SERO-SURVEILLANCE OF RINDERPEST IN LEBANON

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ABSTRACT

Rinderpest caused by a Paramyxoviridae Morbillivirus is considered as a highly contagious disease, which can affect the susceptible cattle. The first step in the serosurveillance consists of subjecting cattle herds to mass vaccination against Rinderpest. The countries that joined the regional programs intending to control and limit any spread of this plague undertook this action. The following step required the monitoring of the rate of immunity in the previously vaccinated cattle by conducting sero-surveillances. Consecutively, other regional programs destined to fulfill this need were planned by the Joint FAO/IAEA division as the project RAW/ 5/ 004 entitled "Support for Sero-Surveillance against Rinderpest" for the west Asia region. Lebanon located in the West Asia region and being twice subject to Rinderpest outbreaks in the last thirty years, joined both WARECC and RAW/5/004 programs. Accordingly, around 37% of the Lebanese cattle were vaccinated in a campaign organized in 1993 by the FAO and implemented by the veterinarians of the Ministry of Agriculture. Some 1249 cattle blood samples were gathered from all Lebanese districts with the help of the local veterinarians. Sera were extracted from these samples and were later on tested in the laboratory using the competitive ELISA technique. The basic principle of this test relies on the specific Rinderpest antibodies detection in the cattle sera. The obtained results following these tests showed that only around 21.6% of the total Lebanese herds are immune against Rinderpest. However, since no disease outbreaks have been reported two years after the vaccination campaign Provisional Declaration of Freedom from Disease is foreseeable in the near future.

Keywords: cattle, humoral immunity, Lebanon, Rinderpest, sero-survey, vaccination

INTRODUCTION

Rinderpest, or cattle plague, is a highly contagious disease of cattle caused by a virus belonging to the Paramyxoviridae genus of the family Morbillivirus. There are many strains of the causative virus but these are immunologically the same, a factor of considerable importance in control (Daubney, 1951). The virus is readily destroyed by heat, by drying and by disinfectants, and it will only survive for few hours outside the animal body (Olvey, 1968).

Rinderpest disease occurs naturally in ruminants and pigs but is primarily important in cattle and buffaloes. Sheep and goats are relatively resistant but can be naturally infected and would be more susceptible to a Rinderpest-like disease known as Peste des Petits Ruminants or PPR (Roeder, 1998). Transmission of RP virus occurs from infected to non-infected animals by close contact and by inhalation.

The normal case of RP has an incubation of 6 to 15 days followed by high fever, anorexia, nasal and lachrymal clear then purulent discharges indicating an inflammation. At this stage severe diarrhea sets in, arising from similar lesions in the abomasums and intestine, causing rapid dehydration. Dyspnea, along with a persistent cough, becomes evident signs before death or recovery of the animal (FAO, 1994).

For the last fifty years, RP outbreaks were recurrent each decade or so in both the African and Middle Eastern regions resulting in more or less great losses in the cattle herds (Zyskowski, 1996). In trying to control these epidemics, local governmental veterinary services conducted each its own massive vaccination campaigns along with quarantine measures. These actions were not efficient as they lacked coordination between neighboring countries and were frequently defeated by cattle importation across their borders (Crowther, 1996). Lebanon was subject to two major Rinderpest outbreaks, one during the seventies and the other during the eighties, as reported by the local Ministry of Agriculture (1971-1974; 1986).

In both instances, efforts were made by the governmental veterinarians to vaccinate the local cattle population but these were of limited efficiency mainly due to the ongoing war condition at the time (Grateau, 1993).

As Rinderpest (RP) was eradicated from most parts of the world and continued to lurk in Africa as well as in West and South Asia, the United Nations Development Program (UNDP) formulated regional projects as part of the Food and Agriculture Organization (FAO) global strategy for RP eradication. Lebanon joined one of the latter projects, namely the West Asia Rinderpest Eradication Campaign Coordination (WARECC), established in 1989. The first phase in RP control consisted of subjecting the local cattle herd to a mass vaccination campaign to eliminate or minimize the incidence of disease then stopping vaccination. In fact, a mass vaccination campaign was conducted during the 1993-1994 period covering 37.3% (16,840 heads) of the local cattle herd estimated to be then of 45.000 heads (85% female and 15% male) (Ministry of Agriculture, 1995). The distribution of the vaccinated cattle heads in the various Lebanese departments is shown in Table 1.

