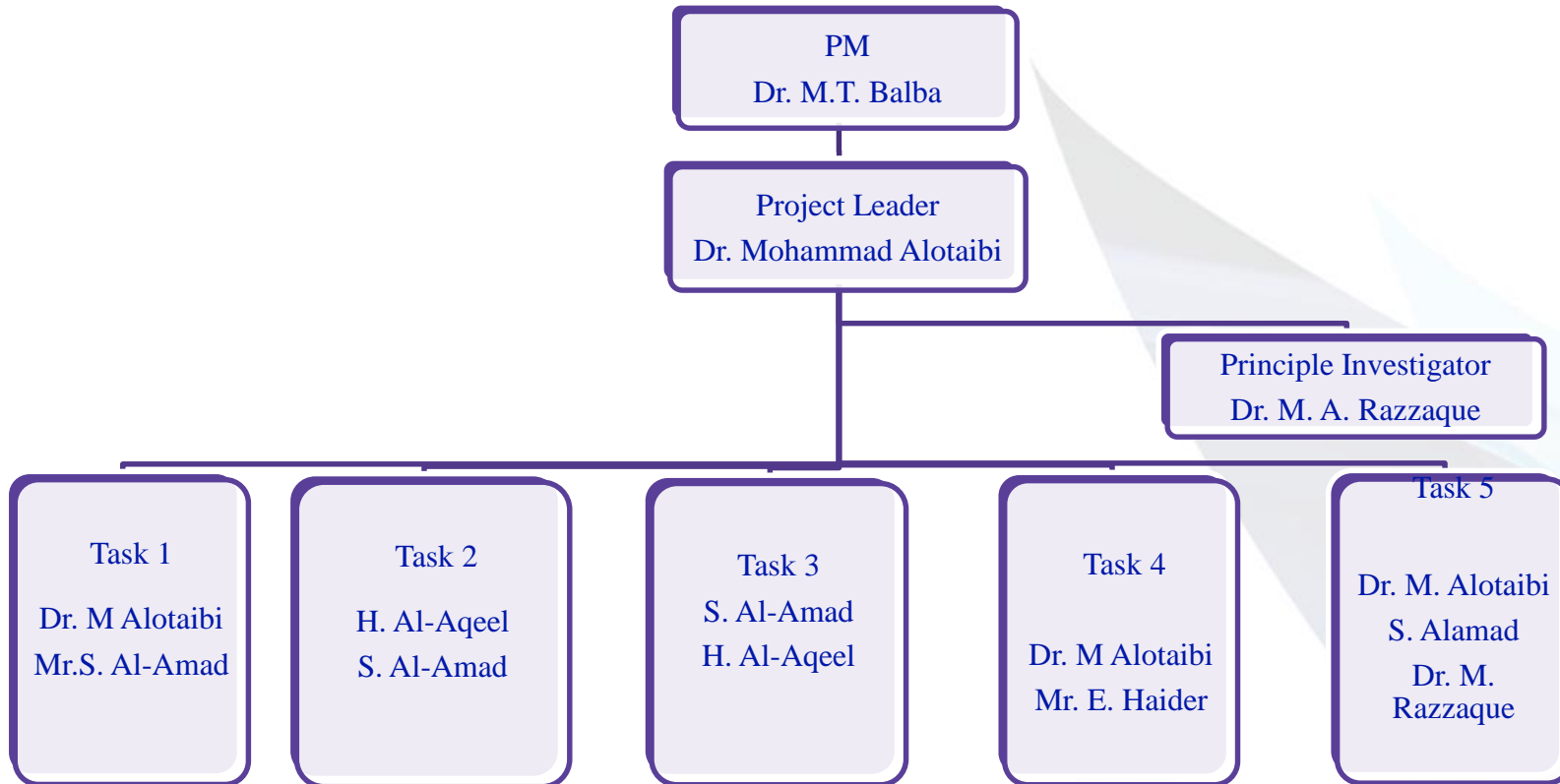
Benefits for Kuwait

- Reduction of morbidity, mortality of calves livestock and increasing the yield of both milk and meat.
- Providing preliminary baseline data (prevalence of rotavirus in calves) that will be useful to the livestock industry in Kuwait.

Project tasks

1. Mobilization, data collection and sample collection from PAAF dairy farms in Kuwait.
2. Preparation of samples for latex agglutination and RNA extraction.
3. Optimization of viral RNA extraction and detection by RT-PCR.
4. Detection of the presence of rotavirus virus antigen, using latex agglutination test, and the viral RNA, using reverse transcriptase-polymerase chain reaction (RT-PCR).
5. Sequence the amplified RNA fragment and blast it in GenBank.
6. Reporting and establishing preliminary baseline data.

Organizational chart



Researchers: Dr. Mohammad A. Alotaibi, Dr. M. A. Razzaque

Mr. Sami Al-Amad

Professionals: Mr. Hamid Al-Aqeel

Mrs. Ebtisam Haider

Technician: Ahmed Bin Hijji

Task Schedule:

Task 1: Mobilisation

Task 2: Optimization of viral RNA extraction and detection by RT-PCR.

Task 3: Isolation, purification and characterization of total viral genomic RNA from calves.

Task 4: Detection of rotavirus by latex agglutination and RT-PCR.

Task 5: Reporting.

Time \ Task	2014/2015												2015/2016											
	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.
I	■	■	■	■																				
II			■	■	■	■																		
III					■	■	■	■	■	■	■	■	■	■	■	■	■	■						
IV								■	■	■	■	■	■	■	■	■	■	■	■	■	■			
V						■						■						■		■	■	■	■	■

Task 1. Mobilisation

Task 2. Optimization of Viral RNA Extraction and Detection by RT-PCR.

Task 3. Isolation, Purification and Characterization of Total Viral Genomic RNA from Calves.

Task 4. Detection of Rotavirus by Latex Agglutination and RT-PCR.

Task 5. Reporting.

Training

- Training of KISR technicians and professionals with new techniques (e.g. Sample collection and handling, RNA isolation and characterization, Primer design and RT-PCR optimization).
- Training veterinaries and professionals in PAAF (e.g. Basics of sample collection, RNA isolation and RT-PCR).

Budget

Table 1. Detailed Budget

Expenses Category	FY 13/14	FY 15/16	TOTAL
A. Salaries:			
- Researchers	13,080	14,170	27,250
- Professionals	3,360	3,360	6,720
- Technicians	0,792	0,264	1,056
B. Support labour contract*	3,000	3,000	6,000
Sub Total	20,232	20,794	41,026
B. Operating Expenses:			
- Experimental lab supplies			
- RNA Extraction Kit	2,500	2,000	4,500
- Primers	250	1,750	2,000
- RT-PCR kit	2,000	-	2,000
- DNA staining reagents	350	-	350
- Gel electrophoresis reagents	1,250	750	2,000
- DNA purification kit	1,150	500	1,650
Subtotal	7,500	5,000	12,500
- Consultant	2,500	2,500	5,000
- Publication cost	-	800	800
- Car Rental	4,020	4,020	8,040
- Travel	1,000	1,000	2,000
- Petty Cash	500	500	1,000
Sub Total	15,520	13,820	29,340
C. Capital Expenses	-	-	-
- Ph meter	750	750	1,500
- PCR machine	6,750	-	6,750
- Refrigerated centrifuge	750	1,000	1,750
Sub Total	8,250	1,750	10,000
GRAND TOTAL	44,002	36,364	80,366

*Support labour contract should be covered by the client

Support letter from PAAF



Public Authority of Agriculture
Affairs & Fish Resources
الهيئة العامة لشئون الزراعة
والثروة السمكية



Date: التاريخ
Ref: المرجع

بقطاع الثروة الحيوانية لإسهامهم الخبرات البحثية والعلمية.
وأمزيج من التنسيق الاتصال بالسيدة/ مها الباتل - مديرة إدارة المفتحات
والبحوث الحيوانية ومدير إدارة الصحة الحيوانية (بالقدم).

تلفون: ٢٢٢٥٢٩٨٠ - موبايل: ٦٦٠٨٣٨٨٨ - فاكس: ٢٤٧٢٥٩٢٤

شاكرين لكم حسن تعاونكم ...