TABLE 1

Department	Total number Vaccinated (Heads)	Percent of existing cattle (%)
Mount Lebanon	2, 846	35.6
South Lebanon	8, 694	66.9
Bekaa Valley	2, 500	20.8
North Lebanon	2,800	23.3
TOTAL	16, 840	37.3

Distribution of the Vaccinated Cattle (Heads) in Lebanon

Source: Ministry of Agriculture, 1995

The next phase in the RP control strategy consisted of accomplishing a national serological survey in order to monitor the immune status of the local cattle herd following the previous vaccination campaign. The survey constituted an early step of a five-year regional project aiming at RP eradication from West Asian countries and coded as RAW /5 / 004 as approved by the Joint FAO/IAEA division (Zyskowski, 1996). Lebanon joined this project in 1995 and consequently an intensive blood sampling operation was started early 1996 (Crowther, 1996). It is the objective of the present study to report the outcome of the accomplished serological survey, a step crucial in determining Lebanon's cattle immune status against Rinderpest disease.

MATERIAL AND METHODS

In 1995, the size of the local cattle population of different ages and gender nearly doubled reaching a number of 77, 000 heads distributed as follows: 19% in Mount Lebanon, 29% in South Lebanon, 30% in the Bekaa Valley and 22% in North Lebanon (Ministry of Agriculture, 1995).

The adopted strategy for blood sampling was based on Dr Allen's recommendations (Allen, 1995) requesting a series of steps and resulting in the determination of 15 cattle heads per farm from, at least, 200 farms all over Lebanon. The adopted strategy would then insure a complete randomization in the blood sampling method. A special questionnaire form was prepared to assist in categorizing the immune background of each sampled farm.

The validity of the sampling size is based on the actual cattle population at the time of the survey (77 000 heads) and the percent vaccinated farms out of the total number of sampled farms. Consequently, specifically designed Epi.Info software by the joint FAO/IAEA division was used to derive the minimum sample size required at the present field conditions at 95 % confidence level. This is based on the following formula:

Sample size = n / [1- (n / population size)], according to Epi info FAO Program. Whereby: n: or Uncorrected Sample Size = Z^2 [P(1-P)] / D^2

Z: is the Standard Normal Deviate corresponding to the required Confidence level.

P: Expected Frequency

D: Deviation between P and Worst Acceptable Frequency

As recommended by the joint FAO/IAEA Division, the adopted serological test for antibody screening against RPV was the Enzyme Linked Immunosorbent Assay or ELISA. All critical reagents and relevant kits were provided through the RAW/5/004 project FAO/IAEA division by DBSL in order to standardize the conditions of running the ELISA in all WANA countries. This measure aimed at ensuring a ground result comparison between the different laboratories of the West Asian countries.

The antibody presence or absence in the collected individual blood samples is detected when running the ELISA (Anderson et *al.*, 1991), (Libeau et *al.*, 1991), basically through reading the optical density (OD) following plate preparation .A special software (EDI 2), provided by the joint FAO/IAEA division, was used to compute the recorded ODs to calculate the Percent Inhibition (PI) value for each well/sample based on the following formula:

PI = 100 - [(OD of each well / Median⁽¹⁾ OD of Cm⁽²⁾) x 100]

A PI value greater than 50 % would report the tested animal as positive (immune against RPV).

⁽¹⁾: Median OD of Cm is calculated by averaging the two intermediate ODs of the fourrecorded data.

⁽²⁾: Cm is the Rinderpest monoclonal antibody from kit.

Based on the questionnaire and PI value, the collected blood samples were to be segregated in a 2x2 table (table 2) arrangements with immunity (+ or -) and vaccination (+ or -) states as parameters. Consequently, data was quantitatively analyzed using the Chi-squared test, the Odds Ratio (OR) and the Cornfield 95% confidence interval for the determined OR to evaluate the significance of the association between immunity and vaccination for the different groups (Allen, 1995). The tests for association significance were conducted using the Epi Info software mentioned earlier.

TABLE 2

Data Used in the Computation of the Chi-Squared Test

		ELISA Results		Totals
		Positive	Negative	
Vaccination	Yes	252	328	580
	No	18	651	669
Totals		270	979	1249

At the end of the blood collection phase, a total of 1249 blood samples, representing 238 cattle farms from a total number of 138 Lebanese villages, were tested for the presence of RPV antibodies (Table 2). The immune background relative to each farm allowed for the identification of those farms previously vaccinated against RPV.

As a result of this sero-surveillance, 238 farms disseminated all over Lebanon were visited and had their cattle sampled (Allen, 1995). The gathering of this information was made possible by filling of specially designed forms for the survey after the sampling was achieved in each of the visited farms.

RESULT

Based on the information noted in the questionnaire and from the ELISA assay, the collected sera were segregated as shown in Tables 3, 4, 5 & 6.

According to Table 3, the reported cumulative percent of surveyed vaccinated farms was 42%.

This table shows that 138 villages, spread nearly all over the country, were visited in the course of the sero-surveillance exercise in which 238 farms were sampled. Exactly, one hundred farms of these (42 %) were previously visited by the Ministry of Agriculture veterinarians in the process of vaccination campaign that took place in 1993. North Lebanon department had the greatest share in farm representation with 100 farms compared to South Lebanon that was represented by 15 farms. 89 and 34 farms were from the Bekaa and Mount Lebanon respectively. This order was reversed in the rate of vaccinated farms where South Lebanon had 93.3% of its sampled farms vaccinated against only 28.0% for North Lebanon. While for Bekaa and Mount Lebanon these rates were 43.8% and 55.9% respectively.

TABLE 3

Distribution of Sampled Farms in	the Various	Lebanese Regions
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District	Caza	Sampled	Sampled	Vaccinated	Vaccinated
		Villages (No.)	Farms (No.)	Farms (No.)	Farms (%)
North	Akkar	33	52	11	21.1
Leb.	Tripoli	15	33	9	27.3
	Koura	5	9	3	33.3
	Zghorta	2	6	5	83.3
	Total	55	100	28	28.0
South	Saida	7	9	8	88.9
Leb.	Tyre	2	3	3	100.0
	Nabatieh	2	3	3	100.0
	Total	11	15	14	93.3
Mount	Metn	5	6	2	33.3
Leb.	Jbeil	4	6	3	50.0
	Aley	4	8	7	87.5
	Chouf	9	10	4	40.0
	Keserwan	3	3	2	66.7
	Baabda	1	1	1	100.0
	Total	26	34	19	55.9
Bekaa	Baalbeck	8	12	10	83.3
Valley	Zahle	6	6	5	83.3
	W. Bekaa	4	4	3	75.0
	Rachaya	25	54	9	16.7
	Hermel	3	13	12	92.3
	Total	46	89	39	43.8
Cumula	tive Total	138	238	100	42.0

Furthermore, Table 4 shows that some 1249 blood samples were gathered from all sampled farms that counted some 4097 cattle heads leading to a percentage of 30.5% of sampling. Whereas, only 580 (46.4%) of these 1249 sampled animals had reportedly been vaccinated. Also, following the c-ELISA tests operated on these samples it was determined that only 270 (21.6%) of the 1249 sampled were immune.

The data shown in Table 5 presents the percentages of animals sampled, previously vaccinated and confirmed as immune against RPV based on ELISA test in the various districts. The information is essential for the determination of the association between vaccination and immune state of the sampled cattle by calculation of both the Chi-squared test and the Odds Ratio test. In the qualitative analysis of data (protection rate in this case) the Chi-squared test is generally used. This test determines the significance of the association between the established four sets of cattle samples detailed in Table 5. Moreover, another test,

the Odds Ratio (OR) is calculated so as to determine which vaccinate status is responsible for this immunity: Positive or negative vaccination.

Nevertheless, 252 (20.2%) cattle were revealed to have immunity from a previous vaccination. And 328 (26.3%) cattle were found not immune though they were vaccinated and they represent 26.3%.