وقضوا بتبريل فاتح الاحرار...

المدير العام

ميداء حياي رقتيل
مديرة المدير العام لشئون الثروة
الحيوانية

Tel: 22255955 - 22253039 Fax: 22253953-22252937 البريد الإلكتروني 222527-22269222 فاكس 22252688 - 222690099
P.O. Box: 21422 Safat - 13075 Kuwait email: anitra@paa.gov.kw شارع البريد ٢٢-٢٦ الكويت



Public Authority of Agriculture
Affairs & Fish Resources
الهيئة العامة لشئون الزراعة
والثروة السمكية



Date: التاريخ
Ref: المرجع

المسيد / مدير عام معهد الكويت للأبحاث العلمية

خبر طيبة ويمن

الموضوع : طلب دعم فني

مديرة الهيئة العامة لشئون الزراعة والثروة السمكية أطيب تحياتها ...

وبالإشارة إلى الموضوع المذكور أعلاه ... وإلى كتابكم مرجع
(م ع/١٣٤٧/١٤/١٣٦٢) بخصوص تسهيل مهمة الدكتور/ محمد العتيبي
في جمع عينات من المخلفات الحيوانية من المزارع التابعة للهيئة وهذا
لمعرفة مدى انتشار فيروس (الروتا) في العجول .

يسرنا إبلاغكم بأن الهيئة العامة لشئون الزراعة والثروة السمكية ومن
منطلق تشجيع الكوادر الوطنية على إجراء البحوث العلمية من أجل تطوير
القطاعات الفنية ، ترحب بالتعاون معكم لإنجاز مشروع البحث .

كما نود أن يساهم في هذا البحث من جانب الهيئة كل من:

الطبيب البيطري/ أحمد زيدان العجمي من إدارة المختبرات والبحوث
الحيوانية والطبيب البيطري/ عيسى ظاهر الغزي من إدارة الصحة الحيوانية

Tel: 22253855 - 22263930 Fax: 22255953-22252937 البريد الإلكتروني 222527-22269222 فاكس 22252688 - 222690099
P.O. Box: 21422 Safat - 13075 Kuwait email: anitra@paa.gov.kw شارع البريد ٢٢-٢٦ الكويت

Support letter from FMPU

Tel. 24673360
Fax : 24675914
P. O. Box. 25390 Safat
Postal Code 13114
Kuwait - Arabia

اتحاد منتجي الالبان الطازجة
UNION OF FRESH DAIRY PRODUCERS

تلفون : ٢٤٦٧٣٣٦٠
فاكس : ٢٤٦٧٥٩١٤
ص.ب. ٢٥٣٩٠ الصفاة
الرمز البريدي 13114
الكويت - بلاد العرب

Date 2013/106/ أم أشارتنا: 01/9/2013

التاريخ 01/9/2013

السادة / معهد الكويت للأبحاث العلمية
المحترمين
تحية طيبة وبعد ،،،

الموضوع / مشروع فيروس الروتا

يهديكم اتحاد منتجي الالبان أطييب تحياتها ، وبالإشارة إلى الموضوع أعلاه ، وبعد اجتماعنا مع الباحث التابع لمعهدكم الموقر الدكتور / محمد العتيبي ، بخصوص المشروع المذكور أعلاه لفحص العجول والعجلات بدولة الكويت ويسرنا إن نتعاون معكم وتزويدكم بالعينات المطلوبة ومساعدتكم في البحث والكشف عن المرض في العجول والعجلات وذلك لما فيه مصلحة بلدنا الحبيبية الكويت للحد من انتشار الأمراض بقطيع الأبقار .

وتفضلوا بقبول فائق الاحترام والتقدير،،،

رئيس مجلس الإدارة
عبد الحكيم أحمد الأحمد



للاستفسار : 99882266
65650228

Future studies

- Transcribe other part(s) of rotavirus RNA and sequence it.
- Second phase of the project to cover other live stock e.g. Sheep or Poultry.
- Study other pathogens e.g. *E. coli*, *Salmonella*.

Thank you