TABLE 4

Vaccination and Immune Status of Samples from the Various Visited Farms

District	Caza	Total No.	%	%	%
21501100	Culu	of Animals	Sampled Vaccinated		Immune
		in Sampled	Animals	Sampled	Sampled
		Farms	1	Animals	Animals
	Akkar	444	$16.4^{1}(73)^{2}$	$30.1^3(22)$	$16.4^4(12)$
North	Tripoli	402	10.7(43)	32.6(14)	23.3(10)
Leb.	Koura	231	7.4(17)	64.7(11)	17.6(3)
	Zgharta	101	11.9(12)	58.3(7)	50.0(6)
	Total	1178	12.3(145)	37.2(54)	21.3(31)
	Saida	1053	26.5(279)	22.6(63)	11.8(33)
South	Tyre	135	49.6(67)	61.2(41)	19.4(13)
Leb.	Nabatieh	58	69.4(40)	75.0(30)	40.0(16)
	Total	1246	31.0(386)	34.7(134)	16.1(62)
	Metn	164	64.0(105)	53.3(56)	8.6(9)
	Jbeil	60	25.0(15)	53.3(80	33.3(5)
Mount	Aley	121	82.6(100)	57.0(57)	19.0(19)
Leb.	Chouf	260	40.8(106)	40.6(43)	13.2(14)
	Keserwan	113	51.3(58)	46.5(27)	31.0(18)
	Baabda	28	10.7(3)	100.0(3)	66.7(2)
	Total	746	51.9(387)	50.1(194)	17.3(67)
	Baalbeck	260	36.9(96)	76.0(73)	25.0(24)
	Zahle	142	44.4(63)	68.2(43)	65.1(41)
Bekaa	W. Bekaa	156	32.7(51)	64.7933)	19.6(10)
	Rachia	285	23.1(58)	15.5(9)	17.2(10)
	Hermel	84	75.0(63)	63.5(40)	39.7(25)
	Total	927	35.7(331)	59.8(198)	33.2(110)
Grand	Total	4097	30.5(1249)	46.4(580)	21.6(270)

 $^{1}73$ / 444 x 100 = 16.4% 2 Actual Number of cattle $^{3}22$ / 73 x 100 = 30.1% $^{4}12$ / 73 x 100 = 6.4%

However, only 18 (1.4%) cattle heads were immune though they did not receive any vaccination. Also, 651(52.1%) of sampled cattle were non-vaccinated and non-immune.

Table 6 reveals the portions of each age category out of the total cattle population sampled. Consequently, it was found that the greater than two years category covers 78% of

the total sample whereas both one to two years and less than two years cover respectively 9% and 13%. These proportions are essentially due to the fact that farmers reluctant whenever blood withdrawal was to be done on their few cattle because of fear of future production losses.

TABLE 5

Detailed Immune Status of Collected Cattle Sera

District	Caza	Vacc./ Imm. (cattle heads)	Vacc./ Non Imm. (cattle heads)	Non Vacc./ Imm. (cattle heads)	Non Vacc./ Non Imm. (cattle heads)
	Akar	12	10	0	51
North	Tripoli	10	4	0	29
Leb.	Koura	3	8	0	6
	Zgharta	6	1	0	5
	Total	31	23	0	91
	Saida	32	31	1	215
South	Tyre	11	30	2	24
Leb.	Nabatieh	15	15	1	9
	Total	58	76	4	248
	Metn	9	47	0	49
	Jbeil	5	3	0	7
Mount	Aley	19	38	0	43
Leb.	Chouf	12	31	2	61
	Keserwan	15	12	3	28
	Baabda	2	1	0	0
	Total	62	132	5	188
	Baalbeck	22	51	2	21
	Zahle	38	5	3	17
	W. Bekaa	10	23	0	18
Bekaa	Rachia	9	0	1	48
	Hermel	22	18	3	20
	Total	101	97	9	124
Grand	Total	252	328	18	651
Percentages (%)		20.2	26.3	1.4	52.1

DISCUSSION

The vaccination campaign against Rinderpest started effectively in May 1993 following the TCP LEB2254 E agreement and ended early May 1994. Nearly two years later (February 1996) a sero-surveillance was initiated. Consequently, and taking these facts into consideration, the minimum age of the previously vaccinated cattle was around 22 months at least since no vaccination could be effected before two weeks of age.

The importance of age distribution resides in the fact that it helps explaining the results previously showed in Table 5. In fact, both sets of vaccinated animals either immune or non-immune representing respectively 20.2% and 26.3% of the sample size belong to the greater than two years category.

TABLE 6

Dept.	Age Group	Set A ⁴	Set B ⁵	Set C ⁶	Set D ⁷	Total
North	<1 Year	0	0	3	1	4
Leb.	1 - 2 Years	0	0	0	4	4
	> 2 Years	31	23	0	86	140
South	<1 Year	0	0	0	35	35
Leb.	1 - 2 Years	0	0	1	35	36
	> 2 Years	58	76	0	178	312
Mount	<1 Year	0	0	5	74	79
Leb.	1 – 2 years	0	0	0	35	35
	> 2 Years	62	132	0	79	273
	<1 Year	0	0	5	40	45
Bekaa	1 - 2 Year	0	0	4	37	41
	> 2 Year	101	97	0	47	245
Total		252	328	18	650	1249

Immune Status of Cattle Sera with Age Distribution

⁴Set A : Vaccinated and Immune Cattle. ⁵Set B : Vaccinated and non Immune Cattle. ⁶Set C : Non Vaccinated and Immune Cattle. ⁷Set D : Non Vaccinated and non Immune Cattle.

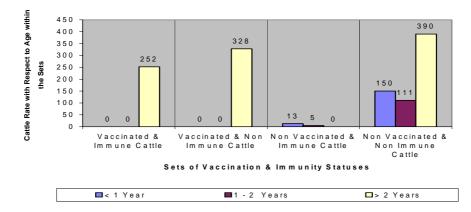


Figure 1 : Correlation Between Age & Vaccination and Immunity Statuses

Figure 1. Correlation between age & vaccination and immunity statuses.

Also, the majority of 14%, representing the non-vaccinated and immune cattle set, belongs to the less than one year category (exactly 13 animals of the 18), whereas the rest (5 animals) belong to the one to two years category. However, the 52.1%, representing the non vaccinated and non immune cattle set, is divided among the three age categories: 150 cattle in the less than one year category, 111 cattle in the one to two years category and 390 in the greater than two years category. Figure 1 presents age, vaccination and immunity statuses sampled cattle.

As expected all sampled cattle aged two years or more, and coming from vaccinated farms were immune, (Table 6). However 328 (33.8%) of this age group (set B0 were negative. This may be due to recently introduced animals to the farm or simply, because these may have not received, a proper vaccine dose during the vaccination campaign (faulty vaccine administration).

As for the calves, (less than one year of age) immunity status varied between immune and non-immune. The immunity of the non-vaccinated calves (Set C: $13/163 \times 100 = 8.0 \%$) was clearly conferred to them through their maternal colostrums. In fact, such immunity is not for life and may last three months to eight months at most depending on the quantity of colostrums fed. (Daubney, 1951) stated that a calf fed by a proper amount of colostrums will enjoy immunity for eight months before the depletion of the antibodies whereas another one fed with smaller quantities of colostrums will be immune for three months only. On the other hand, the calf whose dam was not vaccinated, or which did not receive any immune colostrum, will have no immunity at all. In fact, such situation is illustrated in Table 5, by a percentage of 92.0% (Set D: $150/163 \times 100 = 92.0\%$).

For the sampled cattle aged between one to two years, the majority of negative results were expected whereas a percentage of 95.7% was observed (Set D: $111/116 \times 100 = 95.7\%$). The reason for the non-immune status of this age group is due to non-vaccination of these cattle and their maternal immunity has been depleted long before. Exceptionally, some 4.3% (Set C; $5/116 \times 100 = 4.3\%$) of the sampled animals aged between one to two years had immunity. This fact may be due to recent introduction of imported vaccinated animals.

The official report of vaccination following the 1993/1994 campaign as stated by Hilan (1985) around 16 840 vaccinations were executed at a time when cattle population was about 45000 heads. Accordingly, the expected percent of immune cattle is around 37.33%. The sero-surveillance discussed in the present study was accomplished with the help of the regional veterinarians, who directed most of their sampling toward the relatively accessible and familiar farms. In fact, this was clearly reflected by the rate of sampled vaccinated farms that amounts (42.0%) (Table 1), compared to the percentage of sampled vaccinated cattle (46.4%) (Table 3). Accordingly, the expected frequency of vaccinated animals would be around 50%.

This figure is used in computation of the sample size, which was determined by the Epi-Info software provided by the Joint FAO/IAEA for the statistical analysis of data. Consecutively, some 1052 random samples are sufficient to demonstrate with a 95% confidence that the expected frequency of vaccination lies between 47 and 50%. In fact, exactly 1249 samples (Table 3) were randomly gathered from all Lebanese regions, which confer a high level of representativeness to this surveillance (Allen, 1995).

The immunity rate according to the results of the tests is around 21.6% (Table 3) whereas some 47 to 50% of immunity should have been observed following the vaccination rate. This percentage of 21.6% is not far from reality for two major reasons.

The first reason is that there has been an increase in cattle population due to importation. The declared vaccination rate in 1994, which averaged around 37.33% represented the cattle population that was about 45000 heads. So, a decrease in this rate is expected following the increase in the cattle population to about 77000 heads as due to importation.

The second reason is that most probably not all cattle vaccinations were successful mainly due to the high sensitivity of the Rinderpest vaccine to climatic high temperatures. Indeed, one bottle of diluted vaccine must be preserved at low temperature (5 to 8°C), away from light and from any bacterial contamination during all the vaccination procedure. Also, it must be used within maximum one and a half-hour after it has been diluted. Moreover, this vaccine in its freeze-dried status must be preserved at a constant 4°C temperature (Grateau, 1993). This matter can be easily detected in Table 5 in comparing the rate of vaccinated samples reaching 46.4% while that of the effectively immunized ones reaching 21.6% only.

In the quantitative analysis of data the Chi-squared test is generally used. This test will determine the significance of the association between the established four sets of cattle samples detailed in Table 5.

This test was conducted using the epi-info program as well, where data were introduced to a (2x2) table as in Table 2.

After computing these data, the results of the Uncorrected Chi-Squares test and the Yates corrected Chi-Squares test were respectively 304.57 and 302.17. Knowing that the degree of freedom of a 2x2 table is one and that the critical value for one degree of freedom is alpha 0.01=6.63 and since the Yates corrected values is greater than 6.63, it is concluded that the present association is a highly significant one. Furthermore, both uncorrected and Yates corrected probability values of 0.000000, is smaller than the 0.01, the theoretical value, a fact that ascertains the strength of this association. Explicitly, this strong association indicates that the immunity in the Lebanese herd is essentially linked to its vaccination status.

Moreover, another test, the Odds Ratio (OR) is to be calculated so as to determine which vaccination status is responsible for this immunity: For positive or negative vaccination, the Odds Ratio (OR) value, calculated by the Epi-Info software, is of 27.79 which is greater than one, a fact that implies once more that a positive association exists between both immunity and vaccination statuses. Also, Epi-Info calculates the Cornfield 95% confidence interval for the OR which ranges between 16.56 and 47.20.

Interpretation of these results shows that the immunity status of the Lebanese herd is 27.79 times more due to vaccination than to non vaccination (Acquisition of maternal antibodies in this case). Also, it is 95% confidant that the OR ranges from 16.56 and 47.20 contains the true value.

CONCLUSION

This sero-surveillance has achieved its goals by the fulfillment of all the objectives stated in the RAW5/004 IAEA project.

Furthermore, the work that has been achieved so far is sufficient to put Lebanon on the track for a *Provisional Declaration of Freedom from Disease* state using the OIE Pathway. Since the last vaccination campaign, applied more than two years ago, not a single case of Rinderpest has been reported. Any Rinderpest outbreak can be easily monitored and controlled because of the awareness of the local breeders to the dangers of this disease and the great distance between neighborly farms.

It is recommended to establish a system for confirming diagnosis of Rinderpest and to operate a sero-surveillance on small ruminants (sheep and goats) to determine whether they passively carry the Rinderpest virus that can be easily transmitted to cattle in case of cohabitation.

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